Rat Metabolism of Fenvalerate (Pydrin Insecticide)

Philip W. Lee,* Stephen M. Stearns, and Walter R. Powell

Pydrin insecticide Y rich is an isomerically enriched form of fenvalerate containing an excess ratio of the active diastereomer *SS,RR*, designated as Y, over the less active diastereomer *RS,SR*, designated as X, at a ratio of approximately 85:15. Fenvalerate contains Y:X in a ratio of 45:55. Following a single oral dose (8.4 mg/kg), greater than 90% of the administered radioactivity from the acid moiety (chlorophenyl-^{14}C) and the alcohol moiety (phenoxyphenyl-^{14}C) was eliminated within the initial 24 h. There was no major difference in the elimination rate or the metabolite distribution profile between the two different fenvalerate preparations. Cleavage of the ester linkage was the primary metabolic pathway. The acid and alcohol portions of the parent molecule underwent hydroxylation, oxidation, and conjugation. These metabolic reactions are independent of the isomeric composition of the test material. Tissue residue data showed the lack of bioretention of ^{14}C residues in the various organs.

INTRODUCTION

Fenvalerate [1, cyano(3-phenoxyphenyl)methyl 4-chloro-α-(1-methylethyl)benzeneacetate, Pydrin insecticide] is an effective synthetic pyrethroid insecticide possessing excellent insecticidal activity and, also, favorable environmental stability. Fenvalerate contains two asymmetric carbon atoms, at the acid and alcohol moieties. The most insecticidally active isomer of fenvalerate has the 2-*S*,α-*S* (*SS*) configuration. Fenvalerate contains 22% of the *SS* isomer and a diastereomeric ratio of 45:55 [Y (*SS,RR*)/X (*SR,RS*)].

Metabolism of fenvalerate and the various fenvalerate isomer compositions in laboratory animals has been extensively studied. Different fenvalerate preparations included the racemic, 2*S*,α*RS*, and the resolved *SS* isomer, labeled with carbon-14 at the various positions (carbonyl, α-carbon, and the CN moiety), have been examined by Sumitomo Chemical Company (Ohkawa et al., 1979; Kaneko et al., 1981). To provide additional information to evaluate the potential contribution of stereoisomerism to the metabolic fate of fenvalerate, this report summarizes the comparative metabolism of two different fenvalerate preparations [racemic and the Y diastereomer (*SS,RR*)] in rats following a single oral dose. Special considerations of this study are (1) to identify and quantify significant fenvalerate metabolites, (2) to characterize route and rate of elimination, and (3) to determine tissue residue distribution profile.

MATERIALS AND METHODS

Test Materials and Reference Standards. Radiolabeled fenvalerate and appropriate reference standards were synthesized by the Biological Sciences Research Center, Shell Development Company. The following radiolabeled materials were used: fenvalerate (racemic) and fenvalerate Y rich labeled with carbon-14 in the chlorophenyl (acid) and the phenoxyphenyl (alcohol) moieties. [^{14}C]Fenvalerate Y rich was obtained by the preparative thin-layer chromatographic (TLC) separation of the [^{14}C]fenvalerate racemic material with 1.0-mm preparative silica gel TLC plates after developing six consecutive times in hexane–tetrahydrofuran (97:3). The final specific activity and the isomeric composition of the four test materials are presented in Table I. The specific activity of 5 μCi/mg allows the detection of ^{14}C residues in the animal tissues at a level of 0.5% of the administered dose (8.4 mg/kg). The radiochemical purity of all test materials was greater than 99% as determined by analytical two-dimensional TLC, liquid scintillation counting (LSC), and autoradiography.

Authentic reference standards included CPIA [**2**, 4-chloro-α-(1-methylethyl)benzeneacetic acid], 2-OH-CPIA [the α and β diastereomers **3** and **4** respectively, of 4-chloro-α-(2-hydroxy-1-methylethyl)benzeneacetic acid], α-OH-CPIA [**5**, 4-chloro-α-hydroxy-2-(1-methylethyl)-benzeneacetic acid], 2,α-diOH-CPIA [**6**, 4-chloro-α-hydroxy-α-(2-hydroxy-1-methylethyl)benzeneacetic acid], 2-OH-CPIA lactone [**7**, 3-(4-chlorophenyl)dihydro-4-methyl-2(3*H*)-furanone], PBacid [**8**, 3-phenoxybenzoic acid], 4′-OH-PBacid [**9**, 3-(4-hydroxyphenoxy)benzoic acid], 4′-OH-fenvalerate [**10**, cyano(3-phenoxy-4-hydroxyphenyl)methyl 4-chloro-α-(1-methylethyl)-benzeneacetate]. Methyl esters of **2** [CPIA-Me, **11**], **3**, **4** [2-OH-CPIA-Me, **12**], **5** [α-OH-CPIA-Me, **13**], **8** [PBacid-Me, **14**], and **9** [4′-OH-PBacid-Me, **15**] were prepared by methylation with diazomethane. Structures of the rat metabolites of fenvalerate are presented in Figure 1.

