Methylcarbamates Affect Acylanilide Herbicide Residues in Soil

Simultaneous application of *p*-chlorophenyl methylcarbamate (PCMC) with 3′,4′-dichloropropionanilide (propanil) to soil retards propanil degradation in soil and greatly reduces the formation of 3,3′,4,4′-tetrachloroazobenzene (TCAB). An explanation for this phenomenon was found in enzymatic studies, conducted with a partially purified enzyme isolated from a propanil hydrolyzing soil fungus. Enzymatic hydrolysis of propanil to propionic acid and 3,4-dichloroaniline (DCA) was strongly inhibited by PCMC and 1-naphthyl methylcarbamate (carbaryl), but only weakly inhibited by 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (carbofuran). PCMC did not affect the conversion of DCA to TCAB in soil.

Controlled biodegradation and residue formation of pesticides could be an important feature of pesticide usage and formulation. This report describes the effect of 1-naphthyl methylcarbamate (carbaryl) and *p*-chlorophenyl methylcarbamate (PCMC) on the biodegradation and residue formation of the herbicide 3′,4′-dichloropropionanilide (propanil). Propanil is registered for postemergence application in rice at rates not to exceed 8 lb per acre per season. Previous reports have demonstrated that propanil is microbially degraded to propionic acid and 3,4-dichloroaniline (DCA) (Bartha *et al.*, 1967), two molecules of the DCA then being condensed to 3,3′4,4′-tetrachloroazobenzene (TCAB). The potential hazard of TCAB residues accumulating in soil was questioned (Bartha and Pramer, 1967).

We recently reported that several methylcarbamate insecticides inhibit the biodegradation of the phenylcarbamate herbicide isopropyl *m*-chlorocarbanilate (chlorpropham) in soil (Kaufman *et al.*, 1970). Two- to four-fold increases in soil persistence of chlorpropham were observed when chlorpropham was applied in combination with certain methylcarbamates. Practical application of this phenomenon has proven useful for a more prolonged chlorpropham control of dodder in alfalfa with fewer applications. The addition of PCMC to chlorpropham formulations doubled the period of dodder control by single applications of chlorpropham under greenhouse conditions (Dawson, 1969).

Detailed enzyme studies with a chlorpropham hydrolyzing enzyme isolated from a *Pseudomonas striata* Chester indicate that methylcarbamates lacking steric hindrance of the carbamate linkage are competitive inhibitors of the chlorpropham hydrolyzing enzyme (Kaufman *et al.*, 1970). Chlorpropham is degraded by soil microorganisms to isopropyl alcohol, CO_2, and *m*-chloroaniline. *m*-Chloroaniline is further degraded by ring cleavage with the ultimate evolution of CO_2 from the *m*-chloroaniline ring. The methylcarbamate inhibition of the phenylcarbamate hydrolyzing enzyme is not surprising, since the ester and amidase-like activity of the enzyme has been recognized (Kearney and Kaufman, 1965), and the anticholinesterase activity of some methylcarbamates is well known (Metcalf and Fukuto, 1965).

Since our initial observation of this phenomenon in soil, we have examined its application to other pesticides (Kaufman and Miller, 1970). Preliminary observations on the effect of carbaryl, PCMC, *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl)phosphorothioate (diazinon), and *O,O*-diethyl *S*-(ethylthio)methylphosphorodithioate (phorate) on certain amide, carbamate, and urea herbicides have been made (Kaufman and Miller, 1970). PCMC, as well as carbaryl, inhibited microbial degradation of chlorpropham and increased its persistence in soil under greenhouse conditions. PCMC also inhibited the biodegradation of propanil in microbial culture systems. The possible role of such interactions in affecting the formation of complex pesticide residues in soil prompted us to investigate the effect of PCMC on the biodegradation of propanil in soil.

EXPERIMENTAL

Duplicate 50-g samples of Hagerstown silty clay loam soil were prepared with each of the following treatments: (1) no treatment; (2) propanil, 5 mg; (3) propanil, 50 mg; (4) propanil, 5 mg and PCMC, 0.25 mg; (5) propanil, 50 mg and PCMC, 0.25 mg; (6) propanil, 5 mg and PCMC, 2.5 mg; (7) propanil, 50 mg and PCMC, 2.5 mg; (8) PCMC, 2.5 mg; (9) 3,4-dichloroaniline (DCA), 50 mg; and (10) PCMC, 2.5 mg and DCA, 50 mg. Propanil and DCA were added directly into each soil sample, whereas PCMC was applied in 0.1 ml of acetone from appropriate stock solutions. All samples not receiving PCMC were also treated with 0.1 ml of acetone. The acetone was allowed to evaporate before thoroughly mixing the chemicals into the soil. All soil samples were moistened to 70% of their water holding capacity and incubated at 26° C in biometer flasks. The soil samples were aerated daily for the first week and then every other day for the remainder of the 30-day incubation period.

