matoes nor potatoes contained significant amounts of 2-imidazoline. In contrast, Vonk (1976) found approximately 8% of applied zineb occurred as 2-imidazoline 2 weeks after treatment. The difference between the present result and the positive findings obtained with [^{14}C]zineb on lettuce (Vonk, 1976) may be attributable to differences in plant species or, more likely, due to removal of any imidazoline by rain since the residue is readily removed by washing. Some leaf surfaces apparently retain imidazoline and approximately 8% of the total EBDC residue can occur as imidazoline 2 weeks after the application of [^{3}H]mancozeb (Lyman, 1971).

Small amounts of ethyleneurea were present in tomatoes while none was detectable in potato tubers (Table III). The residues in tomato were confirmed by high-resolution mass spectrometry with single ion monitoring as previously described (Newsome, 1978). The levels in tomatoes were similar to those observed in an earlier survey of commercial products (Newsome, 1978) and appeared independent of the application rate of mancozeb. Much less ethyleneurea was found than was anticipated from other studies (Lyman, 1971; Vonk, 1976) where levels ranged from 10 to 17% of the total residue.

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LITERATURE CITED

Lyman, W. R., *Pure Appl. Chem., Suppl.*, 243 (1971).
Nash, R. G., *J. Agric. Food Chem.* **24**, 596 (1976).
Newsome, W. H., *J. Agric. Food Chem.* **22**, 886 (1974).
Newsome, W. H., *J. Agric. Food Chem.* **24**, 999 (1976).
Newsome, W. H., Panopio, L. G., *J. Agric. Food Chem.* **26**, 638 (1978).
Newsome, W. H., *J. Agric. Food Chem.* **26**, 1325 (1978).
Ripley, B. D., Cox, D. F., *J. Agric. Food Chem.* **26**, 1137 (1978).
Vonk, J. W., *Meded. Fac. Landbouwwet., Rijksuniv. Gent.* **41**, 1383 (1976).

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Metabolism, Tissue Distribution, and Elimination of *cis*-[^{14}C]Chlordane in the Tropical Freshwater Fish *Cichlasoma* sp.

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Tropical freshwater cichlids, *Cichlasoma* sp., weighing 300 g each, were individually placed in 16 L of water with 80 μg of *cis*-[^{14}C]chlordane for 72 h. The fish rapidly absorbed the compound, accumulating 2.8 ppm in visceral fat, 1.2 ppm in bile, and 1.1 ppm in gall bladder. Recovery of the radioactivity added to water at the end of 72 h was estimated to be 64.8 and 6.1% in fish and exposure water, respectively. The rate of elimination of the absorbed radioactivity was about 2.9% per week. Dichlorochlordene, oxychlordane, chlordene chlorohydrin, dihydroxyheptachlor, dihydroxydihydrochlordene plus four unidentified compounds accounted for 12.5% of the radiocarbon recovered from fish and exposure water. The remainder was determined to be unchanged *cis*-chlordane.

Though extensively used for over two decades, work on the metabolism of chlordanes in comparison with other cyclodienes as aldrin, dieldrin, and heptachlor has been slow. Poonawalla and Korte (1964, 1971) showed that *trans*-chlordane administered to rats and rabbits was rapidly metabolized and eliminated. Identification, characterization, and probable pathway of formation of oxychlordane from chlordanes (Schwemmer et al., 1970; Lawrence et al., 1970; Polen et al., 1971; Street and Blau, 1972) was another important development in the toxicology of chlordanes. Detailed investigation of the in vivo metabolism of chlordanes was carried out by Barnett and Dorough (1974). Two other reports on the metabolism of chlordanes in the rat (Tashiro and Matsumura, 1977; Brimfield et al., 1978) have appeared more recently.

