COMPOSITIONAL AND CHIRAL PROFILES OF WEATHERED CHLORDANE RESIDUES IN SOIL

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Abstract—The fate of chlordane and other persistent organic pollutants in the environment is of international concern. The behavior of persistent organic pollutants under both abiotic and biotic conditions must be determined for the comprehensive elucidation of their cycling through the biosphere. Standard analytical methods such as gas chromatography with electron capture detection are adequate for studies of cycling under abiotic conditions. Since two of the main components of technical chlordane, cis-chlordane and trans-chlordane, are optically active, chiral gas chromatography can be used to study the impact of biotic influences on chlordane's fate. We report here the use of chiral gas chromatography interfaced with ion trap mass spectrometry as part of an analytically rigorous method for the simultaneous determination of the compositional and chiral profiles of weathered soil residues of technical chlordane. Using the method described, several patterns in the long-term weathering of technical chlordane in soil are observed.

Keywords—Chlordane Soils Chiral Fate

INTRODUCTION

The pesticide chlordane was widely used in the United States for both agricultural and residential applications from the time of its introduction in the 1940s through its banning by the U.S. Environmental Protection Agency in 1988 [1,2]. Its toxicity and persistence in the environment have led to its being included as one of the 12 persistent organic pollutants whose use is to be curtailed by an international treaty currently under negotiation [3]. As applied, chlordane was not a single compound but a mixture of over 140 different related compounds known as technical chlordane. The three most abundant components of technical chlordane, cis-(or α) chlordane (CC), trans-(or γ) chlordane (TC), and trans-nonachlor (TN), account for approximately 25 weight % of the technical mixture [4].

The environmental persistence of chlordane is well established [5–12]. Estimates of the half-life of technical chlordane in soil range from less than 10 to more than 20 years [5,13,14]. Although technical chlordane is persistent, over time the relative amounts of the components of technical chlordane change because the components are subject to physical transformations (e.g., hydrolysis, volatilization) at different rates. Different transformation rates are due to the different physical properties, such as vapor pressure, Henry's Law constant, and water solubility.

The components also undergo biotic transformations (e.g., microbial degradation) at different rates. It is important to determine if changes in the chlordane profile of a site result from fluxes into and out of a site or from in situ degradation. The distinction is between the chemical alteration of the contaminant in the latter case and concentration changes via dispersal through the environment in the former.

It is now recognized that studies of the environmental fate of pollutants are enhanced by data concerning the chiral pro-
a formulation containing 72 weight % technical chlordane at a rate of 0.0076 L/m² on April 28, 1960. The site remained under turf cover until 1998, when soil cores were collected for analysis. After collection of the cores, the turf cover over part of the site was removed, and this area was tillled in preparation for planting; the turf cover remained intact over the nontilled portion. Additional samples from both the turf-covered and the tilled areas were collected at the end of the 1998 and during the 1999 growing seasons. A total of 35 samples were analyzed from this site (code P): 19 from the turf covered cores, 7 from the tilled area after the first growing season, 9 from the tilled area during the second growing season.

**Agricultural soils**

Samples from the top 10 cm of soil from six Connecticut farms in crop production as of 1997 were collected. One such site had been in tobacco production through 1978, at which point it lay fallow for several years before planted in food crops (code H, four samples); a second site was an ornamental nursery under Taxus production for more than 20 years (code J, six samples). The remaining agricultural soils were from small farms of 5 to 10 acres (code M, three samples, and code A, one sample each from three separate farms).

**Residential soils**

Soil samples from home foundations and lawns of private residences were collected by homeowners. Knowledge of chlordane use by homeowners is problematic; therefore, these samples have been divided into two subcategories based on the amount of chlordane residues found—those containing more than 5,000 ng/g total chlordane compounds were coded F (15 samples), as they came primarily from soils surrounding foundations, and those containing less than 5,000 ng/g total chlordane, which were termed residential samples and coded R (32 samples).

