Degradation of *cis*- and *trans*-Permethrin in Flooded Soil

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The degradation of permethrin [(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate] was studied in a flooded Memphis silt loam soil incubated at 25 °C. [*carbonyl*-^{14}C]-*cis*-, [*carbonyl*-^{14}C]-*trans*-, and [*methylene*-^{14}C]-*cis*-permethrin were added to soil at rates of 0.1 and 1.0 ppm. Soils were analyzed after 0, 4, 8, 16, 32, and 64 days to determine the distribution of ^{14}C in CO_2, solvent-extractable compounds, water-soluble polar compounds, and soil-bound residues. Thin-layer chromatographic analysis of the organic solvent extracts showed that *trans*-permethrin was more rapidly degraded than *cis*-permethrin. Two metabolites, 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (DCVA) and 3-phenoxybenzyl alcohol (PBalc), which resulted from permethrin hydrolysis, were identified. Other metabolites identified were 3-phenoxybenzoic acid (PBacid) and 3-phenoxybenzaldehyde (PBald), an intermediate in the conversion of PBalc to PBacid. Fragmentation of DCVA and PBacid to CO_2 was not extensive, and cumulative $^{14}CO_2$ recoveries were less than 3.5% for all treatments during the 64-day incubation period. Metabolism of *trans*-permethrin resulted in the accumulation of polar compounds in the water. Soil-bound residues gradually increased with time and accounted for 3.3–11.4% of the ^{14}C activity after 64 days. The largest percentage of soil-bound ^{14}C residue was in the fulvic acid fraction.

INTRODUCTION

The aerobic metabolism of permethrin [(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate], a widely used agricultural insecticide, has been extensively studied in soil under laboratory conditions (Kaneko et al., 1978; Kaufman et al., 1977, 1978a,b; Lord et al., 1982; Williams and Brown, 1979). The half-life (time required for 50% degradation) of permethrin in aerobically incubated soil is less than 4 weeks, and the degradation of the trans isomer is more rapid than the cis isomer. The major metabolic pathway involves the hydrolysis of the ester bond of permethrin, producing 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (DCVA) and 3-phenoxybenzyl alcohol (PBalc), the oxidation of PBalc to 3-phenoxybenzoic acid (PBacid) and the decarboxylation of DCVA and PBacid releasing CO_2 (Kaneko et al., 1978; Kaufman et al., 1977,1978b). It was postulated that 3-phenoxybenzaldehyde (PBald) was formed during the conversion of PBalc to PBacid (Kaufman et al., 1977). Hydroxylation of permethrin and DCVA also was reported as a metabolic reaction (Kaneko et al., 1978).

Less information is available on the anaerobic metabolism of permethrin in flooded soils. The degradation of permethrin in flooded Kettering loam and flooded Hagerstown silty clay loam soil was slower than in comparable aerated soils (Kaufman et al., 1977; Lord et al., 1982). After 60 days, less than 1% of the applied [^{14}C]permethrin was recovered as $^{14}CO_2$ from flooded Hagerstown soil while 51% of the applied [^{14}C]permethrin was recovered as $^{14}CO_2$ from aerated Hagerstown soil. The only degradation product identified from flooded Hagerstown soil was DCVA (Kaufman et al., 1977). Sharom and Solomon (1981) studied the degradation of *cis*- and *trans*-permethrin in sodium azide treated and nontreated lake water and flooded sediment. Their study showed that *cis*-permethrin was more persistent than *trans*-permethrin and biological as well as chemical degradation occurred. The purpose of this investigation was to examine the anaerobic metabolism of [*carbonyl*-^{14}C]-*cis*- and *trans*- and [*methylene*-^{14}C]-*cis*-permethrin in flooded soil.

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MATERIALS AND METHODS

[*carbonyl*-^{14}C]-*trans*-, [*carbonyl*-^{14}C]-*cis*-, and [*methylene*-^{14}C]-*cis*-permethrin with specific activities of 54.8, 54.8, and 57.0 mCi/mM, respectively, were supplied by the Agricultural Chemical Division, FMC Corp., Middleport, NY (present address: Princeton, NJ). A Beckman Model LS-3150T scintillation counter and Beckman Ready-Solv Solution VI scintillation cocktail were used to analyze soluble ^{14}C samples. A Packard Tri-Carb sample oxidizer (Model 306), Packard Carbo-Sorb, and Packard Perma flour V cocktail were used to analyze soil-bound ^{14}C residue samples. Brinkman silica gel 60F-254 Chromatoplates (0.25-mm thickness) and Kodak Co. no-screen medical X-ray film (NS-54T) were used for TLC autoradiography analysis. Burdick & Jackson Co. analytical grade solvents were used throughout the study.