Treatment of Animals. Male and female Sprague-Dawley albino rats (SIM:SDf1 strain, 7 weeks old, 175–200 g each) were obtained from Simonsen Laboratories, Gilroy, CA. Animals were fasted for 16 h with free access to water and then administered a single oral dose of 8.4 mg/kg of ^{14}C test compound. Labeled compounds were formulated in corn oil at the concentration of 1.68 mg/mL. Control animals received corn oil only at the rate of 5 mL/kg. Each treatment group consisted of five male and five female test animals. Treated animals were held individually in Nalgene plastic metabolism chambers (Sybron/Nalge Company) which allowed the separate collection of urine and feces. Preliminary data have shown that no [^{14}C]-carbon dioxide was recovered from the respired air of treated animals with either labeled material when maintained in all glass metabolism cages (Stanford Model MC3000, Stanford Glassblowing Company). Animals were given free access to food (Purina Rodent Chow) and water during the remaining holding period. Excreta were collected daily for up to 5 days and were stored at 4 °C prior to analysis.

Animals were sacrificed 5 days after dosing. The following tissues were collected: blood, lung, liver, kidney, fat, muscle, heart, gonad, and brain. All tissues were stored at 4 °C prior to analysis.

Analysis of Urinary Excreta. Daily urinary excreta from individual control and treated animals were adjusted

Biological Sciences Research Center, Shell Development Company, Modesto, California 95352.

0021-8561/85/1433-0988$01.50/0

Table I. Specific Activity and the Isomeric Composition of the Various Fenvalerate Dosing Solutions

preparations	specific activity, μCi	Y/X isomeric ratio	isomeric composition, %			
			SS	*RR*	*SR*	*RS*
[*chlorophenyl*-^{14}C]fenvalerate (racemic)	4.8	44/56	22	22	28	28
[*phenoxyphenyl*-^{14}C]fenvalerate (racemic)	5.1	44/56	22	22	28	28
[*chlorophenyl*-^{14}C]fenvalerate (Y rich)	5.8	90/10	45	45	5	5
[*phenoxyphenyl*-^{14}C]fenvalerate (Y rich)	5.0	90/10	45	45	5	5

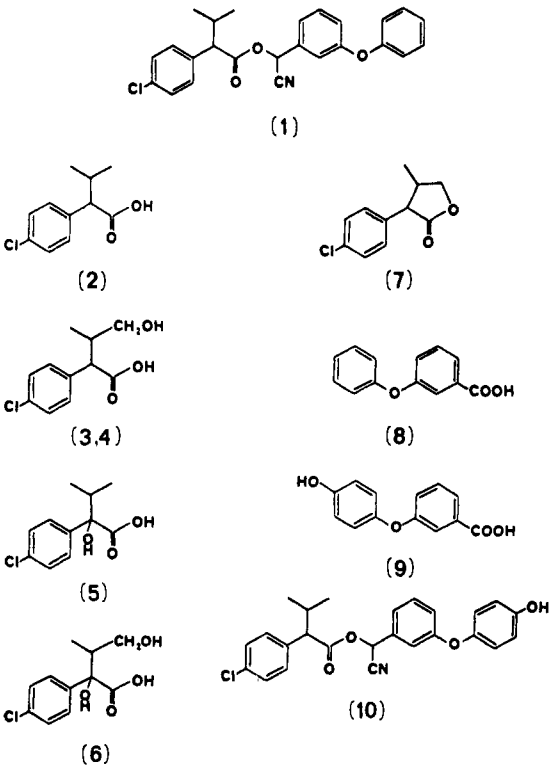

Figure 1. Rat metabolites of fenvalerate.

to a final volume of 25 mL with 0.01 M phosphate buffer (pH 7.4). Triplicated 0.1-mL aliquots were sampled and analyzed by LSC.

For the quantitative and qualitative characterization of urinary metabolites, the pH of the combined 45-h urine was adjusted to pH 4 with 6 N hydrochloric acid and extracted three times with equal volumes of ethyl acetate.

Water-soluble conjugates remaining in the aqueous phase after the initial ethyl acetate extraction were analyzed after enzyme and acid hydrolysis. Enzyme hydrolysis was carried out at 35 °C for 12 h with sulfatase/β-glucuronidase enzyme (Sigma Chemical Company). Subsequent to the enzyme hydrolysis, acid hydrolysis was carried out at pH 1, 90 °C, for 4 h. Radiolabeled materials released after enzyme or acid hydrolysis were recovered by solvent extraction and analyzed by two-dimensional TLC. Radioactivity remaining in the aqueous phase was not further characterized.

Analysis of Fecal Excreta. Fecal excreta from individual control and treated animals was collected daily and lyophilized for 24 h prior to analysis. Dried materials were pulverized in a microanalytical mill (Tekmar Company). The percent of the administered radioactivity recovered in the daily excreta was quantitatively analyzed by combustion and LSC.

For the qualitative and quantitative characterization of fecal metabolites, a 3-g subsample of the combined day 1 and day 2 fecal excreta from each treated animal was analyzed individually. Freeze-dried fecal materials, after maceration, were first extracted three times with 30 mL of methanol–water (9:1 v/v). The combined extract was quantitatively analyzed by LSC for the total amount of extractable metabolites. The methanol–water extract was concentrated to approximately 15 mL by rotary evaporation, adjusted to 30 mL with 0.01 M phosphate buffer, pH 7.4, and extracted three times with equal volumes of ethyl acetate. The organic-extractable fecal metabolites were analyzed by two-dimensional TLC.

