COMMUNICATIONS

Degradation of *cis*-Permethrin in Soil Amended with Sewage Sludge or Dairy Manure

The degradation of labeled [*carbonyl*-^{14}C]-*cis*-permethrin in sewage sludge and dairy manure amended Matapeake silt loam was investigated in the laboratory. Sewage sludge and dairy manure were applied to soil at rates of 0, 50, and 100 metric tons/hectare (t/ha), leached to remove excess soluble salts, and incubated (30 °C) for 14 days prior to pesticide application. [*carbonyl*-^{14}C]-*cis*-Permethrin was added at 1 ppm, and total CO_2 and $^{14}CO_2$ evolution was monitored regularly throughout a 60-day incubation period (25 °C). ^{14}C-Labeled product distribution was determined by soil extraction and thin-layer chromatographic analysis at the end of the incubation. Incorporation of 50 or 100 t/ha sewage sludge or dairy manure increased permethrin breakdown 87 or 149%, or 64 or 134% above that measured in unamended soil, respectively. The pattern of $^{14}CO_2$ recovery indicated that microbial metabolism accounted for a significant percentage of permethrin breakdown in waste-amended soils after an extensive lag period. The rate of sludge or manure additions appeared to largely control the rate of microbially mediated permethrin degradation but had no apparent effect on chemical mechanisms of breakdown. In waste-amended soils a significant portion of the solvent-extracted ^{14}C was distributed in several relatively polar, unidentified compounds.

The breakdown of several newly introduced pyrethroid insecticides in soil and microbial cultures has been reported in recent publications. In degradation studies of permethrin [(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate], Kaufman et al. (1977, 1978a,b) observed that breakdown was rapid in four of five soils, with the trans isomer degrading more rapidly than the cis isomer. The half-life for cis–trans mixtures was less than 28 days in all but one soil. Microbial metabolism was involved in permethrin degradation but was not essential. The major route of inactivation involved hydrolysis of the ester linkage to form 3-phenoxybenzyl alcohol (PBAl) and 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (DCVA). PBAl was then oxidized to the corresponding carboxylic acid (PBAc). Further breakdown of these products was demonstrated but no characterization of the process was reported. Similar results were reported by Kaneko et al. (1978).

The breakdown of other pyrethroid compounds such as cypermethrin [cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate], WL 41706 [(±)-α-cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate], and fenvalerate [cyano(3-phenoxyphenyl)methyl 4-chloro-α-(1-methylethyl)-benzeneacetate] has been shown to be similar to permethrin (Kaufman et al., 1978b; Roberts and Standen, 1977a,b; Ohkawa et al., 1978). The primary mechanism of both cypermethrin and WL 41706 degradation was hydrolysis of the ester linkage. However, some cypermethrin was hydroxylated to give the α-cyano-3-(4-hydroxyphenoxy)benzyl ester before hydrolysis occurred. Hydrolysis, ether cleavage, hydroxylation, and ester cleavage were all involved in the degradation of fenvalerate in soil.

The influence of organic materials on synthetic pyrethroid insecticide degradation in soil has not been studied. This investigation examined the effects of sewage sludge and dairy manure additions on the breakdown of *cis*-permethrin.

MATERIALS AND METHODS

Soil, Sludge, and Manure Preparation. Matapeake silt loam, a Typic Hapludults, with a pH of 5.3 and organic matter, sand, silt, and clay contents of 1.5, 38.4, 49.4, and 12.2%, respectively, was partially air-dried (~10% moisture) and sieved (2 mm). Sewage sludge (60% primary and 40% secondary), from the Blue Plains Waste Treatment Plant of Washington, DC, was lyophilized and ground to a fine powder (0.85 mm) in a Wiley mill. Dairy manure, from the University of Maryland Dairy Forage Research Farm, was processed by the same procedures. The manure contained no straw or other bedding material.