Soil. Memphis silt loam soil, a Typic Hapludalf, collected from an agricultural field near Marion, AK (Crittenden County) and supplied by FMC Corp., was used to study the metabolism of permethrin in flooded soil. The soil was air-dried to approximately 20% moisture and sieved through a 2-mm (No. 10) U.S. standard sieve to remove crop debris. Analysis of this soil revealed the following characteristics: pH 5.8; cation-exchange capacity, 16.8 mequiv/100 g; sand, 20.8%, silt, 54.0%, clay, 25.2%; organic matter content, 0.7%; moisture content ($^1/_3$ bar), 37.6%.

Soil Treatment and Incubation. Treatment and incubation of soil were conducted in biometer flasks (Bartha and Pramer, 1965). [*carbonyl*-^{14}C]-*cis*-, [*carbonyl*-^{14}C]-*trans*-, and [*methylene*-^{14}C]-*cis*-permethrin were each applied at 0.1 and 1.0 ppm to 50 g (dry weight) of soil in 0.1 mL of ethanol. [^{14}C]Permethrin-treated soils were thoroughly mixed in each biometer flask. Anaerobic conditons were established by flooding the soil in each flask with 75 mL of sterile distilled water (1-cm layer of water above the soil surface) and purging the atmosphere with nitrogen. Biometer flasks were incubated in the dark at 25 °C. Carbon dioxide evolved from the incubated soils was trapped in 0.1 N KOH. On days 0, 4, 8, 16, 32, and 64 the water and soil portion from replicate flasks of each

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Table I. ^{14}C Recovered from Flooded Memphis Slit Loam Soil Treated with [^{14}C]Permethrin

		% of appl ^{14}C rec as						
			solvent extractable			residual		
treatment (rate)	day	$^{14}CO_2$	hexane	$CHCl_3$–MeOH	total extr	water	soil	total res	total
carbonyl-cis (0.1 ppm)	0		5.5	82.5	88.0	0.8	1.9	2.7	90.7
	4	0.2	2.9	85.7	88.6	0.9	2.4	3.3	92.1
	8	0.3	3.1	83.6	86.7	1.2	2.5	3.7	90.7
	16	0.6	2.2	85.7	87.9	1.3	3.0	4.3	92.8
	32	0.9	2.5	82.9	85.4	1.4	3.2	4.6	90.9
	64	1.4	2.8	79.0	81.8	1.6	3.4	5.0	88.2
carbonyl-cis (1.0 ppm)	0		12.9	72.5	85.4	1.0	1.7	2.7	88.1
	4	0.2	8.8	75.8	84.6	1.0	2.0	3.0	87.8
	8	0.4	5.9	77.9	83.8	1.2	2.4	3.6	87.8
	16	0.9	4.5	78.5	83.0	1.7	3.4	5.1	89.0
	32	1.5	3.4	75.7	79.1	2.1	5.2	7.3	87.9
	64	1.7	3.6	74.6	78.2	4.5	3.3	7.8	87.7
carbonyl-trans (0.1 ppm)	0		7.8	80.7	88.5	0.8	1.3	2.1	90.6
	4	0.2	4.4	86.1	90.5	1.1	2.6	3.7	94.4
	8	0.4	4.7	79.2	83.9	4.5	3.5	8.0	92.3
	16	1.4	3.2	69.9	73.1	8.5	7.3	15.8	90.3
	32	2.4	2.0	65.8	67.8	14.3	8.9	23.2	93.4
	64	3.1	1.5	63.4	64.9	16.6	11.4	28.0	96.0
carbonyl-trans (1.0 ppm)	0		15.1	69.4	84.5	0.8	1.5	2.3	86.8
	4	0.3	5.4	76.5	81.9	4.0	3.6	7.6	89.9
	8	0.5	6.1	77.4	83.5	4.6	4.7	9.3	93.3
	16	0.8	4.7	78.8	83.5	5.9	4.2	10.1	94.4
	32	1.0	3.7	74.0	77.7	9.7	5.8	15.5	94.2
	64	1.5	1.8	59.7	61.5	22.9	9.1	32.0	95.0
methylene-cis (0.1 ppm)	0		6.7	80.6	87.3	0.8	4.2	5.0	92.3
	4	0.4	2.8	80.8	83.6	1.3	5.6	6.9	90.9
	8	1.0	3.3	79.2	82.5	1.4	6.5	7.9	91.4
	16	2.0	2.4	73.6	76.0	1.3	7.5	8.8	86.8
	32	2.4	1.3	79.4	80.7	2.2	8.2	10.4	93.5
	64	2.8	2.2	74.6	76.8	2.4	10.7	13.1	92.7
methylene-cis (1.0 ppm)	0		11.4	72.3	83.7	1.1	3.8	4.9	88.6
	4	0.4	6.1	75.2	81.3	0.9	6.3	7.2	88.9
	8	0.6	3.2	82.1	85.3	1.4	6.7	8.1	94.0
	16	1.1	3.5	77.8	81.3	1.6	7.5	9.1	91.5
	32	1.6	2.5	76.9	79.4	2.3	9.0	11.3	92.3
	64	3.4	2.3	72.9	75.2	2.5	9.6	12.1	90.7