Radioactivity remaining in the aqueous phase after organic solvent extraction was considered as water-soluble conjugates which were recovered and analyzed after acid hydrolysis. Radioactivity remaining in the solid fecal material was considered as unextractable residue and was not analyzed further.

Chromatography and Radioassay. Radioactivity was quantitated in 15 mL of Aquasol-2 scintillation solution (New England Nuclear) in a Packard Model 2660 liquid scintillation system. Radioactive areas of the TLC plate, after solvent development, were removed by scraping and analyzed in an Aquasol-2/water (11:4 mL) gel system. Radioactive residues associated with the fecal excreta and tissues were analyzed by combusting subsamples (approximately 100 mg) in a Packard Model 306B sample oxidizer. Background and combustion efficiency of individual tissues and excreta were determined with control animals and [^{14}C]fenvalerate solution as the calibration standard. Oxidizer solution included Carbo-Sorb and Permafluor V (Packard Instrument Company) 10:12-mL mixture. All LSC quantitations were corrected for combustion efficiency and quenching. Excreted radiocarbon is expressed as percent of the administered dose, and tissue residues are given as parts per million (ppm) equivalent of the administered ^{14}C labeled compound based upon tissue wet weight.

The ^{14}C residues recovered from the urine and feces were analyzed by two-dimensional TLC (silica gel F-254, 0.25 mm, E. Merck). R_f values of fenvalerate and reference standards in several TLC solvent systems are presented in Table II. The distribution pattern of ^{14}C residues on the TLC plate was visualized by autoradiography on Kodak SB-5 single-coated X-ray film.

Capillary gas-liquid chromatography of isolated metabolites was carried out with a 25 m × 0.37 mm i.d., SE-30 WCOT glass column in a Varian 3700 gas chromatograph equipped with a ^{63}Ni electron-capture detector. Isothermal analysis was carried out at injector, column, and detector temperatures of 280, 245, and 320 °C, respectively. The helium carrier gas and nitrogen makeup gas flow rate through the detector were 3 and 35 mL/min, respectively. The column split ratio was controlled at 10:1 ratio.

Mass spectral analysis was carried out on the Finnigan 3200 GC/mass spectrometer with a 12 m × 0.3 mm SE-30 WCOT glass column. A summary of the mass spectral data of fenvalerate and model metabolites is also included in Table II.

RESULTS AND DISCUSSION

Elimination Rates. Analysis of the excreta from individual test animals (5 animals per treatment group) provided sufficient data to assess the statistical difference in the metabolic fate between the two fenvalerate preparations. The metabolism of fenvalerate and fenvalerate Y rich in male and female rats was rapid after a single oral

Table II. TLC R_f Values and Mass Spectral Data of Fenvalerate and Model Metabolites

	TLC R_f values		
	system 1[a]	system 2[b]	mass spectral data, m/z
fenvalerate (1)	0.78	0.72	419 (M^+), 225, 181, 169, 167, 154, 127, 125
CPIA (2)	0.70	0.38	212 (M^+), 172, 170, 127, 125
2-OH-CPIA α-isomer (3)[c]	0.48	0.09	210 (M^+), 168, 166, 153, 151, 131, 127, 125, 117, 116, 115, 91
2-OH-CPIA β-isomer (4)[c]	0.58	0.13	210 (M^+), 168, 166, 153, 151, 131, 127, 125, 117, 116, 115, 91
2-OH-CPIA (5)	0.62	0.23	
2,α-diOH-CPIA (6)[d]	0.24	0.03	226 (M^+), 182, 169, 167, 147, 141, 139, 113, 111, 55
2-OH-CPIA lactone (7)	0.73	0.68	210 (M^+), 168, 166, 153, 151, 131, 127, 125, 117, 116, 115, 91
PBacid (8)	0.49	0.38	214 (M^+), 196, 169, 168, 141, 115, 77
4′-OH-PBacid (9)	0.41	0.16	
4′-OH-Fenvalerate (10)	0.62	0.42	435 (M^+), 252, 169, 167, 154, 152, 127, 125, 91, 89
CPIA-Me (11)			226 (M^+), 186, 184, 169, 167, 154, 152, 127, 125, 117, 115, 91, 89
2-OH-CPIA-Me (12)			242 (M^+), 186, 184, 154, 152, 127, 125
α-OH-CPIA-Me (13)			242 (M^+), 201, 199, 185, 183, 141, 139, 113, 111, 105, 43
PBacid-Me (14)			228 (M^+), 173, 169, 141, 115, 98, 77
4′-OH-PBacid-Me (15)			244 (M^+), 213, 185, 157, 128, 106, 76

[a] Hexane–acetone–acetic acid (25:25:1). [b] Toluene–ether–acetic acid (75:25:1). [c] The α- and β-isomers of 2-OH-CPIA (3,4) yield the ring closure product 2-OH-CPIA lactone (7) during direct GC–MS analysis. [d] 2,α-diOH-CPIA (6) yields the ring closure product during direct GC–MS analysis.

