Environmental Toxicology and Chemistry, Vol. 23, No. 11, pp. 2756–2762, 2004
© 2004 SETAC
Printed in the USA
0730-7268/04 $12.00 + .00

PLANT UPTAKE AND TRANSLOCATION OF HIGHLY WEATHERED, SOIL-BOUND TECHNICAL CHLORDANE RESIDUES: DATA FROM FIELD AND RHIZOTRON STUDIES

MARYJANE INCORVIA MATTINA,*† BRIAN D. EITZER,† WILLIAM IANNUCCI-BERGER,† WEN-YEE LEE,† and JASON C. WHITE‡
†Department of Analytical Chemistry, ‡Department of Soil and Water, The Connecticut Agricultural Experiment Station, 123 Huntington Street, New Haven, Connecticut 06511, USA

(*Received* 21 *October* 2003; *Accepted* 17 *April* 2004)

Abstract—It has been observed that plants are susceptible to uptake from soil and in planta transport of technical chlordane, in spite of its hydrophobicity and sequestration within the soil matrix due to weathering. Field and rhizotron studies were conducted with Cucurbitaceae planted in highly weathered, chlordane-contaminated soil to investigate details of soil-to-plant contaminant uptake. In the field-work, *Cucurbita pepo* L. (zucchini) was grown in soil at four levels of chlordane contamination: Clean (<limits of quantitation, 5 ng/g), low (average, 370 ng/g), medium (average, 1,951 ng/g), and high (average, 4,572 ng/g). The analysis of plant tissues (root, stem, leaf, and fruit) resulted in the detection of chlordane consistently at the highest concentration in the root tissue at each level of soil contamination. As the soil chlordane concentration increased, the average chlordane concentration in the root tissue increased as follows: Clean, 370 ng/g; low, 8,130 ng/g; medium, 21,800 ng/g; high, 29,400 ng/g. Further analysis of the field-grown plants showed distinct differences in both the proportional distribution of chlordane among the plant tissues and the pattern of the chlordane residues in each tissue type. These differences are attributed to plant uptake from soil versus uptake from air. In the rhizotron studies, uptake of chlordane residues by *C. pepo* L. was compared with that of another Cucurbitaceae, *Cucumis sativus* L. (cucumber). Xylem sap from the rhizotron-grown plants was collected and analyzed for chlordane, in addition to determination of chlordane residues in soil, roots, and aerial plant tissue. Component fractions and enantiomer fractions of both chiral and achiral chlordanes were followed through soil, root, xylem sap, and aerial tissue compartments. They indicate that the xenobiotic residues translocate enantioselectively from the soil matrix into and through the plant environment with genera-specific patterns. The determination of chlordanes at ng/g concentration explicitly for the first time in the xylem sap of plants grown in contaminated soil confirms the presence of a soil-sequestered and highly hydrophobic organic contaminant within the aqueous plant environment.

Keywords—Persistent organic pollutants Soil-to-plant uptake Rhizotrons Xylem sap

INTRODUCTION

The latter half of the 20th century saw the synthesis of a wide range of organo-halogen compounds, significantly increasing the number of such chemicals well beyond their natural occurrence. Some of these anthropogenic chemicals were introduced intentionally into the biosphere as insecticides or termiticides (e.g., DDT and technical chlordane), or as herbicides (e.g., benefin and trifluralin). Some, which were intended for industrial and other applications (e.g., polychlorinated biphenyls [PCBs] and flame retardants, such as the polybrominated diphenylethers), entered the environment accidentally or from casual disposal procedures. The synthesis of other organo-halogens, such as the dioxins and furans, was never intended, but rather resulted from incomplete incineration of a myriad of chemicals or from industrial byproducts.

In spite of the broad structural and compositional variety of the anthropogenic organo-halogens, there is sufficient overlap of the physico–chemical properties of most of these compounds to describe them generically as hydrophobic, highly persistent in certain environmental compartments, and sufficiently volatile for atmospheric transport to disperse them from the site of original application to remote locations. A consequence of such properties is that a large subset of the organochlorines has become the focus of international concern under the designation persistent organic pollutants (POPs), resulting in an international treaty addressing their use and dispersal [1,2].

For several years our laboratory has studied the cycling through the biosphere of POPs originally applied as agrochemicals, specifically the organo-chlorines, DDT, and technical chlordane. For the former chemical, we have focused on the metabolite, 2,2-bis(*p*-chlorophenyl)1,1-trichloroethylene (*p,p′*-DDE), and in the latter case, the multicomponent technical chlordane formulation has permitted us to track both chiral and achiral chlordane component residues as they transfer from one contiguous environmental compartment to another. We have shown that those chlordane components present in the largest amounts in the originally applied technical formulation (*trans*-nonachlor [TN], *trans*-chlordane [TC], and *cis*-chlordane [CC]) have the longest half-lives in sandy loam soil, $t_{1/2}$ = 22 years [3]. All three compounds may be regarded as highly hydrophobic, having $\log K_{ow} > 6$ [4]. Therefore, they are expected to partition strongly to soil organic matter, reinforcing their tendency to be sequestered within the soil matrix as they weather over time [5]. Within the rhizosphere, the physico–chemical properties of the chlordanes are expected to drive their partitioning to the root lipid bilayer from the soil pore water [4]. Although the systemic distribution of some pesticides, which enter plants through the roots, has been re-

* To whom correspondence may be addressed (maryjane.mattina@po.state.ct.us).

ported [6], the transport into and through the aqueous plant system of soil-bound and highly hydrophobic chemicals such as chlordanes would not be anticipated.

