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Natural History Survey
BULLETIN

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CONTENTS

This paper is published by authority of the State of Illinois, IRS Ch. 127, Par. 58.12.

Keturah A. Reinbold is a Research Assistant, Section of Economic Entomology, Illinois Natural History Survey; Dr. Inder P. Kapoor is a Research Assistant, Department of Entomology, University of Illinois; Dr. William F. Childers is an Associate Aquatic Biologist, Section of Aquatic Biology, Illinois Natural History Survey; Dr. Willis N. Bruce is an Entomologist, Section of Economic Entomology, Illinois Natural History Survey; and Dr. Robert L. Metcalf is Professor of Zoology, Entomology, and Agricultural Entomology and Head of the Department of Zoology, University of Illinois.

(30448—5M—6-71)

Frontispiece. — Organisms and compounds used in this investigation. On a background of daphnia swimming in wat
ire (top to bottom) the green sunfish, guppy, tilapia, and snails.

Comparative Uptake and Biodegradability of DDT and Methoxychlor by Aquatic Organisms

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DDT IS SOLUBLE in water to about 0.002 ppm, but it is soluble in animal fats to about 100,000 ppm. Because DDT possesses such a high lipid-to-water partition value and resists attack by multifunction oxidase detoxifying enzymes, this insecticide has become a ubiquitous environmental pollutant and is found in animal tissues everywhere. For example, DDT is present in Lake Michigan bottom muds at about 0.014 ppm. It has been found in concentrations of 3–6 ppm in fishes, such as coho salmon and lake trout, taken from Lake Michigan and in even higher concentrations in the tissues of fish-eating birds living near the lake (Harrison et al. 1970:505). DDT used for spruce budworm control in the Yellowstone River system was found to persist in the aquatic environment for more than 2 years (Cope 1961:242-244). Bridges et al. (1963) found concentrations of DDT in a number of organisms in a farm pond treated with 0.02 ppm of DDT. Fish in aquaria have been shown to eliminate DDT very slowly after a single sublethal exposure (Gakstatter & Weiss 1967:305). Because animals concentrate DDT in their tissues and eliminate it very slowly, aquatic pollution by DDT, whether it results directly from blackfly and mosquito control programs or indirectly from urban treatments for elm bark beetle control or agricultural applications for fruit pests, is particularly deleterious to environmental quality.

The need for a persistent but biodegradable substitute for DDT is urgent. Methoxychlor is being widely considered as such a substitute, especially for control of blackflies, bark beetles, and fruit and garden pests. It has been reported that, after 28 days of exposure to 0.04 ppm of methoxychlor, bluegills had metabolized or excreted most of the compound which they had taken up. After 56 days no methoxychlor was found in the fish (Kennedy et al. 1970:12). Preliminary studies in a laboratory model ecosystem (Kapoor et al. 1970:1151) indicate that methoxychlor does not accumulate in fishes, as does DDT, but reaches a dynamic equilibrium.

The studies reported in this paper were designed to further test the biodegradability of methoxychlor and to compare in a laboratory aquatic environment (i) the uptake of DDT and methoxychlor directly from water by fishes, from water by crustaceans of the genus *Daphnia* and by snails of the genus *Physa*, and from daphnia by guppies in a daphnia-to-fish food chain and (ii) the subsequent elimination of the insecticides from the organisms.

ACKNOWLEDGEMENTS

We are indebted to several members of the Illinois Natural History Survey staff for their contributions to this investigation. Dr. William H. Luckmann, Head of the Section of Economic Entomology, provided encouragement throughout the study and reviewed the manuscript, which was edited by Robert M. Zewadski, Associate Technical Editor. Richard M. Sheets, Technical Illustrator, prepared the graphs and designed the frontispiece, and Wilmer D. Zehr, Technical Photographer, provided most of the photographs. The photograph of the tilapia appearing in the frontispiece was loaned by the Section of Aquatic Biology.

We wish to thank the Natural History Survey and the University of Illinois for the laboratory space and equipment provided for this investigation.

