

group as with mouse microsomes (Soderlund and Casida, 1977b).

There do not appear to be any fundamental qualitative differences in permethrin metabolism between carp liver microsomes and those of rainbow trout although the extent of metabolism was greater with carp. This may mean that carp liver microsomes are more active than rainbow trout liver microsomes; however, no attempt was made in these studies to accurately compare the kinetics of liver microsomal metabolism by these two species of fish.

Fish metabolize a variety of foreign compounds (Dewaide, 1971) and may excrete the metabolites in the bile as conjugates (Lech, 1973). Rainbow trout metabolize permethrin *in vivo* since the bile of trout exposed to *trans*-[<sup>14</sup>C-alcohol]permethrin contains polar metabolites which are probably conjugates(s). The majority of the conjugate fraction is cleaved on treatment with  $\beta$ -glucuronidase, suggesting that most of the polar material is a glucuronide conjugate. This study, establishing that permethrin undergoes *in vitro* and *in vivo* metabolism in fish, lays the background for more detailed investigations as to the identity of metabolites in the bile and the pharmacokinetics of permethrin uptake, metabolism, and excretion.

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## Toxicity and Fate of Nine Toxaphene Fractions in an Aquatic Model Ecosystem

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The acute toxicity (96 h LC<sub>50</sub>) of nine toxaphene fractions to bluegill fish (*Lepomis macrochirus*) varied from 2.6 to 29.0  $\mu\text{g/L}$  (5.0 to 7.8  $\mu\text{g/L}$  for unfractionated toxaphene). Bluegill fish, snails (*Helisoma* sp.), daphnids (*Daphnia magna*), and algae (*Oedogonium cardiacum*) were exposed to two water concentrations of unfractionated toxaphene and three of its fractions (representative of different toxicities) for 1–32 days in aquatic model ecosystems. Only slight differences were observed between the three fractions and unfractionated toxaphene in total amounts accumulated by each species. Bioaccumulation potential for bluegill fish was much higher than for snails (average of 6000 vs. 480 times water content, respectively). Thin-layer chromatographic analysis of fish and snail extracts taken after 3, 15, and 32 days of exposure indicated considerable metabolism by snails of all compounds but little metabolism by fish.

The widely used insecticide, toxaphene, is a complex mixture of at least 177 ten-carbon polychlorinated compounds (Casida et al., 1974). Attempts to fractionate and identify compounds within this mixture have met with limited success. Two compounds that have been identified, commonly referred to as toxicants A and B, are several

times more toxic to mice and houseflies than the toxaphene mixture (Khalifa et al., 1974). The toxaphene mixture is also quite toxic to nontarget organisms, particularly aquatic organisms (Plimmentel, 1971), but little is known about the toxicity of individual compounds in the mixture. Thus, the structure, relative toxicity, and environmental behavior of the compounds in the toxaphene mixture are only beginning to be known. An obvious problem in studying such a complex mixture of compounds is to determine which part of the mixture to investigate first. We therefore initiated this study to determine if different fractions of the toxaphene mixture differed significantly in (1) acute toxicity to fish (test more species only if major differences

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between fractions were found), (2) distribution between components of the ecosystem (soil to water and water to organisms), and (3) extent of degradation in water and organisms. Only toxaphene and fractions 1, 5, and 9 were tested in the ecosystem since these compounds reflected the range of polarities and toxicities (acute toxicity tests were conducted before ecosystem studies). The rest of the fractions would be tested only if major differences between these four compounds were found.