By surveying a wide range of crops, we have demonstrated that plants within the Cucurbitaceae family especially are adept at the uptake and transport of soil-bound DDE and chlordane, in spite of the hydrophobic properties of these POPs [7–10]. In the present report, the focus is on the Cucurbitaceae, specifically *Cucurbita pepo* L. and *Cucumis sativus* L., under two different experimental schemes. The objective of the field study was to determine how the levels of chlordane components in *C. pepo* L. roots and aboveground plant tissues vary as the chlordane levels in the soil vary. The soil chlordane concentrations ranged from below the limits of quantitation ([LOQ] = 5 ng/g) to approximately 4,500 ng/g. In the second scheme, *C. pepo* L., *C. sativus* L., and *Lupinus albus* cv. Homer (lupin) were grown in rhizotrons in a greenhouse environment. The objective of the rhizotron study was to permit controlled and in situ access to soil and plant compartments, allowing us to collect and analyze xylem sap, in addition to the operationally defined root and aerial plant tissues. Chlordane components were detected in the xylem sap of all three plant types. These data reinforce the argument for a soil-to-plant uptake route. In addition, the enantioselective patterns of the chlordane components traced through the soil, root tissue, xylem sap, and aerial tissue compartments have implications for the mechanisms of uptake and in planta transport of xenobiotic, soil-bound compounds.

MATERIALS AND METHODS

2001 field study

During the summer of 2001, a dose/uptake study was established on the campus of the Connecticut Agricultural Experiment Station in a field plot that has been described previously in detail [3]. The experiments were conducted in a portion of this plot that has been under cultivation since 1998 [7,10].

Eight plastic bins, of approximately 100-L capacity, were positioned in the field plot and assigned completely at random to one of four levels of soil contamination. Each bin was prepared by filling with a 2.5-cm layer of marble chips, and overlaid with fiberglass screening and then with approximately 95 L of soil. Drainage holes were drilled in the side of the bins below the level of the screening. The containers were sunk into the tilled portion of the field so that 5 cm of the vessel remained above the soil surface. The soil immediately downslope from each bin was excavated to provide access to the drainage holes.

Four soils were prepared by mixing various amounts of chlordane-contaminated soil from the field plot with a non-contaminated soil. The soils were mixed thoroughly in a small electric cement mixer for 2 min; final concentrations of each composited soil were in good agreement with predicted mixing values. All soil mixtures were classified as sandy loam with organic matter content ranging from 4 to 6.5%. Duplicate bins were prepared at each final chlordane concentration level (clean, $<$ 5 ng/g; low, 323 and 418 ng/g; medium, 1,991 and 1,911 ng/g; high, 4,512 and 4,633 ng/g, all on dry wt basis). The completely randomized design with two replicates at each of four treatments permitted the data to be subject to analysis of variance (ANOVA).

Zucchini seeds, *C. pepo* L. cv. Black Beauty (Seedway, Hall, NY, USA), were sown in the bins on June 1 and thinned to five to eight plants per bin. Fruit were harvested at marketable size (~y 350 g wet wt) and the plants harvested destructively 68 d from the date of sowing. The field soil surrounding the bins, containing approximately 4,000 ng/g chlordane, was well mulched throughout the growing season.

Air samples in the vicinity of the plants were collected throughout the growing season using a high-volume air sampler (HI-Q Environmental Products, San Diego, CA, USA) at 0.6-m height above the soil. The sampler was equipped with a glass fiber filter preceding a polyurethane foam plug. Air samples were collected over 24-h periods at an average flow rate of 0.35 m^3/min.

Rhizotron studies

Rhizotrons [11] were prepared from polyvinylchloride pipe (48 cm lengths $\times$ 12 cm diameter) cut in half lengthwise. A plexiglass semicircle was fitted to the bottom of the rhizotron and a plexiglass cover to the long dimension; each rhizotron was filled with approximately 3.5 kg of chlordane-contaminated soil.

In the first experimental trial, two rhizotrons were filled with chlordane-contaminated soil and each planted with eight to ten plants of white lupine, *L. albus* cv. Homer (seeds supplied by Edzard van Santen, Auburn University, Auburn, AL, USA) or *C. pepo* L. cv. Black Beauty. This trial was used principally for method development. In the second trial, eight rhizotrons were prepared with the chlordane-contaminated soil and planted with either *C. pepo* L. cv. Black Beauty or *C. sativus* L. cv. Marketmore 76 (Seedway). For each of these species, two rhizotrons were planted with approximately ten plants in a dense regime, and two rhizotrons were planted with a single plant in a nondense regime. The rhizotrons were placed in a greenhouse and watered twice a day using an automated system. Plants were grown for eight weeks after which time plant stems were severed and the xylem sap was collected from individual plants. Exudate collection was followed by destructive harvest of the roots.