This study was supported in part by funds supplied by U. S. Department of Agriculture Regional Projects NC-85, Reduction of Hazards Associated with the Presence of Insecticidal Chemicals in the Environment, and NC-96, Environmental Implications of Pesticide Usage, both Hatch projects. These funds were administered by the University of Illinois College of Agriculture. The study was also supported in part by U.S. Public Health Service grants FD-00271 and FR-07030. **and FWQA 16050 EHH.**

MATERIALS AND METHODS

Radiolabeled Compounds

The investigations were conducted with ^{3}H-ring substituted methoxychlor obtained by the procedure of Kapoor et al. (1970:1146). The compound was 99.9+ percent pure according to our evaluation of it by thin-layer chromatography, and it had a specific activity of 4.81 mC/mM. Ring-labeled ^{14}C-DDT was obtained from the World Health Organization of the United Nations, Geneva, Switzerland. The DDT had a specific activity of 5.48 mC/mM and a purity of 99.9+ percent.

Radioassay Methods

The radioactivity in 1-ml water samples containing radiolabeled compounds was counted in 10 ml of ^{3}H scintillation fluid (200 grams of naphthalene, 10 grams of PPO, 0.25 gram of POPOP in dioxane to make 1 liter) in a Beckman S-250 scintillation counter. All organisms used in our investigations were freeze-dried and ground to a powder with a mortar and pestle. Each of three 10-mg portions of each sample of the powdered material was placed in 15 ml of scintillation fluid. The samples were kept in the dark overnight before counting to quench phosphorescence. The radioactivity in each sample was determined by averaging the results of the three counts. Quenching, if any occurred, was corrected for, using the appropriate quench curves. All insecticide concentrations in the organisms were calculated on a dry-weight basis.

Chromatographic Techniques

Thin-layer chromatography (TLC) was performed in the usual manner using glass plates coated with 0.25 mm of silica gel. To determine the relative proportions of parent compounds and their metabolites in tilapia (*Tilapia mossambica*) and green sunfish (*Lepomis cyanellus*), portions of the powdered organisms were extracted with acetonitrile (2 ml for tilapia, 4 ml for green sunfish). About 0.05 ml of the extract from each sample was spotted on a TLC plate. Non-radiolabeled parent compounds and model metabolites were incorporated as internal standards for cochromatography by spotting them on the plate over the acetonitrile extracts of the fishes. Methoxychlor chromatograms were developed in a solvent system consisting of 3 parts diethyl ether and 1 part petroleum ether (b. p. 60° to 68° C), and those of DDT in petroleum ether (b. p. 60° to 68° C) alone.

Radioautographs of the ^{14}C-labeled DDT metabolites were made by exposing Eastman Kodak No Screen Medical X-ray film to the developed chromatograms. Black spots which had developed on the film were matched with the incorporated internal standards to determine the identity of the metabolites. The corresponding areas of silica gel were scraped from the chromatograms and counted by scintillation counting; from these counts the relative percentages of each parent compound and its metabolites were then calculated. The relative proportions of methoxychlor and its metabolites were determined by scraping appropriate spots or strips of silica gel from the chromatograms and radioassaying them.

Bioassay Methods

We used cylindrical Pyrex jars 8 inches deep and 8¼ inches in diameter in all of the experiments reported on here. Each jar contained 5 liters of synthetic hard water (Cairns 1969:8) and was covered by a plate glass lid. The water was constantly aerated by compressed air bubbling through a glass tube extending down the inside wall of the jar. Both DDT and methoxychlor were added to the water in these jars from standard 0.0025-percent solutions in acetone. The water-insecticide solutions were allowed to equilibrate for 24 hours before any aquatic organisms were placed in the jars.

The animals used were daphnia (*Daphnia magna*) (hereafter referred to as "daphnia"), snails (*Physa* sp.) (hereafter referred to as "snails"), tilapia (*Tilapia mossambica*) (hereafter referred to as "tilapia"), green sunfish (*Lepomis cyanellus*), and guppies (*Lebistes reticulatus*). At 2-day, 3-day, or 10-day intervals, the organisms were moved to fresh water or were surface rinsed with clean water and then frozen. Subsequently, the organisms were freeze-dried, powdered, and radioassayed. In all experiments organisms held under the same conditions as were the test animals but not exposed to insecticides were prepared and assayed in the same manner as were the test samples.

