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Application of Capillary Electrophoresis To Study the Enantioselective Transformation of Five Chiral Pesticides in Aerobic Soil Slurries

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The enantiomers of five chiral pesticides of environmental interest, metalaxyl, imazaquin, fonofos (dyfonate), ruelene (cruformate), and dichlorprop, were separated analytically using capillary electrophoresis (CE) with cyclodextrin chiral selectors. For metalaxyl, imazaquin, and fonofos, aqueous slurries of soil samples from two sites in Georgia and one in Ohio were spiked with the racemate of each pesticide at 50−60 mg/L of aqueous phase of the slurry, and CE analyses were performed at various time intervals to determine enantiomer fractions (EF). Metalaxyl underwent enantioselective transformation; in one soil, the half-life of the target active *R*-(+)-enantiomer was 17 days while that for the *S*-(−)-enantiomer was 69 days. Transformation occurred more slowly in the other two soils but was still selective for the *R*-(+)-enantiomer. Imazaquin and fonofos exhibited nonselective enantiomer loss over their 3 months of incubation time; this could have been due to abiotic or nonselective microbial reactions. Ruelene and dichlorprop were transformed selectively in a variety of soils in a previously reported study (*7*) that showed the influence of environmental changes on the transformation of chiral pollutants in soils; analytical methods used in that study are reported here to further illustrate the application of CE. CE is shown to be a simple, efficient, and inexpensive way to follow the transformation of chiral pesticides in laboratory microcosms where concentrations can be made high enough (25−50 mg/L initial racemate concentration) for detection of residual parent enantiomers during most of the process.

KEYWORDS: Capillary electrophoresis; micellar electrokinetic chromatography; pesticides; chiral; enantiomers; enantioselective transformation

INTRODUCTION

While the global problem of pesticide pollution decreases with the development of more environmentally friendly compounds, pesticides are still applied in large quantities and may present pollution and exposure problems. The five pesticides investigated here—metalaxyl, imazaquin, fonofos (dyfonate), ruelene (cruformate), and dichlorprop—are chiral members of different pesticide classes used in modern agriculture. Compound structures are shown in **Figure 1**. Twenty-five percent or more of the members of several classes of pesticides are chiral; that is, they exist as two mirror image isomers called enantiomers. It is known that enantiomers often differ in their biological properties but not in abiotic properties. Metalaxyl is an acetanilide fungicide primarily applied to tobacco, ornamentals, conifers, and turf for control of airborne and soilborne pathogens. Imazaquin, a member of the imidazolinone class of herbicides, is frequently used to control the growth of weeds in soybean crops, turf, and ornamentals. Fonofos, an organophosphorus insecticide also known as dyfonate, is primarily applied to control insects in a number of crops and in turf. Ruelene, also known as cruformate, is an organophosphorus insecticide formerly used on cattle for the control of cattle grubs but is no longer registered for use in the United States. Finally, dichlorprop is a phenoxyalkanoic acid herbicide widely used in different parts of the world for postemergence control of broad-leaved weeds.

These pesticides range in soil persistence from a few weeks to a year or so and range widely in their toxicities. Information on physical and chemical properties, environmental fate, and toxicity of these pesticides may be found in various handbooks and websites (*1*−*3*).

Even when pesticides are applied correctly, accumulation due to field runoff and multiple applications over a given area can easily lead to detectable concentrations in streams, subsurface soils, and groundwater, leading to an increased probability of

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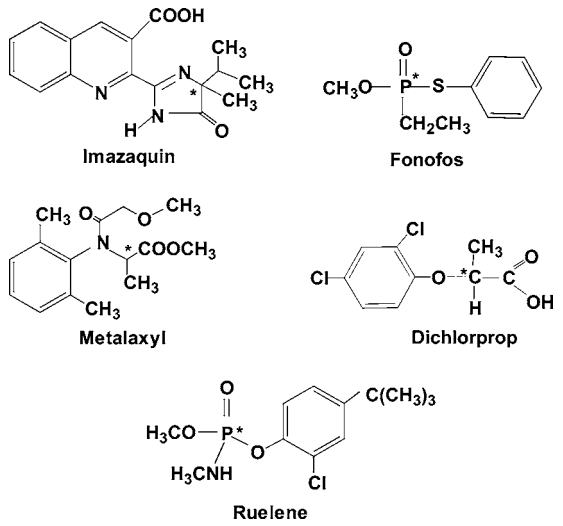

Figure 1. Structures of the chiral pesticides investigated here. Chiral centers are denoted by an asterisk.

adverse exposure effects. In addition, although environmental transformation of pesticides usually results in less harmful products, such products may be more harmful to biological systems or to the environment than the parent pesticide; this has been shown, for example, with DDT (*4*). Because of these possibilities, transformation studies on these and other pesticides are crucial.

Pesticide transformation is the result of both biotic and abiotic processes, the latter of which may include photolysis, hydrolysis, redox, sorption, or other nonselective chemical reactions (*5*). Whereas abiotic processes are normally nonselective, biologically controlled reactions such as microbial transformation are often enantioselective, resulting in increased persistence of one enantiomer. While many studies have been reported on both types of processes, a much smaller amount of data has been collected on biotic enantioselective transformation patterns of chiral pesticides. These data are important in that enantiomers often have different effects on biological systems; one enantiomer may be more active toward the target pest species, but the effect on nontarget species may also differ for each enantiomer (*6*). Finally, characteristics of the environment that affect the microbial population may, in turn, affect enantioselectivity and pesticide activity (*7*−*9*). As one enantiomer is transformed, the activity of the residual pesticide is altered. In this way, biological uptake, effectiveness, and toxicity of the residual pesticide changes over time (*6*, *7*, *10*−*13*).

