amidine hydrochloride treated ones at the 6-h time point (Table III). Furthermore, the results of in vitro study indicate that the formation of *N*-desmethylchlordimeform and *N,N*-didesmethylchlordimeform is much more pronounced in the preparation incubated with chlordimeform than that incubated with sulfamidine (Figure 2).

Throughout the study it has been consistently observed that metabolism of sulfamidine is more rapid than that of chlordimeform. A logical explanation for this phenomenon would be that the rate of enzymatic hydrolytic degradation of chlordimeform is less than that for sulfamidine or sulfamidine sulfoxide. It must be noted that the rate of nonenzymatic hydrolysis for chlordimeform itself was found to be identical with that for sulfamidine and its sulfoxide (data not shown). Also, once *N*-desmethylchlordimeform (V) or *N*-formyl-4-chloro-*o*-toluidine (VII) is formed as a result of oxidative reactions, the subsequent metabolic fate should be identical for both compounds. Since the key difference in metabolism between these two compounds appears to be in the levels of *N*-desmethylchlordimeform (V) formed as judged by the distribution and the in vitro study, it appears to be reasonable to ascribe the overall metabolic difference to the differential degradation of sulfamidine sulfoxide and chlordimeform.

In the data shown in Table V, we can observe that in the presence of NADPH and SKF 525-A the levels of these two precursors for *N*-desmethylchlordimeform (V) are almost identical (54%). Yet, the level of *N*-desmethylchlordimeform (V) was higher in the test with chlordimeform (II) than that with sulfamidine sulfoxide (III). At the same time the level of very polar metabolites (aqueous) in the former test was only one-third as much.

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Comparative Aerobic Soil Metabolism of Fenvalerate Isomers

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An aerobic soil metabolism study was conducted to determine the degradation rate of individual isomers of fenvalerate and to assess the potential influence of the *RS*, *SR*, and *RR* isomers to the metabolism of the most insecticidally active *SS* isomer. Individual [*phenoxyphenyl*-^{14}C]fenvalerate isomers degraded at different rates. The calculated half-lives for the *SR*, *RS*, *SS*, and *RR* isomers in fenvalerate (racemic mixture) were 155, 89, 108, and 178 days, respectively. The resolved *SS* isomer degraded at a faster rate with a calculated half-life of 74 days. Racemization of the resolved *SS* isomer did not occur. A qualitative difference in the chemical nature of soil metabolites between fenvalerate and the resolved *SS* isomer was not observed. Soil degradation products, phenoxybenzoic acid, 3-(4-hydroxyphenoxy)benzoic acid, and 4′-OH- and $CONH_2$-fenvalerate, each accounted for less than 2% of the applied radioactivity. Extensive degradation of these soil metabolites was evident since approximately 50% of the applied radioactivity was recovered as $^{14}CO_2$ and as unextractable bound residues.

Fenvalerate [cyano(3-phenoxyphenyl)methyl 4-chloro-α-(1-methylethyl)benzeneacetate, PYDRIN insecticide] is an effective broad-spectrum pyrethroid insecticide. In addition to its highly selective insecticidal activities, fenvalerate exhibits photolytic stability and an extended field residual activity (Miyamoto and Mikami, 1983; Reed et al., 1983).

Fenvalerate contains two asymmetric carbon atoms and the most insecticidally active stereoisomer has the 2*S*,α*S* (*SS*) configuration (Yoshioka, 1978). Significant qualitative or quantitative difference in soil metabolism between the racemic mixture and the resolved *S*-acid (*SR*, *SS*) diastereomer was not observed (Ohkawa et al., 1978). The primary objective of this investigation was to determine the soil degradation rate of individual fenvalerate isomers

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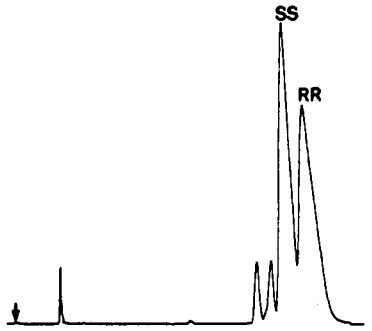
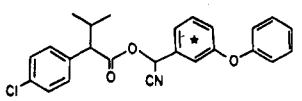


Figure 1. Preparative HPLC separation of the *SS* and *RR* isomers from the preparative TLC isolated *Y* diastereomer (flow rate 0.5 mL/min).

and to assess the potential influence of the *RS*, *SR*, and *RR* isomers to the metabolism of the most insectcidally active *SS* isomer. Isomer separation by both the gas–liquid chromatography (GLC; Horiba et al., 1980a) and high-performance liquid chromatography (HPLC; Horiba et al., 1980b) methods required time-consuming derivatization with *l*-menthol. This analytical difficulty has hindered the study of the comparative metabolism of individual fenvalerate isomers. As a result of this investigation, a rapid and convenient method to separate and quantitate individual fenvalerate isomers using a chiral stationary phase HPLC column (Papadopoulou-Mourkidou, 1985) has been devised.