Comparative uptake of DDT and methoxychlor from water by two fish species, tilapia and green sunfish, was studied at insecticide levels of 0.001, 0.003, and 0.01 ppm. The tilapia experiment was duplicated using three fish (0.6–1.8 grams per fish) in each jar. Two jars of tilapia were used at each of the three levels of each insecticide (12 jars). Only one green sunfish (5–9 grams) was placed in each of three jars at each concentration of each compound (18 jars). One fish was removed from each jar (two tilapia from each insecticide concentration and one green sunfish from each concentration) on the 3rd, 10th, and 31st days, after which the experiment was terminated. On the 10th and 20th days those fishes which remained were transferred to jars containing insecticide concentrations equivalent to those in which the fishes had been placed at the beginning of the experiment.

The uptake of insecticides from water by daphnia (1 gram per jar) was studied at the insecticide levels used in the fish experiments. The daphnia from half of the jars at each concentration of each insecticide were removed on the 3rd day and from the remaining jars on the 6th day. All samples were then radioassayed.

In another experiment tilapia weighing 0.2–1.4 grams each were exposed, five fish per jar in each of five jars for each insecticide, to 0.003 ppm of DDT or methoxychlor for 12 days. At 3-day intervals one fish from each jar was removed for radioassay, and the rest were transferred to water freshly treated with 0.003 ppm of insecticide. After 12 days the remaining fish were moved to water containing no insecticide. Samples of one fish each were then taken after additional periods of 2, 5, 9, 11, 12, and 15 days and assayed for radioactivity to determine the comparative insecticide excretion rates.

Daphnia (2 grams) and snails (1 gram) were exposed together in jars to a level of 0.003 ppm of DDT or methoxychlor for 2, 4, or 6 days. In one series of jars the daphnia and snails were exposed for 2 days, the animals in one jar were removed for radioassay, and the remaining organisms were moved to water without insecticide. They were transferred to fresh water without insecticide every 2 days, and a one-jar sample was removed after 1, 2, 4, or 6 days. The organisms in another series of jars were transferred to water containing the original level of insecticide after 2 and 4 days. They were also sampled for radioassay after 4 days. After 6 days a sample was removed for radioassay, and the remaining organisms were moved to water without insecticide. Again, they were transferred to fresh water every 2 days and were sampled after 1, 3, or 5 days.

Insecticide uptake in a daphnia-to-guppy food chain was studied by holding 2–5 grams of daphnia in water containing 0.003 ppm of DDT or methoxychlor for 48 hours and then feeding them to guppies held in water without any insecticide. Three jars of daphnia exposed to methoxychlor were combined. One-third of these daphnia were analyzed for insecticide content by scintillation counting and the remaining animals were fed to guppies held in two jars, each containing 10 fish. The same procedure was followed with daphnia exposed to DDT and fed to two additional jars of fish. By exposing additional jars of daphnia to the insecticides at 2-day intervals, we repeated the process and fed the fish every other day. Fish were removed from each jar on the 6th, 8th, and 20th days and assayed for radioactivity.

RESULTS AND DISCUSSION

Uptake and Metabolism by Fishes

The results of the investigation of the uptake from water of DDT and methoxychlor by tilapia and green sunfish are presented in Table 1. At the 0.001-ppm level the concentration of methoxychlor in tilapia dropped from 0.8 ppm after 3 days of exposure to 0.2 ppm after 10 days, remaining at 0.2 ppm at the end of 31 days. The DDT concentration decreased from 1.3 ppm after 3 days to 0.7 after 10 days; it then increased markedly to 6.8 ppm at 31 days. The same pattern occurred at the higher insecticide levels. The pattern was repeated in the green sunfish, which differed from the tilapia, however, in having lower DDT-to-methoxychlor ratios. These lower ratios may have been the result of the green sunfish's lower proportion of body fat and its consequent smaller capacity for concentrating DDT in fat tissues.