If the active enantiomer is transformed at a different rate than its counterpart, application and exposure information should be revised to reflect this aspect of pesticide use. For example, the *R*-(+)-enantiomer of metalaxyl has been shown to be the active form, and there are commercially available *R*-enriched or enantiomerically pure *R* products (e.g., Metalaxyl-M). The active enantiomer of the phenoxyalkanoic acid herbicides is also the *R*-(+)-enantiomer; dichlorprop, for example, is available as the separate *R*-product (Dichlorprop-P). In cases such as these, enantiomerically pure products can be used in half the amount of the racemic mixtures to achieve the same effect (*14*). Through the increase in efficiency of the product, the total amount of pesticide released into the environment is greatly reduced. Data on the enantioselective transformation patterns of chiral pesticides are essential in order to maximize effectiveness of the pesticide products while maintaining a safer environmental level.

Traditional methods for the analysis of chiral pesticides in environmental matrices include high-performance liquid chromatography (HPLC) and gas chromatography (GC). Both of these techniques, however, require relatively expensive chiral columns that are specific for separation of a limited number of analyte enantiomers, and GC analysis requires extraction of the pesticide from an aqueous matrix and sometimes derivatization for adequate volatility. In contrast, chiral analysis accomplished with traditional capillary electrophoresis (CE) or the micellar electrokinetic chromatography (MEKC) mode of CE requires only addition to the run buffer of chiral selectors such as cyclodextrins (CDs) to achieve enantiomer separations. These CDs, which are easily incorporated into the existing aqueous electrolyte solutions (run buffers), form complexes with the enantiomers of chiral analytes, resulting in diastereomers that have different CE migration times. As a result, rapid chiral analyses are possible with aqueous samples and yield reliable, reproducible results.

Despite wide use in chiral drug analyses, the application of CE to the analysis of pesticides (*15*) or chiral pesticides (*6*, *7*, *15*−*17*) is relatively new. In past investigations using CE, chiral pesticides and other pollutants such as dichloroprop (*6*, *7*), ruelene (*7*), and bromochloroacetic acid (unpublished work of the authors) have exhibited enantioselective transformation rates. To study enantiomer transformation under a variety of conditions, a CE separation method was developed for the enantiomers of metalaxyl, imazaquin, and fonofos and applied to monitor the transformation of the enantiomers of these pesticides in aerobic soil slurries from three sites. Periodic sampling and analysis of the slurries over 3 months (1 year for metalaxyl) allowed calculation of enantiomer fractions (EF) and observation of any enantioselectivity. In addition, examples of the application of CE to study the enantioselective environmental transformation of ruelene and dichlorprop are provided here. These two applications are from previous research to study the influence of environmental changes on the transformation of chiral pesticides in soils. Environmental data based on these applications are published elsewhere (*7*), but the CE analysis was not discussed there in any detail.

MATERIALS AND METHODS

Chemical Reagents and CE Buffers. Sodium tetraborate (Na-TB), sodium dodecyl sulfate (SDS), HPLC grade acetonitrile (AcN), and the various CDs were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. Pesticide standards were obtained from Chem Service (West Chester, PA) and diluted with methanol to make spiking solutions.

Acetate buffers were made with sodium acetate and adjusted to the desired pH with glacial acetic acid, while borate buffers were made from Na-TB and deionized water. The borate buffer was adjusted to the desired pH with dilute HCl. Metalaxyl, a neutral compound, was analyzed by CE in the MEKC mode in a 30 mM Na-TB, pH 8.5, buffer with 100 mM SDS as the micelle, 15% AcN, and 40 mM γ-CD as the chiral selector. Imazaquin was analyzed by CE as an anion; the buffer was 50 mM acetate, pH 4.5, with 15 mM dimethyl-β-CD as the chiral selector. Fonofos was analyzed as a neutral compound in the MEKC mode with a run buffer of 20 mM Na-TB, pH 8.5, 100 mM SDS, 15% AcN, and 25 mM γ-CD. Ruelene, also neutral, was analyzed in the MEKC mode using a run buffer of 20 mM Na-TB, pH 8.5, containing 100 mM SDS, 20% AcN, and 40 mM 2-hydroxypropyl-β-CD as the selector. Dichlorprop was analyzed by CE as an anion using 25 mM Na-TB, pH 8.5, with 25 mM trimethyl-β-CD as the chiral selector. In these buffers, SDS was the micelle for MEKC analysis of neutral species, while AcN served as an organic modifier to react with the CE column surface and enhance enantiomer separation.

Soil Slurries and Sampling Procedure for Metalaxyl, Imazaquin, and Fonofos. Approximately 2 kg of soil was collected from each of three sites, two in Athens, Georgia, and one in Ohio, known to be free

of pesticide residues in question. The soil characteristics were as follows: Ohio soil, pH 5.68, 1.7% TOC; Horseshoe Bend (Athens site 1), pH 5.46, 1.0% TOC; and USDA (Athens site 2), pH 5.53, 3.0% TOC. The pH was measured in a slurry of 1:2.5 soil to 0.01 M $CaCl_2$ (wt:wt). To separate empty amber bottles (200 mL) was added a known amount of each pesticide in methanol solution to achieve 50−60 mg/L total concentration in the final water−soil slurry; the methanol was then allowed to evaporate. Each soil was dried and sieved to remove large particles, and approximately half of each was autoclaved in order to eliminate microbes and serve as a control sample. An aliquot (e.g., 10 g) of each soil was placed in the bottle with each pesticide residue, and enough deionized water (e.g., 50 mL) was added to provide an adequate equilibrium concentration (e.g., 50 mg/L in the water) of the pesticide for CE or MEKC analysis. The bottles were sealed with rubber septa and crimp caps and placed on a shaker. The entire system was allowed to shake continuously in the dark for 3 days before sampling began to ensure total compound distribution and desorption from the glass bottle surfaces and then were continuously shaken in the dark over the course of the investigation.

The investigation of each of the three pesticides required a total of 15 bottles, which included three active samples (for triplicate analyses) and two autoclaved samples (as duplicate controls) for each of the three soils under investigation. Every second day during the experiments, the bottle caps and septa were removed, the bottles were flushed gently with fresh air to maintain an aerobic environment, and the bottles were recapped.

