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Enantiomeric Differences in Permethrin Degradation Pathways in Soil and Sediment

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Chirality occurs widely in synthetic pyrethroids. Studies have shown significant differences in both aquatic toxicity and degradation rates between enantiomers from the same diastereomer of selected pyrethroids. To better understand chiral selectivity in biodegradation of pyrethroids, ^{14}C-labeled permethrin was used to characterize enantiomeric differences in the formation of transformation intermediates in two soils and a sediment. Individual enantiomers of permethrin were spiked into soil and sediment samples, and transformation products were identified with known standards. Enantioselectivity was observed in most treatments when the dissipation of the parent enantiomers, the amount of intermediates and bound residues formed, and mineralization rates were compared between the enantiomers. The results show that all enantiomers of permethrin hydrolyzed rapidly and that the hydrolysis products were quickly further transformed. The direct hydrolysis products, cyclopropanic acid (Cl_2CA), 3-phenoxybenzyl alcohol (PBalc), and 3-phenoxybenzoic acid (PBacid), were recovered at small percentages, ranging from 1 to 14% for Cl_2CA and from 0.2 to 6% for PBalc and PBacid. The *R*-enantiomer of both *cis*- and *trans*-permethrin was mineralized more quickly than the *S*-enantiomer after hydrolysis. The degradation products from *cis*-permethrin were more persistent than those from *trans*-permethrin. As some transformation intermediates of permethrin may have greater acute and chronic toxicity than the parent compound, enantioselectivity in the formation of degradation intermediates may lead to different overall toxicities and merit further investigation. This study suggests that for chiral compounds, enantioselectivity may be reflected not only in the dissipation of the parent enantiomers but also in the kinetics of formation of intermediate transformation products.

KEYWORDS: Enantioselectivity; chiral selectivity; enantiomer; pyrethroids; transformation pathways

INTRODUCTION

Chirality in modern pesticides has received an increasing amount of attention over the past decade (*1*−*4*). Pyrethroids are synthetic derivatives of the chrysanthemumic acids. They constitute a group of very potent insecticides, which are considered to be good substitutes for organophosphate and carbamate compounds (*5*). Pyrethroids have been widely used for insect control on agricultural crops, on animals, and around households (*5*−*7*). For instance, agricultural use of permethrin in California increased by 50% from 1999 to 2004 (California Pesticide Use Reports, http://www.cdpr.ca.gov/docs/pur/purmain.htm). With the restriction of some organophosphate insecticides, the use of pyrethroids is expected to increase further. Even though pyrethroids show low toxicity to birds and mammals, they are acutely toxic to a wide range of aquatic organisms, including water-column- and sediment-dwelling invertebrates, often at a trace concentration of $<$0.5 ppb (*6*−*8*). This makes contamination of streams by pyrethroids a great environmental concern in both agricultural and urban areas (*9*, *10*).

All pyrethroids are chiral compounds, having two or three chiral centers. Chirality of pyrethroids may arise from the acid moiety, the alcohol moiety, or both (*11*, *12*). However, even though many studies have been carried out to understand the metabolism and environmental fate of pyrethroids (*13*−*15*), essentially all of the earlier studies regarded racemic pyrethroids as single compounds, failing to acknowledge potential enantioselectivity in their biologically mediated environmental processes. Recent studies showed that enantioselectivity may occur in both aquatic toxicity and biodegradation of pyrethroids (*16*, *17*). Evaluation of chiral selectivity in the degradation pathways of pyrethroid enantiomers will be of significant value for improving our understanding of the enantioselective degradation of pyrethroids in the environment. In addition, some metabolites of pyrethroids are known to have enhanced toxicity to certain nontarget organisms when compared to the parent compound. For instance, the effect of permethrin and its 10 degradation products on the growth of fungi and on the growth, photosynthesis, and acetylene-reducing ability of two species of green algae and three species of cyanobacteria was evaluated by Stratton and Corke (*18*). For all of the test organisms, permethrin was found to be relatively nontoxic, whereas some of its metabolites, including 3-phenoxybenzaldehyde, 3-phenoxyben-

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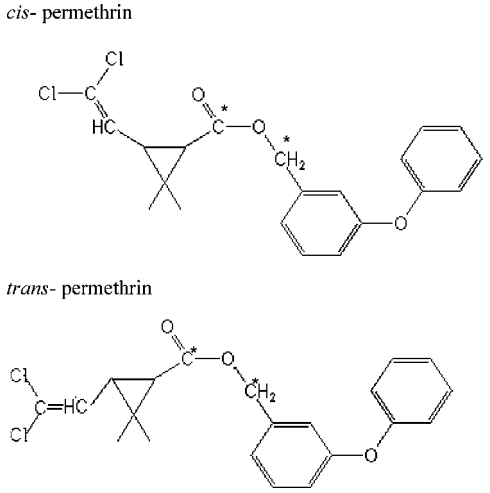

Figure 1. Chemical structures and ^{14}C-labeling positions of permethrin enantiomers. Asterisks indicate labeled positions.

zyl alcohol, 3-hydroxybenzoic acid, and 3-phenoxybenzoic acid, were found to be more toxic. In a study by Tyler et al. (*19*), pyrethroid metabolites of environmental degradation were found to have the potential to interact with steroid hormone receptors. Some metabolites of permethrin displayed both estrogenic and antiandrogenic activities with potencies >100-fold greater than those of the parent compound.