Extraction methods for chlordane in soil, plant tissue, air

Soil, plant tissue, and air samples were extracted by previously published methods [10]. Soil and plant tissue samples were extracted twice; each air sample was extracted once. Briefly, soil was spiked with 50 μl of an internal standard solution containing 4 μg/ml $^{13}C_{10}$ TC + 2 μg/ml $^{13}C_{10}$ TN (Cambridge Isotope Laboratories, Andover, MA, USA) in toluene. Microwave-assisted extraction of 3-g soil was performed using 2:3 hexane:acetone. Plant tissue was spiked with 50 μl of internal standard, extracted with 2-propanol and petroleum ether, washed with water and saturated sodium sulfate, and cleaned up on Florisil® (U.S. Silica, Berkeley Springs, WV, USA).

Air samples collected on polyurethane foam plugs were spiked with 25 μl of internal standard, Soxhlet extracted in petroleum ether for 16 h, concentrated to less than 10 ml, and cleaned up on Florisil. The glass fiber filters used in the high volume air samplers were found to have nondetectable or trace quantities of chlordane and subsequently were not analyzed.

Xylem sap collection

During sap collection, a drip-flow watering system was used to assure that the roots and soil in the rhizotron remained moist. Single plant stems of the rhizotron-grown plants were cut approximately 1 cm above the soil level on an angle; the initial

flow of liquid was wicked carefully onto a tissue. Using a 5-μl Wiretrol capillary (Drummond Scientific, Broomall, PA, USA), the xylem sap was collected into an acid-washed 14-ml glass vial over monitored time periods.

Extraction methods for chlordane in xylem sap

The toluene-based internal standard solution described above was diluted first into methanol, and then into distilled water to produce an aqueous solution containing internal standards at 20 ng/μl of each labeled component. One hundred μl of this solution was transferred to a 1-ml subsample of each sap in acid-washed conical vials and septum sealed. The solution was sampled using solid-phase microextraction (SPME fibers). A 65-μm PDMS-DVB fiber (Supelco, Bellefonte, PA, USA) precleaned (by thermal desorption) was inserted through the septum directly into the sap solution. The fiber and solution were sonicated for 75 min. The fiber was then desorbed thermally in the injection port of the gas chromatograph/mass spectrometer (GC/MS) for subsequent analysis of chlordanes.

Analysis methods for chlordane

Chlordane was quantitated on a Saturn 2000 Ion Trap GC/MS system (Varian, Sugar Land, TX, USA) equipped with a γ-DEX-120 column (Supelco; 30-m × 0.25-mm i.d. × 0.25-μm film thickness). A deactivated silica guard column (0.5 m × 0.25 mm) was attached before and after the analytical column with press-tight connectors (Restek, Bellefonte, PA, USA). The GC oven was programmed as follows: Initial temperature 120°C, hold 1 min; ramped at 20°C/min to 155°C; ramped at 0.5°C/min to 195°C; ramped at 20°C/min to 230°C, hold 21.6 min. The injection port was maintained at 230°C, and a 3-μl splitless injection was used. For the analysis of SPME fibers, the GC program was modified as follows: The injection port temperature was set to 260°C; the injector was set for a 4-min splitless injection; GC oven program was 100°C for 4 min, 20°C/min to 155°C, 0.5°C/min to 196°C, 20°C/min to 225°C, hold for 7.8 min; flow pressure settings: Initial pressure 25.2 pounds per square inch (psi), 41.6 psi/min to 35.6 hold for 3.5, 41.6 psi/min to 25.2, 2 psi/min to 29.2, 0.04 psi/min to 32.4, 2 psi/min to 35.1 hold 10.65 min.

The mass spectrometer conditions remained the same for both sets of GC conditions and were: 38-min filament delay; emission current 60 μA; target total ion current 5,000 counts; maximum ionization time 25,000 μs; multiplier offset +200 V; and scan range *m*/*z* 345 to 425.

A set of calibration standards containing TC, CC, TN (ChemService, West Chester, PA, USA) were prepared in iso-octane at 10, 25, 50, 100, 250, 500, and 1,000 μg/L. Each solution contained TC, CC, and *exo*-heptachlor epoxide (*exo*-HEPX, a chiral chlordane metabolite) at the cited concentration, TN at one-half the cited amount. Every calibration solution also contained 50 μg/L of each labeled component: $^{13}C_{10}$-TN and racemic $^{13}C_{10}$-TC. For each instrumental run of 10 to 20 soil, plant, or air samples, a complete set of standards was injected twice, once before and once after the sample extract injections. Each soil and plant extract was injected once; each air extract was injected twice. Iso-octane was injected after every five to six sample injections in order to lengthen column lifetimes.

For xylem sap analysis, 1-ml aliquots of water containing 0.5, 1, 2.5, and 5 μg/L TN, TC, and CC along with 1 μg/L of $^{13}C_{10}$-TC and $^{13}C_{10}$-TN were prepared. These water-based standards were analyzed using the same SPME technique as the xylem saps described above and used to develop the calibration curves for the sap quantitation.

Chiral assignments of TC, CC, and *exo*-HEPX were made using injections of the pure enantiomers (EQ Laboratories, Atlanta, GA, USA). Because the exact chiral assignment for the enantiomers of MC-5, a chiral, octachlordane, is unknown, the enantiomers are designated MC-5A and MC-5B. Representative chromatograms have been published previously [12,13]. The elution order of the chlordanes of interest in this study under the specified conditions was −*exo*-HEPX, +*exo*-HEPX, TN (achiral), +TC, −TC, −CC, +CC, MC-5A, MC-5B.