MATERIALS AND METHODS

Test Materials and Reference Standards. [*phenoxyphenyl*-^{14}C]Fenvalerate and appropriate reference standards were synthesized by the Biological Sciences Research Center, Shell Development Co. The labeled



Fenvalerate (• ^{14}C)

material had a radiochemical purity of greater than 99.5% as determined by thin-layer chromatography (TLC), autoradiography, and liquid scintillation counting (LSC). The specific activity was 36.0 μCi/mg as determined by LSC and GLC.

Isolation of the *SS* isomer from [*phenoxyphenyl*-^{14}C]-fenvalerate was carried out by a combination of TLC and HPLC. Separation of the two fenvalerate diastereomers [(*SR*, *RS*; designated as *X*) and (*SS*, *RR*; designated as *Y*)] was carried out by one-dimensional preparative TLC (silica gel F-254, 0.25 mm, E. Merck). TLC plates (10 plates, 40 μCi/plate) were developed six consecutive times in the hexane–ether (20:1, v/v) solvent system. The R_f values for the *X* and *Y* diastereomers were 0.49 and 0.46, respectively. The initial TLC separation was necessary since it minimized the amount of material applied to the HPLC column. Radioactivity, visualized by autoradiography, was extracted from the silica gel with ethyl acetate as the eluting solvent. Solvent was removed, and the remaining residual material was redissolved in hexane. An HPLC chromatogram showed an approximate 95% isomeric separation of the *X* and *Y* diastereomers was achieved by the preparative TLC (Figure 1).

The separation of the *SS* and the *RR* isomers (from the isolated *Y* diastereomer) was carried out using a chiral stationary phase HPLC column. Each isomer was collected from the corresponding eluting peak. Adequate separation was obtained at the concentration range of 0.5–1.0 mg of total material/injection. The isomeric compositions for [^{14}C]fenvalerate (*RS* 26.4%, *SR* 26.8%, *SS* 23.7%, *RR* 23.2%; Figure 2) and the resolved [^{14}C] *SS* isomer (total yield 65 μCi, *SS* >98.5%; Figure 2) were determined by a combination of HPLC, fraction collection, and LSC.

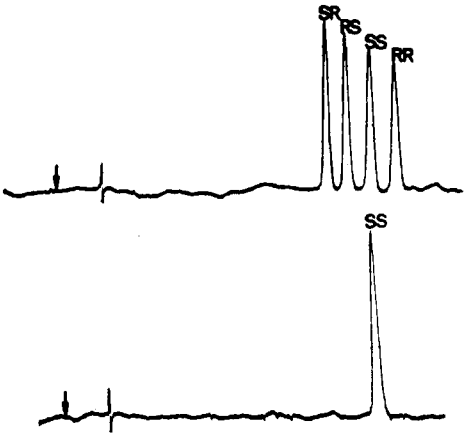


Figure 2. HPLC chromatograms of [*phenoxyphenyl*-^{14}C]fenvalerate and the resolved [^{14}C] *SS* isomer. The HPLC retention times for the *SR*, *RS*, *SS*, and *RR* isomers are 20.5, 22.1, 24.0, and 26.1 min, respectively (flow rate 1 mL/min).

Authentic reference standards included 4′-OH-fenvalerate [cyano(3-phenoxy-4-hydroxyphenyl)methyl 4-chloro-α-(1-methylethyl)benzeneacetate], $CONH_2$-fenvalerate [(aminocarbonyl)(3-phenoxyphenyl)methyl 4-chloro-α-(1-methylethyl)benzeneacetate], PBacid [3-phenoxybenzoic acid], and 4′-OH-PBacid [3-(4-hydroxyphenoxy)benzoic acid].

Aerobic Soil Metabolism. Freshly collected silty loam from Lake Village, AR, was used in this study. The soil collection site, a cotton-growing area, did not have a history of previous exposure to fenvalerate. Air-dried soil samples (24 h at room temperature) were passed through a 2-mm sieve to remove any large debris and stored at 4 °C for approximately 1 week prior to use. This silty loam has the following physical characteristics: cation-exchange capacity, 7.6 mequiv/100 g; total nitrogen, 800 ppm; hydrogen, 1.5 mequiv/100 g; bulk density, 1.1 g/cm^3; pH (soil), 5.7; organic matter, 1.3%; sand, 36%; silt, 48%; clay, 16%; field moisture at $^1/_3$ bar, 18.5%.