**Technical chlordane**

A sample of technical chlordane (lot 51710412) manufactured by Velsicol Chemical (Memphis, TN, USA) was analyzed in duplicate and coded T.

**Analytical procedure**

The soil extraction procedure is described in detail elsewhere [5]. Briefly, each soil was sieved, and a 3-g subsample was extracted with 50 ml 2:3 hexane:acetone using a microwave extraction system. A second subsample was weighed and dried for determination of moisture. The extract was concentrated and solvent exchanged to iso-octane for GC/ITD analysis. The extract was analyzed on a Saturn 2000 Ion Trap GC/MS (Varian, Sugar Land, TX, USA) system fitted with a 30-m × 0.25-mm i.d. × 0.25-µm film thickness GAMMA-DEX-120 column (Supelco, Bellefonte, PA, USA) for the separation of chiral compounds. The GC oven was programmed as follows: initial temperature 120°C, hold 1 min; 20°C/min to 155°C; 0.5°C/min to 195°C; 20°C/min to 220°C, hold 11 min. The injection port was maintained at 230°C, and a 3-µl splitless injection was used. After a 40-min filament delay, the mass spectrometer was turned on under the following conditions: electron impact, emission current 50 µA, target total ion current 5,000 counts, maximum ionization time 25,000 µs, multiplier offset + 200 V, and scan range m/z 265 to 430. All sample extracts were injected twice. Data are reported on the dry-weight basis of the soil.

Racemic standards of CC and TC together with achiral TN (ChemService, West Chester, PA, USA) were used to prepare a series of calibration standards in iso-octane with individual enantiomer concentrations of 5, 12.5, 25, 50, 125, and 250 ng/ml. Each calibration standard also contained oxychlordane (ChemService), a chlordane metabolite, at twice the concentration of the other compounds. Although oxychlordane (OXY) is chiral, its enantiomers were not resolved under the chromatographic conditions specified previously. For each instrumental run, the complete set of standards was injected twice, once before and once after the set of duplicate injections of each sample extract. Enantiomerically pure standards of (+)-TC, (+)-TC, (+)-CC, and (-)-CC (EQ Laboratories, Atlanta, GA, USA) were used to establish the retention order of the enantiomers.

**Quantitation**

Two ions from the most intense chlorine cluster, the (M-Cl)⁺ ion, of each compound were selected. For TC and CC, the ions are m/z = 373, 375; for TN, the ions are m/z = 407, 409; for OXY the ions are m/z = 387, 389. Extracted ion chromatograms were converted into an ASCII text file with ChemSW GC/MS file translator (ChemSW, Fairfield, CA, USA). The files were then imported into PeakFit (Version 4, SPSS, Chicago, IL, USA). The settings for data manipulation in PeakFit were smoothing via a Fourier transform routine and integrating using the AutoFit Peaks II second derivative parameters. The observed isotope ratio from the selected pair of ions (e.g., m/z = 373, 375 for CC and TC) for each analyte was determined for standards and for samples. External standard calibration curves for each analyte were generated from the sum of the two extracted ions from the (M-Cl)⁺ cluster. Enantiomer ratios (ER, +/−) were calculated from each ion in the pair. In addition to CC and TC, soil samples contained a third enantiomeric pair tentatively identified as the MC5 congenor based on the work of Falconer et al. [9]. This was quantified in every sample using the average response factor from the four enantiomers included in the standards, that is, (+)-CC, (-)-CC, (+)-TC, and (-)-TC; because standards were unavailable for these enantiomers, they are labeled as MC5A and MC5B.

Extracted ion chromatograms of the m/z = 373 ion are shown in Figure 1. Each panel shows the ion chromatogram both before and after Fourier transform smoothing. The ion chromatogram for a standard from the middle of the calibration range is shown in panel A. At this concentration, the smoothing process provides minimal improvement. Although the enantiomers are not baseline resolved, sufficient separation exists for good integration. Panel B illustrates the effect of the Fourier transform on a soil sample with individual components present at less than 5 ng/g. The effect of the Fourier transform smoothing is apparent. Panel B also shows the two peaks that are tentatively identified as MC5. For consistency, all samples were smoothed.