The main objective of this study was to determine if enantioselectivity occurred in the biodegradation of pyrethroids in soil and sediment media by comparing the formation of transformation products between enantiomers. Permethrin was selected as a model pyrethroid compound due to its widespread use and the availability of ^{14}C-labeled permethrin and authentic standards of some of its metabolites.

MATERIALS AND METHODS

Chemicals. Racemic [^{14}C]permethrin [3-phenoxybenzyl (*1RS*)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecaboxylate], with ^{14}C labeled in the acid or alcohol moiety, was provided by FMC (Princeton, NJ). The structure and labeling positions of *cis*- and *trans*-permethrin are shown in **Figure 1**. A previously developed HPLC method (*16*) was used for the separation and preparation of individual ^{14}C-labeled permethrin enantiomers. The resolution of enantiomers was achieved on a Sumichiral OA-2500I column (Sumika Chemical Analysis Service, Osaka, Japan) by using 99.5% hexane and 0.5% 1,2-dichloroethane as the mobile phase. The individual enantiomers were manually collected at the HPLC outlet and enriched. The purity of these enantiomers was determined to be >99% by HPLC and/or GC analysis prior to their use. A similar approach was also used to prepare pure enantiomers from nonlabeled permethrin. By mixing ^{14}C-labeled and nonlabeled enantiomers, the radioactivity of each permethrin enantiomer was adjusted to about 5×10^6 dpm mg^{-1}.

Standards of four metabolites, *cis*-3-(2,2-dicholorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (c-Cl_2CA, 98.9%), *trans*-3-(2,2-dicholorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (t-Cl_2CA, 99.3%), 3-phenoxybenzyl alcohol (PBalc, 99.5%), and 3-phenoxybenzoic acid (PBacid, 99.2%), were obtained from FMC. Solvents and other chemicals used in this study were of analytical or HPLC grade.

Soils and Sediment. Two soils and one sediment were used in this study (**Table 1**). The soil samples were collected from the surface layer (0−15 cm) of a field plot planted with a low-growing groundcover (spring cinquefoil, *Potentilla tabernaemontani*) and a field plot planted with tall fescue turfgrass (*Festuca arundinacea*) at the Agricultural Experiment Station near the University of California, Riverside, campus. The two soil samples were selected because they had been planted to a dicot (spring cinquefoil) and a monocot (grass) for an extended time (about 10 years), and the different planting practices may have influenced the soil microbial communities in the rhizosphere. The sediment sample (sandy clay) was collected from the 0−10 cm depth in San Diego Creek in Orange County, California. San Diego Creek is the main drainage channel connecting the inland regions and the Newport Bay estuaries. The soil and sediment samples were air-dried for 24 h at room temperature, homogenized while still slightly moist, and then passed through a 2-mm sieve before use.

Table 1. Textural and Chemical Properties of Soil and Sediment Samples Used in This Study

	pH	OM (%)	sand (%)	silt (%)	clay (%)
San Diego Creek sediment	7.9	1.1	20.0	46.0	34.0
turfgrass soil	7.4	1.7	65.0	22.5	12.5
groundcover soil	7.3	1.6	62.0	24.0	14.0

Incubation Experiments. Ten grams of soil or sediment (dry weight equivalent) was placed in 150-mL glass flasks (for aerobic treatments) or 20-mL glass vials (for anaerobic treatments). For the groundcover and turfgrass soils, the water content was adjusted to about 60% of the field holding capacity (w/w) by adding deionized water. For the San Diego Creek sediment, 6 mL of deionized water was added to each sample to immerse the sediment and form a 0.5-cm layer of water. Individual ^{14}C-carbonyl- or ^{14}C-alcohol-labeled permethrin enantiomers were then spiked into the soil or sediment samples, at a rate of 10 μg of chemical in 50 μL of acetone for each sample. This resulted in an initial pesticide concentration of 1.0 mg kg^{-1} with a radioactivity of 5×10^4 dpm per sample. The treated soil samples were incubated under aerobic conditions, whereas the treated sediment samples were incubated under both aerobic and anaerobic conditions. For aerobic treatments, the sample containers were loosely covered with aluminum foil and kept at room temperature (20 ± 1 °C). For the anaerobic treatment, the sample vials were equilibrated, spiked, crimp sealed, and incubated in a nitrogen-filled plastic chamber at room temperature. All of the samples in the aerobic treatments were checked regularly for water content by weighing and were frequently mixed by hand for aeration.

Duplicate samples were removed from each treatment at 14 and 56 days after the pesticide application and immediately transferred into a freezer (−20 °C) to stop degradation. For extraction, each sample was thawed at room temperature and transferred to a preweighed 250-mL glass centrifuge bottle. The sample was mixed with anhydrous sodium sulfate (25 g for soil samples and 50 g for sediment samples, respectively) and 50 mL of hexane/acetone (1:1, v/v) for 1 h on a mechanical shaker and then centrifuged at 1000 rpm for 20 min. The same extraction step was repeated for a total of three times, and the solvent extracts were combined and evaporated to around 2 mL on a vacuumed rotary evaporator at 50 °C. The residues were transferred to a glass evaporation tube and blown down with nitrogen to 0.5 mL. To determine the organic-soluble radioactivity, a 100-μL aliquot of the final extract was added into 5 mL of Ultima Gold cocktail (Perkin-Elmer, Boston, MA) and the radioactivity was measured on a Beckman LC-5000TD liquid scintillation counter (LSC) (Beckman, Fullerton, CA). The centrifuge bottle with the extracted soil or sediment was weighed again to determine the water content of the solid phase. An aliquot of the extracted soil or sediment was removed, air-dried, and then combusted on a biological oxidizer (OX-500 Biological Oxidizer, R. J. Harvey, Hillsdale, NJ). The released $^{14}CO_2$ was trapped in 15 mL of cocktail, and the radioactivity was measured by LSC to obtain the activity associated with the nonextractable or bound residues. The recovery for the oxidizer was about 80% and was used to correct for the measured activity. The overall recovery of the added radioactivity was 96.1−104.2% on the basis of analysis of soil and sediment samples immediately following spiking.

