standard were applied to each TLC plate on each analysis.

When the 2-, 4-, and 72-h excreta samples were partitioned and chromatographed on columns, about 31–33% (Table IV) of the extractable ^{14}C activity was eluted with benzene–acetone, while another 29–37% eluted with the more polar solvent. Results in Table V show that most of the ^{14}C activity of the benzene–acetone eluates had the same R_f and ultraviolet light reaction as standard [^{14}C]-zearalenone and cochromatographed with it, while the acetone eluates contained more polar, labeled metabolites which separated completely from [^{14}C]zearalenone on cochromatography.

When the pooled bile samples were extracted with ethyl acetate, only about 4% of the ^{14}C was recovered in the extract (Table IV). Of this, only about 8% could be accounted for as unchanged zearalenone on TLC plates (Table V). However, when the previously extracted pooled bile was hydrolyzed with β-glucuronidase (Dorough et al., 1974) 24 h at 37 °C, 82% of the remaining ^{14}C activity became extractable with ethyl acetate. About 25% of this appeared to be [^{14}C]zearalenone, as determined by TLC, while approximately 50% was in the form of polar metabolites which eluted from silica gel columns with acetone and separated into two major spots on thin-layer chromatograms. Neither of these spots cochromatographed with [^{14}C]zearalenone. Further characterization of these metabolites was not possible without appropriate standard compounds to be used for comparison.

Table VI clearly indicates that most of the ^{14}C residues in egg yolk and clutch are lipophilic metabolites (92–93% remaining in the chloroform layer).

Only 50–60% of the ^{14}C residues in liver samples were extractable with ethyl acetate (Table VI) and it is probable that the unextracted portion reflects a continuing production of glucuronide conjugates. Most of the ^{14}C residues extracted from the liver samples were lipophilic metabolites and are probably the source of the radioactivity found in egg yolk and clutch. Only small amounts (1–6%) of the extractable dpm were eluted from silica gel columns with benzene–acetone (95:5) and there was insufficient radioactivity for detection on thin-layer plates. The elution patterns from the columns, however, would suggest the presence of small quantities of unchanged [^{14}C]zearalenone and polar metabolites.

In summary, the following conclusions appear justified based on the data presented: (1) most (about 94%) of a single dose of zearalenone had been excreted within 72 h after administration, (2) about one-third of the dose was excreted as unchanged zearalenone while another one-third was a polar metabolite, (3) zearalenone was readily conjugated with glucuronic acid, and (4) there appeared to be no major retention sites in edible muscle tissues but persistent levels of lipophilic metabolite(s) of unknown composition and toxicological significance were detected in egg yolks for at least 72 h after administration of the parent compound.

Thus, it would appear that after a single exposure of laying hens to feed contaminated with low levels of zearalenone, the health hazard to the human population would be minimal. However, if the exposure time was prolonged, it is possible that significant levels of the lipophilic metabolite(s) might accumulate in egg yolk. Further work to elucidate the structure and toxicity of such metabolite(s) appears justified.

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LITERATURE CITED

Dailey, R. E., Blaschka, A. M., Brouwer, E. A., *J. Toxicol. Environ. Health* **3**, 479–489 (1977).
Dorough, H. W., Cardona, R. A., Lolmaugh, S. C., *Drug Metab. Dispos.* **2**, 129–139 (1974).
Epply, R. M., *J. Assoc. Off. Anal. Chem.* **51**, 74–78 (1968).
Mirocha, C. J., Pathre, S. V., Christensen, C. M., in "Mycotoxins in Human and Animal Health", Rodricks, J. V., Hesseltine, C. W., Mehlman, M. A., Ed., Pathotox Publishers, Inc., Park Forest South, IL, 1977.
Mirocha, C. J., Schauerhamer, B., Pathre, S. V., *J. Assoc. Off. Anal. Chem.* **57**, 1104–1110 (1974).
Steele, J. A., Mirocha, C. J., Pathre, S. V., *J. Agric. Food Chem.* **24**, 89–97 (1976).
Stoloff, L., Henry, S., Francis, O. J., Jr., *J. Assoc. Off. Anal. Chem.* **59**, 118–121 (1976).
Ware, G. M., Thorpe, C. W., *J. Assoc. Off. Anal. Chem.* **61**, 1058–1062 (1978).

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Metabolism of *cis*-[^{14}C]Chlordane and *cis*-[^{14}C]Photochlordane in Bluegill Fish

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Bluegill fish rapidly absorbed *cis*-[^{14}C]chlordane or *cis*-[^{14}C]photochlordane during a 48-h exposure to 5 parts per billion of each insecticide. Elimination of chlordane was linear but slow during the 6-week period, while that of photochlordane was biphasic, being rapid in the early phase (first 3 weeks). Less than 7% of the radioactivity retained in the chlordane-treated fish was in the form of two conjugates which on acid hydrolysis yielded at least eight hydroxylated products. In the case of photochlordane, 16% of the radioactivity in fish was in the form of nine apolar and five polar metabolites. Twenty-five percent of the radioactivity excreted in water by photochlordane-treated fish was in the form of two metabolites, the remaining being unchanged photochlordane.

