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## Fate of Alachlor and Propachlor in a Model Ecosystem

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Ring-<sup>14</sup>C-labeled alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide] and propachlor (2-chloro-N-isopropylacetanilide) were studied in a model ecosystem designed by R. L. Metcalf and associates. These two herbicides were transformed into many compounds in water over the experimental period of 33 days. According to thin-layer chromatographic and radioautographic analyses, at least eight alachlor degradation products and seven propachlor degradation products were

found in the water. Alachlor and propachlor constituted only 1.8 and 0.4%, respectively, of the radioactivity in the water at the end of the experiment. Thus, it appears that these two herbicides are labile under an aquatic environment. No parent compounds were detected in the organisms and there was no evidence to indicate that their metabolites or degradation products were magnified in the food chain.

Alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide] and propachlor (2-chloro-N-isopropylacetanilide) are widely used for weed control in corn, soybeans, and many other crops. The mode of action of these  $\alpha$ -chloroacetamides was described by Jaworski (1969). Inhibition of protein synthesis is believed to be the general locus of interaction, although multiple sites of action are also indicated. Metabolism of propachlor in corn, sorghum, sugar cane, and barley has been reported by Lamoureux et al. (1971). At least three water-soluble metabolites are produced in each species during the first 6 to 24 hr following treatment. Compounds I and II were identified as the glutathione conjugated and  $\gamma$ -glutamylcysteine conjugated propachlor, respectively. Compounds I and II in corn seedlings were shown to be transitory metabolites, and they were not detected 72 hr following treatment.

At the present time, no data are available concerning these two compounds in the food chain. Recently a model ecosystem for the evaluation of pesticide biodegradability and food chain magnification has been developed (Metcalf et al., 1971). The model ecosystem has been used to evaluate several pesticides (Yu et al., 1974a,b, 1975; Sanborn and

Yu, 1973; Booth et al., 1973; Isensee et al., 1973). Based on our past experience, good reproducibility was obtained with this model ecosystem. This paper reports the study of alachlor and propachlor in this model ecosystem and is part of our continuing effort to examine effects of pesticides in the environment.

### MATERIALS AND METHODS

**Labeled Compounds.** Ring-<sup>14</sup>C-labeled alachlor (specific activity 1.0 mCi/mmol) and propachlor (specific activity 4.0 mCi/mmol) were provided by Monsanto Co., St. Louis, Mo. Radiochemical purities were determined to be 99% by thin-layer chromatography and radioautography.

**Model Ecosystem.** The procedures described by Metcalf et al. (1971) were followed with some modification (Yu et al., 1974a). Fifty microcuries of ring-<sup>14</sup>C-labeled alachlor (13.5 mg) or propachlor (2.65 mg) was applied to the base of the 7-day-old sorghum plants in the aquarium. These dosages approximated 2.7 and 0.5 lb/acre, respectively, for alachlor and propachlor based on the land area of the model ecosystem. Experiments were carried out in one aquarium for each compound.

**Sample Preparation.** The procedures, which have been described previously (Yu et al., 1974a), are essentially an acetone extraction for organisms and an ether extraction for water.

**Thin-Layer Chromatography (TLC).** Solvent extracts from water and organisms were analyzed on a silica gel F-254 aluminum plate (0.25 mm thickness, E. Merck AG.) developed in a solvent consisting of methanol-benzene (5:95, v/v).

### RESULTS AND DISCUSSION

Radioactivity in the water was monitored through the

Illinois Natural History Survey and Illinois Agricultural Experiment Station, Urbana, Illinois 61801.

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**Table I. Concentration (Parts per Million) of Alachlor, Metabolites, and Degradation Products in the Unhydrolyzed and Hydrolyzed Water**