Data reduction and data criteria have been described previously [10,12]. PeakFit (SPSS, Chicago, IL, USA) was used to integrate extracted ion chromatograms. Isotope ratios of two ions from the most intense ion cluster, $(M\text{-}Cl)^+$, for each compound were required to be within ± 2 standard deviations of the mean average determined from the two standard sets injected along with the samples. For each ion peak, the S/N ratio exceeded 2.5. If any component did not meet these acceptance criteria, the entire injection was rejected. A standard reference soil (Environmental Research Associates, Arvada, CO, USA) was analyzed periodically for quality control. Percent recovery typically exceeded 95%. All concentrations for soil and plant tissue are reported on a dry weight basis. With the data criteria above, the limits of quantitation for each component were set at: 0.01 ng/m^3 in air; 1 ng/g in soil; 5 ng/g in plant tissue; 0.2 ng/ml in sap. For each injection of an extract, ±TC, ±CC, TN, MC-5A, MC-5B, and ±*exo*-HEPX were quantitated using the ±TC, ±CC, TN components as the chlordane sum for a given sample. Quantitation of the optically active component of technical chlordane, MC-5, and the rationale for the five-component sum, have been discussed elsewhere [3,10].

RESULTS AND DISCUSSION

2001 field study

The 2001 field study was a dose/uptake study, designed to approximate normal field cultivation conditions of *C. pepo* L. as closely as possible. The chlordane residues in four tissue compartments of *C. pepo* (roots, stems, leaves, fruit) across the chlordane concentrations in soil will be discussed from three different perspectives.

For the first perspective we present in Figure 1 log-transformed chlordane concentrations in each of the four plant tissue compartments for each of the three levels of contaminated soil. Plants grown in clean soil are not included in this plot. A tabulation of the raw data set from which this graph was prepared is available in Lee et al. [14]. To support our contention that conditions for the containerized 2001 field study closely resemble typical field conditions, data from the direct field sowing of *C. pepo* L. in the Connecticut Agricultural Experiment Station plot during the 2000 growing season [10] are included in Figure 1.

A two-way factorial ANOVA (soil concentration × tissue type, log-transformed data) showed a significant interaction ($p = 0.008$). Concentration of chlordane in the fruit did not respond to soil concentration in the same manner as the other three tissue types. As is evident in Figure 1, an increase in soil chlordane concentration results in higher concentration of the contaminant in each of the four tissue compartments examined ($p < 0.001$). This increase of chlordane concentration in plant tissue with increased soil concentration differs from a previously reported inverse relationship between these two

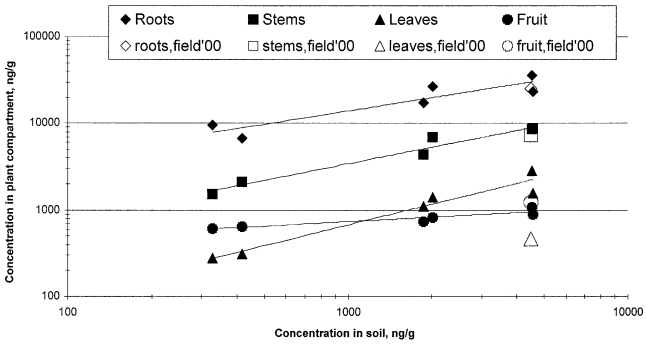

Fig. 1. Log-transformed chlordane concentration in *Cucurbita pepo* L. tissues versus soil concentration for the 2001 bin study and 2000 field study. Regression equations do not include 2000 field data: Roots, $y = 393.6x^{0.516}$; stems, $y = 41.92x^{0.637}$; leaves, $y = 2.769x^{0.796}$; fruit, $y = 223.2x^{0.173}$.

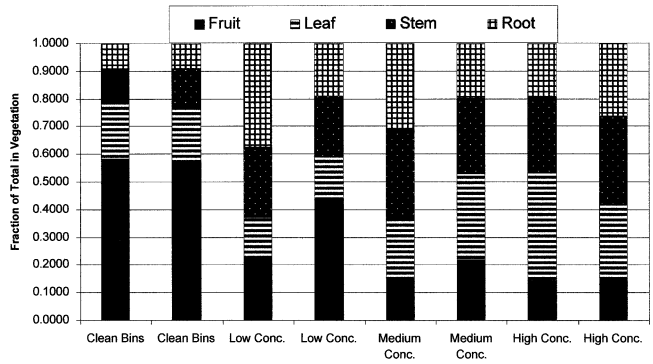

Fig. 2. Proportional amounts of chlordane in *Cucurbita pepo* L. tissues across soil chlordane concentrations.

variables [15]. However, in the previous study, soil chlordane concentrations were less than 200 ng/g and the higher of the two soil concentrations examined was only 1.4 times that of the lower concentration. In the present study, soil chlordane concentrations spanned an order of magnitude. Based on the data presented in Figure 1, the previously reported variability in summer squash tissue chlordane concentration would be anticipated for the examined soil values. The present experiment establishes the positive correlation between chlordane concentration in the soil (for the soil type used in these trials) and that in *C. pepo* L. tissues.