Contamination of aquatic environments with chlorinated cyclodiene insecticides (Brooks, 1974; Matsumura, 1975; Edwards, 1977) and their accumulation and ecological concentration by aquatic food chains (Craig and Rudd, 1974; Metcalf, 1977) can result in high levels of their residues in organisms at higher trophic levels (Rudd, 1964; Woodwell et al., 1967; Sanborn et al., 1976; Blus et al., 1977). The residues of cyclodiene insecticides, because of

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their prolonged and extensive use, will persist even in the United States where their use is restricted/banned for considerable time (Matsumura, 1972). Chlordanes have been used for both domestic and agricultural control of insects. Their residues are now common in the U.S. in water (Lichtenberg et al., 1970; Bevenue et al., 1972; Burns et al., 1975; Mattraw, 1975; Tanita et al., 1976) and bottom sediments (Bevenue et al., 1972; Law and Goerlitz, 1974; Mattraw, 1975). Residues of chlordanes and their bioalteration products have been found in fish (Miles and Harris, 1973), pelicans (Blus et al., 1977), and aquatic invertebrates (Bugg et al., 1967; Godsil and Johnson, 1968; Tanita et al., 1976). Chlordanes are refractive to bioalteration in rats (Barnett and Dorough, 1974; Brimfield et al., 1978) but more so in freshwater goldfish (Feroz and Khan, 1979a) and cichlid (Feroz and Khan, 1979b). Since cyclodienes are extremely toxic to fish (Tarzwell, 1963; Podowski et al., 1979), the knowledge of their fate (persistence, metabolism, and disposition) and effects on various fish species has been needed for a long time. Studies of the metabolism of *cis*-[^{14}C]chlordane (one of the major and more stable constituent of the technical chlordane) were therefore carried out in Lake Michigan fish, bluegill (*Lepomis macrochirus*).

The residues of cyclodiene insecticides have been shown to undergo molecular changes under sunlight and ultraviolet light (Rosen et al., 1966, 1969; Bennon, 1969; Khan et al., 1969; Vollner et al., 1969; Podowski et al., 1979). The photoisomers of cylodienes are generally more toxic than their parents to mammals, fish, and invertebrates. This has been observed with photoaldrin, photodieldrin (Rosen et al., 1966, 1969; Khan et al., 1973), photoheptachlor, and *cis*-photochlordane (Podowski et al., 1979). *cis*-Photochlordane has been observed to be twice as toxic as *cis*-chlordane to bluegill and goldfish (Podowski et al., 1979). In spite of the significance of this terminal environmental residue of the commonly used chlordane, information on the metabolic fate of *cis*-photochlordane is completely lacking. Investigations were therefore undertaken to understand the metabolic fate of *cis*-[^{14}C]photochlordane in bluegills.

MATERIALS AND METHODS

Fish. Bluegill fish (*Lepomis macrochirus*) originating from McGraw Wildlife Foundation, East Dundee, IL, were used in this investigation. The fish were gradually acclimated to warmer temperature (20–22 °C) at the John G. Shedd Aquarium, Chicago, IL, for several weeks. Later, the fish were maintained at the laboratory in aquaria containing dechlorinated tap water for over 4 weeks before exposing them to the insecticide.

Chemicals. *cis*-[^{14}C]Chlordane, 99+% pure, sp. act. 10.9 mCi/mmol, prepared by New England Nuclear, was a gift from Velsicol Chemical Corporation, Chicago, IL. *cis*-[^{14}C]Photochlordane was prepared by exposing equimolor concentrations of *cis*-[^{14}C]chlordane and benzophenon (Fisher Scientific Co.) in acetone to ultraviolet light employing the method described by Podowski et al. (1979). *cis*-[^{14}C]Photochlordane was purified by using 0.5 mm thick silica gel G Prekotes (Applied Science Lab., State College, PA) in three runs of heptane. The final purified product behaved as a single compound as judged by electron-capture detector gas chromatography.

The solvents used were of analytical reagent grades.

Exposure of Fish. (*1*) *For Absorption and Elimination Studies.* A stock solution of 80 μg/mL (2.126 μCi/mL) was prepared in acetone. Final concentration of each insecticide in 6 L of water contained in 10-L glass jars (12 in. diameter) was 5 parts per billion (ppb). The fish were starved for 48 h before the commencement of the exposure; their weight ranged from 72 to 75 g in the case of *cis*-chlordane and from 40 to 60 g in the case of *cis*-photochlordane. Each jar containing only one fish was constantly bubbled with air and its top securely covered with aluminum foil to prevent loss of insecticide due to volatilization. Additional jars, containing 5 ppb of the insecticide, with similar bubbling of air, but without fish served as controls. These runs were made in triplicate. One-milliliter samples of the water were withdrawn from each jar at several intervals following the exposure to determine radioactivity present in water. At the end of the 48-h exposure, the fish were transferred to insecticide-free water to observe the elimination of the absorbed radioactivity in water. One-milliliter samples of water were analyzed over a period of 6 weeks for chlordane which was extended to 15 weeks for *cis*-photochlordane. Feeding was resumed and water was changed every fourth day.