As stated, most of the work on chlordanes yielding important information has so far centered around mammalian species. The fate of these chemicals in other organisms is not well understood. In this area, aquatic organisms require special attention because of their importance in food chain and because contamination of aquatic environments with chlordane residues has been reported. For instance, of 546 samples of surface waters from various parts of the United States during 1964–1968, Lichtenberg et al. (1970) found 1% samples positive for chlordane residues. A survey for pesticide residues in Oahu, Hawaii, in 1970–1971 (Bevenue et al., 1972) showed chlordane residues ranging from 0.004 to 0.009 ppb in nonpotable waters and 190 to 378 ppb in the sediments. A later report from Hawaii Kai Marina (Tanita et al., 1976) indicated widespread occurrence of chlordanes in aquatic environments of Hawaii. Law and Goerlitz (1974) reported very high incidence (92% of the samples) of chlordane residues from sediments of tributaries to San Francisco Bay. Similarly Burns et al. (1975) detected high levels of contamination of water and sediments of Habitant Creek (Nova Scotia, Canada) with chlordanes. Some aquatic environments of Southern Florida were also reported

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positive for chlordane residues (Mattraw, 1975).

Occurrence of chlordanes in aquatic environments has resulted in their residues in fish and other aquatic biota. Miles and Harris (1973) found chlordane residues in muscles of carp, bass, white sucker, lake trout, and cisco from Thames River and Muskoka Lake (Ontario, Canada). The Atlantic menhaden regurgitated by brown pelicans was found to contain chlordanes along with oxychlordane (Blus et al., 1977). Bugg et al. (1967), Casper (1967), Godsil and Johnson (1968), and Tanita et al. (1976) reported chlordane residues in different species of mollusks from various parts of the United States and Canada.

Technical chlordane is a mixture of at least 14 compounds of which *trans*-chlordane, *cis*-chlordane, and heptachlor comprise 24, 19, and 10%, respectively (Saha and Lee, 1969; Brooks, 1974). The data compiled by Brooks (1974) on the toxicity of pure major constituents of technical chlordane indicate that the approximate toxicity ratios for heptachlor, *cis*-chlordane, and *trans*-chlordane in the rat are respectively 1:5:12 (LD_{50} for heptachlor = 100 mg/kg). More or less similar relationship among the toxicities of these insecticides is evident for housefly and mosquito larvae (*Aedes aegypti*). However, the toxicity ratios for heptachlor, *cis*-chlordane, and *trans*-chlordane in bluegills (96 h LC_{50} for heptachlor 64 ppb) and goldfish (96 h LC_{50} for heptachlor 185 ppb) were respectively 1:0.27:2.2 and 1:0.14:2.4 (Podowski et al., 1979), indicating *cis*-chlordane to be the most toxic compound to these species of fish.

In addition to this, *cis*-chlordane appears to be more persistent than *trans*-chlordane in aquatic environments and organisms (Burns et al., 1975; Tanita et al., 1976; Sanborn et al., 1976). Due to their lipophilic nature and stability, chlordanes are concentrated by aquatic biota. Mosquitofish (*Gambusia affinis*) in a model ecosystem concentrated chlordanes by 3061 to 8252 times of the levels in water (Sanborn et al., 1976). Brown pelicans feeding on Atlantic menhaden (containing 12 ppb chlordane residues) showed 240 ppb in their eggs (Blus et al., 1977).

Because of the wide occurrence of chlordanes in water and aquatic animals, the study of accumulation, elimination, and metabolism of *cis*-chlordane in a tropical freshwater fish, *Cichlasoma* sp., was carried out.

MATERIALS AND METHODS

Organisms. The cichlids, *Cichlasoma* sp., from South America, were provided by the John G. Shedd Aquarium, Chicago, IL. The fish weighed 306 ± 9.4 g (n = 6). They were maintained at 26 °C in 20-gal aquaria containing dechlorinated tap water. The animals were acclimated to laboratory conditions for 2 weeks before use. They were fed frozen smelt or small live goldfish at approximately 1–2 g $fish^{-1}$ day^{-1}.

Chemicals and Apparatus. *cis*-[^{14}C]Chlordane (1-*exo*,2-*exo*,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane) was bought from the New England Nuclear Corporation, Boston, MA. This purchase was arranged through the courtesy of the Velsicol Chemical Corporation, Chicago, IL. All carbon atoms in the hexachloronorbornene part of the molecule were labeled. Specific activity of the compound was 10.9 mCi/mmol. It was reported to be 97%+ pure, but when tested in different thin-layer chromatographic (TLC) systems followed by X-ray autoradiography, radiopurity was found to exceed 99%.