Sampling for CE analysis was performed approximately once a week until concentrations of the residual pesticide approached CE detection levels (several months, depending upon pesticide and soil characteristics). Samples were taken by withdrawing 1 mL of the slurry from the bottle with an insulin syringe before the solids settled. The caps were not removed during the sampling process. The 1 mL aliquots were centrifuged to separate soil particles and frozen (without removal of the soil pellet) until CE or MEKC analyses could be performed. For analysis, 80 μL of the thawed supernatant was withdrawn with an automatic pipet and about 40 μL was placed in a plastic CE vial insert (Beckman Instruments, Fullerton, CA) for CE analysis. The soil pellet and residual water were refrozen and saved for possible future analyses. In cases where analyses revealed that the majority of the analyte was adsorbed to the solid material, the soil pellet was separated by decanting the water; it was then rinsed with water, decanted again, and extracted in a vortex mixer with 1 mL of methanol. The mixture was then diluted with 2 mL of water, centrifuged, and analyzed by CE in the same manner as the supernatant.

Soil Slurries and Sampling Procedures for Ruelene and Dichlorprop. One gram each of soils from various locations (*7*) that had been treated either in the field or in the laboratory to simulate various environmental conditions was mixed with a vortex mixer for about 1 min in test tubes with 10 mL of sterile (autoclaved) distilled water containing about 50 mg/L of racemic ruelene or dichlorprop. Controls were devised to account for abiotic degradation and volatilization. The tubes were capped with metal friction caps and incubated in the dark at 25 °C for about 8 months, during which time 100 μL sample aliquots were collected weekly with a syringe for analysis by CE. Samples were centrifuged or passed through nylon syringe filters as necessary to remove solids, and then about 40 μL was placed in a Beckman CE vial insert for analysis.

Instrumentation. All CE and MEKC analyses were carried out on a Beckman P/ACE 5500 or P/ACE 5000 CE (Beckman Instruments) system at 23 °C with UV detection at 200 (fonofos, metalaxyl, and ruelene) or 230 nm (imazaquin and dichlorprop). Applied voltages were 15 (metalaxyl and dichlorprop), 25 (ruelene and fonofos), or 30 kV (imazaquin). Sample injection was hydrodynamic, usually for 5 s. Uncoated fused silica columns (75 μm i.d., 300 μm o.d., 57 cm total length, 50 cm effective length) were used as purchased from MicroSolv Technology Corporation (Long Branch, NJ). Columns were designated for use with either borate or acetate buffer systems in order to preserve electrophoretic resolution and migration times. The CE column was washed with 0.1 M sodium hydroxide, water, and the run buffer, in that order, for about 2 min each between runs. Data were collected and analyzed with Beckman Gold software and Microsoft Excel. Concentration values were calculated based on the peak areas of external standards analyzed daily, although the use of an internal standard would have improved the run-to-run peak area variability. EF values were calculated from measured enantiomer peak areas as follows:

$$\mathrm{EF} = \text{area (+)-enantiomer}/[\text{area (+)-enantiomer} + \text{area (−)-enantiomer}] \quad (1)$$

or, if optical rotation is unknown,

$$\mathrm{EF} = \text{area 1st eluting peak/sum of areas of both peaks} \quad (2)$$

RESULTS AND DISCUSSION

Metalaxyl, Imazaquin, and Fonofos. It was expected that the results of this research might show different transformation rates for each enantiomer of each pesticide, and further, unique transformation rates for the enantiomers with different soil types. In other words, EFs would be dependent upon the particular microbial population present. Autoclaved samples were not expected to show enantioselectivity or degradation at the same rate as their live counterparts; any decrease in concentration in autoclaved samples should be attributable only to sorption, hydrolysis, or abiotic reduction/oxidation processes. In addition, autoclaved and live samples were kept in the dark to prevent photochemical reactions.

Initial work to determine calibration curves and detection limits indicated that the pesticides could be quantified at levels as low as 1−2 mg/L for each enantiomer. Therefore, the spiked slurries, starting at a concentration of 50−60 mg/L of the racemic pesticide, were expected to yield several months of quality data. Persistences of the three pesticides in soil are expressed in the literature (*2*) as a half-life of 1−4 months for fonofos, an average half-life of slightly over 2 months for metalaxyl, and an "average persistence" of 4−6 months for imazaquin.

The most common method of comparison of enantiomer areas is the parameter known as EF, which is defined such that a racemic mixture would have an EF value of 0.5 (*18*). For metalaxyl, the (+)-enantiomer elutes first under the CE conditions used here; separate enantiomers were not available to determine elution orders for imazaquin and fonofos. So, in all three cases, EF values are calculated as the area of 1st eluting enantiomer divided by the sum of the areas of both enantiomers (eqs 1 and 2).

The electropherograms of standards of the three compounds showed fair to excellent enantiomer separation with reasonable migration times and, as expected, EF values of approximately 0.50. Imazaquin enantiomers are easily and completely separated by almost 1 min in less than 12 min analysis time (**Figure 2**). The EF of the imazaquin standard was 0.49. CE analysis of fonofos provided adequate enantiomer separation (**Figure 3**); the EF of the fonofos standard was 0.50. Metalaxyl CE analysis gave almost baseline separation (**Figure 4**), with the EF of the standard as 0.49.