(*2*) *For Metabolism Studies.* Exposure concentration was increased in order to study metabolism. This was done by exposing three fish (30–50 g each) to 25 ppb in 8 L of water for 48 h in the case of *cis*-chlordane. Because of the higher toxicity of *cis*-photochlordane (LC_{50} = 12 ppb), the fish were exposed first for 48 h to 5 ppb and then exposed twice to 2.5 ppb for 48 h each time. The fish receiving three consecutive treatments were transferred to insecticide-free water. These were observed for the following 12 days (giving a change to clean water every fourth day) and then killed and frozen.

In the case of *cis*-chlordane, three fish were used in 8 L of water, as the available fish were generally smaller in size. Following the 48-h exposure, they were frozen (not left for 12 days in clean water as in the case of *cis*-photochlordane) for they showed delayed toxicity symptoms.

Extraction of Radioactivity from Whole Fish. The bluegill exposed to *cis*-[^{14}C]chlordane or *cis*-[^{14}C]photochlordane were processed for solvent extraction as follows: The weighed fish, chopped into small segments, was pulverized to dry powder in a mortar and pestle in the presence of anhydrous sodium sulfate (1:4 w/w). Subsequently, the pulverized powder was extracted three times with diethyl ether (400 mL for 100 g). These extracts, following radioassay, were combined. The residue was further extracted two times with petroleum ether (250 mL for 100 g) in order to extract apolar metabolites. Finally, the residue was extracted three times with methanol (250 mL for 100 g) to obtain polar metabolites. Each of these extracts was individually assayed for radioactivity and then pooled.

Purification. The solvent extracts, concentrated under a stream of nitrogen, containing large amounts of fatty material were cleaned up by repeated preparative thin-layer chromatography (TLC) on 0.5-mm silica gel Prekotes using the following: (1) benzene/ethyl acetate (1:1) for apolar extracts of chlordane-treated fish, (2) two runs of heptane for photochlordane-treated fish extracts, and (3) butanol/acetic acid/water (6:1:1) for polar extracts in both the cases.

Analysis. TLC was employed for separation and identification of metabolities. Samples were spotted on 0.25 mm thick silica gel 60 F-254 (E. Merck, Darmstadt, West Germany) and developed in the respective solvent systems. The radioactive areas of TLC plates were located by exposing the developed plates to Eastman Kodak no-screen films (NS-2T) for periods ranging from 2 to 6 weeks. For quantitative measurements the radioactive areas were scraped and extracted with suitable solvents (diethyl ether or methanol) and aliquots (10 μL to 1 mL) taken into vials

Table I

code	metabolites/zones resolved with TLC from
WE_1 to WE_2	ether extract of water
WM_1 to WM_2	methanol extract of water
FE_I to FE_{VII}	ether extract of fish (zone)
FE_1 to FE_{10}	ether extract of fish upon rechromatography of (zones)
FP_1 to FP_5	petroleum ether extract of fish
FM_1 to FM_6	methanol extract of fish
$FM_{1.1}$ to $FM_{1.5}$	released metabolites following acid hydrolysis of FM_1
$FM_{2.1}$ to $FM_{2.5}$	released metabolites following acid hydrolysis of FM_2

for liquid scintillation counting.

Gas–liquid chromatography (GLC) was performed on Packard Model 7300 series with glass columns and ^3H electron-capture detectors. The column used (3.5 × 2 mm) was packed with 3% SE-30 on 80–100 mesh Gas-Chrom Q. The column temperature was 190 °C and the detector and inlet temperatures were 220 °C. Nitrogen flow rate was 40 mL/min.

Radioassay. A liquid scintillation spectrometer (Packard Instruments, Model 3900 with a Model 544 absolute activity analyzer) was used for radioactive measurements. Samples of solvent extracts (10 μL to 1 mL) and water (1 mL) were counted with 10 mL of Insta-Gel (Packard Instruments). Counting efficiency determined by using chlordane and photochlordane as internal standards was considered for corrections.

Lyophilization. Samples of water containing eliminated metabolites were lyophilized (Virtis, Research Equipment, Gardiner, NY). The dry powder was extracted first with diethyl ether and then with methanol. The concentrated extracts were analyzed by TLC and autoradiography.

Metabolite Designation. Table I shows a summary of the codes used in the text, subsequent tables, and figures to designate the metabolites/radioactive zones extracted from elimination water (W) and exposed fish (F) using solvents ether (E), petroleum ether (P), and methanol (M).

RESULTS

Absorption. Radioassay of the exposure water from experimental and control jars following the exposure was carried out. Decline of activity from the exposure water corrected after deducting the loss in control water was ascribed as the radioactivity absorbed by the fish. The absorption was very rapid and more than 95% of the activity (5 ppb in 6 L) was lost from the experimental jars both with *cis*-[^{14}C]chlordane and *cis*-[^{14}C]photochlordane.