A 100-g subsample of the test soil was transferred to a 500-mL Erlenmeyer flask. Concentrations of 20 and 5 ppm of [^{14}C]fenvalerate and the resolved [^{14}C] *SS* isomer, respectively, were established in the air-dried test soil by applying the appropriate amount of the test material in methanol (1 mL/100 g) directly onto the soil surface. The final specific activities of [^{14}C]fenvalerate and the resolved [^{14}C] *SS* isomer were 2.5 and 10 μCi/mg, respectively. After the evaporation of the solvent, the soil moisture of each treated sample was adjusted to approximately 15% with tap water. Following thorough mixing, flasks were sealed with a glass stopper affixed with a hollow glass tube that extended to within approximately 1.5 cm of the soil surface. A detailed description of the soil incubation conditions, sampling, and extraction procedures has been reported in the soil metabolism study with [*chlorophenyl*-^{14}C]fenvalerate (Lee, 1985). At each sampling interval, the distribution of radioactivity in the $^{14}CO_2$ solvent trap, methanol-extractable fraction, and the bound fraction was determined.

Chromatography and Radioassay. Radioactivity was quantitated in 15 mL of Aquasol-2 scintillation solution (New England Nuclear) with a Packard Model 300 liquid scintillation system. The radioactive area on the TLC plate, after solvent development and autoradiography

Table I. Distribution of the Applied Radioactivity in the Treated Soil at Various Time Intervals

	% of applied radioactivity at day										
	[^{14}C]fenvalerate						[^{14}C] *SS* isomer				
	0	14	30	60	90	180	0	14	30	60	90
$^{14}CO_2$		1.1	3.6	9.4	13.6	25.4		3.7	11.1	17.9	21.5
extractable	104	99.1	88.3	77.1	68.1	43.4	102	89.8	80.2	54.0	51.0
parent	100	98	86	75	59	40	99	85	77	50	45
unextractable	<1	4.6	8.6	14.4	18.3	24.6	<1	10.4	10.7	24.3	27.5
total	104	105	101	101	100	94	102	103	102	96	100

(Kodak SB-5 single-coated X-ray film), was removed by scraping and analyzed in an Aquasol-2–water (11 mL:4 mL) gel system. [^{14}C] residues in the soil samples were analyzed by combusting subsamples (100 mg) in a Packard 306B sample oxidizer prior to LSC. Combustion efficiency was determined on untreated soil and [^{14}C]fenvalerate solution as the calibration standard.

Extractable [^{14}C] residues were analyzed by two-dimensional TLC (silica gel F-254, 0.25 mm, E. Merck). The R_f values of fenvalerate and the reference standard in several TLC solvent systems and the capillary GLC operating conditions have been reported (Lee, 1985; Lee et al., 1985).

The isomeric compositions of [^{14}C]fenvalerate, the resolved [^{14}C] *SS* isomer treatment solutions, and the undegraded parent molecule recovered from aged soil at each sampling interval were examined by HPLC using a chiral stationary phase column [Varian Model 5000 liquid chromatograph; UV detection at 254 nm; 5-μm Bakerbond covalent chiral phase (DNBPG) column (4.6 × 250 mm, J. T. Baker Research Products, Phillipsburg, NJ); ambient; 0.5–1.0 mL/min; solvent system, hexane–dichloroethane–ethanol (400:30:0.15, v/v/v)]. Isomeric composition was determined by collecting column effluents and direct radioassay.

RESULTS AND DISCUSSION

The portions of the applied radioactivity quantified as $^{14}CO_2$, solvent-extractable and -unextractable fractions of the [^{14}C]fenvalerate and [^{14}C] *SS* isomer treated soil are summarized in Table I. A steady decrease of the extractable radioactivity along with a corresponding increase of $^{14}CO_2$ formation and unextractable [^{14}C] residues was observed. The overall recovery of the applied radioactivity throughout this study was consistently close to 100%.

Data presented in Table I showed greater than 99% of the recovered radioactivity was the undegraded parent molecule immediately after soil treatment. HPLC analyses showed that the isomeric composition of the undegraded [^{14}C]fenvalerate and [^{14}C] *SS* isomer, isolated by preparative TLC using the toluene–ether (4:1) solvent system, was unchanged in comparison to the treatment solution. These results demonstrated the efficiency of the extraction procedures and the chemical and isomeric stabilities of [^{14}C]fenvalerate and the resolved [^{14}C] *SS* isomer under the analytical procedures used in this study.