The metalaxyl experiment provided more definitive data than did experiments with imazaquin and fonofos. Aqueous phase samples from the metalaxyl-spiked Ohio soil slurry exhibited marked differences in peak areas and decreasing EF values with time. As shown in **Figure 4**, the enantiomer that elutes from the column first was preferentially transformed in the slurry. Spiking experiments with the pure enantiomer showed this first-eluting enantiomer to be *R*-(+)-metalaxyl. **Figure 5** shows the calculated EF values and concentrations of each metalaxyl enantiomer in the aqueous phase of the Ohio soil slurry over time. The disappearance of metalaxyl was assumed to follow

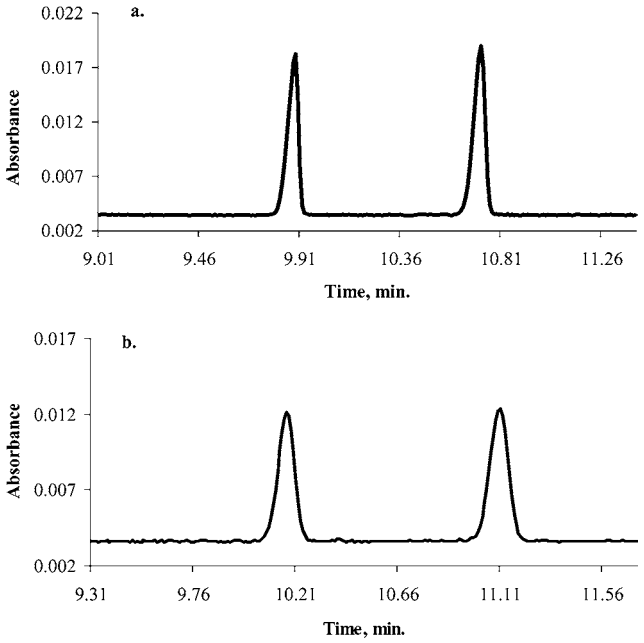

Figure 2. Typical electropherograms of the MEKC separation of imazaquin enantiomers. A decrease in imazaquin concentration occurs from the initial (**a**) to the final (**b**) analysis of Athens site 1 soil, but there is no selectivity.

first-order kinetics, so the rate constant and half-life for each enantiomer were determined by plotting the natural log of the concentration as a function of time. The disappearance rate constants were thus calculated as 0.04 and 0.01 day^{-1} for the *R*- and *S*-enantiomers, respectively, which correspond to half-life values of 17 and 69 days; this compares to the "average" literature value of approximately 70 days for racemic metalaxyl (*2*). It is known that the *R*-enantiomer of metalaxyl is responsible for all of its fungicidal activity; in fact, *R*-metalaxyl is available as a separate product (*19*). For this particular Ohio soil, however, the *S*-enantiomer is more persistent with a half-life four times that of the active enantiomer.

The selectivity of disappearance of the metalaxyl enantiomers in the Ohio soil slurry indicates a microbial reaction. However, the selectivity of this reaction is apparently site specific. **Table 1** shows the transformation kinetic and EF data for the two Athens soil samples as well as the Ohio soil sample. While the data at 55 days show about 50% reduction in total metalaxyl concentration for each of the Athens samples, the EF values are still approximately racemic. This indicates metalaxyl loss by either an abiotic process, perhaps sorption, or by nonselective microbial reactivity. However, extension of incubation time of these samples to about 1 year (data not shown) clearly showed the subsequent occurrence of a selective microbial reaction, with preference for loss of the *R*-enantiomer in both cases.

Buser et al. (*14*) showed selective loss of the *R*-enantiomer of metalaxyl in a Swiss soil. However, in a subsequent publication (*20*), the same research group measured the loss of metalaxyl from a variety of soils in Switzerland and found a striking shift in chiral preference with pH and other soil characteristics. For soils with pH > 5, $kR > kS$; for soils with pH 4−5, $kR \sim kS$; and for soils with pH < 4 and most anaerobic soils, $kR < kS$ (where k = disappearance rate constant). In the case of our Ohio soil, which had a pH of 5.68, the *R*-enantiomer was also transformed faster than the *S*-enantiomer, in agreement with the Swiss research. The pH values of our other two soils, while lower than that of the Ohio soil, were also above 5 (5.46 and 5.53) and also fit the Swiss pattern of pH dependence—but after a considerably longer incubation time.

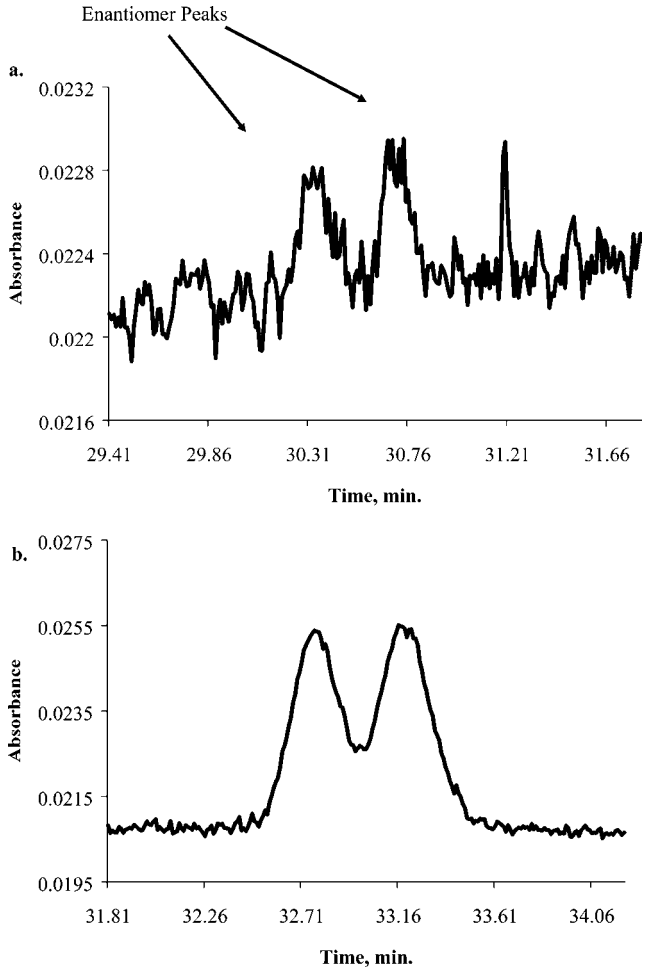

Figure 3. (**a**) MEKC electropherogram of fonofos enantiomers from the supernatant portion of one of the Athens soil slurries collected after 8 weeks of reaction time. (**b**) MEKC electropherogram of the corresponding extract of the soil pellet from the slurry sample. Differences in migration times from **a** to **b** are due to matrix and CE column EOF variations.