Elimination. The bluegill, preexposed for 48 h to 5 ppb of *cis*-[^{14}C]chlordane or *cis*-[^{14}C]photochlordane, were transferred to insecticide-free water, and the water was radioassayed to determine the rate of elimination of radioactivity. Figure 1A gives the percent of absorbed (based on the above data 28.65 μg/fish) radioactivity eliminated in water. Chlordane-treated fish eliminated radioactivity much slower than fish treated with *cis*-photochlordane (Figure 1B). Only about 20% of the absorbed activity was eliminated in about 6 weeks by chlordane-treated fish, while the same amount of radioactivity was eliminated in less than 2 weeks by *cis*-photochlordane treated fish. Elimination was linear over a period of 6 weeks in the case of chlordane, while in the case of *cis*-photochlordane the pattern was biphasic. Subsequent to the 3-week period of linear elimination a much slower period of elimination was observed till about 5 weeks. There was virtually no elimination after a period of 9 weeks. Similar differences in elimination of *cis*-chlordane and *cis*-photochlordane have been observed in goldfish (Ducat and Khan, 1979).

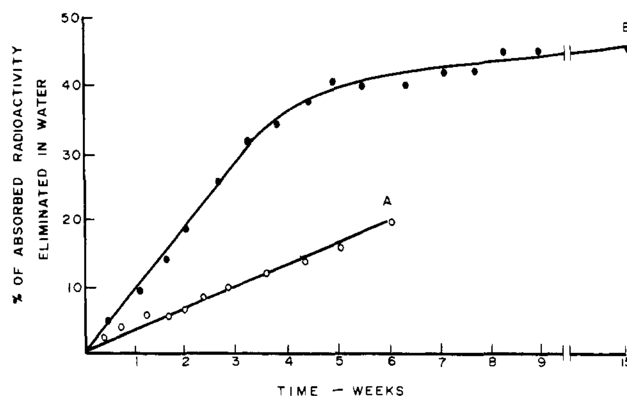

Figure 1. Elimination of radioactivity by bluegill on transfer to clean water following 48 h of exposure to 5 ppb of (A) *cis*-[^{14}C]chlordane (absorbed amount 28.65 μg/fish), (B) *cis*-[^{14}C]-photochlordane (absorbed amount 28.50 μg/fish). Average of three fish, each exposed individually in 6 L. The fish were transferred to clean water every fourth day.

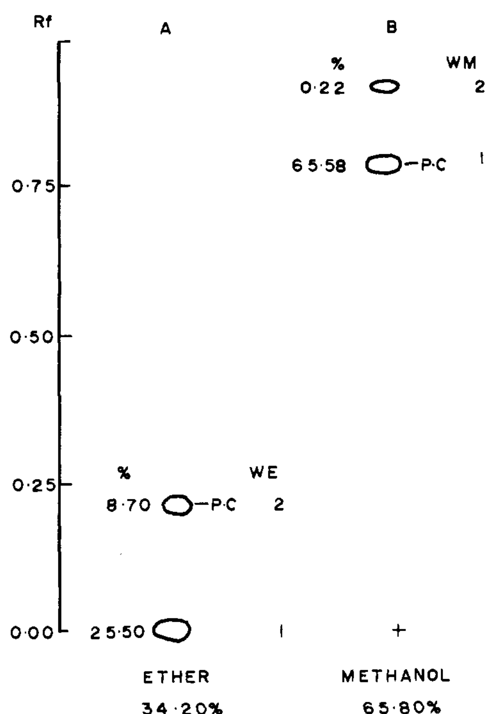

Figure 2. TLC data of the products eliminated in water on transfer of *cis*-[^{14}C]photochlordane-treated fish to water. PC = photochlordane; solvent system: (A) two runs of heptane, (B) butanol/acetic acid/water (6:1:1). Recovery of metabolites is presented as percent activity eliminated in water.

A liter of water containing about 13000 dpm eliminated by one of the fishes in 4 days treated with *cis*-[^{14}C]photochlordane was lyophilized. The dry residue extracted with diethyl ether (3 × 25 mL) and later with methanol (3 × 25 mL) gave the following recoveries shown as percent radioactivity in water: diethyl ether 34.2% and methanol 65.8%. The resolution of these extracts in the TLC system is shown in Figures 2A and 2B.

Extractability of Radioactivity from Whole Fish. Recovery values are shown as percent of the radioactivity present in fish. Diethyl ether, used first in the sequence of solvent extraction of pulverized powder of fish treated with 25 ppb of *cis*-[^{14}C]chlordane, recovered the largest percentage of radioactivity (70.38%). Petroleum ether

Table II. TLC R_f Values and GLC Retention Times (t_R) for *cis*-Chlordane Metabolites and the Authentic Reference Compounds in Indicated Solvent System

standard or metabolite	R_f values on TLC: benzene/ ethyl acetate (1:1)	R_f values on TLC: hexane/ ethyl acetate (9:1)	t_R on GLC,[a] min
standard			
dihydroxychlordene	0.16	0.00	5.1
1,2-dihydroxyheptachlor	0.39	0.05	6.8
1-hydroxychlordene	0.49	0.04	2.5
chlordenechlorohydrin	0.56	0.08	4.1
cis-chlordane	0.66	0.41	3.5
metabolites			
$FM_{1.1}$	0.00	0.00	ND
$FM_{1.2}$	0.02	0.00	ND
$FM_{1.3}$	0.06	0.05	ND
$FM_{1.4}$	0.56	0.08	4.1
$FM_{1.5}$	0.61	0.09	2.7
$FM_{2.1}$	0.00	0.00	ND
$FM_{2.2}$	0.10	0.02	ND
$FM_{2.3}$	0.39	0.05	6.8
$FM_{2.4}$	0.56	0.08	4.1
$FM_{2.5}$	0.61	0.10	3.2
FM_3	0.66	0.41	3.5
FE_1	0.66	0.41	3.5
FP_1	0.66	0.41	3.5