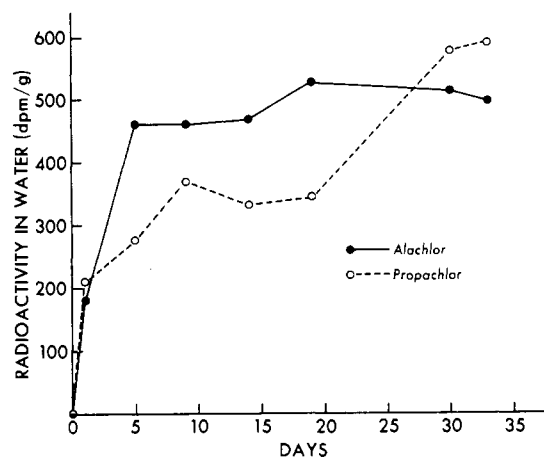
	UHy-H <sub>2</sub> O	Hy-H <sub>2</sub> O	Total (%)
Unknown I (0.70) <sup>a</sup>	0.00027	0.00078	0.00105 (1.8)
Alachlor (0.61)	0.00105	0.0	0.00105 (1.8)
Unknown II (0.51)	0.00190	0.00177	0.00367 (6.2)
Unknown III (0.43)	0.01050	0.00413	0.01463 (24.6)
Unknown IV (0.33)	0.00077	0.00165	0.00242 (4.1)
Unknown V (0.20)	0.00083	0.00966	0.01049 (17.6)
Unknown VI (0.13)	0.00172	0.0	0.00172 (2.9)
Unknown VII (0.07)	0.00069	0.00339	0.00408 (6.9)
Unknown VIII (0.00)	0.00036	0.00516	0.00552 (9.3)
Unextractable	0.04142	0.01487	0.01487 (25.0)
Total <sup>14</sup> C			0.05950

<sup>a</sup> *R<sub>f</sub>* value; silica gel F-254; methanol-benzene (5:95); 0.0, not detected.

**Table II. Concentration (Parts per Million) of Propachlor, Metabolites, and Degradation Products in the Unhydrolyzed and Hydrolyzed Water<sup>a</sup>**

	UHy-H <sub>2</sub> O	Hy-H <sub>2</sub> O	Total (%)
Unknown I (0.69)	0.0	0.00007	0.00007 (0.5)
Unknown II (0.62)	0.00006	0.00012	0.00018 (1.3)
Propachlor (0.55)	0.00006	0.0	0.00006 (0.4)
Unknown III (0.40)	0.00126	0.00214	0.00340 (24.7)
Unknown VI (0.27-0.15)	0.00014	0.00031	0.00045 (3.3)
Unknown V (0.10)	0.00008	0.00021	0.00029 (2.1)
Unknown VI (0.03)	0.00002	0.00033	0.00035 (2.5)
Unknown VII (0.00)	0.00004	0.00433	0.00437 (31.8)
Unextractable	0.01209	0.00459	0.00459 (33.4)
Total <sup>14</sup> C			0.01375

<sup>a</sup> See footnote a to Table I.

**Figure 1. Radioactivity in water monitored through the experimental period (33 days).**

33-day experimental period (Figure 1). Radioactivity increased steadily during the first 5-10 days and then leveled off. Radioactivity in the water did not decrease or only decreased slightly at the end of the experiment.

Analyses of water at the end of the experiment were presented in Tables I and II. Ether extracts of the unhydrolyzed water consisted of only 1.8 and 0.4% of parent alachlor and propachlor, respectively. Upon acid hydrolysis (0.025 N HCl at 70°, 20 hr), no further release of the parent compounds was detected. The unextractable radioactivity after acid hydrolysis consisted of 25 and 33% of the total radioactivity for alachlor and propachlor, respectively. The ether extractable radioactivity could be separated by TLC and detected by radioautography. There were at least eight degradation products for alachlor and seven degradation products for propachlor.

None of the parent compounds or degradation products could be detected in the organisms except that snails con-

**Table III. Concentration (Parts per Million) of Alachlor, Metabolites, and Degradation Products in the Organisms**

	Organisms						
	Algae	Crab	Daphnia	Elodea	Fish	Mosquito	Snail
Alachlor (0.61) <sup>a</sup>		0.0	0.0	0.0			0.0
Unknown I (0.27)		0.0	0.0	0.0			0.658
Unknown II (0.00)		0.321	0.0	1.767			0.185
Unextractable	0.569	0.524	0.422	2.961	0.106	0.244	0.544
Total <sup>14</sup> C	0.659	0.845	0.422	4.728	0.232	0.289	1.387

<sup>a</sup> *R<sub>f</sub>* value; silica gel F-254; methanol-benzene (5:95). No value indicates the extract was not chromatographed because it was less than 500 cpm; 0.0, not detected.