Transformation of these pesticides may also proceed by abiotic processes such as photolysis, hydrolysis, and reduction/oxidation as well as microbial transformation and may be affected by sorption. In our studies, photolytic processes were eliminated by the use of amber vials and the fact that the slurries were kept in the dark throughout the course of investigation. Hydrolytic, redox, and sorption processes related to intrinsic soil slurry characteristics such as pH and soil composition would have occurred in both live and autoclaved slurries, although autoclaving could conceivably change the soil characteristics and thus the reaction rates. Autoclaved samples were analyzed at the beginning and end of the experiment and compared with live samples to determine the extent of these abiotic processes.

Comparisons among the metalaxyl-spiked Ohio samples revealed that faster overall transformation rates with definitive enantioselectivity occurred in the live samples relative to the autoclaved ones (**Table 1**). For the autoclaved sample, the initial EF was 0.49, the same as for the metalaxyl standard, and the enantiomer concentrations were 33.5 and 36.5 mg/L. The same autoclaved sample had an EF of 0.50 when analyzed at day 47, which indicates no significant difference in peak areas, and corresponding concentrations of 24 and 25.5 mg/L, which point toward considerably less overall loss, about 30% (total of both enantiomers), as compared to the live sample, which had lost about 64% in concentration at day 47 (72% at day 55 when the experiment was terminated) with an EF of 0.08.

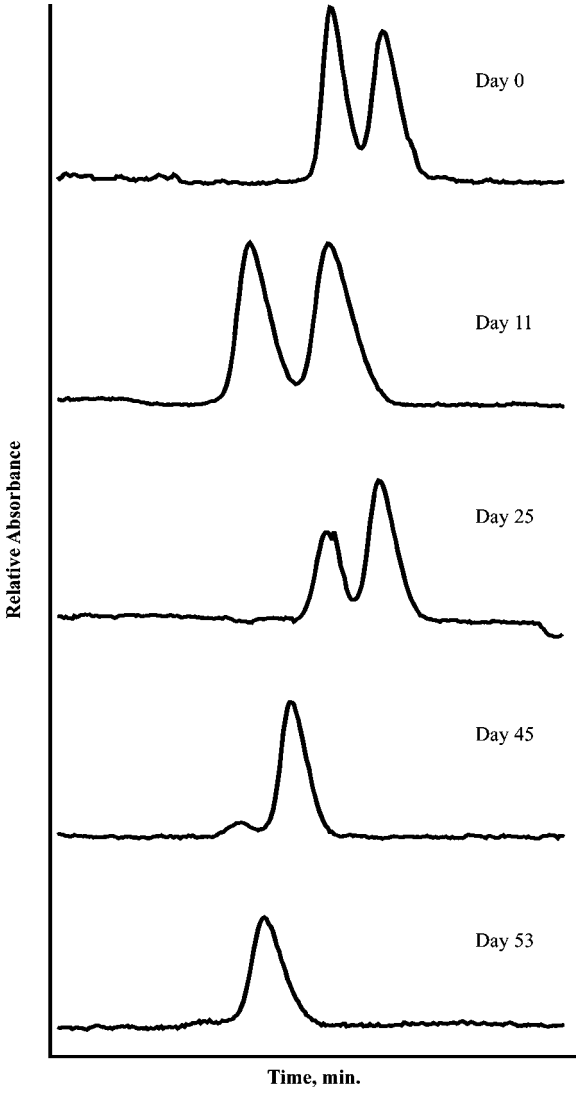

Figure 4. Electropherograms of metalaxyl enantiomers in a series of slurry samples revealing the change in enantioselective degradation pattern with incubation time. The first peak is *R*-(+)-metalaxyl. Migration times vary with time due to changes in the CE column EOF.

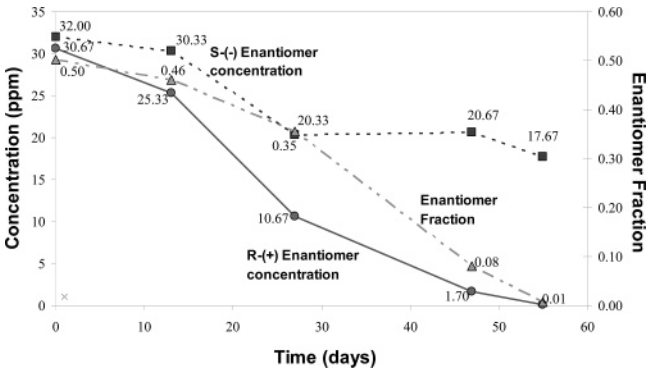

Figure 5. Graphical representation of the degradation of metalaxyl enantiomers in the Ohio soil slurry. The concentrations and EF values are the averages of triplicate samples. The degradation trends follow first-order rate kinetics.

In the case of imazaquin, analysis of the aqueous phases and the separated soils showed all detectable herbicides to be completely contained in the aqueous phases throughout the 3 month time of the experiment. As shown in **Figure 2**, live samples of imazaquin in Athens site 1 soils decreased in aqueous phase concentrations without enantiomeric preference; this was also the case with imazaquin in Athens site 2 soils. Analysis of the separated soil phase showed imazaquin to be below the detection level, so sorption was not the mechanism of loss. This is as expected—the imazaquin would be negatively charged ($pK_a = 3.8$) at the pH of the soils studied here and therefore would not be attracted to the soil surface, while imazaquin's water solubility of 60 mg/L would maintain solubility. In addition, use of amber bottles prevented photolytic processes. The concentration decreases are possibly due to hydrolysis or abiotic redox reactions. However, an additional possibility is that the microbial population present transforms both enantiomers at approximately the same rate. If the rates were slightly different, a longer incubation time might have shown selectivity to appear after several months. Because the half-life of imazaquin can be as long as 6 months (*2*), longer incubation times for these experiments would possibly reveal more information.