[a] GLC glass column 3.5 ft × 2 mm packed with 3% SE-30 on 80–100 mesh Gas-Chrom Q; column temperature, 190 °C, detector and inlet temperature, 220 °C; nitrogen gas flow rate, 40 mL/min; sensitivity, 3×10^{-9}; ND, not determined.

recovered an additional 2.60%. The residue when further extracted with methanol recovered 16.52% of the radioactivity. The total radioactivity recovered in these three solvent extracts amounted to 89.50% of the activity retained in fish.

The fish exposed to multiple treatments of 5, 2.5, and 2.5 ppb of *cis*-[^{14}C]photochlordane when extracted similarly showed 84.14% recovery of the retained activity. 74.93% was in diethyl ether and petroleum ether and 9.21% in methanol extracts.

The total recoveries of 89.50% in the case of chlordane and 84.14% in the case of photochlordane by the above procedure (pulverization and the sequential extraction with the above mentioned solvents) was considered adequate. The losses of 10.50 and 15.86% of the respective activities either remained totally unextractable as bound to residue or presumably lost as CO_2 which was left untrapped.

Metabolities from Whole Fish. *cis-[^{14}C]Chlordane.* The diethyl ether (FE_1) and petroleum ether (FP_1) extracts of fish analyzed by TLC in hexane/ethyl acetate (9:1) system revealed to consist entirely of unchanged chlordane (Figure 3A) as confirmed by cochromatography using TLC (indicated solvent systems) and GLC (Table II). The methanol extract separated into three radioactive spots (FM_1 to FM_3) in butanol/acetic acid/water (6:1:1) system (Figure 3B). The radioactive spot FM_3, comprising 9.93% activity, was identified as unchanged chlordane. FM_2 and FM_1 together, constituting 6.59%, were assumed to be conjugates considering that they were unextractable with petroleum ether or hexane.

Conjugates FM_1 and FM_2 were subjected to acid hydrolysis by incubating known amounts with 1 mL of 1 N HCl for 2 h at 80 °C. The results of recovery of radioactivity when extracted with diethyl ether are shown in Table III. The released radioactivity of FM_1 and FM_2 when chromatographed on TLC in benzene/ethyl acetate (1:1) separated into five spots each designated as $FM_{1.1}$ to $FM_{1.5}$ and $FM_{2.1}$ to $FM_{2.5}$ (Figures 4A and 4B).

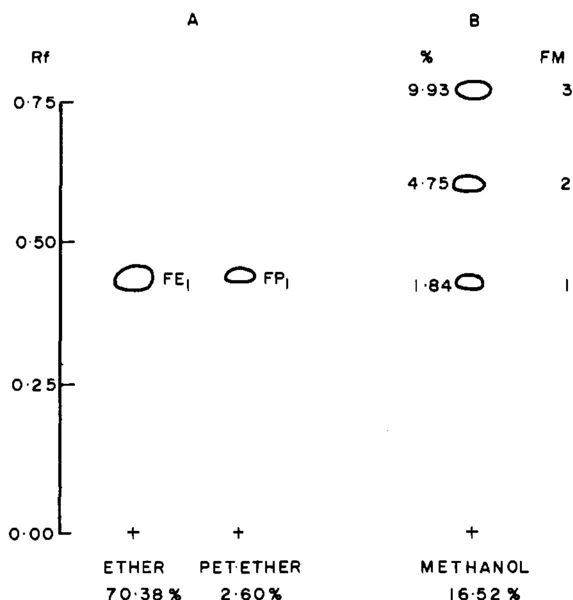

Figure 3. TLC data of extracts of bluegill exposed to *cis*-[^{14}C]chlordane for 48 h. Solvent system (A) hexane/ethyl acetate (9:1), (B) butanol/acetic acid/water (6:1:1). FE_1, FP_1, and FM_3 identified as *cis*-chlordane.