**Limits of quantitation**

At a S/N > 3, the instrument detection limit (IDL) for these analyses was 3 ng/ml (6 ng/ml for OXY). All sample extracts were initially reduced to a final volume of 10 ml. As necessary, the extract was either diluted with iso-octane or concentrated under a gentle stream of nitrogen in order to produce an extract within the calibration range of the standards. After concentration, the limits of quantitation in the soil were approximately...
cause of the favorable comparison between these sample sets, those determined from the stored and re-extracted soil. Determined from the stored, frozen extract were within 1% of two results was less than 25%. The enantiomeric ratios temperature. The average difference in concentrations between the freezer-stored extracts for seven soil samples were compared to chiral GC/ITD results for the re-extraction of those prepared times selected for quantitation were used to monitor instrument performance and possible chromatographic interferences. Acceptance criteria for these ratios were established using all injections made of the mixed standard over the entire calibration range during the course of this project. For low concentration samples (<85ng/ml), the isotope ratio criteria required samples to be within 15% of the theoretical value; for high concentration samples (>85ng/ml), the criteria were within 4% of the theoretical value for all compounds except OXY, discussed in the following. If the measured isotope ratios for any analyte were outside the acceptance criteria, the sample data were excluded from the data analysis.

Oxychlordane data were somewhat problematic. Although present in the standards at twice the concentration level of other compounds, the OXY data in the low-level standards are both less accurate and less precise than the data for the other compounds. For OXY, one of the two monitored isotope masses is the same as a bleed ion from the chiral capillary column. At very low concentrations, this results in excess noise and bias in the system. At the higher concentrations, the effect is not observed, and the measured isotope ratio approaches the theoretical ratio. Alternate ions from the cluster are much less intense. For these reasons, detection limits are higher, and acceptance criteria are wider for oxychlordane than for the other compounds.

The ER can be calculated for each pair of enantiomers using each ion selected from the cluster. Since each standard is injected twice, once before and once after the sample set, the ER is calculated four times at each concentration level. The measured values differed only slightly from the theoretical value of 1, with less than a 2% difference from theoretical values when averaged over the entire time period of the project. These data show that ER can be measured with good precision even at low concentrations. In fact, for the samples that met the isotope ratio criteria, well over half the samples had a RSD of less than 2% for their four measurements of ER; in only three samples were RSDs greater than 5% when the sample was deemed acceptable by the isotope ratio criteria.

**Quantitation comparisons**

The capability of the entire analytical procedure including chiral GC/ion trap MS to perform quantitative analysis was tested with two procedures. First, a certified check sample containing TC and CC in soil (ERA, Arvada, CO, USA; cat. no. 720, lot 355) was analyzed a total of six times during the four months of the study. This sample was certified only for the total amount of a racemate, not for individual enantiomers. The sum of the measured racemates was approximately 85% of the certified value for both TC and CC, well within the acceptance limits, and confirmed the quality of the concentration data generated by this method.
Profiles of soil chlordane residues

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Second, we compared the data collected using the current chiral GC/ITD method with those collected using the previous achiral GC/ECD method. A total of 79 samples analyzed during our previous work by the GC/ECD procedure were also analyzed in the current work (either fresh soil extraction or analysis of frozen extract) using the chiral GC/ITD method. Although a good correlation exists between the two methods ($r^2 = 0.88$), for most of the samples ITD analysis resulted in higher concentrations. The absolute value of the percentage difference for each sample was determined by Equation 2:

$$\text{% difference} = \frac{\text{absolute value}[100 \times (\text{ITD} - \text{ECD})/\text{average}]}{2}$$

The average % difference was 34%. This is quite good agreement given the concentration range of various samples, the different instrumental procedures employed, the use of stored sample extracts, and comparison of samples analyzed years apart. Together with our analyses of the certified soil, these measurements gave us confidence in the quantitative capability of the technique.