Thin-Layer Chromatography (TLC). Silica gel glass TLC plates (60 F254, 10 × 20 cm, 0.25-mm thickness, Merck, Darmstadt, Germany) were used for the separation and tentative identification of permethrin and metabolites in the organic-soluble fraction. The solvent system used for developing the TLC plates was hexane/acetone (3:2, v/v). The R_f values of the four metabolites and *cis*- and *trans*-permethrin are given in **Table 2**. For each sample, 100 μL of the final sample extract was spiked on the TLC plate at about 2 cm from the base with

Table 2. R_f Values of Permethrin and Metabolites on Thin-Layer Silica Gel Plates Developed in Hexane/Acetone (3:2, v/v)

	^{14}C-acid-labeled		^{14}C-alcohol-labeled	
compound	*cis*	*trans*	*cis*	*trans*
c-Cl_2CA	0.64			
t-Cl_2CA		0.61		
Pbalc			0.62	0.62
Pbacid			0.53	0.53
cis-PM	0.81		0.81	
trans-PM		0.80		0.80

a microsyringe. Nonlabeled standards of the metabolites and permethrin were spiked in parallel with the sample on the same plate. The spiked plate was then developed in the solvent mixture to about 17 cm from the baseline. After the developed plate was air-dried, silica gel at the same positions as the reference standards was carefully scraped using a spatula. The silica gel was transferred to 5 mL of Ultima Gold cocktail solution (Perkin-Elmer) in 7-mL scintillation vials and mixed at high speed on a mechanical shaker for 30 min. After the silica gel had settled, the sample vials were measured for radioactivity on LSC. The silica gel remaining in the same channel was also scraped and the total radioactivity counted on LSC. This activity was defined as unidentified metabolites in this study.

RESULTS AND DISCUSSION

Disappearance of Parent Enantiomer. The fractions of the parent permethrin enantiomer as percent of the applied radioactivity after 14 and 56 days of incubation are given in **Table 3**. Paired *t* test showed that there was no difference in the residual parent enantiomer fractions between the two labeling positions for the same treatment. Under aerobic conditions, degradation of the parent enantiomers was consistently faster in the two soils than in the sediment (**Tables 4** and **5**). In the San Diego Creek sediment, all permethrin enantiomers were degraded more slowly under anaerobic conditions than under aerobic conditions (**Table 5**). Paired *t* test for the same time points between the aerobic and anaerobic treatments yielded a *p* value of 0.04 for the ^{14}C-carbonyl-labeled permethrin enantiomers and <0.01 for the ^{14}C-alcohol-labeled permethrin enantiomers. The limited time points in this study precluded the possibility for calculating a half-life value. However, it may be estimated that the half-life of all permethrin enantiomers was <14 days in all treatments, with the only exception being the enantiomers from *cis*-permethrin in the sediment incubated under anaerobic conditions, for which the half-life was estimated to be around 14 days. Moderate persistence was previously reported for permethrin in soils, with average half-lives of 30 days for aerobic conditions and 108 days for anaerobic conditions (*20*). The half-life of racemic permethrin in aged sediments was found to be much longer, at 160 days under aerobic conditions and 240 days under anaerobic conditions (*21*). The short persistence of permethrin in this study suggests that the soil and sediment samples were likely to be more microbially active and that freshly spiked permethrin was more susceptible to microbial transformations than aged pesticides (*13*, *15*, *21*).

In all treatments, enantiomers from the *trans*-permethrin diastereomer were always degraded more rapidly than the *cis*-permethrin enantiomers under the same conditions, and the difference was always significant at $\alpha = 0.05$ (**Table 3**). For instance, after 14 days of incubation, only 9.6−13.7% of the applied activity was recovered as *trans*-permethrin enantiomers in the soils, whereas 21.1−31.4% of the spiked activity remained as *cis*-permethrin enantiomers. These results are consistent with previous studies, in which more rapid degradation was consistently found for *trans*-permethrin than for *cis*-permethrin in soils, in aqueous systems and in plant (*11*, *12*, *21*−*26*).

Differences in transformation rates were further observed between the enantiomers from the same diastereomer. Paired *t* test showed significantly slower degradation of *R*-*cis*-permethrin than of *S*-*cis*-permethrin in the two soils under aerobic conditions. However, the difference between these two enantiomers was found to be insignificant for the San Diego Creek sediment under aerobic conditions ($p = 0.31$). Paired *t* test further showed significant enantioselectivity in the San Diego Creek sediment under anaerobic conditions, but the direction of selectivity was reversed from that in the aerobic soils, with *S*-*cis*-permethrin degrading more slowly than *R*-*cis*-permethrin. A similar comparison was also performed for the two enantiomers of *trans*-permethrin. The results showed that in groundcover and turfgrass soils, *R*-*trans*-permethrin was significantly more persistent than *S*-*trans*-permethrin ($p = 0.03$). However, in the San Diego Creek sediment, no difference was found under aerobic conditions ($p = 0.91$), whereas *R*-*trans*-permethrin was observed to be preferentially transformed over *S*-*trans*-permethrin under anaerobic conditions ($p = 0.01$). These observations indicate that enantioselectivity frequently occurred in permethrin degradation in the soil and sediment samples, but the direction of the selectivity varied with the matrix and incubation conditions. In this study, the groundcover soil and the turfgrass soil were taken from adjacent field plots, and the soils were therefore of the same origin (**Table 1**). Planting of the soil to different plants (i.e., dicot versus monocot) did not result in different enantioselectivity in the degradation of permethrin. In contrast, the San Diego Creek sediment was from a freshwater creek and displayed different enantioselectivity from the soils. Even in the same sediment, enantioselectivity seemed to be influenced by oxygenation, suggesting oxygen supply may play an important role in the enantioselective degradation of pyrethroids, likely by influencing the indigenous microbial communities.