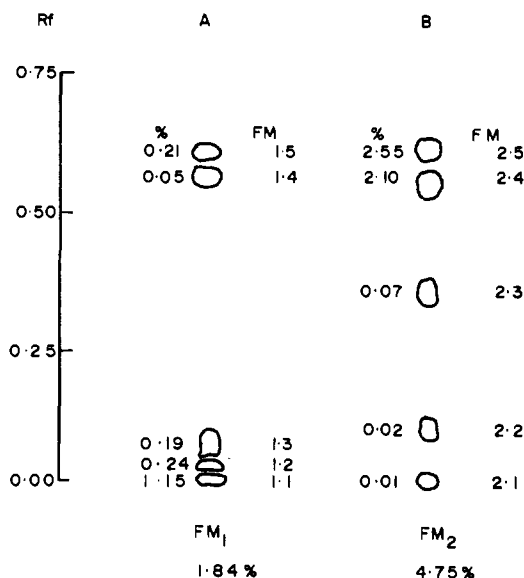

Figure 4. TLC data of the acid hydrolystate of conjugates FM_1 and FM_2 (from Figure 3B) from methanol extract of bluegill exposed to *cis*-[^{14}C]chlordane. FM_1 and FM_2 separately incubated with 1 mL of 1 N HCl for 2 h at 80 °C. The ether extracts of released radioactivity chromatographed in benzene/ethyl acetate (1:1), $FM_{1.1}$ to $FM_{1.5}$ resolved from FM_1 and $FM_{2.1}$ to $FM_{2.5}$ resolved from FM_2. $FM_{1.4}$ and $FM_{2.4}$ are tentatively identified as chlordenechlorohydrin and $FM_{2.3}$ as heptachlorodiol.

Table III. Recovery of Radioactivity in Diethyl Ether following Acid Hydrolysis of Conjugates FM_1 and FM_2

	dpm incubated	dpm recov	% recov
FM_1	41450	32750	79.01
FM_2	60750	54960	90.46

Metabolities $FM_{2.4}$ and $FM_{1.4}$, together amounting to 2.15%, matched with authentic chlordene chlorohydrin both in TLC and GLC behaviors (Table II). $FM_{2.3}$, found in small amount (0.07%) was tentatively identified as 1,2-dihydroxyheptachlor by similar cochromatography. The spots remaining at the origin ($FM_{1.1}$ and $FM_{2.1}$) being very polar are speculated to be the trihydroxy derivatives,

Table IV. TLC R_f Values of Metabolites of *cis*-Photochlordane Extracted from Bluegill

metabolites	solvent systems			
	heptane, 2 runs	benzene/ethyl acetate, (3:1), 2 runs	butanol/acetic acid H_2O (6:1:1)	chloroform/methanol (9:1)
FE_1	0.01	0.86	0.62	0.80
FE_2	0.04	0.88	0.64	0.83
FE_3	0.06	0.90	0.70	0.85
FE_4	0.10	0.91		
FE_5	0.15	0.91		
FE_6	0.22	0.92	0.78	0.91
FE_7	0.29	0.92		
FE_8	0.37	0.92		
FE_9	0.43	0.92		
FE_{10}	0.50	0.93		
FP_1	0.01	0.86		
FP_2	0.04	0.88		
FP_3	0.22	0.92	0.78	0.91
FP_4	0.43	0.92		
FP_5	0.50	0.93		
FM_1	0.00	0.00	0.25	0.00
FM_2	0.00	0.00	0.40	0.006
FM_3	0.00	0.00	0.48	0.01
FM_4	0.00	0.00	0.54	0.05
FM_5	0.00	0.00	0.57	0.09
FM_6	0.22	0.92	0.78	0.91
WE_2	0.22	0.92		
WM_1	0.22	0.92		
cis-photochlordane	0.22	0.92	0.78	0.91

Table V. GLC Retention Times (t_R) for *cis*-Photochlordane and Its Metabolites (Conditions Described in Table I)

metabolites	t_R, min
FE_1	
FE_2	
FE_3	2.3
FE_4	3.2
FE_5	4.8
FE_6	6.7
FE_7	
FE_8	
FE_9	
FE_{10}	8.5
cis-photochlordane	6.7
WE_2	6.7
WM_1	6.7

while $FM_{1.2}$, $FM_{1.3}$, and $FM_{2.2}$ considering that they did migrate from the origin could be dihydroxy in nature. On the basis of TLC and GLC characteristics, $FM_{1.5}$ and $FM_{2.5}$ are assumed to be monohydroxy derivatives.

cis-[14C]Photochlordane. The TLC of ether extracts of the fish in two runs of heptane revealed seven radioactive zones (FE_I to FE_{VII}), which further resolved into ten spots, designated Fe_1 to Fe_{10} upon rechromatography (Figure 5A). Table IV gives the R_f values of these metabolites in several solvent systems. The GLC retention times (t_R values) are presented in Table V.

The fish extract obtained with petroleum ether resolved into five spots, FP_1 to FP_5, which correspond to FE_1, FE_2, FE_6, FE_9, and FE_{10}, respectively (Figure 5B and Table IV).

The methanol extract when chromatographed in a solvent system comprising of butanol/acetic acid/water (6:1:1) separated into six spots (Figure 5C).