The results show far greater contrasts when we examine the concentration factor (Fig. 1 and 2). While

Table 1. — Comparative uptake from water of ^{14}C-DDT and ^{3}H-methoxychlor by tilapia and green sunfish.

Insecticide Concentration in Water in ppm	*Days of Exposure to Insecticide*	*Insecticide Concentration*[a] *in ppm in Tilapia*		*Ratio of DDT to Methoxychlor*	*Insecticide Concentration*[a] *in ppm in Green Sunfish*		*Ratio of DDT to Methoxychlor*
		Methoxychlor	DDT		Methoxychlor	DDT	
0.001	3	0.8	1.3	1.6	0.8	0.5	0.6
	10	0.2	0.7	3.5	0.3	0.6	2.0
	31	0.2	6.8	34.0	0.2	3.9	19.5
0.003	3	1.8	4.4	2.4	. .	2.2	. . .
	10	0.3	2.5	8.3	0.6	1.7	2.8
	31	0.6	12.0	20.0	0.6	10.2	17.0
0.01	3	9.0	16.4	1.8	7.4	. . .	. . .
	10	1.0	13.9	13.9	1.9	8.6	4.5
	31	2.0	106.0	53.0	2.7	40.2	14.9

[a]All such concentrations were calculated on a dry-weight basis.

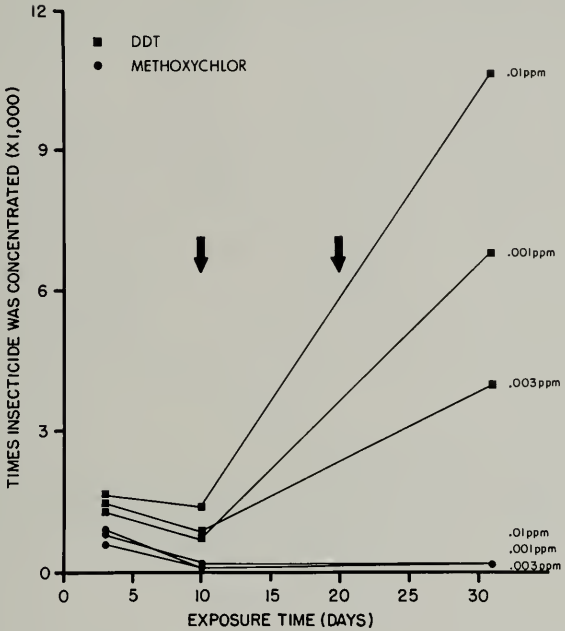

Fig. 1. — Comparative uptake from water of ^{14}C-DDT and ^{3}H-methoxychlor by tilapia. The vertical arrows indicate the days when fish were transferred to jars containing insecticide levels equivalent to those which the fish had been placed in at the beginning of the experiment.

the insecticide concentration in methoxychlor-exposed fishes reached an equilibrium, which indicated that the compound was being metabolized and excreted, DDT was increasingly concentrated in the fishes. In tilapia exposed to the 0.01-ppm insecticide level at the end of 31 days, even after the DDT had been concentrated 10,600 times it had not reached a steady state. Furthermore, when tilapia were held in water containing 0.003 ppm of insecticide for 12 days and subsequently were left for 15 days in jars containing water with no insecticide, the radioactive compounds in methoxychlor-exposed fish were rapidly excreted (Fig. 4). At the end of 15 days in uncontaminated water there was a 10,000-fold difference between the concentrations in tilapia of methoxychlor and of DDT (Table 4).

The metabolic pattern (Table 2) indicates that both fishes metabolized methoxychlor to a greater extent than they did DDT. Tilapia metabolized DDT to a greater extent than green sunfish metabolized it. Tilapia contained DDD as a major metabolite, but the major metabolite of the green sunfish was DDE. Methoxychlor was rapidly metabolized by tilapia, which contained considerably higher proportions of the mono-*O*-demethylated product [2-(*p*-methoxyphenyl)-2-(*p*-hydroxyphenyl)-1, 1, 1-trichloroethane] but only slightly greater proportions of the bis-*O*-demethylated metabolite [2, 2-bis-(*p*-hydroxyphenyl)-1, 1, 1-trichloroethane] than did the green sunfish.