Authentic reference standards (heptachlor, dichlorochlordene, oxychlordane, *cis*-chlordane, heptachlor epoxide, chlordene chlorohydrin, dihydroxyheptachlor, dihydroxydihydrochlordene) were a gift from the Velsicol Chemical Corp. Solvents and other chemicals were of reagent or analytical grade. Makes and types of the equipment used are mentioned at relevant places in the text.

Treatment of Fish and Radioanalysis of Water. Six fish were individually placed in 20-L Pyrex glass jars containing 16 L of water with 80 μg of *cis*-[^{14}C]chlordane delivered in 0.5 mL of acetone. Two control tanks without fish were similarly prepared. Final concentration of *cis*-[^{14}C]chlordane in water was 5 ppb (which is within the range of its solubility; Metcalf, 1977; Glooschenko and Lott, 1977). The water was aerated at the rate of 8–10 mL/min, and tops of the jars were covered with aluminum foil. Fish were not fed during the exposure period which lasted for 72 h. Samples (5 mL) of the exposure and control water were taken at 0, 2, 4, 6, 9, 12, 24, 36, 48, 60, and 72 h after starting the experiment and the radioactivity present in water determined by liquid scintillation counting as described elsewhere (Feroz and Khan, 1979a,b,c).

At the end of the 72-h exposure period, the fish were divided into two groups of three fish each. The fish in one group were killed and frozen to await analyses for tissue residues, total radioactivity, and identification of metabolites as detailed below. The exposure water from these fish was also frozen. The fish in the other group were used for study of elimination of absorbed radioactivity.

Handling of Fish and Sampling of Water in Elimination Study. The three treated fish were individually transferred to clean water (16 L in 20-L jars) at the end of 72-h exposure period. Feeding was resumed and water in their containers was changed every 6–8 days. Water samples (5 mL) were taken on a daily basis for 20 weeks and radioassayed (Feroz and Khan, 1979a,b,c). Water lost by evaporation was replaced.

Estimation of Tissue Residues. The three frozen fish were thawed and dissected and their organs were removed and weighed wet. Small samples (100–200 mg) were taken for determination of radioactive content by solubilization and liquid scintillation counting (Feroz and Khan, 1979b).

Extraction, Cleanup, and Identification of Metabolites in Fish. After tissue sampling, whole fish (three) were separately cut into small pieces and bones and skin (hard parts) separated from the other parts (soft parts) which were then extracted. An outline of the procedure for extraction of metabolites is given in Figure 1. The soft parts were homogenized in 1.5 L of 1:1 mixture of acetonitrile–water in a Sorvall Omnimixer (Ivan Sorvall, Norwalk, CT) at maximum speed for 15 min, and the homogenate was filtered. The filtrate was partitioned against 2 × 1.5 L of ethyl acetate and 1 × 1.5 L of hexane. The organic extract (ca. 5.2 L) was dried over sodium sulfate and reduced in volume to near dryness on a rotary evaporator. Viscous residue was made up to 100 mL in hexane and partitioned against 3 × 200 mL of acetonitrile. Acetonitrile was evaporated and this fraction pooled with the fraction detailed below.

Assessment of radioactivity in the residue showed high radioactive content. Residue was therefore pooled with bones and skin, dried, and pulverized in a pestle and mortar to a powder. It was then Soxhlet-extracted with 1 L of petroleum ether for 4 h at 40 °C. The extract was dried over sodium sulfate, and solvent evaporated, and the residue was taken in 160 mL of hexane which was partitioned against 3 × 320 mL of acetonitrile. Acetonitrile was evaporated and the residue chromatographed (in portions) on 60–100 mesh deactivated (Velsicol Chemical Corp., 1972) Florisil (Fisher Scientific Co., Fair Lawn, NJ).