Distribution of Permethrin Residues in Different Phases. Ester cleavage is generally considered to be the predominant process in environmental degradation of permethrin, which results in the production of the cyclopropanic acid (Cl_2CA) and 3-phenoxybenzyl alcohol (PBalc). The 3-phenoxybenzyl alcohol fragment may be further oxidized to 3-phenoxybenzoic acid (PBacid). Other minor pathways with each isomer can lead to the formation of esters with one hydroxyl substituent at the 2′- or 4′-position of the phenoxy group or the *gem*-dimethyl group, an ester with hydroxylation at both 4′-phenoxy and *gem*-dimethyl sites, an acid moiety hydroxylated at either methyl group, or conjugates of these acids. Further decarboxylation of the cyclopropanic acid and phenoxylbenzoic acid may also occur, which ultimately result in the evolution of CO_2 (*23*−*26*). The overall pathways are depicted in **Figure 2** to facilitate the following discussion.

In this study, permethrin residues were recovered in the organic-soluble and nonextractable or soil-bound fractions (**Tables 4** and **5**). The results show that 15−30% of the applied radioactivity was bound to the soil or sediment phase following solvent extraction. In all treatments, significantly more ($p = 0.01$) bound residues were formed with the *S*-enantiomer than for the *R*-enantiomer, and the difference was observed for both *cis*- and *trans*-permethrin. The bound ^{14}C residues of pyrethroids are generally associated with organic matter and are considered not to be bioavailable and therefore a pathway for detoxification (*26*).

The difference between the applied radioactivity and the recovered activity is assumed to be the loss of radioactivity as

Table 3. Recovered Activity as the Parent Permethrin Enantiomer in Soils Incubated under Aerobic Conditions and Sediment Incubated under Aerobic or Anaerobic Conditions (Percent of Applied Activity)

	14 days				56 days			
	cis		*trans*		*cis*		*trans*	
	R	*S*	*R*	*S*	*R*	*S*	*R*	*S*
				^{14}C-Carbonyl-Labeled Permethrin				
groundcover soil	30.2 ± 0.2	25.0 ± 0.5	10.9 ± 1.7	9.6 ± 1.0	4.3 ± 0.1	3.8 ± 0.2	2.6 ± 0.0	1.5 ± 0.1
turfgrass soil	31.4 ± 0.2	21.1 ± 0.2	12.0 ± 0.7	9.9 ± 0.0	7.0 ± 0.0	0.8 ± 0.0	4.6 ± 0.1	2.2 ± 0.1
sediment, aerobic	34.6 ± 0.5	36.6 ± 1.8	22.9 ± 0.4	21.3 ± 0.7	15.3 ± 1.3	14.6 ± 0.5	11.2 ± 1.1	14.2 ± 0.6
sediment, anaerobic	50.7 ± 0.1	53.3 ± 0.6	28.8 ± 0.4	34.3 ± 0.1	26.4 ± 2.2	30.3 ± 0.3	13.4 ± 0.7	21.3 ± 2.0
				^{14}C-Alcohol-Labeled Permethrin				
groundcover soil	28.8 ± 0.4	23.1 ± 1.9	10.8 ± 1.8	11.2 ± 0.0	4.2 ± 1.1	3.3 ± 0.2	2.8 ± 0.3	1.6 ± 0.3
turfgrass soil	29.7 ± 1.2	23.7 ± 1.6	13.7 ± 0.2	12.3 ± 1.1	5.4 ± 0.6	2.2 ± 0.2	3.3 ± 0.0	1.7 ± 0.2
sediment, aerobic	32.6 ± 1.2	37.2 ± 3.9	23.8 ± 1.0	18.8 ± 0.5	15.5 ± 0.3	14.5 ± 0.4	11.0 ± 1.4	14.4 ± 0.9
sediment, anaerobic	45.2 ± 2.5	51.3 ± 1.8	25.9 ± 2.1	30.3 ± 0.1	24.8 ± 0.3	30.2 ± 0.7	14.4 ± 0.0	24.4 ± 1.0

Table 4. Recovered Activity in the Organic-Soluble and Soil-Bound Phases after Incubation of ^{14}C-Labeled Permethrin Enantiomers in Soils under Aerobic Conditions (Percent of Applied Activity)