The data from these experiments in-

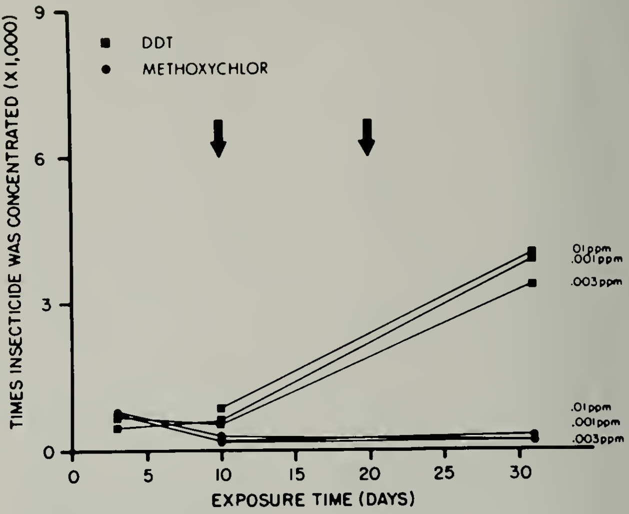

Fig. 2. — Comparative uptake from water of ^{14}C-DDT and ^{3}H-methoxychlor by green sunfish. The vertical arrows indicate the days when fish were transferred to jars containing insecticide levels equivalent to those which the fish had been placed in at the beginning of the experiment.

Table 2. — Distribution of ^{14}C-DDT and ^{3}H-methoxychlor and their metabolites in tilapia and green sunfish following exposure to water containing these insecticides.

Insecticide	*Insecticide Concentration in Water in ppm*	*Days of Exposure to Insecticide*	*Relative Percentages of Insecticides and Their Metabolites Found in Fishes*					
			In Tilapia			In Green Sunfish		
DDT			DDT	DDD	DDE	DDT	DDD	DDE
	0.001	3	62.5	37.5	0.0	100.0	0.0	0.0
		10	62.0	38.0	0.0	100.0	0.0	0.0
		31	66.0	29.0	5.0	91.0	1.0	8.0
	0.003	3	77.0	23.0	0.0	100.0	0.0	0.0
		10	56.0	37.0	7.0	100.0	0.0	0.0
		31	47.0	44.0	9.0	75.0	4.0	21.0
	0.01	3	76.0	24.0	0.0	...	...	...
		10	60.0	36.5	3.5	92.5	2.5	5.0
		31	76.0	20.0	4.0	90.0	3.0	7.0
Methoxychlor			A[a]	B[b]	C[b]	A[a]	B[b]	C[b]
	0.01	3	43.4	45.2	11.4	59.0	29.8	11.2
		31	13.9	71.3	14.8	66.3	26.9	6.8

[a]Parent material: $CH_3OC_6H_4HCCCl_3C_6H_4OCH_3$
[b]Metabolite B: $HOC_6H_4HCCCl_3C_6H_4OCH_3$
Metabolite C: $HOC_6H_4HCCCl_3C_6H_4OH$

dicate that methoxychlor is readily biodegradable in tilapia and green sunfish. It seems likely that this insecticide is also biodegradable in other fish species.

Uptake and Metabolism by Daphnia and Snails

Daphnia also concentrated both methoxychlor and DDT taken up from water. The DDT-to-methoxychlor ratio (Table 3) in daphnia after 3 days of exposure at the three insecticide levels used in these experiments ranged from 1.8 to 2.5, a pattern similar to that displayed by the fishes we studied.

When daphnia and snails were held together in jars containing 0.003 ppm of insecticide, daphnia concentrated the two insecticides at about the same rate, but the snails concentrated me-

Table 3. — Comparative uptake from water of ^{14}C-DDT and ^{3}H-methoxychlor by daphnia.

Insecticide Concentration in Water in ppm	*Days of Exposure to Insecticide*	*Insecticide Concentration[a] in ppm in Daphnia*		*Ratio of DDT to Methoxychlor*
		Methoxychlor	DDT	
0.001	3	11	28	2.5
	6	9	15	1.7
0.003	3	37	66	1.8
	6	22	32	1.5
0.01	3	143	316	2.2
	6	...	83	..