Compositional profiles. Eight analytes were quantitated in each sample: (+)-TC, (−)-TC, (+)-CC, (−)-CC, TN, OXY, MC5A, and MC5B. These analytes are summed to determine the total chlordane residue. Foundation samples (F) ranged from 5,000 to 94,000 ng/g. Connecticut Agricultural Experiment Station (CAES) plot samples (P) from 1,000 to 18,000 ng/g, residential lawn/garden samples (R) from 35 to 4,000 ng/g, and agricultural samples (A, H, J, M) from 22 to 700 ng/g. In order to compare comprehensive compositional profiles of samples whose chlordane concentrations differ by orders of magnitude, for each sample the individual concentration of each component was normalized to the sum of all components. This normalized data set was then subjected to principal components analysis (PCA). The first two factors of the PCA account for 90% of the variability in the data set. These factors are plotted in Figure 2, in which each sample is represented by its site code. It should be remembered that the farther apart two samples are in the PCA plot, the more differences in data patterns between the samples. Some comments about this plot should be made. The two technical chlordane analyses, group I, show the position of the component profile in the originally applied material. The sample set II consists of residential soils that had greater than 5,000 ng/g total chlordane and therefore were labeled as coming from home foundations (code F). Although these 15 samples came from 13 different homeowners and, therefore, 13 unrelated sites, they have a relatively consistent pattern as indicated by their occurrence in the same region of the principal components plot. The fact that this group resides next to the technical chlordane samples on this plot indicates that few changes have occurred in the compositional profile. It appears, therefore, that the use of high concentrations of chlordane around home foundations creates a chlordane source that undergoes few changes in compositional profile during weathering, regardless of soil characteristics. The remainder of homeowner-submitted soils (code R, <5,000 ng/g) do not group into any consistent pattern and are scattered throughout the PCA plot. These low levels may result from alternate sources, such as atmospheric deposition and substantially different weathering characteristics. The other grouped sample sets each represent a set of samples from a single location; thus, each set likely has its own source(s) or weathering characteristics to produce its unique compositional profile. It is, therefore, not surprising that each forms its own cluster. These observations lead us to the conclusion that sample source is one important determinant of the profile of these eight chlordane analytes.

To illuminate the changes that occurred during weathering, the samples of each group were averaged, except for the nonfoundation residential samples (code R) that the principal component plot showed to have no consistency. The bar chart of these average profiles is shown in Figure 3. Table I lists the $+/-$ enantiomer ratios for TC and CC associated with this figure. As noted previously, the F soils have relative concentrations of the chlordane components similar to (T), and their ERs are close to racemic. We conclude that the TC, CC, TN, and MC5 residues in the foundation soils closely resemble those in technical chlordane. The high total concentration (5–94 ng/g), small amount of OXY, and enantiomeric ratios racemic or very close to racemic in the F soils suggest that minimal enantioselective degradation has occurred in these soils. It is possible that some abiotic weathering has occurred, as some minor changes in the compositional profile appear to be evident.