treatment were extracted and each biometer flask side arm was emptied and recharged with 0.1 N KOH. Duplicate aliquots (1 mL) of the 0.1 N KOH solution were analyzed by liquid scintillation counting (LSC) for $^{14}CO_2$ content.

Analysis of the Water and Soil Samples. The water and soil were placed in a stainless-steel beaker and extracted with 150 mL of hexane by blending with a heavy-duty laboratory stirrer. The soil layer was removed and further blended consecutively with 3:1 methanol–chloroform (150 mL) and methanol (150 mL). The hexane and water were partitioned, and the water was extracted with chloroform (200 mL). Duplicate aliquots (1 mL) of the above extracts were analyzed for ^{14}C content by LSC. The extracted soil was air-dried, and a 2.0-g subsample was ground to a powder with a mortar and pestle. Duplicate subsamples (200–300 mg) of each extracted soil were analyzed for bound ^{14}C residues by combustion (Packard Tri-Carb oxidizer) and LSC. Organic matter fractionation was performed by extracting 2.0 g of soil with 0.5 N NaOH (10 mL) for 24 h. The fulvic and humic acids were separated by acidification of the extract with 5 N HCl (1 mL) for 2 h. The insoluble humic acid was washed with 1 N HCl (2 mL) and dissolved in 0.1 N NaOH (2 mL). The ^{14}C contents of the fulvic and humic acids were determined by LSC. The ^{14}C activity incorporated into the humin was determined by combustion of the alkali-extracted soil.

Product Identification. For each treatment, each rate, and each sampling day, the replicate chloroform extracts of the water were combined with the replicate methanol–chloroform and methanol soil extracts. For each treatment, both rates, and all sampling days, the replicate hexane extracts of the soil and water were combined. Extracts were reduced in volume on a flash evaporator and concentrated under nitrogen to 0.5 mL for further analysis. Each concentrate was spotted on silica gel chromatographic plates (0.25-mm thickness), and products were separated by thin-layer chromatography (TLC) using the following solvent systems for two-dimensional development: benzene (saturated with formic acid)–ether (10:3) in the first direction (R_f 0.63 for *trans*-permethrin, 0.59 for *cis*-permethrin, 0.34 for PBalc, 0.42 for PBacid, 0.56 for PBald, 0.44 for DCVA) and three developments with hexane–benzene–acetone (7:3:0.1) in the second direction (R_f 0.57 for *trans*-permethrin, 0.67 for *cis*-permethrin, 0.24 for PBalc, 0.29 for PBacid, 0.40 for PBald, 0.02 for DCVA). No-screen medical X-ray film was used to autoradiograph TLC plates. The spots containing ^{14}C activity were scraped from the TLC plates and counted by LSC for quantitative determination of the parent compound and its metabolites. Products were identified by comparing R_f values with analytical standards.