[a]All such concentrations were calculated on a dry-weight basis.

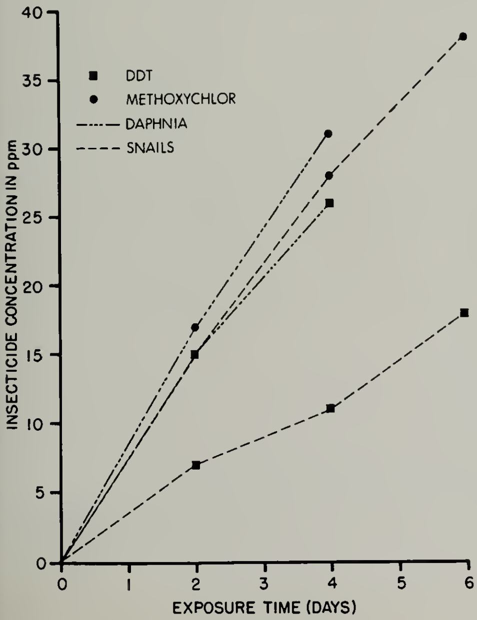

Fig. 3. — Comparative uptake of ^{14}C-DDT and ^{3}H-methoxychlor by snails and daphnia following exposure to a 0.003-ppm insecticide level in water.

Table 4. — Comparative uptake and excretion of ^{14}C-DDT and ^{3}H-methoxychlor by tilapia, daphnia, and snails on exposure to water containing 0.003 ppm of insecticide and subsequently to water with no insecticide.

Days of Exposure to Insecticide	*Days in Clean Water Following Exposure*	*Insecticide Concentration[a] in ppm in Tilapia*		*Ratio of DDT to Methoxychlor*	*Insecticide Concentration[a] in ppm in Daphnia*		*Ratio of DDT to Methoxychlor*	*Insecticide Concentration[a] in ppm in Snails*		*Ratio of DDT to Methoxychlor*
		Methoxychlor	DDT		Methoxychlor	DDT		Methoxychlor	DDT	
2	0	...	...	...	17.0	15.0	0.9	15.0	7.0	0.5
	1	...	...	...	11.0	10.0	0.9	14.0	5.0	0.4
	2	...	...	...	8.0	8.0	1.0	12.0	3.0	0.3
	4	...	...	...	2.0	5.0	2.5	12.0	3.0	0.3
	6	...	...	...	...	...	..	11.0	4.0	0.4
3	0	2.0	5.0	2.5	...	...	..	...	...	..
4	0	...	...	...	31.0	26.0	0.8	28.0	11.0	0.4
6	0	4.0	8.0	2.0	...	...	..	38.0	18.0	0.5
	1	...	...	...	...	...	..	...	13.0	..
	3	...	...	...	...	...	..	36.0	...	..
	5	...	...	...	...	...	..	23.0	10.0	0.4
9	0	5.0	11.0	2.2	...	...	..	...	...	..
12	0	8.0	13.0	1.6	...	...	..	...	...	..
	2	2.0	13.0	6.5	...	...	..	...	...	..
	5	2.0	...	...	...	...	..	...	...	..
	9	2.0	6.0	3.0	...	...	..	...	...	..
	11	...	4.0	...	...	...	..	...	...	..
	12	0.2	...	...	...	...	..	...	...	..
	15	0.0001	1.0	10,000.0	...	...	..	...	...	..

[a]All such concentrations were calculated on a dry-weight basis.

thoxychlor much more than they concentrated DDT—18 ppm of DDT:38 ppm of methoxychlor after 6 days of exposure to the insecticides (Table 4 and Fig. 3). When daphnia and snails were held in water free of insecticide after exposure to DDT or methoxychlor, both organisms excreted radioactive compounds. Daphnia excreted methoxychlor more rapidly than DDT, while the opposite was true of the snails (Table 4 and Fig. 4). The snails apparently cannot readily metabolize methoxychlor and, as a result, retain relatively persistent, high concentrations of that insecticide once they have taken it up. This observation agrees with that of Kapoor et al. (1970:1151). The snails, like the other organisms studied, also tend to retain DDT.