#### MATERIALS AND METHODS

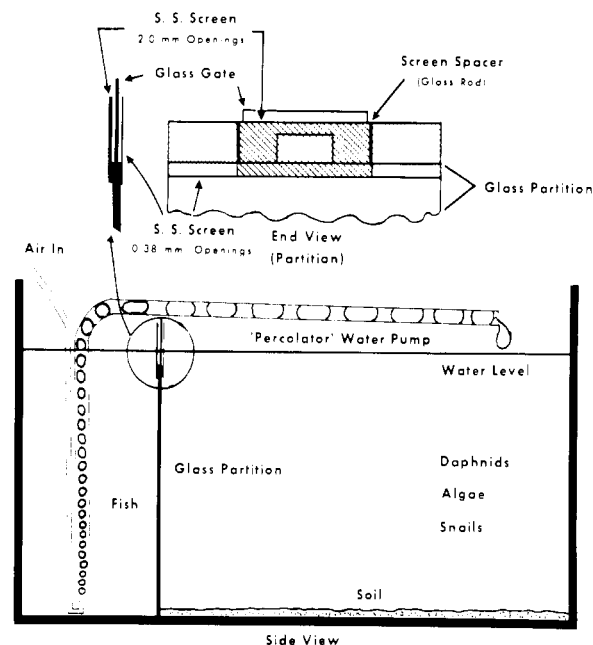
**Radioactive Compounds.** Synthesis of the [ $^{14}\text{C}$ ]toxaphene (6.14  $\mu\text{Ci}/\text{mg}$ ) used in this study has been described (Oliver, 1977). Toxaphene has a water solubility of 3.0 ppm (Gunther, 1968); solubilities of the fractions are not known. The synthesized material was separated into nine fractions of approximately equal radioactivity by dry column chromatography using 120 g of silica gel packed into a column 50 cm long by 2 cm i.d. and developed with cyclohexane. The developed column was divided into nine sections of varying predetermined lengths (so that each section contained comparable amounts of  $^{14}\text{C}$ ), cut with a razor blade, and the cyclohexane was allowed to evaporate from the silica gel. The silica gel portions were then extracted with benzene, and the extracts were filtered into 50-mL volumetric flasks and made up to volume. The nine fractions and toxicant A were spotted separately on silica gel plates (20  $\times$  20 cm GF-254, E. Merck, Darmstadt) and then developed in hexane saturated with formic acid for 16.5 cm, removed, dried, and developed again in the same solvent. The range in  $R_f$  values for fractions 1-9 and toxicant A were 0-0.27, 0.26-0.36, 0.33-0.42, 0.38-0.45, 0.43-0.49, 0.45-0.53, 0.50-0.56, 0.55-0.61, 0.60-0.67, and 0.45-0.50, respectively. Unlabeled toxaphene was synthesized and fractionated by the same procedure used for the [ $^{14}\text{C}$ ]toxaphene. TLC and GC (45.7 m  $\times$  0.51 mm Dexsil 300 SCOT, 125  $\rightarrow$  260  $^{\circ}\text{C}$  at 1  $^{\circ}\text{C}/\text{min}$ , 11 psi at 125  $^{\circ}\text{C}$  No Split, 15 mL/min make up temperature program started 8 min after injection) analysis did not differ between the labeled and unlabeled compounds.

**Acute Toxicity.** Bluegill (*Lepomis macrochirus*) were exposed to toxaphene and each of the nine fractions to determine the acute toxicity of these compounds to fish. A 96-h static jar test was used, as described by McCann and Jasper (1972) and Stephan (1975). Culture and treatment techniques were as follows: Bluegill, obtained from the Harrison Lake National Fish Hatchery, Charles City, VA, averaged 33.7 mm in length and 0.5 g in weight. No fish was larger than twice the length of the smallest fish. They were kept in recirculated, reconstituted soft water (19  $^{\circ}\text{C}$ ) for at least 10 days prior to use where they were acclimated to test conditions and fed a commercial trout chow. During this period they were observed for disease and condition. Less than 3% died during acclimation and holding periods. Only healthy fish were used in the tests.

Reconstituted water was used during the acclimation, holding, and test periods and was formulated by adding 2 mg of KCl, 30 mg of  $\text{MgSO}_4$ , 30 mg of  $\text{CaSO}_4$ , and 48 mg of  $\text{NaHCO}_3/\text{L}$  of demineralized water with resistivity of 1 million ohms (Stephan 1975). The water was continuously recirculated, aerated, and filtered.

The fish were fed a commercial trout chow which was obtained from a supplier whose feed in the past had been pesticide free; however, this particular lot was not specifically evaluated for residue levels.

Before testing, the fish were held in untreated test water for 3 days without food. The tests were conducted at 18  $^{\circ}\text{C}$  in 15 L of water in 19-L wide-mouth glass jars. The



**Figure 1.** Recirculating static model ecosystem. Tank dimensions: 41  $\times$  20  $\times$  24 cm; glass partition 18 cm high. Tank volume 16 L with a 1-cm water depth over glass partition.

fish were acclimated for 16 h in the test containers before being exposed to toxaphene and its fractions. There were 10 fish per concentration during the tests on each fraction. Acetone was the solvent used and did not exceed 1.8 mL in 15 L of water.