In the second approach to examining the data, we compared the proportional distribution of the contaminant among the various plant tissues (root, stem, leaf, fruit) across the chlordane concentrations in the soil. This approach was prompted by the small, but quantifiable, amount of chlordane residues in the plant tissues of *C. pepo* L. grown in the noncontaminated, containerized soil in the 2001 field study (see Table 2 in Lee et al. [14]). We were interested in determining if the data from the 2001 field study indicate any differences in the chlordane distribution pattern in plant tissues related to the two uptake routes, air-to-plant versus soil-to-plant.

For plants growing in each bin, the absolute amount of chlordane (ng) in each tissue type was determined from the concentration in that tissue and the total dry weight of that tissue. The proportionate amount in the tissue type was then calculated from the formula given in Equation 1

$$\text{Proportion in tissue type } j = \frac{\text{ng in tissue type } j}{\sum_j \text{ng in tissue type } j} \quad (1)$$

This data set provides the relative amount of chlordane stored in each plant tissue type for *C. pepo* plants grown in each of the eight bins in the trial. The 32 entries in this data set are presented in Figure 2. As seen in Figure 2, the proportion of the total concentration of chlordane present in the fruit from plants grown in the clean bins is higher than for the fruit grown in any of the contaminated soils. This proportion of chlordane in the fruit changes from an average of 0.15 for plants grown in the high-concentration soil to an average of 0.58 for plants grown in the clean soil. Single-factor ANOVA shows that this proportionate amount of chlordane in the fruit is significantly different ($p < 0.05$) among the four treatments. If ANOVA is repeated omitting the values from the clean soil, the differences for plants grown in the three levels of contaminated soil are not significant ($p > 0.05$). Because the plants grown in the clean bins are contaminated primarily by the air-to-plant route, we conclude that the air-to-plant route impacts the distribution of the contaminant to *C. pepo* L. fruit very differently and proportionately more than does the soil-to-plant route.

The third perspective for examining the data also was intended to distinguish airborne chlordane uptake from soil-bound uptake. In this case we examined whether the pattern of the chlordane residues within each plant tissue type in *C. pepo* L. differs between one uptake route versus the other. Atmospheric chlordane concentrations above the soil within the vicinity of the bins were determined at 0.6-m height above the field surface throughout the growing season (detailed examination of the atmospheric concentrations above the field plot have been published elsewhere, see Eitzer [16]). All plants grew within this 0.6-m height. Comparisons are made based on component fractions (CF), defined in Equation 2 where C represents concentration, and presented in Figure 3.

$$\text{CF} = \frac{C_{\text{single component}}}{C_{(+)\text{TC}} + C_{(-)\text{TC}} + C_{(+)\text{CC}} + C_{(-)\text{CC}} + C_{\text{TN}}} \quad (2)$$

Figure 3A shows the average data from the plants grown in the duplicate bins filled with clean soil, which are contaminated primarily by air-to-plant transfer; and Figure 3B averages data from plants grown in duplicate bins containing high-concentration, chlordane-contaminated soil, which are contaminated primarily by soil-to-plant transfer. The results in panel B are typical for plants grown in high, medium, and low chlordane-contaminated soil. Although there was no significant difference ($p > 0.05$) in the root, leaf, and fruit biomass for plants grown in soils at the four different chlordane concentrations, there is a distinct difference in the CF patterns across the four tissue types between the two panels of Figure 3, in particular for TN. Clearly, in planta processes for the two routes of exposure produce different component fractions in the vegetative tissues.

Cucurbita pepo L. tissues likely are impacted by both uptake routes, a soil-to-plant as well as an air-to-plant route [14]. The absolute and relative chlordane concentrations in the plant tissues will reflect uptake via both these independent routes [17]. We hypothesize that the relative magnitude of the two routes for a given plant species depends on several factors, including but not limited to: The quality and quantity of root exudates [18]; soil-contaminant concentration versus air-contaminant concentration; leaf surface area; and plant health.