	14 days				56 days			
	cis		*trans*		*cis*		*trans*	
	R	*S*	*R*	*S*	*R*	*S*	*R*	*S*
				Groundcover Soil				
				^{14}C-Carbonyl-Labeled Permethrin				
organic-soluble	59.0 ± 1.3	50.1 ± 2.5	28.6 ± 3.1	28.5 ± 2.3	20.5 ± 0.3	30.0 ± 1.7	12.3 ± 0.1	11.9 ± 0.6
bound residues	21.2 ± 0.7	22.9 ± 1.1	24.4 ± 1.1	29.5 ± 0.7	21.3 ± 0.7	25.4 ± 0.3	13.1 ± 0.3	18.0 ± 0.9
total recovered	80.2 ± 0.6	73.0 ± 3.6	52.8 ± 4.2	58.2 ± 1.4	42.5 ± 1.1	55.4 ± 1.6	25.3 ± 0.4	29.0 ± 1.6
				^{14}C-Alcohol-Labeled Permethrin				
organic-soluble	55.1 ± 0.5	45.6 ± 2.6	31.1 ± 3.3	28.6 ± 0.0	19.4 ± 1.5	24.7 ± 1.1	11.7 ± 0.1	11.3 ± 0.6
bound residues	21.1 ± 0.9	25.0 ± 0.1	23.7 ± 0.2	30.6 ± 0.0	20.8 ± 1.4	25.4 ± 0.5	14.6 ± 0.5	18.6 ± 1.6
total recovered	76.2 ± 0.4	70.6 ± 2.7	54.8 ± 3.3	59.2 ± 0.0	40.6 ± 0.1	49.6 ± 0.6	25.3 ± 0.4	28.5 ± 2.3
				Turfgrass Soil				
				^{14}C-Carbonyl-Labeled Permethrin				
organic-soluble	54.9 ± 0.2	46.2 ± 1.4	29.6 ± 1.0	24.5 ± 0.9	26.8 ± 0.5	31.0 ± 2.4	13.0 ± 0.1	12.1 ± 0.9
bound residues	22.2 ± 0.3	25.0 ± 1.2	25.0 ± 0.6	31.5 ± 0.2	22.0 ± 0.8	25.4 ± 0.3	13.0 ± 0.9	17.1 ± 0.1
total recovered	77.1 ± 0.1	71.2 ± 2.6	54.6 ± 0.4	55.9 ± 0.7	26.1 ± 1.0	30.1 ± 0.4	26.1 ± 1.0	30.1 ± 0.4
				^{14}C-Alcohol-Labeled Permethrin				
organic soluble	55.1 ± 1.3	48.3 ± 4.3	30.0 ± 0.6	28.6 ± 2.1	22.6 ± 0.8	31.5 ± 3.1	12.3 ± 0.1	11.5 ± 0.5
bound residues	22.9 ± 0.2	24.4 ± 0.1	25.1 ± 0.1	30.1 ± 0.2	21.1 ± 0.8	24.9 ± 0.3	13.6 ± 0.6	17.2 ± 3.0
total recovered	78.0 ± 1.5	77.7 ± 4.2	55.1 ± 0.5	58.9 ± 1.9	43.4 ± 1.6	56.9 ± 2.8	26.9 ± 0.7	30.1 ± 1.2

$^{14}CO_2$, which ranged from 20 to 75% of the originally applied radioactivity for the different treatments. The fraction of ^{14}C potentially lost to mineralization was consistently greater for *trans*-permethrin than for *cis*-permethrin, suggesting that the derivatives of *trans*-permethrin were more readily mineralized than those of *cis*-permethrin. Between the two enantiomers, the loss of the *R*-enantiomer to mineralization was greater than that for the *S*-enantiomer, and the difference was significant for both *cis*- and *trans*-permethrin at 56 days ($p < 0.05$). This suggests that even though *R*-enantiomers were not always transformed more quickly than the *S*-enantiomers initially, their subsequent transformations and mineralization steps could be more rapid.

Degradation Products in the Organic-Soluble Phase. Four major transformation intermediates that were identified in previous studies (*23*−*26*) were analyzed for each permethrin enantiomer. The relative fractions of these metabolites obtained from the four permethrin enantiomers during incubation of soils and sediment samples are summarized in **Tables 6** and **7**. In soils spiked with ^{14}C-carbonyl-labeled permethrin, Cl_2CA was detected in all of the samples, ranging from 1.4 to 13.9% of the applied activity. In all of the samples treated with individual *cis*-permethrin enantiomers, Cl_2CA was formed in significantly ($p < 0.05$) larger fractions for *S*-*cis*-permethrin than *R*-*cis*-permethrin. However, between the two enantiomers from the *trans*-permethrin, no significant difference was found in their relative fractions. In the soils spiked with ^{14}C-alcohol-labeled permethrin, PBalc and PBacid were detected in most cases at small percentages, usually <5% of the applied activity. In *cis*-permethrin, PBalc and PBacid were detected at a greater or similar percentage for the *R*-enantiomer than for the S-enantiomer, whereas in *trans*-permethrin an opposite trend was observed. However, statistical analysis did not show a significant difference for either *cis*- or *trans*-permethrin. This may partly be attributed to the relatively rapid transformation of PBalc to PBacid and further to simpler derivatives in the environment (*25*). From a comparison of the data from the 14 and 56 day treatments, it is also evident that with *cis*-permethrin enantiomers, the amount of all three hydrolysis products increased over the incubation time, whereas it decreased or remained unchanged for *trans*-permethrin. It is known that the hydrolytic metabolites of permethrin may exhibit potentially higher toxicity to certain nontarget organisms than permethrin (*18*, *19*). The above finding suggests that hydrolysis products of *cis*-permethrin are more persistent than those of *trans*-permethrin and thus may retain biological activity for a longer time in the environment.

A large fraction of the recovered radioactivity was defined as unidentified metabolites in this study due to the lack of chemical standards for all possible transformation metabolites.