Because of the difficulty in obtaining sufficient toxaphene fractions there was no replication of tests on the individual fractions. The nine fractions of toxaphene and the original technical toxaphene were tested individually. Because of the difference in toxicity of technical toxaphene and its nine fractions, fish were exposed to concentrations of 0.42-180 ppb to determine  $\text{LC}_{50}$ 's (concentration of toxicant lethal to 50% of the test organisms over the exposure period) after 24, 48, and 96 h of exposure. The  $\text{LC}_{50}$  determinations and confidence limits were calculated by using log/probits of concentrations and toxicity (Litchfield and Wilcoxon, 1949).

**Model Ecosystem.** The overall procedures and design of a recirculating static model ecosystem, as described by Isensee et al. (1976), were slightly modified for this study (Figure 1). Compartmental design allows simultaneous exposure of different trophic level organisms representative of two food chains. The screen allows water passage, yet prevents daphnids (*Daphnia magna*) from being consumed by bluegill fish (no daphnids were fed to fish during the experiment). Fish were fed daily a commercial trout chow. The percolator water pump ensures uniform mixing of water between the two compartments.

For the ecosystem study, unfractionated toxaphene and fractions 1, 5, and 9 (all  $^{14}\text{C}$ -labeled) were absorbed to Matapeake silt loam soil (pH 5.3; organic matter content 1.5%; sand, silt, and clay contents of 38.4, 49.4, and 12.2%, respectively) at the rate of 0.1 and 1.0 ppm. (These treatment rates represent soil residues often found following normal applications of toxaphene and assuming possible erosion of treated soil into a pond or lake). Treated soil (400 g) was placed in the large compartment of ecosystem tanks. Controls received 400 g of untreated soil. Duplicates of each treatment and rate were prepared, then flooded with 16 L of water. The next day, 16 bluegill fish, 11 snails (*Helisoma* sp), about 200 daphnids, and 1 g of algae (*Oedogonium cardiacum*) were added to each

tank. Fish were placed in the small compartment; all other organisms in the large compartment. Tissue samples (two fish, one or two snails, 20–40 mg of daphnids and 30–50 mg of algae) were taken 1, 3, 7, 15, and 32 days after the start of the experiment. After day 32 the experiment was terminated and the remaining fish, snails, and algae were placed in untreated water. These organisms were harvested after 3 and 10 days of "desorption". Snails and bluegill were homogenized whole in methanol and the homogenizate was assayed directly by scintillation counting. Daphnids were rinsed with distilled water, patted dry with cheesecloth (to remove surface moisture), weighed, and added to 15 mL of scintillation cocktail (composed of 750 mL of Triton X-100, 2250 mL of *p*-xylene, 16.5 g of 2,5-diphenyloxazole, and 1.2 g of 1,4-bis[2-(5-phenyloxazolyl)benzene] for direct counting. Algae were rinsed with distilled water and patted dry; subsamples were weighed directly into combustion boats and immediately combusted in a stream of O<sub>2</sub>. The <sup>14</sup>CO<sub>2</sub> was dried by passing through a column of anhydrous CaSO<sub>2</sub> and trapped in 10 mL of monoethanolamine/2-methoxyethanol (1:7, v/v). A 5-mL aliquot of the trapping solution was assayed for radioactivity by standard liquid scintillation methods.

Fish and snail extracts (1.0-ppm treatment rates) representative of each compound were spotted on silica gel TLC plates and developed twice (same direction) in hexane saturated with formic acid. Control fish and snail extracts were also spotted, then spiked with authentic standards and developed as above. Each plate was autoradiographed for 1 week with Kodak No-Screen medical X-ray film. The developed zone for each spot was divided into 1-cm zones (except larger areas for the parent material), scraped, and counted by standard liquid scintillation methods.

Dual water samples were taken on day 1, 3, 7, 15, and 32. Duplicate 1-mL samples were taken for direct liquid scintillation counting and 10-mL samples were extracted once with 5 mL of hexane (initial extraction efficiency for toxaphene was 90%). Duplicate 2-mL hexane samples were concentrated to near dryness, then analyzed by liquid scintillation counting. On day 28, two additional water samples (500 and 300 mL) were taken from the control and the 1.0 ppm rate of each treatment (one replicate only). The 500-mL samples were extracted twice with 250 mL of hexane, then concentrated to 50 mL. The 300-mL samples were extracted twice with 100 mL of ethyl acetate/hexane (7:3), then concentrated to 100 mL. Twenty milliliters of the hexane and ethyl acetate/hexane extracts (control and treatments) were concentrated, spotted, developed, and analyzed as described above.