**Table IV. Concentration (Parts per Million) of Propachlor, Metabolites, and Degradation Products in the Organisms<sup>a</sup>**

	Organisms						
	Algae	Clam	Daphnia	Elodea	Fish	Mosquito	Snail
Propachlor (0.55)							0.0
Unknown I (0.30)							0.015
Unknown II (0.00)							0.060
Unextractable	0.186	0.009	0.048	0.087	0.009	0.134	0.177
Total <sup>14</sup> C	0.207	0.015	0.057	0.089	0.015	0.160	0.252

<sup>a</sup> See footnote a to Table III.

tained an unknown ( $R_f$  0.27) in the alachlor experiment and an unknown ( $R_f$  0.30) in the propachlor experiment (Tables III and IV). According to Lamoureux et al. (1971) and Sharp (1972), these herbicides in the plants were rapidly conjugated with nature products as transitory metabolites. Two of them have been identified as conjugates of glutathion and  $\gamma$ -glutamylcystein. Since model metabolites were not available at the time and these metabolites and degradation products did not accumulate in the food-chain organisms (Tables III and IV), no attempt was made to identify them.

Total  $^{14}\text{C}$  was higher in organisms than in water. For example, snail accumulated 24 times more alachlor metabolites than did the water. We do not know whether this constitutes an environmentally acceptable level, but we do know that the accumulation is much less than that of organochlorine pesticides such as DDT which is concentrated over several thousand times more in organisms than in water (Metcalf et al., 1971).

In summary, alachlor and propachlor were rapidly degraded in the water. There was no evidence to indicate that these two herbicides and their degradation products were magnified in the food chain. For example, the total radioactivity in a food chain from algae  $\rightarrow$  mosquito  $\rightarrow$  fish decreased from 0.66 to 0.23 ppm in alachlor, and from 0.21 to 0.015 ppm in propachlor.

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## Polybrominated Biphenyls: Tissue Distribution and Effect on Hepatic Microsomal Enzymes in Japanese Quail

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Japanese quail were fed polybrominated biphenyls (PBB) at 0, 10, 20, 100, or 1000 ppm in a semi-purified diet for 9 weeks. They would not accept the diet containing 1000 ppm. Although feed intake and growth were similar in quail fed 0-100 ppm of PBB, egg production was reduced with none of the eggs hatching from quail hens fed 100 ppm of PBB. Tissue residues of PBB were generally higher in males than females. The liver micro-

somal enzymes, aniline hydroxylase, aminopyrine *N*-demethylase, *N*-methylaniline *N*-demethylase, and *p*-nitroanisole *O*-demethylase were all induced by dietary PBB. Male quail had the highest induction at 20 ppm of PBB, while in female quail peak enzyme induction occurred at 100 ppm of PBB. Post-mortem examinations showed no gross or microscopic lesions in the birds fed up to 100 ppm of PBB.

Polybrominated biphenyls (PBB) have been estimated to have recently contaminated about one million chickens with tissue residues in excess of 1 ppm and egg residues of approximately 0.3 ppm (*Food Chem. News*, 1974). These contaminated flocks have been detected in Michigan as well as other states and are of growing concern to state and federal regulatory and health agencies. The PBB are probably not now present in the ecosystem in as large amounts as polychlorinated biphenyls (PCB) (Peakall and Lincer, 1970) since they have been manufactured as textile flame retardants only since 1970, but their potential toxicity may be greater than PCB (*Food Chem. News*, 1974).

This investigation was undertaken to study the tissue and egg distribution and excretion of PBB and the effect on reproductive performance and hepatic microsomal enzyme induction in Japanese quail.

#### EXPERIMENTAL SECTION

**Animals and Diet.** Japanese quail, 1-day old, obtained from the Department of Poultry Science, Cornell University, were divided into 5 groups, 24 unsexed quail per group within a Petersime brood unit. They were fed ad libitum a semi-purified isolated soybean protein-cornstarch diet (Stoewsand and Robinson, 1970). At 3 weeks of age, calcium and phosphorus were increased in the diet from 1.3 to 3.4% and 0.8 to 1.3%, respectively. PBB (containing about 75% hexabromobiphenyl) was dissolved in corn oil and added to each diet at 0, 10, 20, 100, or 1000 ppm and fed for an additional 9 weeks.

**Analytical Procedure.** Analytical procedures for the

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