Table 5. Recovered Activity in the Organic-Soluble and Bound Phases after Incubation of ^{14}C-Labeled Permethrin Enantiomers in the San Diego Creek Sediment under Aerobic or Anaerobic Conditions (Percent of Applied Activity)

	14 days				56 days			
	cis		*trans*		*cis*		*trans*	
	R	*S*	*R*	*S*	*R*	*S*	*R*	*S*
Aerobic Conditions								
^{14}C-Carbonyl-Labeled Permethrin								
organic-soluble	49.2 ± 0.5	54.9 ± 3.0	41.8 ± 0.1	43.7 ± 0.7	38.5 ± 1.1	42.7 ± 0.3	28.2 ± 2.6	34.5 ± 0.9
bound residues	19.6 ± 1.5	22.5 ± 0.3	17.3 ± 0.3	18.4 ± 0.1	16.7 ± 0.9	20.6 ± 0.5	16.5 ± 0.6	19.9 ± 0.7
total recovered	68.8 ± 1.9	77.4 ± 2.7	59.1 ± 0.3	62.1 ± 0.5	55.2 ± 4.2	63.3 ± 1.6	44.7 ± 0.6	54.4 ± 0.1
^{14}C-Alcohol-Labeled Permethrin								
organic-soluble	50.6 ± 0.4	61.4 ± 3.4	48.1 ± 1.5	46.2 ± 2.0	38.4 ± 1.5	44.8 ± 0.9	26.8 ± 3.2	35.8 ± 2.8
bound residues	20.5 ± 0.1	22.3 ± 0.4	16.8 ± 0.5	20.1 ± 1.0	18.0 ± 0.4	22.6 ± 0.8	13.2 ± 1.8	13.1 ± 0.1
total recovered	71.1 ± 0.3	83.7 ± 6.7	64.9 ± 1.0	66.3 ± 0.0	56.4 ± 2.0	67.4 ± 1.0	40.0 ± 5.0	48.9 ± 2.7
Anaerobic Conditions								
^{14}C-Carbonyl-Labeled Permethrin								
organic-soluble	66.6 ± 2.0	70.9 ± 2.2	54.5 ± 0.2	60.9 ± 0.1	51.1 ± 1.4	65.6 ± 0.4	30.0 ± 1.8	39.8 ± 3.7
bound residues	20.0 ± 2.2	23.0 ± 0.7	17.3 ± 0.8	19.3 ± 0.0	19.1 ± 1.0	22.9 ± 0.4	14.5 ± 0.0	19.9 ± 0.4
total recovered	86.6 ± 4.2	93.9 ± 1.6	71.8 ± 0.6	80.2 ± 0.1	70.2 ± 0.4	87.9 ± 0.8	44.5 ± 1.2	59.7 ± 3.3
^{14}C-Alcohol-Labeled Permethrin								
organic-soluble	65.4 ± 3.0	72.7 ± 0.7	50.0 ± 3.1	55.1 ± 1.8	50.5 ± 0.8	64.8 ± 0.3	33.7 ± 2.3	44.3 ± 0.2
bound residues	20.2 ± 0.1	21.1 ± 0.4	16.5 ± 1.4	20.8 ± 1.0	18.8 ± 1.1	22.7 ± 0.9	17.2 ± 0.7	22.5 ± 1.4
total recovered	85.6 ± 3.8	93.8 ± 1.1	66.5 ± 4.5	75.9 ± 2.7	69.3 ± 1.9	87.5 ± 0.6	50.9 ± 3.0	66.8 ± 1.1

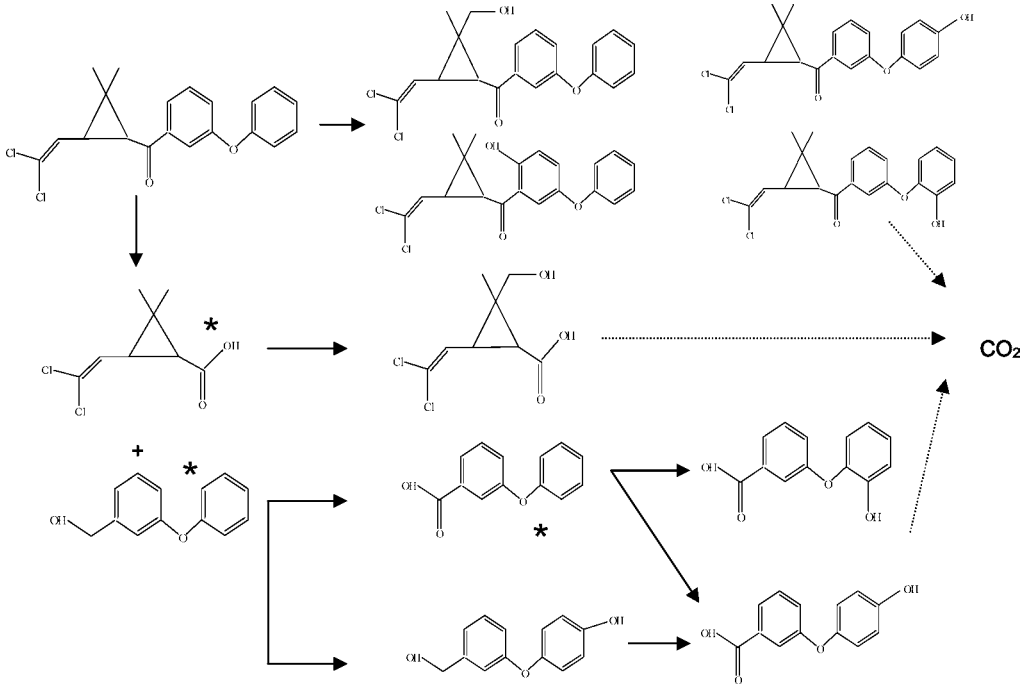

Figure 2. Degradation pathways of permethrin in soil. Asterisks indicate the metabolites identified in this study.