## RESULTS AND DISCUSSION

**Acute Toxicity.** The acute toxicity of unfractionated toxaphene and nine fractions of toxaphene are shown in Table I. The 96-h LC<sub>50</sub>'s of between 5.0 and 7.8 ppb for toxaphene resembled those obtained by Mayer et al. (1975) for yearling brook trout (*Salvelinus fontinalis*), 10.8 ppb after 4 days, 4.8 ppb after 8 days. The nine fractions can be roughly divided into two groups according to toxicity; i.e., fractions 1, 2, 8, and 9 were far less toxic than fractions 3–7. Fractions 3–7 were all within the same toxicity range (or lower) than unfractionated toxaphene, indicating that some compounds in the toxaphene mixture are considerably more toxic than others. This is particularly true for fractions 5 and 6, which were consistently more toxic than unfractionated toxaphene. Based on TLC data, toxicant A is located in fractions 5 and 6, which may account for their higher toxicity.

Table I. Acute Toxicity of Toxaphene and Nine Fractions of Toxaphene as Measured by 24- and 96-h LC<sub>50</sub> for Bluegill Fish in a Static Jar Exposure

treatment	24-h LC <sub>50</sub> (95% CL), <sup>a</sup> μg/L	96-h LC <sub>50</sub> (95% CL), <sup>a</sup> μg/L
toxaphene <sup>b</sup> (first test)	19.0 (14.6–24.7)	5.0 (3.7–6.7)
toxaphene <sup>b</sup> (second test)	22.5 (17.2–29.5)	7.8 (5.7–10.7)
fraction 1	87.0 (65.6–115.4)	25.0 (20.0–31.3)
2	49.5 (34.8–70.5)	17.0 (12.1–23.9)
3	20.5 (14.1–29.9)	7.3 (5.0–10.6)
4	22.8 (18.3–28.5)	5.7 (3.9–8.3)
5	8.9 (6.2–12.9)	3.4 (2.6–4.3)
6	7.5 (5.2–10.9)	2.6 (2.1–3.2)
7	27.0 (22.3–32.7)	7.8 (5.7–10.8)
8	62.0 (51.6–74.5)	39.0 (32.4–46.9)
9	90.0 (67.0–120.8)	28.0 (21.2–36.9)

<sup>a</sup> CL = 95% confidence limits. <sup>b</sup> Toxaphene and fractions 1–6 were run in the first test (average fish size 0.46 g) and toxaphene and fractions 7–9 were run in the second test (average fish size 0.5 g).

**Model Ecosystem.** The concentration of toxaphene and fractions 1, 5, and 9 in water is shown in Table II. For the direct count (DC) analysis, all compounds generally increased in concentration up to 32 days at the 1.0-ppm rate and up to 15 days (followed by a general decrease) at the 0.1-ppm rate. However, for the hexane extraction (HE) analysis, the concentrations remained nearly constant with time (both treatment rates). These results suggest that some metabolites were forming with time. All <sup>14</sup>C in solution (parent compounds plus metabolites) would be detected with the DC analysis, while primarily the parent compounds or nonpolar metabolites would be measured with the HE analysis. By day 32, the DC analysis indicated concentrations about three–five times greater than the HE analysis. The concentrations found in water were about one order of magnitude higher for the 1.0-ppm rates than for the 0.1-ppm rate. Between compounds, fraction 1 consistently had the highest water concentration followed by toxaphene, fraction 5, then fraction 9. These results were consistent with TLC behavior of the fractions, i.e., fraction 1 was most polar, fraction 9 least polar.

Hexane and a combination of ethyl acetate/hexane (70:30, v/v) extracted considerably different amounts (about 14 and 70%, respectively) of total <sup>14</sup>C from water samples taken on day 28. TLC analysis indicated that much more of the hexane extracted <sup>14</sup>C was located at the same R<sub>f</sub> as the parent compounds (toxaphene 21%, fraction 1 38%, and fractions 5 and 9 33%) than for the ethyl acetate/hexane extracted <sup>14</sup>C (5, 10, 9, and 5%, respectively). On the basis of the TLC data, only 3–6% of the <sup>14</sup>C found in water on day 28 (by either extraction method) was the parent toxaphene or fractions 1, 5, or 9.

The concentrations of toxaphene and fractions 1, 5, and 9 in the various organisms is shown in Table III. The tissue concentrations were directly related to the amounts available in water, i.e., organisms at the 1.0-ppm rate contained about ten times more (of all compounds) than did organisms exposed to the 0.1-ppm rates.