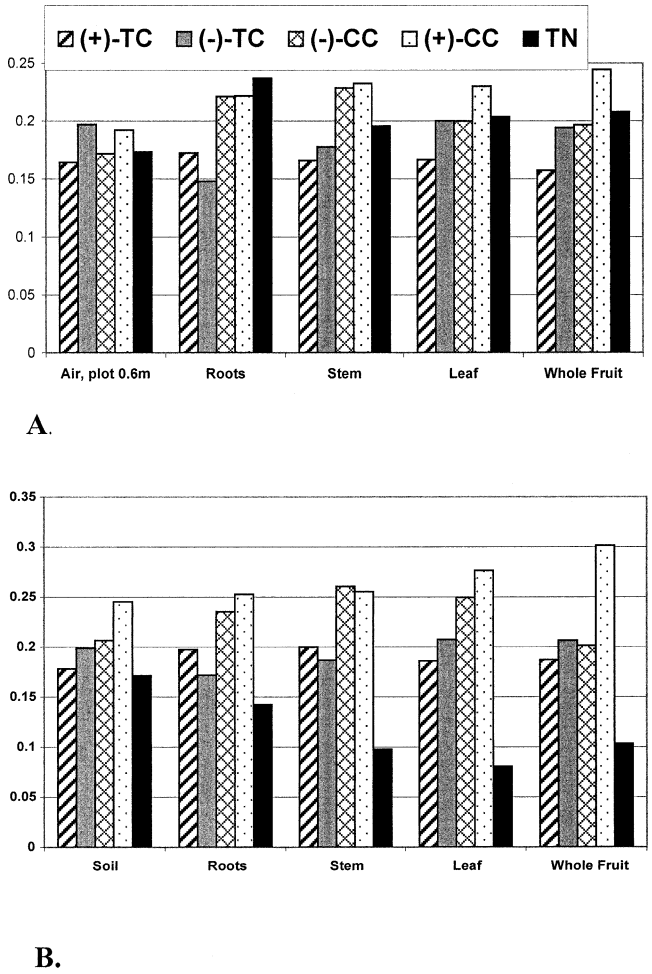

Fig. 3. Component fractions (see text) for *Cucurbita pepo* L. tissues. (**A**) Plants grown in clean soil. (**B**) Plants grown in high-chlordane–contaminated soil. TN = *trans*-Nonachlor, TC = *trans*-chlordane, and CC = *cis*-chlordane.

Rhizotron studies

Although growing conditions for *C. pepo* L. grown in rhizotrons differ from those grown in field soil, the chlordane concentrations observed for rhizotron-grown plants are in reasonable agreement with those shown on the dose-uptake curves of Figure 1. The soil used in the rhizotron studies was contaminated with chlordane at 3,350 ng/g; the *C. pepo* L. roots from 37,600 ng/g to 52,000 ng/g, and the *C. pepo* L. aerial tissue from 2,220 ng/g to 3,900 ng/g in the rhizotron-grown plants. The aerial tissue concentrations from the rhizotron plants fall between the dose-uptake curves for stems and leaves from the 2001 field study in Figure 1.

The collection of xylem sap via capillary action from severed stems [19,20] permits the analysis for chlordane in a plant matrix other than whole plant tissue for the first time. As seen in Table 1, significant quantities of chlordane compounds are present in these saps, from *C. pepo* L. and *C. sativus* L., under both dense and nondense cultivation regimes. It also should be noted that in addition to its presence in sap from the Cucurbitaceae, chlordane was identified in the xylem sap from *L. alba* cv. Homer, although it was not quantitated.

Not only are the quantities of chlordane in the xylem sap significant, but the patterns of the chlordane components differ for *C. pepo* L. versus *C. sativus* L. xylem sap, illustrated in Figure 4. This figure shows a pair of selected ion mass spectral traces for typical *C. pepo* L. and *C. sativus* L. xylem sap samples for comparison with the components in the rhizotron soil and in the root and aerial tissues. At the top of the figure, the enantiomeric pattern for *exo*-HEPX is presented. For both *C. pepo* L. and *C. sativus* L. root tissues, the pattern in the soil and that in the tissue is the same. Examination of the trace for the *C. sativus* L. sap, however, indicates a clear shift in enantiomeric fraction (EF), defined in Equation 3,

$$\mathrm{EF} = \frac{C_{+\mathrm{enantiomer}}}{C_{+\mathrm{enantiomer}} + C_{-\mathrm{enantiomer}}} \tag{3}$$

toward more of the −*exo*-HEPX enantiomer, while the EF in *C. pepo* L. sap slightly favors enhanced +*exo*-HEPX quantities. The EF values for *exo*-HEPX for both *C. pepo* L. and *C. sativus* L. in the soil were not significantly different from those in the root ($p > 0.05$). However, there was a significant difference in *exo*-HEPX EF values for both *C. pepo* L. and *C. sativus* L. root versus sap ($p < 0.05$).

At the bottom of the figure are shown the selected ion traces for the achiral TN and the chiral components TC, CC, and MC-5. Once again, for whole root tissues of *C. pepo* L. and *C. sativus* L., the pattern is the same as that in the soil. However, there is a notable shift in EF for the MC-5 components in the *C. pepo* L. xylem sap away from racemic, while the MC-5 components in the *C. sativus* L. xylem sap are well below LOQ. Although less dramatic, shifts in the EF for the CC enantiomers also are noted in *C. pepo* L. versus *C. sativus* L. xylem sap. The EF values for CC for both *C. pepo* L. and *C. sativus* L. in the soil were not significantly different from those in the root ($p > 0.05$). Again, there was a significant difference in EF values for CC for both *C. pepo* L. and *C. sativus* L. root versus sap ($p < 0.05$).

From the chlordane patterns in the whole root tissue, it is likely that the residues derive largely from these compounds that are adsorbed to the hydrophobic root exterior. The residues in the sap, however, derive from the chlordane components that have been enantioselectively displaced from the soil into the plant interior.

Table 1. Chlordane concentration (ng/ml) in xylem sap averaged across rhizotrons by species and growing regime

	ZND[a]	ZD	CND	CD[b]
+TC	7.59	2.68	3.94	2.35
−TC	7.29	3.31	3.93	3.27
−CC	12.65	3.58	7.39	5.00
+CC	15.91	5.29	6.71	4.18
TN	4.50	1.93	5.19	3.96
+*exo*-HEPX[d]	32.0	7.93	6.90	9.69
−*exo*-HEPX	17.67	4.80	8.45	13.02
MC-5A	2.57	1.07	[c]	[c]
MC-5B	5.97	2.27	[c]	[c]

[a] Z = Zucchini; ND = nondense regime.
[b] C = Cucumber; D = dense regime.
[c] Four of five samples were nondetect and one was close to limits of quantitation.
[d] *exo*-HEPX = *exo*-heptochlorepoxide.