Qualitative differences in the chemical nature of soil metabolites between [^{14}C]fenvalerate and the resolved [^{14}C] *SS* isomer were not observed. Greater than 85% of the solvent-extractable [^{14}C] residues at the various sampling intervals were characterized as the undegraded parent molecule by TLC. Several minor degradation products were observed, however each accounting for less than 2% of the applied radioactivity. The rapid generation of $^{14}CO_2$ showed the susceptibility of the [*phenoxyphenyl*-^{14}C]-labeled metabolic products to undergo extensive soil microbial degradation (Mikami et al., 1984). Minor metabolites retaining the intact parent structure included 4′-OH-fenvalerate and $CONH_2$-fenvalerate, both having been identified as soil degradation products in a study with [*chlorophenyl*-^{14}C]fenvalerate (Lee, 1985). PBacid and 4′-OH-PBacid were products resulting from the cleavage of the parent ester linkage (Mikami et al., 1984).

Table II. Isomeric Composition of Undegraded [^{14}C]Fenvalerate and the Resolved [^{14}C] *SS* Isomer Recovered from the Treated Soil at Various Time Intervals

	isomeric composition,[a] %				
	[^{14}C]fenvalerate				
	SR	*RS*	*SS*	*RR*	resolved *SS*
treatment soln[b]	26	27	24	23	98
zero time	26	26	24	24	97
14 days	27	26	23	24	98
30 days	28	24	23	25	98
60 days	29	22	22	27	97
90 days	30	21	22	28	96
180 days	31	18	21	31	nd[c]

[a] Determined by peak collection from the HPLC and LSC. [b] See Figure 1. [c] Not determined.

Unextractable radioactivity remaining in the [^{14}C]fenvalerate and the resolved [^{14}C] *SS* isomer treated soils at the 90-day sampling interval accounted for 18 and 28% of the applied radioactivity, respectively. Only a trace level of radioactivity was recovered after repeated extraction of the bound residue with the various organic solvents (hexane, chloroform, acetone, acetonitrile). A negligible level of the bound [^{14}C] residues was released when the extracted soil was further treated with 0.01 N HCl.

[^{14}C]Fenvalerate and the resolved [^{14}C] *SS* isomer, recovered from the test soil by methanol extraction, were isolated at various time intervals by preparative TLC using toluene–ether (4:1) as the developing solvent. The isomeric composition of the undegraded parent molecule was examined by HPLC. Table II summarizes the isomeric compositions of the undegraded [^{14}C]fenvalerate and the resolved [^{14}C] *SS* isomer. It is apparent that the racemization of the resolved [^{14}C] *SS* isomer to other isomers did not occur in the test soil since greater than 96% of the recovered material was *SS* isomer, with 45% overall fenvalerate recovery. The isomeric purity of the [^{14}C] *SS* isomer recovered at zero time was 97%.

Table II shows a tentative change in the isomeric composition of [^{14}C]fenvalerate at the various sampling intervals. These changes (up to 9% for the *RS* isomer) suggest the potential of a stereoselective rate difference in the degradation of individual fenvalerate isomers.

Apparent differences in the degradation rates between the four isomers in the racemate were evident. The *RS* isomer degraded at a faster rate than the *SS*, *SR*, and *RR* isomers (Table III). The soil degradation rates of individual fenvalerate isomers are graphically illustrated in Figure 3. A plot of the log concentration of individual isomers recovered at each sampling interval vs. time gave a straight-line ($r^2 > 0.97$) correlation. By using the first-order rate equation, the observed soil degradation rate constants (k) for each fenvalerate isomer and the resolved *SS* isomer were calculated. The calculated half-lives for

Table III. Concentrations (ppm) of [^{14}C]Fenvalerate and the Resolved [^{14}C] *SS* Isomer at Various Time Intervals

	[^{14}C]fenvalerate					
	total	*SR*	*RS*	*SS*	*RR*	[^{14}C] *SS* isomer
zero time	20.2 (100)[a]	5.41 (100)	5.62 (100)	4.99 (100)	4.79 (100)	4.91 (99)
14 days	19.8 (98)	5.15 (95)	5.15 (92)	4.75 (95)	4.75 (99)	4.19 (85)
30 days	17.4 (86)	4.86 (90)	4.17 (74)	4.00 (80)	4.34 (91)	3.84 (77)
60 days	15.2 (75)	4.39 (81)	3.33 (59)	3.33 (67)	4.09 (85)	2.50 (50)
90 days	11.9 (59)	3.58 (66)	2.50 (45)	2.62 (53)	3.34 (70)	2.19 (45)
180 days	7.9 (39)	2.43 (45)	1.40 (25)	1.63 (33)	2.46 (51)	nd[b]

[a] Values in parentheses expressed as percent of total isomer at zero time. [b] Not determined.