Table III. Elimination of the Administered [*chlorophenyl*-^{14}C]Fenvalerate and Fenvalerate Y Rich in the Urinary and Fecal Excreta of Male and Female Test Animals[a,b]

	administered radioactivity, %							
	fenvalerate				fenvalerate Y rich			
day	male urine	male feces	female urine	female feces	male urine	male feces	female urine	female feces
1	28.4 ± 6.0	43.1 ± 6.3	20.7 ± 3.7	53.8 ± 7.7	28.2 ± 12.8	51.8 ± 12.0	29.2 ± 7.0	51.3 ± 10.2
2	7.7 ± 1.7	7.6 ± 3.6	5.3 ± 2.4	5.2 ± 2.6	5.9 ± 1.6	4.1 ± 1.7	4.0 ± 1.4	8.0 ± 8.6
3	1.7 ± 0.5	1.2 ± 0.5	0.9 ± 0.2	0.5 ± 0.1	1.3 ± 0.2	0.6 ± 0.2	1.0 ± 0.2	0.4 ± 0.1
4	0.9 ± 0.3	0.4 ± 0.2	0.5 ± 0.1	0.2 ± 0.1	0.6 ± 0.1	0.2 ± 0.7	0.6 ± 0.1	0.2 ± 0.1
5	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.0	0.4 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	0.1 ± 0.0
total	39.1 ± 5.2	52.6 ± 6.1	27.7 ± 5.0	59.8 ± 8.3	36.4 ± 14.6	56.8 ± 10.3	35.2 ± 8.3	60.0 ± 10.2

[a] Five male and five female rats. [b] Mean ± standard deviation (n = 5).

Table IV. Elimination of the Administered [*phenoxyphenyl*-^{14}C]Fenvalerate and Fenvalerate Y Rich in the Urinary and Fecal Excreta of Male and Female Test Animals[a,b]

	administered radioactivity, %							
	fenvalerate				fenvalerate Y rich			
day	male urine	male feces	female urine	female feces	male urine	male feces	female urine	female feces
1	36.9 ± 6.5	56.0 ± 11.3	30.6 ± 7.4	62.8 ± 9.6	34.4 ± 2.6	61.1 ± 5.5	31.3 ± 17.6	58.8 ± 20.9
2	2.4 ± 1.2	1.6 ± 0.7	2.1 ± 0.5	3.5 ± 3.0	2.5 ± 3.0	2.6 ± 0.9	3.1 ± 2.0	3.2 ± 2.6
3	0.8 ± 0.2	0.8 ± 0.1	0.7 ± 0.4	0.4 ± 0.3	0.8 ± 0.1	0.4 ± 0.1	0.9 ± 0.5	0.3 ± 0.2
4	0.5 ± 0.2	0.2 ± 0.1	0.4 ± 0.2	0.2 ± 0.2	0.5 ± 0.1	0.2 ± 0.1	0.5 ± 0.2	0.2 ± 0.1
5	0.3 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.4 ± 0.1	0.2 ± 0.1	0.5 ± 0.2	0.1 ± 0.1
total	40.9 ± 7.9	58.7 ± 10.6	34.0 ± 7.7	67.0 ± 4.0	38.6 ± 2.4	64.5 ± 6.4	36.3 ± 20.1	62.6 ± 19.3

[a] Five male and five female rats. [b] Mean ± standard deviation (n = 5).

dose and greater than 95% of the administered radioactivity from the acid and alcohol moieties were completely eliminated from the body within 5 days after dosing. The majority of the administered radioactivity was eliminated during the initial 24 h posttreatment. Fecal excretion was the major route of elimination, accounting for approximately 60% of the administered dose. Elimination data are summarized in Tables III and IV.

Sex difference in the urinary elimination rate was observed when test animals received an oral dose of [*chlorophenyl*-^{14}C]fenvalerate. Male test animals eliminated a greater amount of the applied radioactivity in the urine; however, due to individual variability, significant difference in the fecal elimination between the male and female test animals at the 95% confidence level was not observed. When treated with [*chlorophenyl*-^{14}C]fenvalerate Y rich, [*phenoxyphenyl*-^{14}C]fenvalerate, or [*phenoxyphenyl*-^{14}C]fenvalerate Y rich, differences in the elimination rates between the male and female test animals were not observed. There was no significant stereoselective difference in the elimination rate of the racemic fenvalerate or the fenvalerate Y rich in the male and female test animals.

Urinary Metabolite Distribution Profile. There was no observable difference in the chemical nature of the urinary and fecal metabolites recovered from the male and female test animals after treatment with either fenvalerate or fenvalerate Y rich. Summaries of the distribution of urinary and fecal metabolites are presented in Tables V and VI. The primary metabolic pathway was the cleavage of the ester linkage. Degradation products from both the alcohol and the acid moiety underwent metabolic modifications, including oxidations and conjugations (with glucuronide, sulfate, amino acids, etc.). Urinary metabolites in the organic solvent-extractable fraction and as water-soluble conjugates (released by sulfatase/β-glucuronidase enzyme hydrolysis) were analyzed separately. Characterization of the chemical nature of the intact conjugate was not attempted.

Autoradiograms of the radioactivity recovered in the organic-extractable fraction are presented in Figure 2.