Table 1. Metalaxyl Concentrations (μg/mL) and EF Values with Time for Three Soils

sample location	day	average concn of enantiomer *R*-(+)	average concn of enantiomer *S*-(–)	average EF ±	SD EF ($n = 3$)
Ohio site	0	30.67	32.00	0.50	0.04
	13	25.33	30.33	0.46	0.05
	27	10.67	20.33	0.35	0.04
	47	1.70	20.67	0.08	0.01
	55	0.10	17.67	0.02	0.01
	0-autoclaved	33.50	36.50	0.49	0.11
	47-autoclaved	24.00	25.50	0.50	0.07
Athens site 1	0	46.67	49.67	0.47	0.01
	55	23.33	24.33	0.49	0.01
Athens site 2	0	37.33	36.00	0.51	0.01
	55	19.00	18.67	0.51	0.02

Fonofos analysis was considerably more difficult than for imazaquin and eventually required extraction of the soil as well as the aqueous phase. Analysis of the supernatant samples collected after 8 weeks showed aqueous concentrations of the first and second eluting enantiomers to be only 1.2 and 1.3 mg/L, respectively (**Figure 3a**). However, analysis of the extract of the soil pellets gave concentrations of 21 and 23 mg/L (**Figure 3b**), a total of 44 mg/L relative to the initial 60 mg/L concentration of fonofos in the soil slurry. This soil concentration is not surprising, considering that the log K_{ow} of fonofos is 3.9. However, the loss of about 25% of the fonofos initially spiked into the soil slurry is unexplained by our experimental data. Apparently some abiotic reaction occurred, or microbial transformation with no selectivity, since the EF after 8 weeks was the same as that of the standard.

Ruelene and Dichlorprop. CE with CD selectors was used for the enantiomeric analysis of hundreds of environmental soil samples spiked with the racemates of ruelene and dichlorprop to follow their microbial transformation kinetics (*7*). This was done to probe the microbial population character of disturbed/treated and control soil samples from field plots located in Brazil, North America, and Norway. Water slurries of soils from these plots were spiked and analyzed with time for the ruelene and dichlorprop enantiomers by CE. Because ruelene is a neutral compound, it was analyzed by the MEKC mode, while dichlorprop was analyzed as an anion.

Pesticide concentrations decreased by >80% during this time period for most of the soil slurries. **Figure 6** shows the decrease in EF with time during the transformation of 50 mg/mL of ruelene in a slurry of soil from an upland plateau in Risdalsheia, Norway. CE peak patterns at the beginning of the experiment

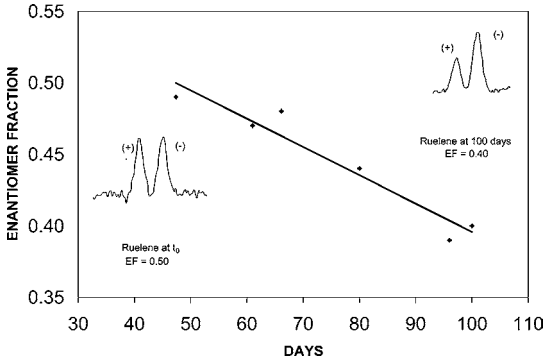

Figure 6. Decrease in EF with time during the microbial transformation of ruelene in a soil slurry. The first data point (47 days) corresponds to the last data collected before enantioselective transformation began. CE peak patterns show the change in EF from t_0 to 100 days.

Table 2. CE Parameter Means and Precision Data for Ruelene and Dichlorprop

	migration time (min)		peak area		EF
enantiomer	+	−	+	−	(±)
	Ruelene (25 μg/mL) ($n = 8$)				
mean	13.386	13.547	2.724	2.747	0.5
SD	0.462	0.473	0.058	0.068	0.01
RSD	3.452	3.489	2.131	2.471	2
	Ruelene (100 μg/mL) ($n = 8$)				
mean	20.123	20.422	14.667	15.881	0.48
SD	0.2	0.208	0.698	0.934	0.01
RSD	0.995	1.016	4.756	5.88	2.08
	Dichlorprop (50 μg/mL) ($n = 6$)				
mean	7.897	7.791	2.451	2.481	0.5
SD	0.061	0.059	0.106	0.105	0.01
RSD	0.77	0.76	4.325	4.247	2

and after 100 days are also depicted. The first migrating peak is the (+)-enantiomer, which preliminary experiments showed to be four times more toxic than the (−)-enantiomer to tent caterpillars (*Malacosoma disstria*) (*7*).

Ruelene was of racemic composition at the beginning, with an EF of 0.50. Both enantiomers disappeared as the same rate for almost 2 months; enantioselective transformation was not obvious before that time. After that, time intervals for analysis were adjusted to observe changes in enantiomer ratios, usually at intervals of about 10 days. During that time, in the case of this sample from Norway, the (+)-enantiomer was preferentially lost by microbial transformation, resulting in an EF (area of first peak/area of both peaks) of about 0.40 after 100 days. When CE detection levels for ruelene (about 5 mg/L injected concentration) were reached, analysis was stopped and reaction rates were calculated based on the first-order rate model, using data beginning with the first analysis that showed enantioselectivity (the first data point in **Figure 6**). For this sample from Norway, the half-life for the (+)-enantiomer was about 17 days, while that for the (−)-enantiomer was about 24 days. Interestingly, all naturally occurring soil samples from Norway preferentially removed the (+)-enantiomer but, of soils in parallel plots that had been warmed to 5° above ambient temperature for 4 years, 22% preferred the (−)-enantiomer.

Table 2 presents statistics for the analysis of ruelene standard solutions. Migration times can be variable because of changes with time in the buffer or column surface conditions. However, for eight consecutive runs of both a 25 μg/mL and a 100 μg/mL solution, the RSD for migration times of each enantiomer is good—about 3.5 for the lower concentration and about 1 for the higher. This degree of migration time reproducibility also shows that the resolution between enantiomer peaks is constant, which serves as one indicator of correct analyte identification. The RSDs of peak area are also good, less than 2.5 for each enantiomer at the low concentration but higher, about 4.8 and 5.9, for the high concentration. The RSDs of the EF values calculated from these enantiomer peak areas are about 2 in both cases, indicating good reproducibility. The average EF calculated from these data is 0.50 for the low and 0.48 for the high concentrations.