The unidentified residues may derive from further transformation of the hydrolysis products and other oxidation reactions of permethrin. Previous studies (*26*, *27*) showed that the most important pathway for both *cis*- and *trans*-permethrin is ester cleavage. However, more $^{14}CO_2$ and ester cleavage products were formed in the soils treated with the less persistent *trans*-permethrin, whereas oxidation products retaining the ester linkage such as diphenyl ether bond-cleavage products and ring-hydroxylated products were more predominant in soils treated with the more persistent *cis*-permethrin. In our study, the amount of unidentified products was always smaller with the enantiomers of *trans*-permethrin than *cis*-permethrin, which implies that the derivates from the oxidization of *cis*-permethrin may be more difficult to be further transformed in soils than the hydrolysis products. For both *cis*- and *trans*-permethrin, the unidentified metabolites accounted for a greater fraction for the *S*-enantiomer than for the *R*-enantiomer, and paired *t* test yielded *p* values of 0.05 and 0.04 for *cis*- and *trans*-permethrin, respectively. In this study, as Cl_2CA, PBalc, and PBacid were always found in relatively small fractions, whereas the unidentified metabolites were recovered in much greater fractions, it may be concluded that Cl_2CA, PBalc, and PBacid were rapidly transformed to other products. From a comparison of the data from the 14 and 56 day treatments, it is apparent that for all enantiomers, the amount of unidentified products in the ground-cover soil and turfgrass soil decreased over the incubation time. However, in the sediment samples, the amount of unidentified residues remained the same or even slightly increased. The difference in the fraction of unidentified residues may be partly attributable to the different rates of subsequent mineralization

Table 6. Recovered Activity as the Parent Compound and Metabolites in the Organic-Soluble Fraction after Incubation of ^{14}C-Labeled Permethrin Enantiomers in Soils under Aerobic Conditions (Percent of Applied Activity)

	14 days				56 days			
	cis		*trans*		*cis*		*trans*	
	R	*S*	*R*	*S*	*R*	*S*	*R*	*S*
				Groundcover Soil				
				^{14}C-Carbonyl-Labeled Permethrin				
Cl_2CA	2.3 ± 0.1	3.6 ± 0.7	10.1 ± 0.7	9.0 ± 1.2	8.8 ± 0.8	13.3 ± 0.1	1.5 ± 0.3	1.6 ± 0.0
permethrin	30.2 ± 0.2	25.0 ± 0.5	10.9 ± 1.7	9.6 ± 1.0	4.3 ± 0.1	3.8 ± 0.2	2.6 ± 0.0	1.5 ± 0.1
unidentified	26.5 ± 1.2	21.5 ± 2.3	7.5 ± 0.8	10.0 ± 0.1	11.0 ± 0.5	16.8 ± 1.8	6.9 ± 0.4	8.3 ± 0.7
				^{14}C-Alcohol-Labeled Permethrin				
PBalc	2.8 ± 0.1	1.8 ± 0.1	5.3 ± 1.2	4.6 ± 0.0	4.9 ± 0.3	6.2 ± 0.5	2.8 ± 0.2	2.1 ± 0.2
PBacid	0.3 ± 0.0	0.2 ± 0.0	4.5 ± 0.8	4.0 ± 0.0	4.7 ± 0.1	7.7 ± 0.9	2.4 ± 0.2	2.5 ± 0.2
permethrin	28.8 ± 0.4	23.1 ± 1.9	10.8 ± 1.8	11.2 ± 0.0	4.2 ± 1.1	3.3 ± 0.2	2.8 ± 0.3	1.6 ± 0.3
unidentified	23.2 ± 0.8	20.5 ± 0.5	10.5 ± 2.6	8.7 ± 0.0	7.6 ± 0.6	15.5 ± 0.1	4.0 ± 0.2	5.2 ± 0.4
				Turfgrass Soil				
				^{14}C-Carbonyl-Labeled Permethrin				
Cl_2CA	1.4 ± 0.1	2.5 ± 0.1	10.7 ± 0.5	9.8 ± 0.8	8.8 ± 0.6	13.9 ± 0.7	5.3 ± 0.1	5.5 ± 0.1
permethrin	31.4 ± 0.2	21.1 ± 0.2	12.0 ± 0.7	9.9 ± 0.0	7.0 ± 0.0	0.8 ± 0.0	4.6 ± 0.1	2.2 ± 0.1
unidentified	22.2 ± 0.6	22.6 ± 0.9	6.9 ± 0.3	4.8 ± 0.1	7.3 ± 0.0	12.3 ± 1.7	4.4 ± 0.0	4.8 ± 0.5
				^{14}C-Alcohol-Labeled Permethrin				
PBalc	5.8 ± 0.6	3.5 ± 0.2	2.2 ± 0.4	4.2 ± 0.3	5.6 ± 0.5	3.9 ± 0.5	4.0 ± 1.4	1.9 ± 1.0
PBacid	0.4 ± 0.0	0.2 ± 0.1	5.2 ± 0.1	5.1 ± 0.6	4.6 ± 0.4	5.8 ± 1.7	2.3 ± 0.1	2.6 ± 1.2
permethrin	29.7 ± 1.2	23.7 ± 1.6	13.7 ± 0.2	12.3 ± 1.1	5.4 ± 0.6	2.2 ± 0.2	3.3 ± 0.0	1.7 ± 0.2
unidentified	19.3 ± 0.5	23.3 ± 2.8	8.8 ± 0.1	7.2 ± 0.1	5.1 ± 0.0	11.6 ± 0.7	2.6 ± 0.4	5.2 ± 0.1