Table V. Distribution of Metabolites in the Urinary and Fecal Excreta from the Male and Female Test Animals[a] after a Single Oral Dose Administration of [*chlorophenyl*-^{14}C]Fenvalerate and Fenvalerate Y Rich[b]

	administered radioactivity, %							
	fenvalerate				fenvalerate Y rich			
	male urine	male feces	female urine	female feces	male urine	male feces	female urine	female feces
fenvalerate (1)		30.5 ± 10.2		46.2 ± 8.3		35.1 ± 12.4		38.6 ± 8.5
4′-OH-fenvalerate (10)		3.8 ± 2.1		2.2 ± 0.8		4.9 ± 1.1		3.9 ± 1.7
2-OH-CPIA lactone (7)	2.6 ± 4.0		1.6 ± 1.3		1.7 ± 0.5		1.3 ± 0.3	
CPIA (2)								
(free)	10.8 ± 4.0	3.4 ± 0.5	10.8 ± 4.8	3.1 ± 0.5	7.9 ± 5.2	4.4 ± 1.7	8.6 ± 3.8	3.6 ± 1.2
(conjugates)	2.9 ± 1.7		2.7 ± 4.3		2.0 ± 1.2		1.0 ± 0.9	
α-OH-CPIA (5)								
(free)	0.3 ± 0.1		0.3 ± 0.1		0.5 ± 0.1		*c*	
(conjugates)	1.1 ± 0.2		0.6 ± 0.2		0.4 ± 0.2		0.3 ± 0.1	
2-OH-CPIA (3)								
(free)	2.8 ± 2.2		2.3 ± 1.2		5.6 ± 2.9		4.9 ± 1.5	
(conjugates)	1.9 ± 1.5		0.2 ± 0.1		*c*		*c*	
2-OH-CPIA (4)								
(free)	0.7 ± 0.5		1.6 ± 1.0		1.6 ± 0.8		2.2 ± 0.9	
(conjugates)	1.0 ± 0.5		0.1 ± 0.1		*c*		*c*	
2,α-diOH-CPIA (6)								
(free)	0.4 ± 0.3		0.3 ± 0.1		3.1 ± 1.1		4.5 ± 0.4	
(conjugates)	0.2 ± 0.1		*c*		2.0 ± 0.8		1.1 ± 0.9	
unidentified	10.4 ± 1.5	8.7 ± 7.2	4.5 ± 2.0	4.0 ± 0.7	5.6 ± 2.0	8.9 ± 1.2	7.5 ± 1.6	8.1 ± 1.6
unextractable	0.6 ± 0.1	3.1 ± 1.7	1.0 ± 0.4	3.5 ± 4.3	3.7 ± 4.3	3.5 ± 1.1	2.6 ± 1.3	5.1 ± 1.2
total	35.7 ± 4.6	49.5 ± 6.5	26.0 ± 5.5	59.0 ± 8.3	34.1 ± 14.2	56.8 ± 10.5	34.0 ± 9.2	59.3 ± 9.2

[a] Five individual test animals. [b] Mean ± standard deviation. [c] Not detected.

Table VI. Distribution of Metabolites in the Urinary and Fecal Excreta from the Male and Female Test Animals[a] after a Single Oral Dose Administration of [*phenoxyphenyl*-^{14}C]Fenvalerate and Fenvalerate Y Rich[b]

	administered radioactivity, %							
	fenvalerate				fenvalerate Y rich			
	male urine	male feces	female urine	female feces	male urine	male feces	female urine	female feces
fenvalerate (1)		37.7 ± 7.2		44.0 ± 12.0		31.9 ± 11.7		34.9 ± 27.1
4′-OH-fenvalerate (10)		3.8 ± 1.0		4.0 ± 0.9		2.4 ± 0.6		2.5 ± 0.8
PBacid (8)	2.0 ± 0.4		1.6 ± 0.9		2.2 ± 0.9		1.9 ± 1.0	
4′-OH-PBacid (9)								
(free)	1.2 ± 0.4	1.5 ± 0.3	3.1 ± 2.1	3.5 ± 1.1	1.0 ± 0.2	1.9 ± 0.7	2.8 ± 1.1	3.6 ± 1.2
(conjugates)	28.9 ± 6.9		21.3 ± 5.2		27.5 ± 3.7		25.2 ± 15.5	
unidentified	3.8 ± 0.4	7.4 ± 1.7	4.4 ± 1.2	6.9 ± 1.8	3.1 ± 0.5	12.6 ± 3.4	3.9 ± 1.1	13.0 ± 3.5
unextractable	3.3 ± 0.4	7.2 ± 4.9	2.3 ± 3.0	7.9 ± 3.0	3.1 ± 0.5	16.7 ± 6.2	2.6 ± 1.0	7.9 ± 5.3
total	39.2 ± 7.5	57.6 ± 10.7	32.7 ± 7.4	66.3 ± 11.6	36.9 ± 2.5	65.5 ± 6.5	36.4 ± 18.8	61.9 ± 19.2

[a] Five individual test animals. [b] Mean ± standard deviation.

Metabolites were tentatively identified by their authentic standards. Metabolites were isolated by preparative TLC and their structures were subsequently confirmed by GC/mass spectroscopy.