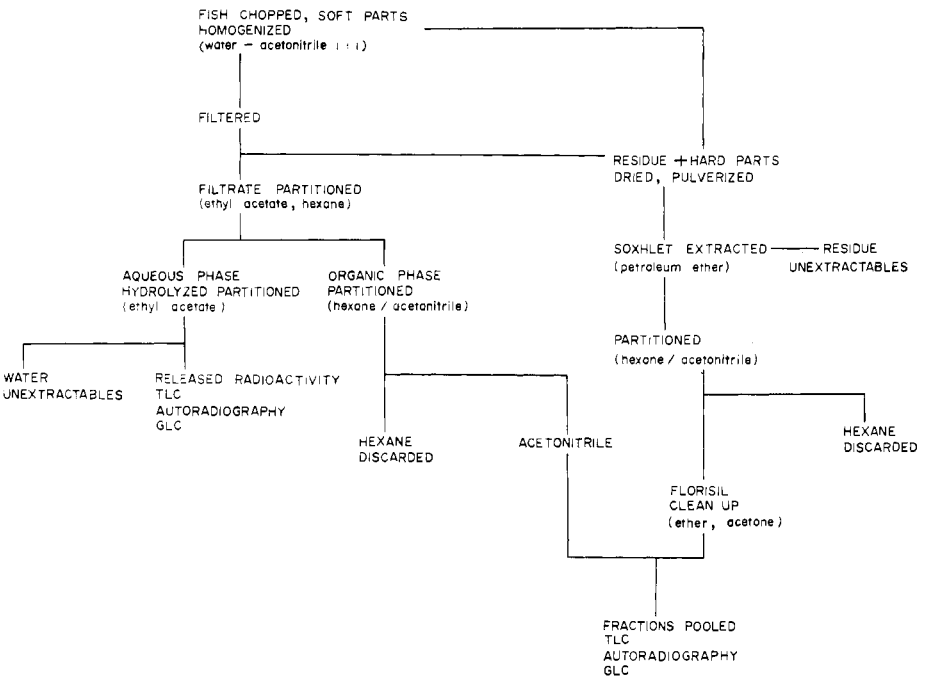

Figure 1. Flow diagram for the extraction of metabolites from treated fish. The metabolites in exposure water were extracted according to the procedure indicated for the aqueous phase from fish.

For each portion, the adsorbent (17.5 g) was dry packed in 1.0-cm glass column and covered with 5.0 g of anhydrous sodium sulfate. The column was prewetted with 50 mL of diethyl ether and eluted with 200 mL of diethyl ether, followed by the same volume of acetone. The eluates were pooled and evaporated under nitrogen, and the residue was combined with the fraction detailed above, for analysis by TLC and gas–liquid chromatography (GLC).

TLC was carried out on silica gel G, F-254 plates (Brinkmann Instruments, Des Plaines, IL) without any pretreatment. Even after hexane/acetonitrile partitioning and Florisil cleanup, the extract contained interfering coextractives which were removed by repeated TLC. Solution of the extract was streaked on chromatoplates and autoradiographed (Feroz and Khan, 1979a,b,c). The radioactive areas were marked, scraped, and eluted, and the process was repeated five or six times in each of the solvent systems shown in Table III. The compounds thus purified were cochromatographed with reference standards for comparison of R_f values. Reference standards were visualized by spraying the plates with acetonic silver nitrate and exposing them to intense UV light (Podowski et al., 1979).

GLC was performed on a Packard gas chromatograph Series 7300 (Packard Instruments, Downers Grove, IL). The chromatograph was fitted with two tritium electron-capture detectors and two glass columns. One of the columns (107 cm × 2 mm i.d.) was packed with 3% SE-30 on 80–100 mesh Gas-Chrom Q and the other (152 cm × 2 mm i.d.) with 3% QF-1 on 80–100 mesh Chromosorb W,HP. Operating conditions were as follows: temperature (°C), inlet 220; column 190; detector 220; with nitrogen as the carrier gas flowing at the rate of 40 and 30 mL/min through SE-30 and QF-1 columns, respectively. Presence or absence of hydroxyl groups on metabolites was tested by derivatization with Tri-Sil Z (Pierce Chemical Co., Rockford, IL), followed by GLC analysis (Feroz and Khan, 1979a,b,c).

Aqueous phase from the fish homogenate was lyophilized, acidified with concentrated HCl to pH 2 and allowed to stand for 6 h at 70 °C. After neutralization with sodium hydroxide, the hydrolysate was extracted with an equal volume of ethyl acetate. Organic extract was dried over sodium sulfate, evaporated and the residue subjected to chromatographic analysis.