Uptake and Metabolism by Guppies in a Daphnia-to-Fish Food Chain

The results of the daphnia-to-guppy food chain study are shown in Table 5

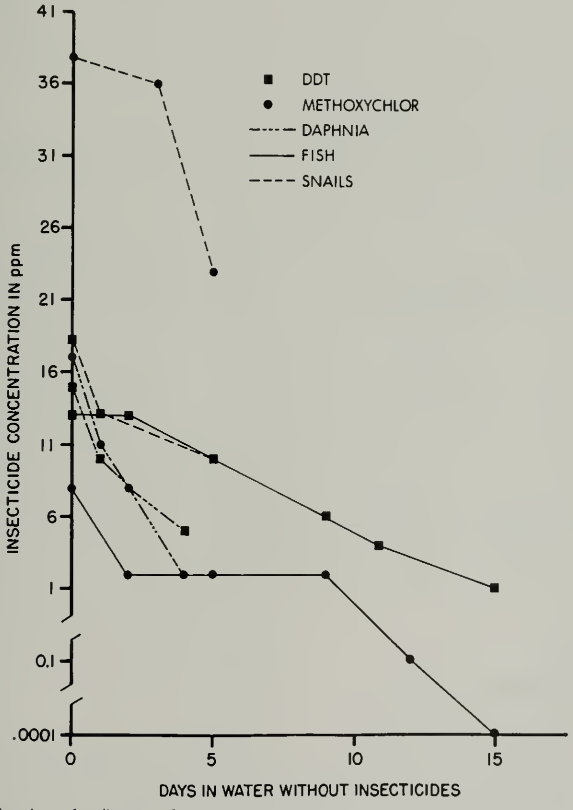

Fig. 4. — Loss of radioactivity from tilapia, snails, and daphnia in water without insecticides following exposure to 0.003 ppm of DDT or methoxychlor in water. Periods of exposure to insecticides: fish, 12 days; snails, 6 days; daphnia, 2 days.

Table 5. — Uptake of ^{14}C-DDT and ^{3}H-methoxychlor in a daphnia-to-guppy food chain.

Insecticide Concentration[a] *in Daphnia in ppm*	*Days Fish Fed on Daphnia Exposed to Insecticide*	*Insecticide Concentration*[a] *in Fish in ppm*	*Rate of Insecticide Uptake From Food By Fish* $\left(\frac{\text{ppm in Fish}}{\text{ppm in Daphnia}}\right)$	*Ratio of Rates of Uptake by Fish, DDT to Methoxychlor*
Methoxychlor				
22.6	6	0.14	0.006	. . .
21.3	8	0.07	0.003	. . .
21.7	20	0.17	0.008	. . .
DDT				
25.1	6	3.10	0.124	21.0
25.9	8	3.69	0.143	48.0
26.6	20	7.80	0.293	38.0

[a]All such concentrations were calculated on a dry-weight basis.

and Fig. 5. There was little difference in the uptake of the two insecticides from water by the daphnia. However, when the daphnia entered the food chain, the methoxychlor content in the fish rapidly reached a steady state, while the concentration of DDT continued to increase. From the 6th to the 20th day the methoxychlor level in the guppies increased only from 0.14 to 0.17 ppm, but the DDT level increased from 3.10 to 7.80 ppm. The differences in concentration of the two compounds are shown by the relative values of the rates of uptake by the fish from the daphnia (column 4 of Table 5) and by the ratios of the rates of uptake (column 5). The difference in accumulation is due to the presence of alkoxy groups on the aryl rings in methoxychlor, which cause it, unlike DDT, to be subject to attack by multifunction oxidases of the fish and, therefore, to be rapidly metabolized and excreted.