RESULTS

The distribution of ^{14}C in CO_2, organic solvent extracts, residuals, and the total ^{14}C recoveries are given in Table I. Cumulative recoveries of ^{14}C as $^{14}CO_2$ from flooded Memphis silt loam soil treated with ^{14}C-labeled permethrin at 0.1 and 1.0 ppm were less than 3.5% after 64 days of incubation. Cumulative $^{14}CO_2$ recoveries were variable, and no consistent differences were noted between treatments or between application rates within treatments. For all treatments, the ^{14}C products extracted from the soil and

Table II. ^{14}C Distribution in Soil Organic Matter Fractions for Flooded Memphis Slit Loam Soil Treated with [^{14}C]Permethrin

treatment	rate, ppm	day	% of resid ^{14}C present in fulvic acid	humic acid	humin
carbonyl-cis	0.1	0	75.7	1.0	23.3
	0.1	4	69.7	0.8	29.5
	0.1	8	63.2	6.3	30.5
	0.1	16	65.7	3.6	30.7
	0.1	32	61.0	3.4	35.5
	0.1	64	61.2	1.4	37.4
carbonyl-cis	1.0	0	73.3	1.7	25.0
	1.0	4	70.5	1.9	27.5
	1.0	8	61.9	2.9	35.2
	1.0	16	62.0	2.3	35.7
	1.0	32	61.5	2.6	35.9
	1.0	64	59.0	2.2	38.9
carbonyl-trans	0.1	0	72.3	5.4	22.3
	0.1	4	70.8	3.6	25.6
	0.1	8	61.8	7.0	31.2
	0.1	16	67.5	4.0	28.5
	0.1	32	70.3	4.0	25.7
	0.1	64	73.8	3.4	22.8
carbonyl-trans	1.0	0	78.1	2.4	19.5
	1.0	4	74.1	3.7	22.2
	1.0	8	72.6	4.6	22.8
	1.0	16	72.2	4.2	23.6
	1.0	32	72.2	4.4	23.4
	1.0	64	76.2	3.6	20.3
methylene-cis	0.1	0	70.6	8.1	21.3
	0.1	4	69.7	5.8	24.5
	0.1	8	64.5	9.1	26.4
	0.1	16	58.7	9.4	31.9
	0.1	32	65.1	9.0	25.9
	0.1	64	67.6	5.8	26.6
methylene-cis	1.0	0	72.9	4.9	22.3
	1.0	4	68.5	6.5	25.1
	1.0	8	66.9	5.4	27.7
	1.0	16	65.2	5.9	28.9
	1.0	32	67.1	5.2	27.7
	1.0	64	64.6	4.9	30.5

water fractions with hexane decreased with time. This trend was not apparent in all cases for the chloroform–methanol extracts. However, the total solvent extractable (^{14}C activity in hexane extract + ^{14}C activity in chloroform–methanol extract) decreased with time. The decreases in total solvent-extractable ^{14}C products for the flooded soil treated with [*carbonyl*-^{14}C]-*trans*-permethrin applied at 0.1 and 1.0 ppm were 23.6 and 23.0%, respectively. In comparison, decreases in the total solvent-extractable ^{14}C products for the [*carbonyl*-^{14}C]-*cis*-permethrin applied at 0.1 and 1.0 ppm were 6.2 and 7.2%, respectively, and for the [*methylene*-^{14}C]-*cis*-permethrin applied at 0.1 and 1.0 ppm were 10.5 and 8.5%, respectively. Concurrent with this decrease in solvent-extractable ^{14}C activity was an increase in ^{14}C residual products remaining in the water. These ^{14}C polar products were not extracted from the water with chloroform or hexane. The greatest increase in ^{14}C products remaining in the water occurred with flooded soil treated with [*carbonyl*-^{14}C]-*trans*-permethrin. Recoveries of ^{14}C products at 0.1 and 1.0 ppm were 16.6 and 22.9%, respectively.

Soil-bound residues gradually increased during the experiment but did not exceed 11.4% for any treatment. Soil-bound residues from the flooded soil treated with [*carbonyl*-^{14}C]-*cis*-permethrin at 0.1 and 1.0 ppm accounted for only 3.4 and 3.3% of applied ^{14}C after 64 days. Total residual ^{14}C values were highest with [*carbonyl*-^{14}C]-*trans*-permethrin and lowest with [*carbonyl*-^{14}C]-*cis*-permethrin. The distribution of ^{14}C-labeled compounds in the fulvic acid, humic acid, and humin fractions of the soil-bound residues is presented in Table II. For all treatments and all sampling periods recovery of residual ^{14}C was highest in the fulvic acid fraction, lower in the humin fraction, and lowest in the humic acid fraction.