For most organisms and treatments, we observed a general increase in tissue content over the first 7 to 15 days. The increase was generally most pronounced and larger for the 1.0-ppm rate as compared with the 0.1-ppm rate. The larger variability observed at the 0.1-ppm rate was probably due to the lower amounts of radioactivity measured.

The tissue concentrations generally decreased after 7 or 15 days (except for algae and snails, treated with toxaphene). The reason for this decrease is not known since

Table II. Concentration of Toxaphene and Three Toxaphene Fractions Found in Water as a Function of Treatment Rate, Time, and Analytical Procedure

compound	treat- ment rate ppm <sup>b</sup>	days														
		1			3			7			15			32		
		DC, ppt	HE, ppt	DC, ppt	HE, ppt	DC, ppt	HE, ppt	DC, ppt	HE, ppt	DC, ppt	HE, ppt	DC, ppt	HE, ppt			
toxaphene	0.1	45 ± 45 <sup>c</sup>	33 ± 14	5 ± 5	35 ± 6	55 ± 55	22 ± 7	545 ± 53	37 ± 13	108 ± 28	28 ± 18	108 ± 28	37 ± 13	108 ± 28	28 ± 18	
	1.0	280 ± 56	237 ± 26	543 ± 90	258 ± 27	973 ± 175	251 ± 23	1598 ± 57	232 ± 26	1568 ± 59	322 ± 2	1568 ± 59	232 ± 26	1568 ± 59	322 ± 2	
fraction 1	0.1	35 ± 24	52 ± 11	90 ± 33	43 ± 6	318 ± 130	86 ± 13	520 ± 60	12 ± 12	285 ± 79	44 ± 36	285 ± 79	12 ± 12	285 ± 79	44 ± 36	
	1.0	830 ± 76	401 ± 75	1381 ± 176	715 ± 297	1798 ± 204	488 ± 34	2750 ± 180	378 ± 111	2935 ± 83	557 ± 73	2935 ± 83	378 ± 111	2935 ± 83	557 ± 73	
fraction 5	0.1	28 ± 17	13 ± 13	93 ± 66	142 ± 0	68 ± 68	57 ± 51	275 ± 161	62 ± 2	30 ± 21	42 ± 16	30 ± 21	62 ± 2	30 ± 21	42 ± 16	
	1.0	150 ± 29	194 ± 36	255 ± 63	170 ± 2	228 ± 68	211 ± 15	848 ± 68	177 ± 13	998 ± 43	344 ± 35	998 ± 43	177 ± 13	998 ± 43	344 ± 35	
fraction 9	0.1	13 ± 13	40 ± 1	138 ± 51	14 ± 14	ND <sup>f</sup>	20 ± 17	340 ± 122	31 ± 11	83 ± 35	54 ± 18	83 ± 35	31 ± 11	83 ± 35	54 ± 18	
	1.0	148 ± 86	121 ± 39	328 ± 33	182 ± 47	790 ± 177	211 ± 21	1148 ± 92	160 ± 34	1425 ± 49	356 ± 90	1425 ± 49	160 ± 34	1425 ± 49	356 ± 90	

<sup>a</sup> Days after start of experiment. <sup>b</sup> Concentration of toxaphene or fractions in 400 g of soil in each tank. <sup>c</sup> DC = direct count of water samples; HE = extractions of water samples with hexane. <sup>d</sup> ppt = parts per trillion. <sup>e</sup> Standard error of the mean. <sup>f</sup> ND = not detectable.

the water concentration did not decrease over this time period. The reduction in tissue concentrations ranged from 2 to 75% (average 42%) but many were not statistically lower (standard error of the means overlapped). Also, there was no general trend for one species to decrease more than another.

The residue left in the organisms after "desorbing" for 3 and 10 days is also shown in Table III (last two columns). After 3 days of desorption, only 25% of the samples were significantly lower than the concentration at 32 days, while after 10 days, 75% were significantly lower. Thus, organisms desorbed slowly with the average total loss ranging from 60 to 77% of the residue found after 32 days. However, some samples did not desorb. The rate of loss of chlorinated insecticides apparently varies widely between compounds. For example, channel catfish (*Ictalurus punctatus*) lost 95% of their accumulated endrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,endo-5,8-dimethanonaphthalene) in 13 days (Argyle et al., 1973), while goldfish (*Carassius auratus*) lost only 50% of their accumulated DDT [2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane] in 29 days (Grezenda et al., 1970).