Although F and J samples have similar chlordane profiles, as seen in Figures 2 and 3, the samples have very different total chlordane concentration ranges. While soil samples from F sites contain tens of thousands of ng/g, those from site J have only 200 to 700 ng/g. From Figure 3, it may be seen that the relative concentrations of the components in J samples are slightly different from technical chlordane: more oxychlordane exists than in F soils, but again ERs are essentially racemic. The presence of oxychlordane suggests that a degradative process is occurring, though the racemic ER suggests that it is either an abiotic process or a nonenantioselective biotic process. The small changes in the compositional profile suggest that these changes are occurring slowly. Thus, relatively slow degradative processes can occur at both low or high soil concentrations. In the remaining three groups of soil samples, P, H, and M, the relative concentrations of the components have changed markedly from that in technical chlordane, OXY is
present in higher relative amounts, and ERs are significantly different from 1. It would appear that in these soils, biotic degradation has altered the technical chlordane pattern substantially. Additional abiotic weathering could also have occurred. Total chlordane concentrations in these soils range from 1,000 to 18,000 ng/g in site P, 20 to 700 ng/g in site H, and 30 to 54 ng/g in site M. From the component profiles summarized in Figure 3, we may conclude that compositional and chiral profiles are not determined solely by total chlordane concentration and that compositional and chiral profiles alter in tandem; that is, larger changes in component ratios (CR, e.g., CC/TC) are accompanied by larger changes in ER. Several other relationships exist between the variables that are apparent from Figure 3. From sites P, H, and M, it is noted that while the concentration of (+)-TC is enhanced relative to its enantiomer, it is (+)-CC that is enhanced relative to its enantiomer. This trend of decreasing trans-chlordane ER with in-

Table 1. Average enantiomeric ratio (+/−) of samples sorted by type. Superscripts after ratio represent groups that are significantly different based on a one-way analysis of variance on rank transform data. Multiple comparisons were calculated using the Bonferroni multiple comparison test at the 0.05 significance level.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Code</th>
<th>(+)/− TC</th>
<th>(+)/− CC</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technical chlordane</td>
<td>T</td>
<td>0.99 ± 0.00b</td>
<td>1.02 ± 0.01c</td>
<td>2</td>
</tr>
<tr>
<td>Foundation</td>
<td>F</td>
<td>0.98 ± 0.02b</td>
<td>1.02 ± 0.01c</td>
<td>15</td>
</tr>
<tr>
<td>Farm 2</td>
<td>J</td>
<td>1.00 ± 0.02b</td>
<td>1.03 ± 0.01c</td>
<td>6</td>
</tr>
<tr>
<td>CAES plot</td>
<td>P</td>
<td>0.85 ± 0.04c</td>
<td>1.18 ± 0.06b</td>
<td>35</td>
</tr>
<tr>
<td>Farm 1</td>
<td>H</td>
<td>0.87 ± 0.02c</td>
<td>1.18 ± 0.02c</td>
<td>4</td>
</tr>
<tr>
<td>Farm 3</td>
<td>M</td>
<td>0.69 ± 0.06b</td>
<td>1.21 ± 0.06c</td>
<td>3</td>
</tr>
</tbody>
</table>

TC = trans-chlordane, CC = cis-chlordane, n = number.

Fig. 3. Average chlordane component profile for each site code producing a distinctive group in the principal components analysis. Site codes as follows: P = CAES plot; F = Foundation (homeowner submitted >5,000 ng/g); H, J, M = agricultural sites; TC = trans-chlordane; CC = cis-chlordane; TN = trans-nonachlor; OXY = oxychlordane.
creases cis-chlordane ER is supported by the plot in Figure 4, the ER of trans-chlordane versus the ER of cis-chlordane for all samples. The data reported by Aigner et al. [11] for soils from Pennsylvania, Ohio, Indiana, and Illinois have also been plotted in Figure 4. The similarity between the data sets for all samples. The data reported by Aigner et al. [11] for soils from Pennsylvania, Ohio, Indiana, and Illinois have also been plotted in Figure 4. The similarity between the data sets for all samples. The data reported by Aigner et al. [11] for soils from Pennsylvania, Ohio, Indiana, and Illinois have also been plotted in Figure 4. The similarity between the data sets for all samples. The data reported by Aigner et al. [11] for soils from Pennsylvania, Ohio, Indiana, and Illinois have also been plotted in Figure 4. The similarity between the data sets for all samples.

Oxychlordane is a metabolite of both trans- and cis-chlordane diastereomers [18]. For the plot of (CC/TC) versus the relative OXY concentration in the soil samples from the current study shown in Figure 5, the coefficient of determination is $r^2 = 0.68$. This is consistent with a more rapid conversion of TC to OXY than the conversion of CC to OXY. This observation is consistent with previous reports; for example, Beeman and Matsumura [19] have shown that the rate of metabolism in vitro of trans-chlordane by a soil microbe is three times more rapid than the rate for cis-chlordane.