Before pesticide application, the soil was amended with $CaCO_3$, moistened, and incubated at 30 °C for 14 days to allow pH equilibration at 6.1. Manure or sludge was mixed into the soil to establish application rates of 0, 50, and 100 t/ha. The soils were leached with distilled water to remove excess soluble salts until the conductivity of the leachate was ≤2 mmho and then incubated moist for an additional 14 days at 30 °C.

Soil Treatment and Incubation. [*carbonyl*-^{14}C]-*cis*-Permethrin was applied at 1 ppm to 40 g (dry weight) of soil in 0.1 mL of chloroform. Permethrin-treated soils were thoroughly mixed in the incubation flasks after solvent evaporation, watered to 26.25% moisture content, and attached to a flow-through incubation unit. All treatments were run in duplicate. Pesticide-treated soils were incubated in the dark at 25 °C for 60 days. Each sample was aerated with CO_2-free air. Carbon dioxide evolved from the incubated soils was trapped in 0.25 N NaOH. CO_2 traps were replaced every 2 days and subsampled for liquid scintillation counting of $^{14}CO_2$. Portions of the remaining trapping solution were titrated to determine total CO_2 evolved (Stotzky, 1965).

Soil Extraction and Product Identification. After incubation, the soil was extracted twice by shaking for 2 h with methanol–chloroform (3:1), followed by shaking for 15 min with methanol alone. The extracts were combined,

Table I. ^{14}C Recovered from [*carbonyl*-^{14}C]-*cis*-Permethrin-Treated Soil Amended with Dairy Manure or Sewage Sludge

soil amendment, t/ha	% ^{14}C recovered in						
			residual				
	$^{14}CO_2$	solvent extract	fulvic acid	humic acid	humin	total residual	total
unamended	0.1d[a]	86.0a	1.0c	0.0b	0.5d	1.6c	87.8
sludge, 50	3.8b	72.5b	12.7a	0.4a	2.1c	14.6a	90.9
sludge, 100	5.8a	65.6b	14.4a	0.5a	2.7a	17.0a	88.4
manure, 50	1.9c	74.6b	8.0b	0.5a	1.9c	9.9b	86.4
manure, 100	5.7a	64.8b	14.3a	0.9a	2.4b	16.8a	87.3

[a] All values are geometric means. Column values for each parameter not followed by the same letter are significantly different at the 5% probability level, as evaluated by the Student–Newman–Kuel multiple range test.

counted for radioactivity, and reduced in volume to 0.5 mL on a flash evaporator. This concentrate was spotted on silica gel chromatographic plates (0.25 mm, silica gel 60 F-254, E. M. Laboratories), which were developed in two directions according to Kaufman et al. (1977). One-dimensional thin-layer chromatography (TLC) plates were also used to identify relatively polar products in the extract by using the method described Gaughan et al. (1977). Autoradiographs were used to locate radioactive spots. Products were identified by comparing R_f values with authentic standards. The ^{14}C-containing products were quantified by scraping spots from the TLC plate directly into counting cocktail for liquid scintillation counting.

After solvent extraction, soils were freeze-dried and finely ground. A subsample was combusted (Packard Tri-Carb oxidizer) to determine the total residual ^{14}C. Organic matter fractionation was performed by extracting the soil with 0.5 N NaOH for 12 h. Fulvic acid was separated from humic acid by acidification of the extract with HCl. The radioactivity remaining in the alkali-extracted soil was determined by combustion and was assumed to be the ^{14}C incorporated into humin.

All the ^{14}C data, as well as the total CO_2 data, lacked homogeneity of variance. Although variances did not vary as a function of means, the log transformation most consistently reduced the nonhomogeneity and thus all data were analyzed as log (DATA + 1). Analyses of variance were run by the GLM procedure of Statistical Analysis System programs (SAS Institute, Inc., Raleigh, NC) utilizing the type III method for sums of squares. Where statistical significance (5% level of probability) was indicated, the Student–Newman–Kuel multiple range test was used to compare means. Means reported herein are geometric means. Comparison of $^{14}CO_2$ recovery vs. time plots was performed on nontransformed data by the GLM procedure.