A general trend in the amounts of the various compounds accumulated by daphnids and fish was: fraction 9 ≥ toxaphene ≥ fraction 5 ≥ fraction 1. This trend was found only for the 1-ppm treatment rate and was not evident for snails and algae at either treatment rate. The accumulation trend was about the reverse of the water concentration, indicating that the differences in polarity between compounds may have influenced the amounts accumulated.

The TLC analysis for fish and snails (1.0 ppm rate for 3, 15, and 32 day samplings) indicated a much higher recovery of parent materials than were found in water. For the fish extracts, an average of 72, 59, 70, and 63% of the recovered <sup>14</sup>C was found at the same R<sub>f</sub> as toxaphene and fractions 1, 5, and 9, respectively. We noted little variation between sampling dates. Most of the remaining <sup>14</sup>C was found at the origin and was presumed to be metabolites. The distribution of <sup>14</sup>C in snail extracts changed with time as recovery of <sup>14</sup>C located at the parent compound R<sub>f</sub> was highest on day 3 (78, 38, 54, and 57% for toxaphene and fractions 1, 5, and 9, respectively) and then decreased to 49, 31, 26, and 25%, respectively, on day 32. The amount of <sup>14</sup>C recovered at the origin increased in direct proportion to the reduction in parent compound <sup>14</sup>C. These TLC results strongly suggest that snails were able to metabolize toxaphene and the three fractions. These results were somewhat surprising since snails have a lower metabolic potential than fish.

Bioaccumulation ratios (BR, concentration in tissue/concentration in water) were calculated for fish and snails, day 32 only (Table IV). (Calculations of BR for other days and organisms were made, but could give misleading results since they were based on total residues in both water and tissue and are therefore not presented). The water and tissue concentrations shown in Table IV are based on the TLC analysis of the hexane and ethyl acetate/hexane water extracts (average of the two) on day 28 and on the analysis of the fish and snails on day 32 (percent recoveries from the 1.0-ppm rate were also used for the 0.1-ppm rate). The fish accumulated more toxaphene and all other fractions than did snails, which is consistent with the TLC analysis of the tissue. However, in another study (Metcalf and Sanborn, 1975) it was found that snails accumulated more toxaphene than fish (BR values of 4250, 9600, and 6900 for fish, snails, and algae, respectively). We do not

Table III. Distribution of [<sup>14</sup>C]Toxaphene and Three Toxaphene Fractions in an Aquatic Model Ecosystem