Table 7. Recovered Activity as the Parent Compound and Metabolites in the Organic-Soluble Fraction after Incubation of ^{14}C-Labeled Permethrin Enantiomers in the San Diego Creek Sediment under Aerobic or Anaerobic Conditions (Percent of Applied Activity)

	14 days				56 days			
	cis		*trans*		*cis*		*trans*	
	R	*S*	*R*	*S*	*R*	*S*	*R*	*S*
				Aerobic Conditions				
				^{14}C-Carbonyl-Labeled Permethrin				
Cl_2CA	3.1 ± 0.0	3.5 ± 0.5	9.4 ± 0.4	10.8 ± 0.3	8.6 ± 0.7	12.6 ± 2.0	6.8 ± 0.3	7.0 ± 0.0
permethrin	34.6 ± 0.5	36.6 ± 1.8	22.9 ± 0.4	21.3 ± 0.7	15.3 ± 1.3	14.6 ± 0.5	11.2 ± 1.1	14.2 ± 0.6
unidentified	11.5 ± 0.0	14.9 ± 0.7	9.6 ± 0.1	11.6 ± 0.4	15.3 ± 0.1	14.6 ± 1.6	11.2 ± 1.1	13.4 ± 0.6
				^{14}C-Alcohol-Labeled Permethrin				
PBalc	2.5 ± 0.3	1.1 ± 0.1	1.9 ± 0.4	3.2 ± 0.3	3.0 ± 0.3	2.8 ± 0.3	5.3 ± 0.8	7.7 ± 0.1
PBacid	3.5 ± 0.3	3.3 ± 0.1	3.5 ± 0.3	3.3 ± 0.5	2.3 ± 0.1	2.0 ± 0.5	1.2 ± 0.3	2.1 ± 0.9
permethrin	32.6 ± 1.2	37.2 ± 3.9	23.8 ± 1.0	18.8 ± 0.5	15.5 ± 0.3	14.5 ± 0.4	11.0 ± 1.4	14.4 ± 0.9
unidentified	12.1 ± 0.8	19.8 ± 2.4	18.9 ± 1.2	20.8 ± 0.7	17.6 ± 1.0	25.4 ± 0.5	9.2 ± 0.7	11.6 ± 1.0
				Anaerobic Conditions				
				^{14}C-Carbonyl-Labeled Permethrin				
Cl_2CA	2.5 ± 0.3	2.8 ± 0.1	10.2 ± 0.3	11.9 ± 0.0	10.0 ± 1.0	12.0 ± 0.4	6.6 ± 0.4	8.0 ± 0.9
permethrin	50.7 ± 0.1	53.3 ± 0.6	28.8 ± 0.4	34.3 ± 0.1	26.4 ± 2.2	30.3 ± 0.3	13.4 ± 0.7	21.3 ± 2.0
unidentified	13.3 ± 0.3	14.9 ± 1.5	15.5 ± 0.6	14.6 ± 0.8	26.4 ± 2.2	30.1 ± 0.3	13.4 ± 0.7	20.9 ± 2.0
				^{14}C-Alcohol-Labeled Permethrin				
PBalc	1.7 ± 0.2	1.0 ± 0.1	2.1 ± 0.0	2.5 ± 0.1	4.0 ± 0.2	3.6 ± 0.2	4.5 ± 0.1	5.8 ± 0.0
PBacid	4.0 ± 0.1	3.6 ± 0.3	2.7 ± 0.4	2.0 ± 0.3	1.3 ± 0.2	1.0 ± 0.0	5.2 ± 1.2	6.3 ± 0.2
permethrin	45.2 ± 2.5	51.3 ± 1.8	25.9 ± 2.1	30.3 ± 0.1	24.8 ± 0.3	30.2 ± 0.7	14.4 ± 0.0	24.4 ± 1.0
unidentified	14.6 ± 0.7	16.8 ± 0.7	19.3 ± 0.6	20.3 ± 2.1	20.4 ± 0.8	30.0 ± 1.2	9.7 ± 1.1	7.8 ± 1.6

of the hydrolytic intermediates that was influenced by the incubation conditions.

In conclusion, findings from this study show that selective degradation occurred not only between the *cis* and *trans* diastereoisomers of permethrin but perhaps also between the *R*- and *S*-enantiomers from the same diastereoisomer. The direction and rate of the enantioselective degradation may be due to selectivity in both hydrolysis and subsequent transformation pathways. Different enantiomers may undergo hydrolysis at different rates when incubated under different conditions. The *R*-enantiomer of both *cis*- and *trans*-permethrin was mineralized more rapidly than the corresponding *S*-enantiomer following hydrolysis, resulting in the formation of more $^{14}CO_2$ and fewer unidentified residues. The degradation products from *cis*-permethrin were more persistent than those from *trans*-permethrin under the same conditions. Such selectivity may contribute to a longer toxicity of the transformation products to certain nontarget organisms from *cis*-permethrin. This study suggests that for chiral compounds, enantioselectivity may be reflected not only in the environmental dissipation of the parent enantiomers but also in the kinetics of formation of intermediate transformation products. As some of the transformation intermediates of synthetic pyrethroids may possess chronic toxicity (e.g., endocrine disruption), it is important to understand enantioselectivity in the rate of metabolite formation in the environment.

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