RESULTS AND DISCUSSION

Recovery of ^{14}C as CO_2 from soil treated with carbonyl-labeled *cis*-permethrin suggested that additions of either sewage sludge or dairy manure increased the rate of pesticide breakdown. Cumulative $^{14}CO_2$ recoveries from unamended soil were minimal during the 60-day incubation (Figure 1). However, in the waste-amended soils, a lag period of 28–38 days, during which essentially no ^{14}C was evolved as CO_2, was followed by a rapid increase to a constant rate of $^{14}CO_2$ evolution. Soil amended with 100 t/ha of either dairy manure or sewage sludge had the highest rates of $^{14}CO_2$ evolution, 0.22 and 0.21% ^{14}C/day, respectively. The $^{14}CO_2$ production rates from the 50 t/ha sludge and manure treatments were successively lower, 0.15 and 0.11% ^{14}C/day, respectively.

Recovery of ^{14}C carbon in the solvent extract and in the bound residue reflected the treatment effects observed in the $^{14}CO_2$ values (Table I). The unamended soil where permethrin degradation was slowest, as indicated by $^{14}CO_2$ recoveries, had the highest ^{14}C recovery in the extract and the lowest ^{14}C recoveries in the residue. Residual ^{14}C values over all treatments were proportional to $^{14}CO_2$ recoveries (R = 0.931), whereas the solvent-extracted ^{14}C varied inversely with $^{14}CO_2$ (R = 0.921).

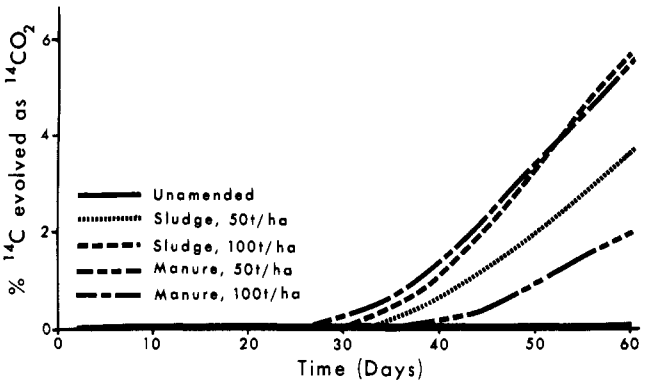

Figure 1. The cumulative percent ^{14}C recovered as $^{14}CO_2$ from [*carbonyl*-^{14}C]-*cis*-permethrin in soil amended with dairy manure or sewage sludge.

Table II. Distribution of Solvent-Extracted ^{14}C in Nonpolar (A) to Polar (E) Products

soil amendment, t/ha	% of applied ^{14}C recovered in zone[a]				
	A	B	C	D	E
unamended	0.4	81.3	0.8	1.6	0.5
sludge, 50	0.1	65.0	0.9	2.1	8.2
sludge, 100	0.1	53.5	0.5	1.9	15.4
manure, 50	0.0	69.4	1.2	1.9	4.0
manure, 100	0.0	56.2	0.7	1.8	11.5

[a] Columns A-E represent TLC zones of metabolites from least to most polar (Kaufman, 1977, 1978b). Zone B contains both cis and trans isomers of the parent permethrin. Zone D is primarily comprised of the DCVA.

Incorporation of ^{14}C into the soil organic matter was also proportional to $^{14}CO_2$ recovery. Most of the residual ^{14}C was in the fulvic acid fraction (80.7%), whereas humic acid and humin accounted for only 3.8 and 15.5% of the soil-bound radioactivity, respectively. The recovery of ^{14}C in fulvic acid was directly proportional to $^{14}CO_2$ evolution (R = 0.961).