Undegraded fenvalerate (1) was not detected in the urinary extract of any test animal. The following compounds were detected as the primary organic-extractable metabolites from the acid portion of the parent molecule: CPIA (2) was the major product and it was identified as its corresponding methyl ester [CPIA-Me (11)]. The α and β diastereomers of 2-OH-CPIA (3 and 4) were identified as the corresponding methyl esters [2-OH-CPIA-Me (12)]. It is interesting to note that the α isomer of 2-OH-CPIA (3) decomposed readily to 2-OH-CPIA lactone (7) during sample preparation and TLC development. However, minimal decomposition of the β isomer (4) occurred. Both 3 and 4 cyclized to yield the lactone (7) during GC–MS analysis. α-OH-CPIA (5), a minor product, was identified as its corresponding methyl ester [α-OH-CPIA-Me (12)]. Derivatization of 2,α-diOH-CPIA (6) with diazomethane resulted in the formation of the lactonization product and the methyl ester of 2,α-diOH-CPIA (6) was not observed during the GC–MS analysis. There were also 7–8 other unidentified minor products (each accounted for less than 1% of the administered dose). Radioactivity remaining in the aqueous phase after the initial organic extraction was analyzed after enzyme hydrolysis which resulted in the recovery of the above metabolites. In the study of Ohkawa et al. (1979), a series of products consisting of an unsaturated acid, its lactone, and an anhydride was identified. They were reported as potential artifacts during analysis or generated via the cyclization of appropriate precursors in the body prior to excretion (Gaughan et al.,

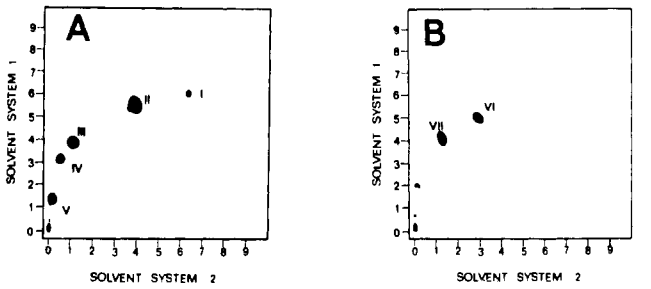

Figure 2. Thin-layer autoradiograms of the organic-extractable urinary metabolites recovered after [*chlorophenyl*-^{14}C]fenvalerate (A) and [*phenoxyphenyl*-^{14}C]fenvalerate (B) treatment. Product designations: I, 2-OH-CPIA-lactone (7); II, CPIA (2); III, 2-OH-CPIA (3); IV, 2-OH-CPIA (4); V, 2, α-diOH-CPIA (6); VI, PBacid (8); VII, 4′-OH-PBacid (9).

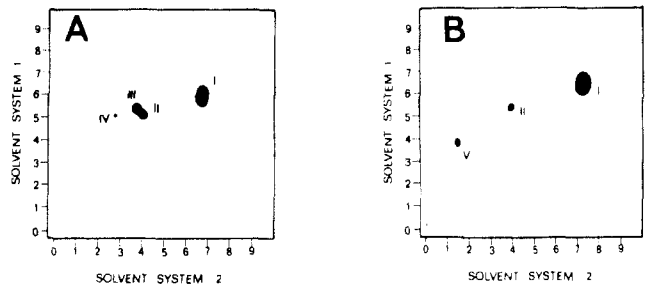

Figure 3. Thin-layer autoradiograms of the organic-extractable fecal metabolite recovered after [*chlorophenyl*-^{14}C]fenvalerate (A) and [*phenoxyphenyl*-^{14}C]fenvalerate (B) treatment. Product designations: I, fenvalerate (1); II, 4′-OH-fenvalerate (10); III CPIA (2); IV, α-OH-CPIA (5); V, 4′-OH-PBacid (9).

1977). The formation of these products could have occurred during the freeze drying of the acidified urinary samples or during TLC analyses. Since these products are potential artifacts and were not detected in the present study, they were not considered to be in vivo metabolic products of fenvalerate in laboratory animals under the experimental conditions tested.

Quantitatively, a significant difference was observed in the amount of conjugated urinary metabolites between the male (19.2 ± 4.2%) and the female (7.2 ± 4.7%) after [*chlorophenyl*-^{14}C]fenvalerate treatment. However, this difference was not observed when animals were administered [*chlorophenyl*-^{14}C]fenvalerate Y rich. 2,α-diOH-CPIA (6) accounted for approximately 0.5 and 5% of the administered dose after fenvalerate and fenvalerate Y rich treatments, respectively. This is the only stereoselective difference observed in this study.

In animals treated with [*phenoxyphenyl*-^{14}C]fenvalerate, PBacid (8) and 4′-OH-PBacid (9) [identified as 4′-OH-PBacid-Me (15)] were detected as the major organic-extractable urinary products from the alcohol portion of the parent molecule (see Figure 2). 4′-OH-PBacid (9) was the only major conjugated product recovered after enzyme hydrolysis. 2′-OH-PBacid (free and conjugated), which was reported in earlier studies (Ohkawa et al., 1979; Kaneko et al., 1981), could be present as a minor metabolite and was not identified in this study. There was no significant difference in the distribution of urinary metabolites between the male and female animals after the administration of [*phenoxyphenyl*-^{14}C]fenvalerate or fenvalerate Y rich.