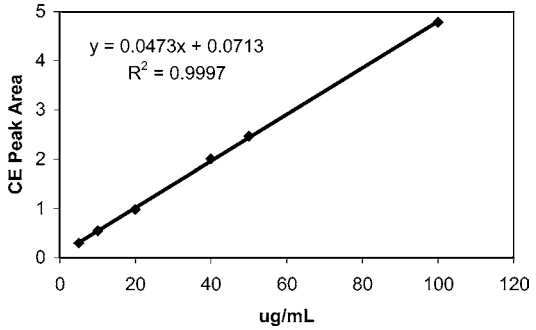

Figure 7. CE standard curve for calculation of dichlorprop concentrations in soil slurries; concentrations range from 5 to 100 μg/mL.

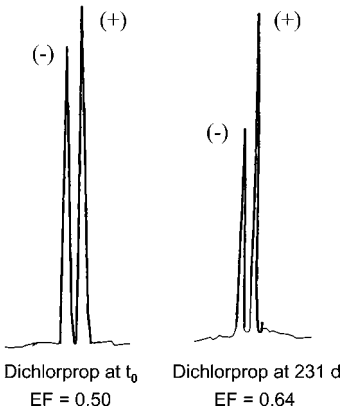

Figure 8. Partial electropherograms of dichlorprop at t_0 and after 231 days of incubation in a soil slurry.

Experimental conditions for dichlorprop were just as described for ruelene except that, because this compound is an anion under normal CE conditions, it is analyzed in the normal CE mode instead of the MEKC mode so does not require the addition of a micelle to the run buffer. **Figure 7** shows the typical linear standard curve, $R^2 = 0.9997$, used for calculation of dichlorprop concentration in these soil slurries, with standard concentrations ranging from 5 to 100 μg/mL. **Table 2** includes precision data for migration times and peak areas of the standard analyzed six times at 50 μg/mL. The RSD for migration time of each peak is about 0.76, while the RSD for the area of each peak is about 4.3. On the basis of these data, the average EF for the dichlorprop standard was 0.50, with a RSD of 2. It was determined by spiking the pure (+)-enantiomer that the (−)-enantiomer migrates first under these CE conditions, so EF = area second peak/area of both peaks.

CE analysis of dichlorprop was conducted at intervals of about 1 week, 2 weeks, 2 months, and then every 2 months after t_0. Approximately baseline separation was routinely obtained for the two enantiomers in these soil samples under these CE conditions (**Figure 8**). The detection limit for dichlorprop was estimated to be 3 μg/mL in the injected solution.

Enantioselective degradation of dichlorprop was not observed for several months, although there was some equal loss of both

enantiomers with each analysis. **Figure 8** depicts partial electropherograms of a soil slurry sample, prepared from soil collected from a deforested pasture area of the Fazenda Nova Vida in Rondonia, Brazil, at t_0 and after 231 days of incubation. The EF values for these two sets of enantiomers are 0.50 and 0.64, respectively. The half-life of the (+)-enantiomer is about 20 days, while that for the (−)-enantiomer is about 17 days; i.e., the active (+)-enantiomer is slightly more persistent. For comparison, in an earlier investigation, the half-life of the dichlorprop (+)-enantiomer in tilled soil samples collected after field application of the herbicide was 8.7 days, while that of the (−)-enantiomer was 4.4 days (*6*); that is, the (−)-enantiomer was also preferred in that case.

The study from which the above illustration was taken (*7*) showed that the transformation of dichlorprop varied both in enantiomer loss rate as well as in enantiomer preference from soil to soil and treatment to treatment in the Brazil samples. In forest soils from Brazil, the transformation was nonselective in 50% of the soils, while in 38% the (−)-enantiomer was transformed faster and in 13% the (+)-enantiomer was preferred. Soils from pastures that had been converted from forests were much more likely to transform the (−)-enantiomer; all of these soils transformed dichlorprop selectively, 93% of them preferring the nonherbicidal (−)-enantiomer.

In conclusion, CE with UV detection is a simple, fast, and relatively inexpensive way to analytically separate and follow the disappearance of pesticide enantiomers in laboratory microcosms where the concentrations can be made high enough (25−50 mg/L initial concentration of racemate) for enantiomer detection during the last part of the transformation process, during which concentrations may approach 1 mg/L. Even though 25−50 mg/L is not directly relevant to environmental concentrations, such concentrations are not too high for valid transformation studies unless the relevant microbes are inactivated by the pesticide. This relatively high concentration requirement could be significantly reduced by the use of more sensitive CE detectors or by concentration of the analyte during sample preparation. For example, CE with UV detection was used in a field study to follow the transformation kinetics of dichlorprop in soil after enrichment of samples by solvent extraction/evaporation; in that case, soil concentrations started at about 1 mg/kg and decreased to about 0.05 mg/kg after several weeks (*6*). Similarly, the soil fraction, as well as the water, of soil slurries in laboratory microcosms can also be analyzed by CE.

An important advantage of CE over GC techniques for the type of laboratory transformation studies described here is the possibility of direct analysis of the aqueous microcosm matrix, with centrifugation or syringe filtration the only cleanup step and no need for extraction. Equally important is the fact that enantiomer separation by CE is simpler and cheaper than with the chiral columns necessary for HPLC or GC. In addition, because it uses only low milliliter volumes of organic solvents, CE is a green chemistry technique. Finally, it is sometimes possible to identify target metabolites produced during microbial transformation, or abiotic reaction products, by matching product peaks with standards (*6*).