CAES plot. It is useful to examine the data from the CAES plot in somewhat more detail than the other sites. This is the only site where the application history of technical chlordane is known with a high degree of accuracy [5]. Relatively high levels of chlordane were applied to this site in 1960, after which the site remained under turf cover until the spring of 1998. In 1998 and 1999, food crops were grown on a portion of this site [6]. The profile pattern of chlordanes across this site remains consistent, from the 1997 samples through the 1999 samples, as demonstrated by its tight grouping in the PCA plot of Figure 2, even though variation exists in the absolute concentration across the site ranging from 1,000 to 18,000 ng/g total chlordanes.

Table 2 compares some subtle differences in samples of soil taken from untilled areas of the CAES site with samples taken from tilled and planted areas. Although the total concentration (or the concentration of any individual component, not shown) is lower in the tilled samples than in the untilled samples, these differences are not significant. However, significant differences in compositional (CRs) and chiral (ERs) profiles have emerged. First, the ERs have changed. The ER for soils from the tilled-planted area differs more from racemic for both CC and TC, with a consistent directional change from racemic for the untilled and tilled soils. It appears that tilling the soil and growing crops produced enantioselective changes. A second change that has occurred is that CR has changed in the tilled versus untilled samples. Both TC/TN and CC/TN are significantly lower in the tilled soil than in the untilled soil. However, the CC/TC has remained the same. Proportionately, more trans-nonachlor is present in the samples from the tilled and planted areas than in samples from the untilled areas. Octanol–air partition coefficients ($K_{oa}$) for these compounds are known: $\log K_{oa} = 8.05$ for TN, $\log K_{oa} = 8.40$ for TC, and $\log K_{oa} = 8.45$ for CC [10]. From these values, we would expect TN to volatilize more quickly than CC and TC. However, the elevated amount of TN versus both TC and CC observed in P soils suggests that other processes have a larger impact on the chlordane profiles than abiotic differential fluxes. The impact of vegetation on the CRs and ERs is under investigation in our laboratory.

CONCLUSIONS

These results show that it is feasible to measure simultaneously compositional and chiral profiles of chlordane using chiral GC interfaced to ion trap mass spectrometry. The results show that biotic degradation in addition to abiotic weathering is occurring in the soils studied. Further monitoring of these sites will allow us to use these measurement techniques to gauge the rate at which some of these transformations occur.

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REFERENCES


Table 2. Comparisons of untilled and tilled-planted soils from the CAES plot

<table>
<thead>
<tr>
<th>Soil</th>
<th>$n$</th>
<th>Total conc. ng/g</th>
<th>ER TC</th>
<th>ER CC</th>
<th>CC/TC</th>
<th>TC/TN</th>
<th>CC/TN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untilled</td>
<td>19</td>
<td>8,100 ± 5,100</td>
<td>0.86 ± 0.05</td>
<td>1.16 ± 0.07</td>
<td>1.16 ± 0.13</td>
<td>2.31 ± 0.50</td>
<td>2.62 ± 0.39</td>
</tr>
<tr>
<td>Tilled</td>
<td>16</td>
<td>7,200 ± 3,200</td>
<td>0.83 ± 0.03</td>
<td>1.20 ± 0.04</td>
<td>1.16 ± 0.04</td>
<td>1.98 ± 0.21</td>
<td>2.28 ± 0.19</td>
</tr>
</tbody>
</table>

*Data are average ± standard deviation. Differences are deemed significant based on $t$ test assuming unequal variances at the .05 significance level, with 27 degrees of freedom. $n$ = number, ER = enantiomer ratios, TC = trans-chlordane, CC = cis-chlordane, TN = trans-nonachlor.