Identification of these metabolities using cochromatography was not possible due to lack of any known standards. Few of the standards of the well known *cis*-chlordane metabolities when chromatographed by TLC did not match with any of the metabolities of *cis*-photochlordane

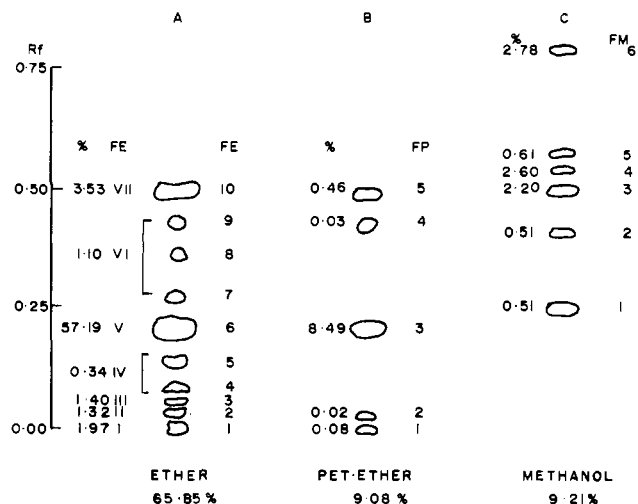

Figure 5. TLC data of extracts of bluegill exposed to *cis*-[14C]photochlordane for 48 h. Solvent systems: (A and B) two runs in heptane, (C) butanol/acetic acid/water (6:1:1). FE I to FE VII are the seven radioactive zones of diethyl ether extract which resolved into ten spots FE_1 to FE_{10} upon rechromatography. FP_1 to FP_5 resolved from petroleum ether extracts, FM_1 to FM_6 resolved for methanol extract. FE_6, FP_3, FM_6 identified as *cis*-photochlordane.

Table VI. TLC R_f Values of *cis*-Chlordane Metabolite Standards for Comparison with Those of *cis*-Photochlordane Metabolites (Table III)

standard chlordane metabolites	R_f values in		
	heptane, 2 runs	benzene/ethyl, 2 runs	acetate (3:1), 1 run
1,2-dihydroxyheptachlor	0.0	0.23	0.16
oxychlordane	0.24	0.86	0.75
1,2-dichlorochlordene	0.49	0.87	0.77
α-chlordane	0.26	0.86	0.76
γ-chlordane	0.24	0.85	0.77
1-hydroxychlordane	0.00	0.58	0.43

(Table VI). Any spectroscopic method could not be attempted to characterize these compounds because of the low amounts recovered after several purifications. Metabolites FE_6, FP_3, and FM_6 (Figure 5) were identified by TLC (Table IV) and GLC (Table V) as the parent *cis*-photochlordane, which altogether constituted 68.46% of the radioactivity. The other most abundant metabolites were FE_{10} + FP_5 (3.99%), FM_4 (2.60%), and FM_3 (2.20%).

DISCUSSION

Both *cis*-[14C]chlordane and *cis*-[14C]photochlordane, at a level of 5 ppb, were readily absorbed by bluegill as judged by the disappearance of almost total activity from the exposure water in the first 24 h. However, two additional exposures of photochlordane at levels of 2.5 ppb reduced the absorption rate.

The bluegill exposed to [14C]chlordane eliminated 20% of the absorbed radioactivity linearly at a rather slow rate over a period of about 6 weeks. Similar slow rate of elimination was reported by Feroz and Khan (1979b) in tropical cichlid. The African clawed frog, *Xenopus laevis*, eliminated, under similar experimental conditions, three times as much activity as bluegill (Sudershan and Khan, 1979). Because of low levels of radioactivity, the nature of the products excreted in water was not investigated.

Photochlordane-exposed fish exhibited a biphasic pattern with a relatively faster linear elimination of about 30% of the radioactivity during the first 3 weeks, followed by a period of much slower elimination till about 9 weeks, beyond which time there was practically no elimination

over a period of 15 weeks. About 45% of the absorbed radioactivity was eliminated during 6 weeks as compared with 20% seen with *cis*-chlordane. The data are indicative that more than 50% of the absorbed activity is retained at the end of 15 weeks. An examination of elimination products in water (Figure 2) reveals that about 75% of the excreted activity was attributable to the unchanged parent compound, while the rest was due to a hydroxylated or conjugated metabolite with a small amount of a compound more apolar than photochlordane.

From the data on metabolites, bluegill degrades less than 7% of chlordane in 48 h to hydroxylated and conjugated products, including chlordene chlorohydrin (which was in appreciable amounts) and heptachlor diol. Goldfish (Feroz and Khan, 1979a) was less efficient than bluegill in degrading chlordane (less than 1% chlordane was metabolized in whole fish). Bluegills appear to metabolize about 16% of photochlordane to several free and conjugated products. It may, however, be pointed out that this higher degradation could have resulted during the 12-day post-exposure period during which the bluegill were kept in clean water.

The striking feature of the metabolism of chlordane in bluegill is the formation of conjugates of the hydroxy derivatives and the complete absence of apolar metabolites. The bluegill differ from other animals in the total absence of oxychlordane, which is one of the predominant metabolities in rats (Barnett and Dorough, 1974; Tashiro and Matsumura, 1977) and cockroach (Feroz and Khan, 1979c). Thus the absence of epoxidation and the presence of a mechanism of hydroxylation followed by conjugation seems to be the most active mode of metabolism of chlordane in bluegill.

A consideration of the number of products formed from photochlordane allows one to infer that there is a great divergence in the pathways of metabolic reactions undergone by this compound in bluegill. The lack of standard compounds and the low amounts of the recovered metabolities poses a difficult situation to identify these products. But judging from the chromatographic behavior, it may be speculated that products with low R_f values are preseumably hydroxylated ones. The other products could arise by oxidative and dechlorination processes. The metabolites separated in butanol/acetic acid/water system are apparently the conjugated products. Bluegills seem to resemble rats in the pathway of metabolism of *cis*-photochlordane. However, the metabolism of *cis*-photochlordane is more extensive in rats (in 5 days) (Feroz and Khan, 1980) than in bluegills.