At the conclusion of the incubation period, all soil samples were extracted and analyzed by glc, tlc, and mass spectrometry. Each soil was extracted for 2 min with 100 ml of spectrometric grade acetone on a Waring Blendor. The extract was filtered and the acetone evaporated in a rotary flash evaporator. The remaining water was washed with benzene, the water discarded, and the benzene reduced to a volume suitable for analysis. The glc column conditions were identical to those described by Chisaka and Kearney (1970). Mass spectral analysis of the products was performed with a Perkin-Elmer Model 270 combination gas chromatograph-mass spectrometer.

A *Fusarium* sp. capable of degrading propanil was mass cultured in a fermentor on media containing 0.2 g of K_2HPO_4, 0.3 g of NH_4NO_3, 0.2 g of $CaSO_4$, 0.2 g of $MgSO_4 \cdot 7H_2O$, 1 mg of $FeSO_4 \cdot 7H_2O$, 1.0 g of yeast extract, 2.0 g of sucrose,

25 mg of chloromycetin, 25 mg of streptomycin sulfate, and 50 of propanil per l. of distilled H_2O. Mycelia were harvested by filtration (Whatman paper No. 1), washed with distilled H_2O, frozen, lyophilized, and stored under desiccation at −5° C. Lyophilized material prepared and stored in this manner retained its enzyme activity for at least 1 year without any significant loss of activity. Before use, the lyophilized material was resuspended in buffer, centrifuged, and the supernatant examined for activity. Partial purification was accomplished by $(NH_4)_2SO_4$ (50%) precipitation from the supernatant. The active material was collected by centrifugation and resuspension of the precipitate in buffer. Chemicals examined as possible enzyme inhibitors included PCMC, carbaryl, and 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (carbofuran).

RESULTS AND DISCUSSION

Propanil, DCA, and TCAB were detected in all soil samples treated with either propanil or propanil and PCMC (Table I). The molar percentage of propanil converted to TCAB decreased with increasing PCMC concentration. The amount of propanil (actual) recovered from the soil appeared directly related to PCMC concentration, whereas the amount of TCAB recovered from the soil was inversely related to PCMC concentration. These effects were observed in soils at both low and high propanil concentrations. An inverse relationship between the DCA and PCMC concentrations was also apparent in soils treated with 5 mg of propanil, but not in soil treated with 50 mg of propanil.

PCMC did not have any effect on TCAB formation in soil originally treated with DCA. The fact that PCMC does not significantly affect the conversion of DCA to TCAB in soils suggests that some other mechanism of inhibition is functional in reducing the formation of TCAB from propanil in soil. Kearney *et al.* (1969) demonstrated that the condensation of DCA to TCAB increased logarithmically as the initial DCA concentration is soil increased. Their data indicate that the initial DCA concentration present in soil may ultimately affect the amount of TCAB residues formed. Propanil is rapidly degraded to DCA and propionic acid in soil (Bartha *et al.*, 1967; Bartha and Pramer, 1967; Chisaka and Kearney, 1970; Kearney *et al.*, 1969). Rapid degradation of large propanil applications would result in relatively high initial concentrations of DCA. Partial inhibition of the degradative mechanism would result in only low concentrations of aniline present in the soil at any given time, or at least a slower release of DCA into the soil environment. A short term experiment using soil treatments 1, 2, and 4 was set up and incubated for 4 hr to test this hypothesis. At this time the samples were extracted and analyzed as before. Approximately 100% of the propanil could be accounted for in this experiment. Analysis of these samples revealed that 78.1% of the propanil in soils receiving propanil only had been hydrolyzed to DCA, whereas in soil receiving both propanil and PCMC, only 4.3% of the propanil had been hydrolyzed to DCA. These results demonstrate that PCMC does inhibit the rapid hydrolysis of propanil to DCA.

We demonstrated earlier that the biodegradation of propanil is inhibited by PCMC in pure cultures systems (Kaufman and Miller, 1970). An attempt was made in this investigation to determine whether or not the mechanism of this interaction is similar to the chlorphropham-carbaryl interaction described earlier (Kaufman *et al.*, 1970). Both PCMC and carbaryl proved to be strong inhibitors of the propanil hydrolyzing enzyme (Table II). Carbofuran, a soil insecticide under current investigation for use in rice soil, was not a strong inhibitor of this enzyme system. The site of this inhibition thus appears to be similar to that which we observed earlier with carbaryl-chlorpropham interactions (Kaufman *et al.*, 1970) and to what other investigators have observed with other propanil hydrolyzing enzymes (Frear and Still, 1968).