organism	treatment rate, <sup>a</sup> ppm	tissue content, ppb						
		treatment days <sup>b</sup>					desorption days <sup>c</sup>	
		1	3	7	15	32	3	10
Toxaphene								
algae	0.1	26 ± 17 <sup>d</sup>	34 ± 9	50 ± 7	65 ± 11	69 ± 12	35 ± 8	31 ± 6
	1.0	202 ± 88	334 ± 67	623 ± 81	663 ± 41	932 ± 321	684 ± 217	416 ± 47
snails	0.1	10 ± 4	5 ± 1	5 ± 0	19 ± 7	5 ± 1	9 ± 2	5 ± 2
	1.0	27 ± 3	51 ± 7	47 ± 1	117 ± 15	81 ± 2	85 ± 4	64 ± 2
daphnids	0.1	33 ± 8	49 ± 15	47 ± 14	56 ± 0	28 ± 0		
	1.0	283 ± 65	504 ± 234	793 ± 399	709 ± 20	379 ± 10		
fish	0.1	13 ± 1	37 ± 2	39 ± 8	55 ± 8	30 ± 7	29 ± 4	19 ± 5
	1.0	180 ± 6	302 ± 25	545 ± 63	599 ± 104	527 ± 97	329 ± 97	287 ± 76
Fraction 1								
algae	0.1	59 ± 15	28 ± 12	54 ± 15	111 ± 3	73 ± 26	69 ± 18	68 ± 2
	1.0	340 ± 70	467 ± 53	669 ± 24	877 ± 5	654 ± 100	469 ± 123	568 ± 23
snails	0.1	4 ± 0	3 ± 1	17 ± 13	15 ± 4	9 ± 1	49 ± 33	3 ± 1
	1.0	22 ± 5	46 ± 6	57 ± 13	117 ± 24	115 ± 6	88 ± 0	87 ± 7
daphnids	0.1	23 ± 1	30	57 ± 2	21 ± 7	17 ± 4		
	1.0	131	229	198 ± 47	461 ± 15	135 ± 26		
fish	0.1	17 ± 3	24 ± 1	14 ± 7	49 ± 3	19 ± 6	33 ± 18	7 ± 1
	1.0	145 ± 13	307 ± 9	347 ± 55	279 ± 51	239 ± 27	209 ± 101	80 ± 22
Fraction 5								
algae	0.1	71 ± 15	36 ± 17	56 ± 1	51	51 ± 0	81 ± 29	20 ± 2
	1.0	242 ± 15	300 ± 9	647 ± 11	471 ± 7	288 ± 4	265 ± 67	200 ± 32
snails	0.1	3 ± 1	4 ± 1	6 ± 5	18 ± 4	7 ± 2	14 ± 8	2 ± 0
	1.0	25 ± 7	61 ± 5	37 ± 13	90 ± 34	78 ± 10	53 ± 5	44 ± 1
daphnids	0.1	31 ± 3	52	36	69 ± 11	20 ± 1		
	1.0	335 ± 49	262	135 ± 1	494 ± 187	340 ± 85		
fish	0.1	30 ± 2	44 ± 3	38 ± 13	68 ± 10	32 ± 7	22 ± 4	30 ± 9
	1.0	218 ± 20	415 ± 63	626 ± 39	565 ± 141	351 ± 68	226 ± 47	169 ± 51
Fraction 9								
algae	0.1	54 ± 14	49 ± 11	65 ± 7	54 ± 19	53 ± 3	49 ± 7	37 ± 2
	1.0	392 ± 61	447 ± 39	1052 ± 174	1101 ± 37	487 ± 38	350 ± 52	305 ± 56
snails	0.1	5	3 ± 1	8 ± 5	10 ± 3	5 ± 1	6 ± 2	0
	1.0	33 ± 4	95 ± 14	84 ± 9	110 ± 12	87 ± 4	89 ± 9	47 ± 6
daphnids	0.1	53 ± 16	81 ± 22	46 ± 1	69 ± 1	31 ± 4		
	1.0	361 ± 1	839 ± 415	337 ± 90	1411 ± 341	353 ± 2		
fish	0.1	19 ± 3	25 ± 5	26 ± 4	44 ± 5	35 ± 4	25 ± 10	37 ± 6
	1.0	153 ± 28	314 ± 17	485 ± 56	790 ± 101	423 ± 54	579 ± 94	370 ± 112

<sup>a</sup> Concentration of treatment compound in 400 g of soil in each tank. <sup>b</sup> Days after start of experiment. <sup>c</sup> Organisms harvested after 3 and 10 days in untreated water. <sup>d</sup> Mean tissue content ± standard error for two replications. Numbers without standard error values based on one sample only.

Table IV. Distribution of <sup>14</sup>C Toxaphene and Three Toxaphene Fractions in Water, Fish, and Snails

	treatment rate, <sup>c</sup> ppm	water and tissue content, ppb, and bioaccumulation ratios			
		treatments			
		toxaphene	fraction 1	fraction 5	fraction 9
water	0.1	0.004 ± 0.001	0.018 ± 0.005	0.002 ± 0.001	0.003 ± 0.001
	1.0	0.051 ± 0.002	0.178 ± 0.004	0.055 ± 0.002	0.058 ± 0.002
fish	0.1	118.9 ± 6.2	10.8 ± 3.2	21.6 ± 5.0	20.2 ± 2.5
	1.0	4700	600	10800	6700
		412 ± 76	150 ± 57	237 ± 46	243 ± 31
		8100	840	4300	4200
snails	0.1	2.7 ± 0.7	2.7 ± 0.4	1.9 ± 0.6	1.1 ± 0.1
	1.0	680	150	950	370
		40 ± 1.0	35.2 ± 1.8	20.1 ± 2.4	21.9 ± 1.1
		780	200	360	380

<sup>a</sup> Based on TLC analysis of water extracts from day 28 and TLC analysis of fish and snail extracts from day 32. <sup>b</sup> Bioaccumulation ratios (concentration in tissue/concentration in water). <sup>c</sup> Concentration of treatment compound in 1000 g of soil in each tank.

know if the different species of fish and snails used between their study and ours contributed to the difference in results.

The amounts of toxaphene and fractions 1, 5, and 9 lost from soil after 32 days of submersion were (average of two treatment rates) 8.3, 21.6, 7.7, and 15.9%, respectively. The residue in the biomass and in water largely accounted for the amount "lost" from soil but we made no attempt to measure the total residue in the biomass (all organisms were not harvested). The <sup>14</sup>C recovered in the analyzed

organisms and as measured in water nearly equaled the difference in the amount of <sup>14</sup>C present in the soil before and after submersion.