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LITERATURE CITED

Barnett, J. R., Dorough, H. W., *J. Agric. Food Chem.* 22, 612 (1974).

Benson, W. R., *Ann. N.Y. Acad. Sci.* **160**, 7 (1969).

Bevenue, A., Hylin, J. W., Kawano, Y., Kelley, T. W., *Pestic. Monit. J.* **6**, 56 (1972).

Blus, L. J., Neely, B. S. Jr., Lamont, T. G., Mulhern, B., *Pestic. Monit. J.* 11, 40 (1977).

Brimfield, A. A., Street, J. C., Futrell, J., Chatfield, D. A., *Pestic. Biochem. Physiol.* **9**, 84 (1978).

Brooks, G. T., "Chlorinated Insecticides", Vol. 2, CRC Press, Cleveland, OH, 1974, pp 149–150.

Bugg, J. C., Jr., Higgins, J. C., Robertson, E. A., Jr., *Pestic. Monit. J.* **1**, 9 (1967).

Burns, B. G., Peach, M. E., Stiles, D. A., *Pestic. Monit. J.* **9**, 34 (1975).

Craig, R. B., Rudd, R. L., "Survival in Toxic Environments", Khan, M. A. Q., Bederka, J. P., Ed., Academic Press, New York, 1974, p 1.

Ducat, D. A., Khan, M. A. Q., *Arch. Environ. Contam. Toxicol.* 8, 409 (1979).

Edwards, C. A., "Pesticides in Aquatic Environments", Khan, M. A. Q., Ed., Plenum Press, New York, 1977, p 11.

Feroz, M., Khan, M. A. Q., *Bull. Environ. Contam. Toxicol.*, **23**, 64 (1979a).

Feroz, M., Khan, M. A. Q., *J. Agric. Food Chem.*, **27**, 1190 (1979b).

Feroz, M., Khan, M. A. Q., *J. Agric Food Chem.* **27**, 95 (1979c).

Feroz, M., Khan, M. Q. Q., *J. Agric. Food Chem.*, in press (1980).

Godsil, P. J., Johnson, W. C., *Pestic. Monit. J.* **1**, 21 (1968).

Khan, M. A. Q., Rosen, J. D., Sutherland, D. J., *Science* **164**, 318 (1969).

Khan, M. A. Q., Stanton, R. H., Sutherland, D. J., Rosen, J. D., Maitra, N., *Arch. Environ. Contam. Toxicol.* 1, 159 (1973).

Law, L. M., Goerlitz, D. F., *Pestic. Monit. J.* **8**, 33 (1974).

Lichtenberg, J. J., Eichelberger, J. W., Dressman, R. C., Longbottom, J. E., *Pestic. Monit. J.* **4**, 71 (1970).

Matsumura, F., "Toxicology of Insecticides", Plenum Press, New York, 1975, p 355.

Matsumura, F., "Environmental Toxicology of Pesticides", Matsumura, F., Boush, G. M., Misato, T., Eds., Academic Press, New York, 1972, p 33.

Mattraw, H. F., Jr., *Pestic. Monit. J.* **9**, 106 (1975).

Metcalf, R. L., "Pesticides in Aquatic Environments", Khan, M. A. Q. Ed., Plenum Press, New York, 1977, p 127.

Miles, J. R. W., Harris, C. R., *Pestic. Monit. J.* **6**, 363 (1973).

Podowski, A. A., Banerjee, B. C., Feroz, M., Dudek, M. A., Willey, R. L., Khan, M. A. Q., *Arch. Environ. Contam. Toxicol.*, **8**, 509 (1979).

Rosen, J. D., Suterland, D. J., Lipton, G. R., *Bull. Environ. Contam. Toxicol.* **1**, 133 (1966).

Rosen, J. D., Sutherland, D. J., Khan, M. A. Q, *J. Agric. Food Chem.* **17**, 404 (1969).

Rudd, R. L., "Pesticides and the Living Landscape", University of Wisconsin Press, Madison, WI, 1964, p 248.

Sanborn, J. R., Metcalf, R. L., Bruce, W. N., Lu, P. Y., *Environ. Entomol.* **5**, 533 (1976).

Sudershan, P., Khan, M. A. Q., unpublished data, Biological Sciences, University of Illinois, Chicago, IL, 1979.

Tanita, R., Johnson, J. M., Chun, M., Maciolek, J., *Pestic. Monit. J.* **10**, 24 (1976).

Tarzwell, C. M., "Pesticides—Their Use and Effect", Swanson, G. A., Ed., N.Y. State Legislative Symp., Albany, NY, 1963, p 30.

Tashiro, S., Matsumura, F., *J. Agric. Food Chem.* **25**, 872 (1977).

Vollner, L., Klein, W., Korte, F., *Tetrahedron Lett.* **34**, 2967 (1969).

Woodwell, G. M., Wurster, C. F., Jr., Isaacson, P. A., *Science* **156**, 821 (1967).

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