TLC autoradiogram analysis of the solvent extracts showed that the major products extracted from flooded soils were the ^{14}C-labeled permethrin parent compounds, as either the *trans*- or *cis*-permethrin. Small amounts of the trans isomer were extracted from soil treated with the [*carbonyl*-^{14}C]- and [*methylene*-^{14}C]-*cis*-permethrin and the cis isomer from soil treated with [*carbonyl*-^{14}C]-*trans*-permethrin (Tables III and IV). The chromatography systems were capable of separating the cis and trans isomers of DCVA, and both isomers were detected in extracts of soil treated with *cis*- and *trans*-permethrin. However, separation of these isomers was not always

Table III. Distribution of ^{14}C Compounds Extracted (Chloroform–Methanol) from a Flooded Memphis Slit Loam Soil Treated with [^{14}C]Permethrin

treatment	rate, ppm	day	% of appl ^{14}C rec as unknown	DCVA	*trans*-permethrin	*cis*-permethrin
carbonyl-cis	0.1	0	0.3	1.5	2.0	77.1
	0.1	4	0.2	1.4	1.8	74.1
	0.1	8	0.3	1.5	1.7	78.5
	0.1	16	0.3	2.0	1.9	80.0
	0.1	32	0.2	2.1	1.3	77.2
	0.1	64	0.2	2.8	0.9	73.4
carbonyl-cis	1.0	0	0.3	1.8	2.0	66.4
	1.0	4	0.2	1.4	2.2	69.4
	1.0	8	0.2	1.5	1.7	73.1
	1.0	16	0.2	3.2	1.5	72.3
	1.0	32	0.2	5.4	1.0	67.6
	1.0	64	0.2	2.5	0.8	69.8
carbonyl-trans	0.1	0	0.2	1.7	76.1	1.1
	0.1	4	0.2	5.7	77.6	1.2
	0.1	8	0.1	8.0	68.7	1.1
	0.1	16	0.2	11.0	55.9	1.1
	0.1	32	0.2	15.1	47.7	1.2
	0.1	64	0.1	26.9	34.2	1.0
carbonyl-trans	1.0	0	0.3	1.5	65.2	0.9
	1.0	4	0.2	6.9	67.1	1.0
	1.0	8	0.1	9.4	64.9	1.0
	1.0	16	0.1	14.0	62.4	1.1
	1.0	32	0.1	23.0	48.6	1.1
	1.0	64	0.1	27.2	30.3	1.0

Table IV. Distribution of ^{14}C Compounds Extracted (Chloroform–Methanol) from a Flooded Memphis Silt Loam Soil Treated with [^{14}C]Permethrin

			% of appl ^{14}C rec as				
treatment	rate, ppm	day	PBalc	PBacid	PBald	*trans*-permethrin	*cis*-permethrin
methylene-cis	0.1	0	1.7	4.8	0.4	3.5	66.2
	0.1	4	1.7	3.1	0.3	2.8	68.0
	0.1	8	1.7	4.0	0.4	2.6	66.6
	0.1	16	1.2	4.7	0.2	2.2	62.5
	0.1	32	1.0	5.5	0.2	2.3	67.3
	0.1	64	1.0	3.9	0.2	1.4	65.0
methylene-cis	1.0	0	1.7	3.8	0.8	2.5	58.9
	1.0	4	1.2	3.0	0.4	2.7	63.6
	1.0	8	1.3	4.0	0.5	2.8	69.3
	1.0	16	1.3	4.1	0.3	2.6	67.0
	1.0	32	1.2	4.6	0.2	2.0	65.8
	1.0	64	1.2	6.5	0.2	1.4	61.5

complete. Therefore, DCVA in this text refers to both isomers, and *cis*- and *trans*-DCVA are presented as one product in Table III. Analysis of chloroform–methanol extracts of flooded soil treated with [*carbonyl*-^{14}C]-*trans*-permethrin showed an increase with time in DCVA concentration and a concomitant decrease in the concentration of the parent compound (Table III). In contrast, the formation of DCVA in flooded soil treated with [*carbonyl*-^{14}C]-*cis*-permethrin was minimal. The flooded soils treated with [*methylene*-^{14}C]-*cis*-permethrin produced three ^{14}C-extractable metabolites: PBacid, PBalc, and PBald (Table IV).