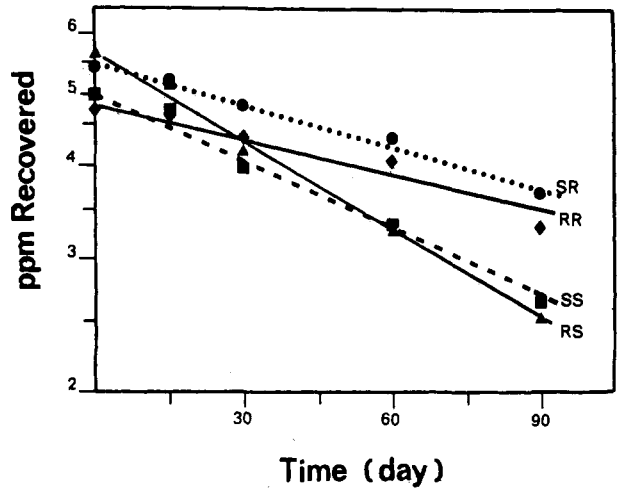


Figure 3. Soil dissipation rates of individual isomers in the *fenvalerate racemic mixture under laboratory aerobic conditions.*

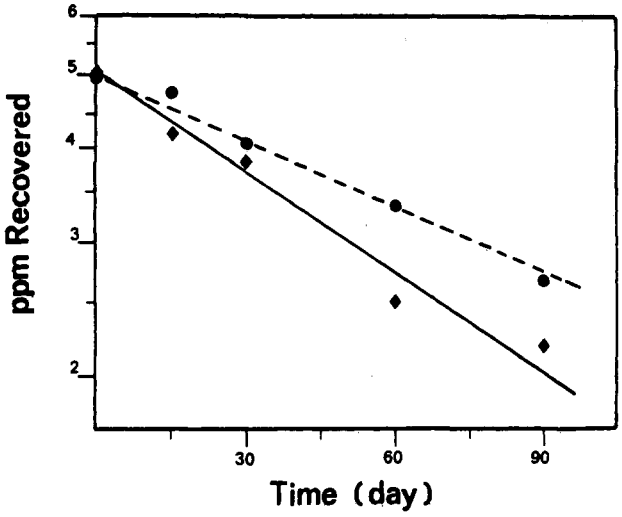


Figure 4. Dissipation rate of the *SS* isomer in the fenvalerate racemic mixture (●) and the resolved test material (♦).

the individual *SR*, *RS*, *SS*, and *RR* isomers were 155, 89, 108, and 178 days, respectively (Table IV). The overall half-life for racemic [^{14}C]fenvalerate was 127 days.

Results presented in Table IV and Figure 4 show the resolved *SS* isomer (half-life of 74 days) appeared to be degraded at a faster rate than the *SS* isomer in the racemic mixture (half-life of 108 days). This difference could be attributed to the presence of the other three fenvalerate isomers (*SR*, *RS*, *RR*) as alternative substrates for soil microorganisms.

Table IV. Degradation Rate Constants and Dissipation Half-Lives of Fenvalerate Isomers and the Resolved *SS* Isomer

	degradn rate ($k \times 10^3$ day^{-1})	half-life, days
fenvalerate (racemic)	5.4 (0.99)[a]	127
SR	4.5 (0.99)	155
RS	7.8 (0.99)	89
SS	6.4 (0.99)	108
RR	3.9 (0.98)	178
resolved *SS* isomer	9.3 (0.97)	74

[a] Regression coefficient (r^2).

Since the resolved *SS* isomer degraded at a faster rate in the absence of the *SR*, *RS*, and *RR* isomers, attempts to remove the other less insecticidally active isomers in the commercial product of fenvalerate could reduce the actual amount of test material required for pest control and to reduce its impact to the environment.

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Registry No. (*RS*)-Fenvalerate, 66267-77-4; (*SR*)-fenvalerate, 67614-32-8; (*RR*)-fenvalerate, 67614-33-9; (*SS*)-fenvalerate, 66230-04-4; 3-phenoxybenzoic acid, 3739-38-6; 3-(4-hydroxyphenoxy)benzoic acid, 35065-12-4; 4′-OH-fenvalerate, 107384-27-0; $CONH_2$-fenvalerate, 67685-93-2.

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