Results reported here show that microbial transformation of chiral pesticides can give preference to one enantiomer, leading to greater persistence of the other. It is obvious from this and earlier studies (*7*, *20*) that this phenomenon of enantioselectivity is site specific and depends on the character of the microbial population at each sample site. In some cases, even the enantiomer preference is reversed at different sites. Future studies of pesticide transformation in soil slurries should include soils with varying pH and other characteristics from different geographical regions. In this manner, general trends in microbial degradation may be observed, leading toward a capability to predict enantioselectivity (*20*).

In some cases, what are experimentally designed to be microbial reactions are not selective, resulting in an equal rate of loss of each enantiomer. Autoclaved (or otherwise sterilized) control samples can undergo abiotic reactions, including sorption, so monitoring of both autoclaved and live samples periodically would allow conclusions regarding microbial vs abiotic reactions in the live samples. In some cases with soil slurries, it would be important to also analyze the soil as well as the water to measure sorbed pesticide, which may give more insight into the transformation process. It is possible that both abiotic and microbial reactions occurred during incubation of the pesticides studied here. In cases where selectivity was not observed, as with imazaquin and fonofos, which were incubated for only 3 months, longer incubation times could have eventually shown some differences in enantiomer loss rates, indicating the occurrence of some level of microbial reactivity.

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LITERATURE CITED

(1) Roberts, T., Ed. *Metabolic Pathways of Agrochemicals*; The Royal Society of Chemistry: Cambridge, United Kingdom, 1998; Parts 1 and 2.

(2) The Pesticide Management Education Program at Cornell University, http://pmep.cce.cornell.edu/.

(3) Kurihara, N., Miyamoto, J., Eds. *Chirality in Agrochemicals*; John Wiley & Sons Ltd.: Chichester, England, 1998.

(4) van den Hoop, M.; Kreule, P.; Loch, J. Sorption kinetics and transformation of DDT in sediment. *Water, Air, Soil Pollut.* **1999**, *110*, 57−66.

(5) Ragnarsdottir, K. Environmental fate and toxicology of organophosphorus pesticides. *J. Geol. Soc.* **2000**, *157*, 859−876.

(6) Garrison, A.; Schmitt, P.; Martens, D.; Kettrup, A. Enantiomer selectivity in the environmental degradation of dichlorprop as determined by high-performance capillary electrophoresis. *Environ. Sci. Technol.* **1996**, *30*, 2449−2455.

(7) Lewis, D.; Garrison, A.; Wommack, K.; Whittemore, A.; Steudler, P.; Melillo, J. Influence of environmental changes on degradation of chiral pollutants in soils. *Nature* **1999**, *401*, 898−901.

(8) Ludwig, P.; Gunkel, W.; Huhnerfuss, H. Chromatographic separation of the enantiomers of marine pollutants: Enantioselective degradation of phenoxycarboxyclic acid herbicides by marine microorganisms. *Chemosphere* **1992**, *24*, 1423−1429.

(9) Tett, V.; Willetts, A.; Lappinscott, H. Enantioselective degradation of the herbicide mecoprop [2-(2-methyl-4-chlorophenoxy) propionic acid] by mixed and pure bacterial cultures. *FEMS Microbiol. Ecol.* **1994**, *14*, 191−199.
(10) Mueller, M.; Schlabach, M.; Oehme, M. Fast and precise determination of alpha-hexachlorocyclohexane enantiomers in environmental samples using chiral high-resolution gas chromatography. *Environ. Sci. Technol.* **1992**, *26*, 566−569.
(11) Armstrong, D.; Reid, G.; Hilton, M.; Chang, C. Relevance of enantiomeric separations in environmental science. *Environ. Pollut.* **1993**, *79*, 51−58.
(12) Huhnerfuss, H.; Faller, J.; Kallenborn, R.; Konig, W.; Ludwig, P.; Pfaffenberger, B.; Oehme, M.; Rimkus, G. Enantioselective and nonenantioselective degradation of organic pollutants in the marine ecosystem. *Chirality* **1993**, *5*, 393−399.
(13) Buser, H.; Mueller, M. Isomer and enantioselective degradation of hexachlorocyclohexane isomers in sewage sludge under anaerobic conditions. *Environ. Sci. Technol.* **1995**, *29*, 664−672.
(14) Buser, H.; Mueller, M.; Poiger, T.; Balmer, M. Environmental behavior of the chiral acetamide pesticide metalaxyl: Enantioselective degradation and chiral stability in soils. *Environ. Sci. Technol.* **2002**, *36*, 221−226.
(15) Hernandez-Borges, J.; Frias-Garcia, S.; Cifuentes, A.; Rodriguez-Delgado, M. Pesticide analysis by capillary electrophoresis. *J. Sep. Sci.* **2004**, *27*, 947−963.
(16) Penmetsa, K.; Leidy, R.; Shea, D. Enantiomeric and isomeric separation of herbicides using cyclodextrin-modified capillary zone electrophoresis. *J. Chromatogr. A* **1997**, *790*, 225−234.
(17) Schmitt, P.; Garrison, A.; Freitag, D.; Kettrup, A. Applications of cyclodextrin-modified micellar electrokinetic chromatography to the separation of selected neutral pesticides and their enantiomers. *J. Chromatogr. A* **1997**, *792*, 419−429.
(18) Wong, C.; Garrison, A.; Smith, P.; Foreman, W. Enantiomeric composition of chiral polychlorinated biphenyl atropisomers in aquatic and riparian biota. *Environ. Sci. Technol.* **2001**, *35*, 2448−2454.
(19) Spindler, F.; Fruh, T. Chiral acylanilides and chiral triazole-related fungicides. In *Chirality in Agrochemicals*; Kurihara, N., Miyamoto, J., Eds.; John Wiley & Sons Ltd.: Chichester, England, 1998; p 155.
(20) Buerge, I.; Poiger, T.; Mueller, M.; Buser, H. Enantioselective degradation of metalaxyl in soils: Chiral preference changes with soil pH. *Environ. Sci. Technol.* **2003**, *37*, 2668−2674.

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