TLC autoradiogram analysis of the extracts over time indicated that the parent [*methylene*-^{14}C]-*cis*-permethrin degraded to PBalc, which was further degraded to PBacid with PBald an intermediate compound formed in this conversion (Table IV). The PBacid metabolite accumulated to 3.9 and 6.5%, over the 64-day incubation period, for application rates of 0.1 and 1.0 ppm, respectively. However, PBacid levels for 0.1 ppm rate fluctuated over time, suggesting further degradation of this metabolite. The accumulation of PBacid at day 0 indicates initial rapid degradation of [*methylene*-^{14}C]-*cis*-permethrin possibly due to oxidative respiration and cell-free enzyme metabolism.

The levels of *trans*-permethrin extracted from soil treated with [*carbonyl*-^{14}C]- and [*methylene*-^{14}C]-*cis*-permethrin at application rates of 0.1 and 1.0 ppm decreased over time, and it is suggested that degradation to DCVA, PBalc, and PBacid occurred (Tables III and IV).

The ^{14}C products identified from the hexane extracts of the flooded soil were the same as those extracted with chloroform–methanol for the corresponding [^{14}C]permethrin treatment. The [^{14}C]permethrin treatment, ^{14}C product, and the percent of the hexane extract were as follows: [[*methylene*-^{14}C]-*cis*-permethrin] *cis*-permethrin (91.2%), *trans*-permethrin (3.6%), PBacid (0.3%), PBalc (1.3%); [[*carbonyl*-^{14}C]-*trans*-permethrin] *trans*-permethrin (91.7%), DCVA (4.8%); [[*carbonyl*-^{14}C]-*cis*-permethrin] *cis*-permethrin (89.5%), *trans*-permethrin (4.3%), DCVA (3.1%).

DISCUSSION

A comparison of data from this study and previous investigations (Kaneko et al., 1978; Kaufman et al., 1977, 1978a,b; Lord et al., 1982; Williams and Brown, 1979) shows that the metabolism of *cis*- and *trans*-permethrin in flooded soil was incomplete and much slower than in aerated soil. In this study, *cis*- and *trans*-permethrin were hydrolyzed to DCVA and PBalc and PBalc subsequently oxidized to PBacid with PBald identified as an intermediate. DCVA and PBacid accumulated in the flooded soil, and further metabolism to CO_2 was minimal. In contrast, *cis*- and *trans*-permethrin in aerated soil rapidly degraded to DCVA and PBacid and was followed by extensive fragmentation of these moieties to CO_2.

Our results showed that in flooded soil *trans*-permethrin was degraded more rapidly than *cis*-permethrin and agree with previous reports (Lord et al., 1982; Sharom and Solomon, 1981). In aerated soil, *trans*-permethrin also was degraded more rapidly than *cis*-permethrin (Carroll et al., 1981; Chapman and Harris, 1981; Harris et al., 1981; Kaufman et al., 1977, 1978a,b; Lord et al., 1982; Williams and Brown, 1979).

However, the half-lives of *cis*- and *trans*-permethrin in aerated soil were shorter than in flooded soil. In this study, the half-lives (as estimated from the disappearance curves) of [*carbonyl*-^{14}C]-*trans*-permethrin applied at 0.1 and 1.0 ppm were 32 and 34 days, respectively, while the half-lives for all other [^{14}C]-*cis*-permethrin treatments were greater than 64 days. In contrast, Kaneko et al. (1978) reported that the half-life for *cis*-permethrin labeled at either [carbonyl-^{14}C] or [methylene-^{14}C] positions was approximately 12 days and for *trans*-permethrin was 6–9 days in aerated soil. Other researchers reported that *cis*,*trans*-permethrin had a half-life of less than 4 weeks in various aerobically incubated soils (Kaneko et al., 1978; Kaufman et al., 1977, 1978a,b; Williams and Brown, 1979).