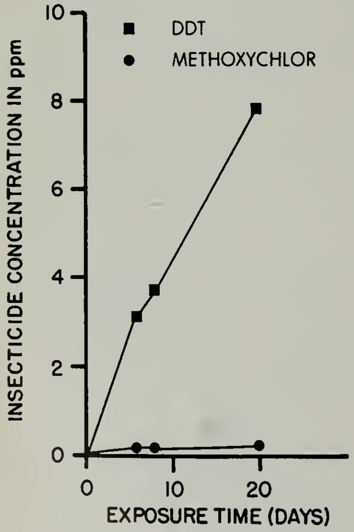

Fig. 5. — Uptake of ^{14}C-DDT and ^{3}H-methoxychlor by guppies in a daphnia-to-guppy food chain. The daphnia were held in water containing 0.003 ppm of DDT or methoxychlor for 48 hours before being fed to the fish.

In general, our investigations show that methoxychlor appears to be readily biodegradable in fishes and in some, though not all, other aquatic organisms. Thus, it seems to be a safer insecticide than DDT to use in or near aquatic environments.

SUMMARY

Comparative studies were made of the uptake and metabolism by three species of fishes, by daphnia, and by a snail of radiolabeled methoxychlor and DDT. Tilapia and green sunfish exposed over a 31-day period to the

radiolabeled insecticides at levels of 0.001, 0.003, and 0.01 ppm in water concentrated DDT as much as 10,600-fold and methoxychlor about 200-fold. However, when tilapia were transferred to water with no insecticide following 12 days of exposure to 0.003 ppm of insecticide in water, the residues of methoxychlor decreased within 15 days from 8 ppm to 0.0001 ppm and DDT from 13 to 1 ppm, a 10,000-fold difference between the concentrations of DDT and methoxychlor.

Significant differences were found between the rates at which tilapia and green sunfish metabolized DDT and methoxychlor. Tilapia metabolized DDT to a greater extent and contained DDD as a major metabolite, while green sunfish contained only small amounts of DDD. Tilapia also metabolized methoxychlor more rapidly than green sunfish metabolized it and contained higher amounts of mono- and bis-phenols produced by *O*-demethylation.

Daphnia in water containing DDT or methoxychlor concentrated DDT at nearly the same rate at which they concentrated methoxychlor to about twice that rate. When daphnia containing either radiolabeled DDT or methoxychlor were fed to guppies to complete a food chain, DDT was rapidly concentrated in the fish, reaching levels of about 8 ppm in 20 days, while methoxychlor concentrations never rose beyond 0.17 ppm. Thus, methoxychlor appears readily biodegradable in fishes. However, the snail used in this investigation could not rapidly metabolize either DDT or methoxychlor and accumulated both to high levels.

LITERATURE CITED

BRIDGES, W. R., B. J. KALLMAN, and A. K. ANDREWS. 1963. Persistence of DDT and its metabolites in a farm pond. American Fisheries Society Transactions 92(4): 421–427.

CAIRNS, JOHN, JR. 1969. Fish bioassays—reproducibility and rating. Revista de Biologia 7(1-2):7–12.

COPE, OLIVER B. 1961. Effects of DDT spraying for spruce budworm on fish in the Yellowstone River system. American Fisheries Society Transactions 90(3):239–251.

GAKSTATTER, JACK H., and CHARLES M. WEISS. 1967. The elimination of DDT-C^{14}, dieldrin-C^{14}, and lindane-C^{14} from fish following a single sublethal exposure in aquaria. American Fisheries Society Transactions 96(3):301–307.

HARRISON, H. L., O. L. LOUCKS, J. W. MITCHELL, D. F. PARKHURST, C. R. TRACY, D. G. WATTS, and V. J. YANNACONE, JR. 1970. Systems studies of DDT transport. Science 170(3957):503–508.

KAPOOR, INDER P., ROBERT L. METCALF, ROBERT F. NYSTROM, and GURCHARAN K. SANGHA. 1970. Comparative metabolism of methoxychlor, methiochlor, and DDT in mouse, insects, and in a model ecosystem. Agricultural and Food Chemistry Journal 18(6):1145–1152.

KENNEDY, HARRY D., LAFAYETTE L. ELLER, and DAVID F. WALSH. 1970. Chronic effects of methoxychlor on bluegills and aquatic invertebrates. U.S. Department of the Interior, Bureau of Sport Fisheries and Wildlife Technical Paper 53. 18 p.

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