CONCLUSION

These studies confirm that uncomplexed, soil-bound, anthropogenic chlordane is taken up and transported within the aqueous plant interior in spite of its highly hydrophobic nature. Furthermore, the transport mechanisms in planta of the residues reflect enantioselective pathways that may be species-

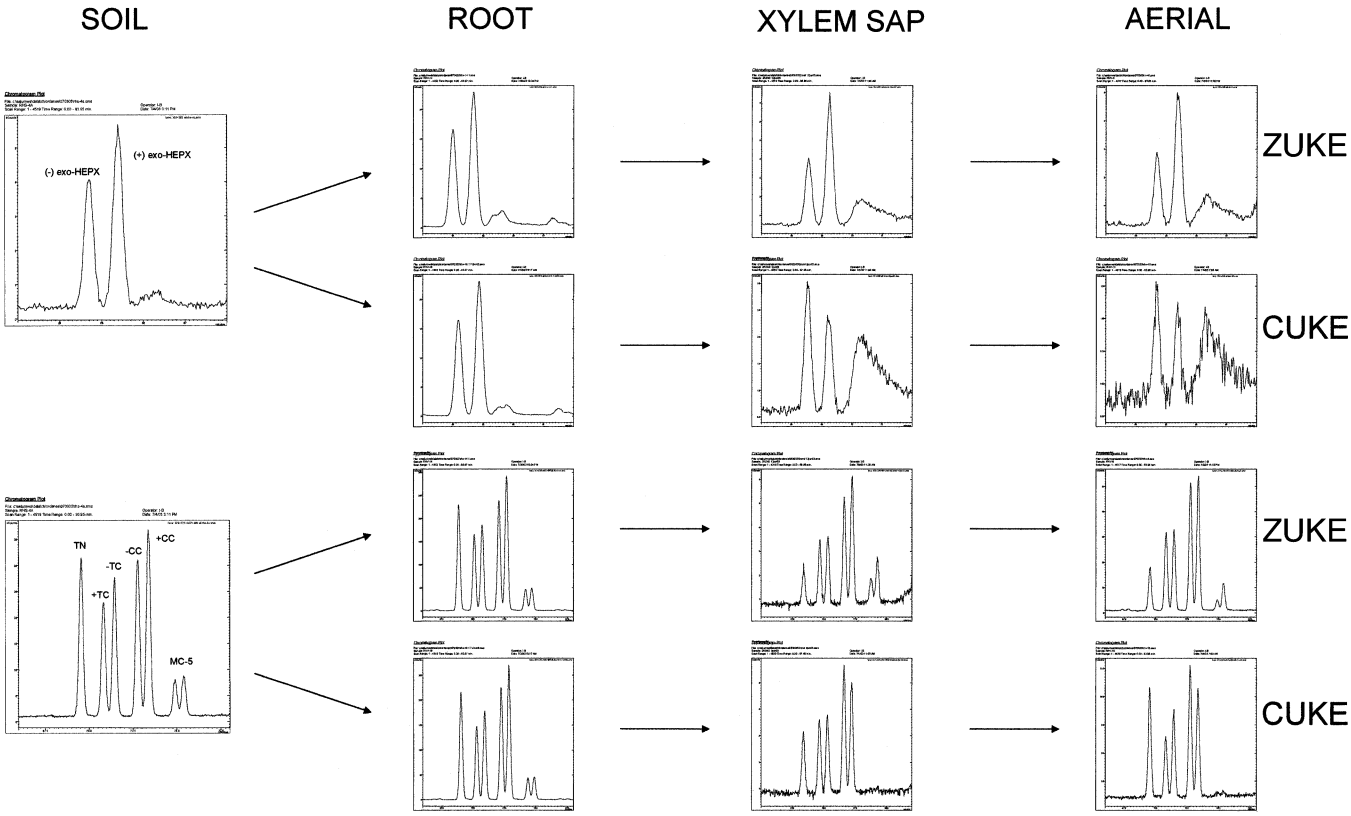

Fig. 4. Selected ion traces from the chiral gas chromatography/mass spectrometry analysis of soil, whole root tissue, xylem sap, and whole aerial tissue from the rhizotron studies. Top portion of figure shows selected ion traces for *exo*-heptochlorepoxide (*exo*-HEPX); lower portion of figure shows selected ion traces for *trans*-nonachlor (TN), *trans*-chlordane (TC), *cis*-chlordane (CC), and MC-5 chlordane components.

specific. Root exudation may play a role in uptake and transport of soil-bound contaminants by plants [18,19,21,22] (reference [22] is available upon request from the corresponding author); studies to determine details of the mechanisms of plant uptake and transport currently are underway in our laboratory.