The increased rates of permethrin degradation attributed to waste amendments were confirmed by the analysis of the soil extracts (Table II). Additions of 50 and 100 t/ha sludge and manure increased permethrin degradation 87 and 149% and 64 and 134% above that in unamended soil, respectively. The percent ^{14}C recovered as the parent compound was inversely proportional to the rate of $^{14}CO_2$ evolution (R = 0.991). Conversion from the cis to the trans isomer was minimal but did occur. Greater than 98% of the recovered permethrin was in the cis configuration. Recovery of known metabolites was restricted by the position of the ^{14}C label to the parent compound and to

Table III. Total Cumulative CO_2 Evolved from Control and *cis*-Permethrin-Treated Soil Amended with Dairy Manure or Sewage Sludge

soil amendment, t/ha	carbon evolved as CO_2, mg	
	control	FMC-35171
unamended	10.0f[a]	12.8f
sludge, 50	34.5e	54.0d
sludge, 100	89.2cd	104.2bc
manure, 50	98.1bc	63.8d
manure, 100	161.8a	142.2ab

[a] All values are geometric means. Values not followed by the same letter are significantly different at the 5% probability level, as evaluated by the Student-Newman-Kuel multiple range test.

3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic (DCVA). Degradation products with polarity (as determined by position on the plates) between that of DCVA and that of the parent molecule did not vary significantly. Metabolites with polarities lower than permethrin contained from 0.0 to 0.4% of the total ^{14}C. The DCVA levels were low and did not vary appreciably with waste treatments. Apparently this product was degraded or altered as quickly as it was produced. However, products more polar than DCVA were significantly increased by the organic waste amendments.

In the unamended soil, permethrin could have been degraded by a combination of biological and chemical mechanisms. However, the low recovery of $^{14}CO_2$ from this treatment was similar to that reported by Kaufman et al. (1977) for soil sterilized with NaN_3, suggesting that permethrin breakdown in the unamended soil could have been the consequence of chemical action. The insignificant amount of ^{14}C incorporated into the soil organic matter of unamended soil is further evidence that permethrin degradation may not have been associated with biological mechanisms.

In waste-amended soils, the increased evolution of $^{14}CO_2$ after an extensive lag period indicated that microbial activity stimulated by the sludge and manure treatments was primarily responsible for the more rapid permethrin breakdown in these soils. The native microbial populations apparently lacked the necessary numbers or types of organisms or enzymes for permethrin metabolism and more than 28 days was required for the development of an enriched population. The final rate of $^{14}CO_2$ evolution correlated to the total microbial activity, as measured by total CO_2 production (R = 0.903) (Table III). However, the length of the lag period before $^{14}CO_2$ evolution started was not influenced by total microbial activity. It could be hypothesized that the type and amount of organic amendment determined the size of the microbial population involved in permethrin breakdown, although the time needed for the microbial population to become capable of permethrin metabolism was independent of waste treatments.

As previously noted, a substantial percentage of the ^{14}C was recovered as polar products (category E, Table II) in the solvent extract of amended soil. We believe that these products ultimately resulted from the microbial breakdown of DCVA. Only trace quantities of polar products were extracted from unamended soil, where microbial breakdown of permethrin was minimal. Preliminary attempts to identify these products were accomplished by comparing R_f values from TLC with the R_f values for permethrin metabolites isolated from rats (Gaughan et al., 1977). Although minor quantities of both cis and trans isomers of 3-(2,2-dichlorovinyl)-2-hydroxy-2-methylcyclopropanecarboxylic acid were tentatively identified, most of the ^{14}C associated with the polar products was distributed among several other unidentified compounds.

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Richard C. Doyle[1]
Donald D. Kaufman*[2]
Gordon W. Burt[3]
Larry Douglass[4]

[1]Agronomy Department
University of Maryland
College Park, Maryland 20142
Present address: Atlantic Research Corp.
Alexandria, Virginia 22314
[2]Beltsville Agriculture Research Center—West
Agriculture Research Service
U.S. Department of Agriculture
Beltsville, Maryland 20705
[3]Agronomy Department
University of Maryland
College Park, Maryland 20142
[4]Animal Science Department
University of Maryland
College Park, Maryland 20142

Received for review May 5, 1980. Accepted October 17, 1980. Computer time for data processing was funded by the University of Maryland, Computer Science Center, and the Washington Computer Center.