The lower recovery of ^{14}C in the soil-bound residues in the flooded soil than in aerated soil (Kaufman et al., 1977, 1978a,b) is further evidence of slower permethrin degradation. The distribution of residual ^{14}C in the fulvic acid, humic acid, and humin soil-bound residue fractions from flooded soil was similar to the distribution of ^{14}C residues in [^{14}C]permethrin-treated soil sterilized with sodium azide (Kaufman et al., 1977). These results indicate that, in flooded soil, microbial metabolism was not important in the production of compounds associated with the soil organic matter fractions.

A substantial percentage of the ^{14}C products in the water portion of the [*carbonyl*-^{14}C]-*trans*-permethrin-treated flooded soil were not partitioned into nonpolar organic solvents (hexane, chloroform). It is suggested that the increased residual activity in the aqueous phase is due to the formation of highly polar products. The accumulation of ^{14}C polar products in the water from the flooded soil is similar to that reported by Kaufman et al. (1977) for flooded Hagerstown silty clay loam soil treated with [^{14}C]-*cis*,*trans*-permethrin.

It is suggested that the characteristics of permethrin degradation in flooded soil are the same as those described for carbon mineralization in flooded soil (Alexander, 1977). Thus, when permethrin-treated soil was flooded, there was a shift from aerobic to anaerobic metabolism. The in-

complete metabolism of permethrin in flooded soil was due to anaerobic transformations as oxygen was metabolized and lost from the soil. The accumulation of organic acids, especially the DCVA in flooded soil treated with [*carbonyl*-^{14}C]-*trans*-permethrin, is evidence of the fermentative character of the microorganisms responsible for permethrin metabolism in flooded soil.

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Identification of the Initial Metabolites of Acetochlor in Corn and Soybean Seedlings

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The initial metabolism of acetochlor (2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide), a selective preemergent herbicide that is used to control problem grass and some broadleaf weeds, was examined in tolerant corn and soybean seedlings in order to delineate the detoxification pathway for this herbicide. Acetochlor was rapidly absorbed and metabolized by etiolated corn seedlings to the glutathione (GSH; glutamylcysteinylglycine) conjugate. Acetochlor was also rapidly absorbed and metabolized by etiolated soybean seedlings. However, in this case the initial metabolite was the homoglutathione (hGSH; glutamylcysteinyl-β-alanine) conjugate and not the glutathione conjugate. The two initial detoxification metabolites were isolated by high-performance liquid chromatography (HPLC), and identification was based upon mass spectral methods, especially fast atom bombardment mass spectrometry (FAB MS).

INTRODUCTION

Acetochlor (1) is a preemergent herbicide used to control grass weeds and some problem broadleaf weeds in a variety of crops such as corn and soybeans. This paper describes the isolation and identification of the initial metabolites of acetochlor in tolerant corn and soybean seedlings. These metabolites were identified in order to delineate the pathway used by tolerant plants to detoxify this herbicide.

The initial corn metabolite of the chloroacetanilide herbicide propachlor [2-chloro-*N*-(1-methylethyl)-*N*-phenylacetamide] was previously identified as the glutathione conjugate on the basis of chromatographic comparisons with synthetic standards (Lamoureux et al., 1971). It has recently been reported that propachlor (Lamoureux and Rusness, 1981) and a related chloroacetanilide [2-chloro-*N*-(2,3-dimethylphenyl)-*N*-(1-methylethyl)acetamide] are converted to similar conjugates in soybeans (Hussain et al, 1983). One of the objectives of the present study was to isolate the initial acetochlor metabolites and confirm the structural assignments by mass spectrometry. Peptide thiol conjugates have been difficult to analyze by mass spectrometry for several reasons (Hudson, 1976). However, the recent introduction of fast atom bombardment mass spectrometry (FAB MS) has greatly aided the mass spectral identification of these nonvolatile conjugates (Frear et al., 1985). The results of the identification of the initial metabolites of acetochlor in tolerant corn and soybean seedlings are detailed below.

EXPERIMENTAL SECTION

Materials. Isotopically labeled acetochlor [[U-^{14}C]-phenyl; specific act. 9.8 mCi/mmol; ^{13}C at the carbon containing the chlorine] was prepared and purified at Monsanto Co. The radiochemical purity was greater than

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