The largest difference between the nine fractions of toxaphene were in acute toxicity to fish (fraction 6 was 15 times more toxic than fraction 8). The amounts accumulated by all organisms were not very different in actual concentration, but the BR for fish and snails were far smaller for fraction 1 than fraction 5 and 9 and toxaphene. Also, snails accumulated less and apparently metabolized

more than fish; results that are the reverse of findings by Metcalf and Sanborn (1975). Thus, this investigation has shown that significant differences exist between the nine fractions of toxaphene tested and that future environmental research should probably be directed toward the two most toxic fractions (5 and 6).

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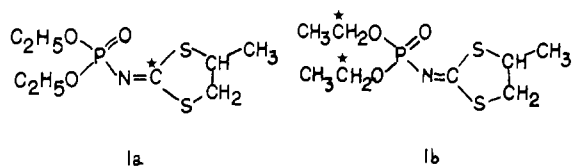
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## Photodegradation of Cytrolane (Mephosfolan) Systemic Insecticide in the Aquatic Environment Using Carbon-13 as a Mass Tracer

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Photodegradation of mephosfolan (Cytrolane, a registered trademark of the American Cyanamid Co.) was studied by exposing approximately equimolar mixtures of either  $^{12}\text{C}/^{13}\text{C}$ -imido-labeled or  $^{12}\text{C}/^{13}\text{C}$ -ethyl-labeled material to sunlight. Use of the  $^{12}\text{C}/^{13}\text{C}$  mixtures facilitated identification of photoproducts by gas chromatography chemical ionization mass spectroscopy (GC-CIMS) because molecular and fragment ions in the mass spectra appear as doublets. Solvent artifacts and volatile rice paddy water constituents gave mass spectra containing only singlet ions and were ignored. Irradiations were conducted in distilled water, "natural" water obtained from a flooded rice paddy, and a 2% acetone-water solution. The half-lives for mephosfolan were 18, 14, and 7 days, respectively, and indicated that photosensitization by paddy water constituents and acetone was occurring. The major products identified were cyclic *S,S*-propylene dithiocarbonate, 2-imino-5-methyl-1,3-dithiolane, and diethyl phosphate. Minor products were ethyl phosphate, phosphoric acid, and diethyl methyl phosphate.

Cytrolane systemic insecticide [mephosfolan; (diethoxyphosphinyl)dithioimidocarbonic acid, cyclic propylene ester] is used for pest control on cotton, corn, rice, sorghum, and sugar cane, especially in the Middle East and Asia. As part of the study on the environmental chemistry of mephosfolan (Ku et al., 1978), we examined its behavior upon exposure to sunlight. This study was conducted by using approximately equimolar mixtures of either  $^{12}\text{C}/^{13}\text{C}$ -imido-labeled (Ia) or  $^{12}\text{C}/^{13}\text{C}$ -ethyl-labeled material (Ib), where the asterisks denote  $^{13}\text{C}$ . Use of the  $^{12}\text{C}/^{13}\text{C}$



mixtures facilitated identification of photoproducts by

GC-CIMS because molecular and fragment ions in the mass spectra appeared as doublets. Solvent artifacts and volatile rice paddy water constituents gave mass spectra containing only singlet ions and were ignored.

#### EXPERIMENTAL SECTION

**Synthesis of [ $^{13}\text{C}$ ]Mephosfolan.** (1) *Imido Carbon Labeled (IL)*. One gram of potassium [ $^{13}\text{C}$ ]cyanide (90% enriched, Merck), 738 mg of sublimed sulfur, and 15 mL of 1,2-dimethoxyethane were refluxed for 1 h and then stirred overnight at room temperature. Diethyl chlorophosphate (2.65 g) was added and the mixture was stirred at room temperature for 1 h. Next, 1.66 g of 1,2-propylenedithiol, 2.3 g of potassium bicarbonate, 75 mL of 1,2-dichloroethane, and 15 mL of water were added. The two-phase reaction mixture was refluxed for 3 h and stirred overnight at room temperature. The organic phase was separated and the aqueous phase was extracted twice with 150 mL of 1,2-dichloroethane. The combined organic phases were back-washed with 30 mL of 5% potassium bicarbonate. After separation, the organic phase was dried over anhydrous magnesium sulfate, filtered, and evaporated under reduced pressure. The crude product, a yellow oil, was obtained in 59% yield. The oil was dissolved in

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