Table I. Effect of PCMC on Propanil Degradation and Residue Formation in Soil

Propanil applied	PCMC applied	Residue extracted			Propanil converted to TCAB
		Propanil	3,4-DCA	TCAB	
mg	mg	μg	μg	μg	%
5	0.00	66.3	418.5	438.4	11.8
5	0.25	914.5	361.3	130.3	3.5
5	2.50	2105.0	296.5	32.1	0.9
mg	mg	mg	mg	mg	%
50	0.00	0.3	0.9	23.9	64.3
50	0.25	12.5	2.3	14.1	38.0
50	2.50	21.6	1.0	0.1	0.3

Table II. Effect of Several Methylcarbamates on Inhibition of a Propanil Hydrolyzing Enzyme[a]

Inhibitor	% Inhibition
None	0
p-Chlorophenyl methylcarbamate (PCMC)	100
1-Naphthyl methylcarbamate (carbaryl)	90
2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (carbofuran)	4

[a] Enzyme inhibitor tests were performed in 25 ml Erlenmeyer flasks containing 1 μM propanil and 1 μM inhibitor in 2.9 ml 0.1*N* glycine buffer (pH 10), and 0.1 ml enzyme preparation. The reaction was terminated by the addition of the reagents necessary for DCA determination (Pease, 1962).

The practical applications and problems of these data are multifold. The potential of this and similar combinations for controlled pesticide biodegradation and residue formation is very real. The ultimate fate and nature of alternative residues must be determined, however. Whether or not the TCAB residues in PCMC-propanil treated soils would eventually reach levels similar to those in propanil treated soils is not known. The fate of DCA is not thoroughly understood. DCA appears to be somewhat recalcitrant to biodegradation (Bartha *et al.*, 1967; Bartha and Pramer, 1967). Recent work of Linke and Bartha (1970) indicate that DCA may be chemically attached to the soil organic matter. High propanil concentrations favored the formation of solvent extractable metabolites such as TCAB, whereas low ones favored the formation of humic complexes. A more regulated release of DCA during the biodegradation of propanil may favor formation of DCA humic complexes and result in the reduced TCAB formation such as we have observed in our investigation here. The significance of DCA-humic complexes, however, might ultimately require additional investigation.

Whether or not propanil-PCMC combinations could be safely used on crop plants is not known. Both organophosphate and certain methylcarbamate insecticides enhance the herbicidal activity of propanil on rice. Frear and Still (1968) have demonstrated that a partially purified propanil hydrolyzing enzyme from rice was inhibited by several methylcarbamate insecticides. It is conceivable, however, that the acylamidase activity of some crop plants is sufficiently high

to permit limited inhibition with low levels of PCMC sufficient for regulating propanil degradation in soil. Although many of the above problems remain to be answered, the results of this investigation indicate that it is feasible to control pesticide biodegradation and residue formation in soil.

LITERATURE CITED

Bartha, R., Lanzilotta, R. P., Pramer, D., *Appl. Microbiol.* **15,** 67 (1967).
Bartha, R., Pramer, D., *Science* **156,** 1617 (1967).
Chisaka, H., Kearney, P. C., J. AGR. FOOD CHEM. **18,** 5 (1970).
Dawson, J. H., *Weed Sci.* **17,** 295 (1969).
Frear, D. S., Still, G. G., *Phytochemistry* **7,** 913 (1968).
Kaufman, D. D., Kearney, P. C., Von Endt, D. W., Miller, D. E., J. AGR. FOOD CHEM. **18,** 513 (1970).
Kaufman, D. D., Miller, D. E., *WSSA Abstracts*, No. **158** (1970).
Kearney, P. C., J. AGR. FOOD CHEM. **13,** 561 (1965).
Kearney, P. C., Kaufman, D. D., *Science* **147,** 740 (1965).
Kearney, P. C., Plimmer, J. R., Guardia, F. B., *Amer. Chem. Soc. Mtg. Abstr.* **No. 31** (1969).
Linke, H. A. B., Bartha, R., *Bacteriol. Proc.* **70,** 9 (1970).
Metcalf, R. L., Fukuto, T. R., J. AGR. FOOD CHEM. **13,** 220 (1965).
Pease, H. L., J. AGR. FOOD CHEM. **10,** 279 (1962).

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