Acknowledgement—The authors acknowledge helpful discussions with F. Ferrandino and W. Elmer of the Department of Plant Pathology and Ecology (Connecticut Agricultural Experiment Station [CAES], CT, USA) regarding statistical treatment of the data and additional conversations with N. Schultes (CAES), and M. Gent (CAES). Portions of this work were supported by U.S. Environmental Protection Agency's (U.S. EPA) Science to Achieve Results Program through Grant R828174; the research has not been subject to U.S. EPA review and does not reflect views of the Agency; no official endorsement should be inferred.

REFERENCES

1. Rayl AJS. 2002. Pollutants without borders. *The Scientist* 16:16–19.
2. Wania F, Mackay D. 1996. Tracking the distribution of persistent organic pollutants. *Environ Sci Technol* 30:390A–396A.
3. Mattina MJI, Iannucci-Berger W, Dykas L, Pardus J. 1999. Impact of long-term weathering, mobility, and land use on chlordane residues in soil. *Environ Sci Technol* 33:2425–2431.
4. Schnoor JL. 2002. Phytoremediation of soil and groundwater. Technology Evaluation Report TE-02-01. Groundwater Remediation Technology Analysis Center, Pittsburgh, PA, USA.
5. Alexander M. 2000. Aging, bioavailability, and overestimation of risk from environmental pollutants. *Environ Sci Technol* 34: 4259–4265.
6. Sicbaldi F, Sacchi GA, Trevisan M, Del Re AAM. 1997. Root uptake and xylem translocation of pesticides from different chemical classes. *Pestic Sci* 50:111–119.
7. Mattina MJI, Iannucci-Berger W, Dykas L. 2000. Chlordane uptake and its translocation in food crops. *J Agric Food Chem* 48: 1909–1915.
8. White JC. 2001. Plant-facilitated mobilization and translocation of weathered 2,2-bis(*p*-chlorophenyl)1,1-dichloroethylene (*p,p′*-DDE) from an agricultural soil. *Environ Toxicol Chem* 20:2047–2052.
9. White JC, Mattina MJI, Eitzer BD, Iannucci-Berger W. 2002. Tracking chlordane compositional and chiral profiles in soil and vegetation. *Chemosphere* 47:639–646.
10. Mattina MJI, White JC, Eitzer BD, Iannucci-Berger W. 2002. Cycling of weathered chlordane residues in the environment: Compositional and chiral profiles in contiguous soil, vegetation, and air compartments. *Environ Toxicol Chem* 21:281–288.
11. Leigh MB, Fletcher JS, Fu X, Schmitz FJ. 2002. Root turnover: An important source of microbial substrates in rhizosphere remediation of recalcitrant contaminants. *Environ Sci Technol* 36: 1579–1583.
12. Eitzer BD, Mattina MJI, Iannucci-Berger WA. 2001. Compositional and chiral profiles of weathered chlordane residues in soil. *Environ Toxicol Chem* 20:2198–2204.
13. Ulrich EM, Hites RA. 1998. Enantiomeric ratios of chlordane related compounds in air near the Great Lakes. *Environ Sci Technol* 32:1870–1874.
14. Lee WY, Iannucci-Berger WA, Eitzer BD, White JC, Mattina MJI. 2003. Plant uptake and translocation of airborne chlordane and comparison with the soil-to-plant route. *Chemosphere* 53:111–121.
15. Haapala JJ. 1996. In Waltz E, ed, *Organic Farming Research Foundation Information Bulletin Fall 1996*, Vol 3. Organic Farming Research Foundation, Santa Cruz, CA, USA, pp 4–5.
16. Eitzer BD, Iannucci-Berger W, Mattina MJI. 2003. Volatilization of weathered chiral and achiral chlordane residues from soil. *Environ Sci Technol* 37:4887–4893.
17. Hülster A, Müller JF, Marschner H. 1994. Soil–plant transfer of poylchlorinated dibenzo-*p*-dioxins and dibenzofurans to vegetables of the cucumber family (*Cucurbitaceae*). *Environ Sci Technol* 28:1110–1115.

18. White JC, Mattina MJI, Lee WY, Eitzer BD, Iannucci-Berger W. 2003. Role of organic acids in enhancing the desorption and uptake of weathered *p,p′*-DDE by Cucurbita pepo. *Environ Pollut* 124:71–80.
19. Reinbott TM, Blevins DG. 1999. Phosphorus nutritional effects on root hydraulic conductance, xylem water flow, and flux of magnesium and calcium in squash plants. *Plant and Soil* 209: 263–273.
20. Tatár E, Mihucz VG, Kmethy B, Záray G, Fodor F. 2000. Determination of organic acids and their role in nickel transport within cucumber plants. *Microchem J* 67:73–81.
21. Campanella B, Paul R. 2000. Presence in the rhizosphere and leaf extracts of zucchini (*Cucurbita pepo* L.) and melon (*Cucumis melo* L.), of molecules capable of increasing the apparent aqueous solubility of hydrophobic pollutants. *Int J Phytoremediat* 2:145–158.
22. Neumann G, Hülster A, Marschner H. 1997. Identifiziering PCDD/F- mobilisierender Verbindungen in Wurselexsudaten von Zucchini. In Merbach W, ed, *Rhisospharenprozesse, Umweltstress und Okosystemstabilitat.* B.G. Teubner Verlagsgesellschaft Stuttgart, Leipzig, Germany, pp 167–175.