Fecal Metabolite Distribution Profile. There was no significant difference in the distribution of fecal metabolites in the male and female test animals after an oral dose of [*chlorophenyl*-^{14}C]- or [*phenoxyphenyl*-^{14}C]fenvalerate and fenvalerate Y rich. In addition to the undegraded parent molecule (30–45% of the administered dose), 4′-OH-fenvalerate (10), CPIA (2) [from the acid portion], and 4′-OH-PBacid (9) [from the alcohol portion] were recovered as primary fecal degradation products (Figure 3). α-OH-CPIA (5) was recovered as a minor fecal metabolite of [*chlorophenyl*-^{14}C]fenvalerate. Fecal solid materials, after solvent extraction, contained 3–16% of the administered dose and were characterized as unextractable bound residues.

Tissue Distribution Profile. There was no significant difference in the level of ^{14}C residues in the male and female test animals after an oral dose of [*chlorophenyl*-^{14}C]- or [*phenoxyphenyl*-^{14}C]fenvalerate and fenvalerate Y rich. Low levels of ^{14}C residues at the detection limit (0.05 ppm) were observed in most of the tissues examined. Highest levels of ^{14}C residues were observed in the liver (approximately 0.2 ppm) and fat (1.5 ppm) tissues. A majority of the hexane extractable residues (>90% of the total fat tissue residues) was characterized as the parent molecule by capillary GLC, two-dimensional TLC, and autoradiography.

CONCLUSION

After a single oral dose, the metabolism of fenvalerate and fenvalerate Y rich was extensive and greater than 90% of the administered dose was eliminated in the initial 24 h. The overall metabolic profile of fenvalerate is very similar to other cyano pyrethroids such as cypermethrin (Cole et al., 1982; Crawford et al., 1981), decamethrin (Ruzo et al., 1978), fluvalinate (Quistad et al., 1983), fenpropanate (Crawford and Hutson, 1977), deltamethrin, tralomethrin, and tralocythrin (Cole et al., 1982). Except for some minor qualitative differences in the characterization of minor metabolites, results from this study are consistent with the earlier reported studies with different radiolabeled materials or various isomeric compositions (Ohkawa et al., 1979; Kaneko et al, 1981).

A complex metabolic degradation pattern of fenvalerate was observed and metabolic reactions could be classified into the following categories.

Reaction 1: Hydroxylation of the Intact Molecule. In addition to the undegraded parent molecule, 4′-OH- and 2′-OH-fenvalerate were recovered as metabolites (in the feces only) which retained the intact molecular structure. The formation of these hydroxylation products, probably due to intestinal microorganisms, was not significantly affected by the stereoisomeric composition of the test material.

Reaction 2: Ester Cleavage. Cleavage of the ester linkage is the initial step and the primary metabolic pathway of fenvalerate in the rat. The acid and the alcohol portion of the parent molecule, upon ester cleavage, underwent further hydroxylation, oxidation, and conjugation reactions prior to elimination. Based on the sum of all the degradation products derived from the acid and alcohol moiety (data from this study, Ohkawa et al., 1979; Kaneko et al., 1981), this reaction is apparently not stereoselective.

Reaction 3: Hydroxylation and Oxidation of the Acid Portion of the Fenvalerate Molecule. The acid moiety [CPIA (2), the asymmetric carbon was retained after ester cleavage] was excreted as free and conjugated products. Hydroxylation at the methyl and/or benzyl position yielded several free and conjugated products. The chemical natures of the acid metabolites were identical regardless of the isomeric compositions of the test material. Although a slight difference in the amount of the various hydroxylated acid metabolites was evident as the *SS* isomer content increased (see Table V), such differences were also observed between the male/female and also the high/low dose studies (Kaneko et al., 1981).

Reaction 4: Hydroxylation and Oxidation of the Alcohol Portion of the Fenvalerate Molecule. The resultant metabolites from the alcohol portion of fenvalerate were rapidly eliminated. The asymmetric center of the alcohol moiety is lost following ester cleavage and the loss of the cyano moiety. The formation of PBacid (8), 2′-OH-, and 4′-OH-PBacid (9) was not affected by the isomeric contents of the test material.

Reaction 5: Oxidation of the Cyano Moiety. The cleavage of the fenvalerate ester linkage was shown to lead to the release of cyanide which was rapidly converted to thiocyanate (localized in the skin) and to a smaller extent as carbon dioxide. Slight differences in the amount of thiocyanate were observed between the various isomeric compositions (Ohkawa et al., 1979). The possible contribution of stereoselectivity to this observed difference

was not demonstrated, primarily due to the large experimental variability.

Reaction 6: Conjugates. The acidic metabolites resulting from ester cleavage underwent conjugation with glucuronic acid, sulfate, and glycine, followed by elimination via urinary excretion. Results did not indicate any significant difference between the various isomer preparations.

Based on the data from this and other studies of the metabolism of fenvalerate, there is no apparent qualitative or quantitative difference in the disposition of racemic fenvalerate or its various isomer combinations.

ACKNOWLEDGMENT

We thank W. B. Burton and T. D. Hoewing for the preparation of labeled materials, E. J. Silveira for GC/mass spectral analysis, and Gerri Solano for the preparation of this manuscript.

Registry No. 1 (isomer 1), 67890-40-8; 1 (isomer 2), 67890-39-5; 2, 55291-27-5; 5, 97635-01-3; 6 (isomer 1), 97635-02-4; 6 (isomer 2), 97635-06-8; 7, 72061-37-1; 8, 3739-38-6; 9, 35065-12-4; 10 (isomer 1), 97635-03-5; 10 (isomer 2), 97635-07-9; 11, 97635-04-6; 12 (isomer 1), 97644-32-1; 12 (isomer 2), 97635-08-0; 13, 97635-05-7; 14, 50789-43-0; 15, 63987-15-5; *p*-$ClC_6H_4CH(CO_2H)CH(CH_3)CH_2OH$ (isomer 1), 97634-99-6; *p*-$ClC_6H_4CH(CO_2H)CH(CH_3)CH_2OH$ (isomer 2), 97635-00-2.

LITERATURE CITED

Cole, L. M.; Ruzo, L. O.; Wood, E. J.; Casida, J. E. *J. Agric. Food Chem.* **1982**, *30*, 631.
Crawford, M. J.; Hutson, D. H. *Pestic. Sci.* **1977**, *8*, 579.
Crawford, M. J.; Croucher, A.; Hutson, D. H. *Pestic. Sci.* **1981**, *12*, 399.
Gaughan, L. C.; Unai, T.; Casida, J. E. *J. Agric. Food Chem.* **1977**, *25*, 9.
Kaneko, H.; Ohkawa, H.; Miyamoto, J. *J. Pestic. Sci.* **1981**, *6*, 317.
Ohkawa, H.; Kaneka, H.; Tsuji, H.; Miyamoto, J. *J. Pestic. Sci.* **1979**, *4*, 143.
Quistad, G. B.; Staiger, L. E.; Jamieson, G. C.; Schooley, D. A. *J. Agric. Food Chem.* **1983**, *31*, 589.
Ruzo, L. O.; Unai, T.; Casida, J. E. *J. Agric. Food Chem.* **1978**, *26*, 918.

Received for review October 10, 1984. Accepted May 20, 1985.

Fate of Fenvalerate (Pydrin Insecticide) in the Soil Environment

Philip W. Lee

The fate of fenvalerate (Pydrin insecticide) in the soil environment was examined. The half-lives of fenvalerate under laboratory aerobic or outdoor conditions in sandy loam and silty clay loam soils are approximately 75–80 days. In addition to degradation products resulting from the cleavage of the ester linkage, $CONH_2$-and 4′-OH-fenvalerate were detected. Further degradation of the soil metabolites was evident by the generation of $^{14}CO_2$ and unextractable residues. The degradation of fenvalerate in the soil environment was primarily by microbial action. Lettuce, beets, and wheat were planted at 30 days, 120 days, and 1 year after the soil was treated with [^{14}C]fenvalerate at a rate equivalent to 2 lb/acre. The crops were harvested at maturity and were found to contain low levels of [*chlorophenyl*-^{14}*C*]- and [*phenoxyphenyl*-^{14}*C*]fenvalerate equivalent residues (below or 1–3 times the limit of detection). Little downward movement of radioactivity was observed in the soil container. It is concluded that under test conditions fenvalerate is relatively nonpersistent in the soil environment. In addition, rotational crops planted at various time intervals after soil treatment contained low, if any, significant residue levels of fenvalerate or its metabolites.

INTRODUCTION

Fenvalerate [1, cyano(3-phenoxyphenyl)methyl 4-chloro-α-(1-methylethyl)benzeneacetate], known as Pydrin insecticide (Shell Chemical Company), Sumicidin (Sumitomo Chemical Company), and Belmark (Shell International Chemical Company), is an effective broad spectrum synthetic pyrethroid insecticide. In addition to its highly selective insecticidal activities, fenvalerate exhibits improved photolytic stability and an extended field residual activity compared to other commercial pyrethroids. The increased wide use pattern and the longer residual activities of fenvalerate warrant the need of understanding the fate of this compound and its degradation products in the environment. As a part of this comprehensive evaluation, this report summarizes (1) the fate of fenvalerate in the soil environment and (2) the uptake and the accumulation potential of fenvalerate and its metabolites in various agricultural crops (lettuce, beets, and wheat) that occurred under field rotational crop conditions.

Biological Sciences Research Center, Shell Development Company, Modesto, California 95352.

MATERIALS AND METHODS

Test Materials and Reference Standards. Radiolabeled fenvalerate and appropriate reference standards were synthesized by the Biological Sciences Research Center (BSRC), Shell Development Company. Two preparations of [^{14}C]fenvalerate, labeled at the chlorophenyl and the other at the phenoxyphenyl moiety, had a radiochemical purity of greater than 99.5% as determined by thin-layer chromatography (TLC), autoradiography, and liquid scintillation counting (LSC). Authentic standards included CPIA [2, 4-chloro-α-(1-methylethyl)-benzeneacetic acid], 4′-OH-fenvalerate [3, cyano(3-phenoxy-4-hydroxyphenyl)methyl 4-chloro-α-(1-methylethyl)-benzeneacetate], $CONH_2$-fenvalerate [4, (aminocarbonyl)(3-phenoxyphenyl)methyl 4-chloro-α-(1-methylethyl)benzeneacetate], PBacid [5, 3-phenoxybenzoic acid], and 4′-OH-PBacid, [6, 3-(4-hydroxyphenoxy)benzoic acid]. Chemical structures of these compounds are pres-

0021-8561/85/1433-0993$01.50/0