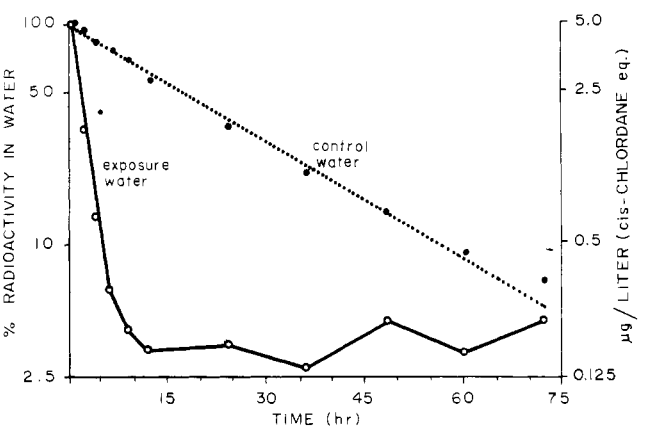

Figure 2. Radioactivity in exposure water (containing fish) and control water at various intervals during the treatment time. Amount added was 80 μg of *cis*-[^{14}C]chlordane/jar. The figure depicts the loss of *cis*-[^{14}C]chlordane from water due to absorption by fish and by volatilization.

Analysis of Exposure Water. Frozen exposure water from each tank was separately reduced in volume by lyophilization (to ca. 3 L), and extracted twice with ethyl acetate and once with hexane (equal to the volume of water). After drying over sodium sulfate, the pooled organic extract was evaporated to near dryness and analyzed by TLC and GLC. The aqueous phase of exposure water was processed as that from fish homogenate (i.e., further reduction in volume by lyophilization, acid hydrolysis, extraction, and analysis). Unextractable radioactivity was determined in all fractions.

RESULTS AND DISCUSSION

Uptake of *cis*-Chlordane. The loss of radioactivity from the exposure water and control water during the 72-h exposure period is shown in Figure 2. The half-life of the radioactivity in the exposure water and control water was calculated to be 1.15 and 17.5 h, respectively.

Table I. Recoveries of *cis*-[^{14}C]Chloride from Cichlids and Exposure Water after 72 h

	fish		water	
	μg^a	$\%^b$	μg^a	$\%^b$
organic extract	48.1 (±3.2)	60.1	1.8 (±0.2)	2.2
aqueous extract	0.5 (±0.1)	0.6	2.3 (±0.3)	2.9
unextractable	3.3 (±0.7)	4.1	0.8 (±0.2)	1.0

[a] Equivalents of chlordane. Figures represent mean (SE) with $n = 3$ in all cases. [b] Percent of *cis*-[^{14}C]chlordane (80 μg) added to water. Conditions stated in text.

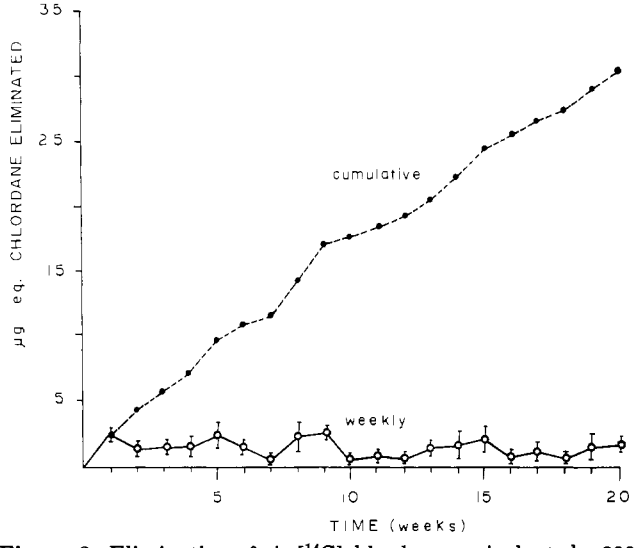

Figure 3. Elimination of *cis*-[^{14}C]chlordane equivalents by 300 g of cichlids after 72 h of exposure to 80 μg of *cis*-chlordane in 16 L of water. Elimination study was conducted in 16 L of water free of insecticide.

Loss of radioactivity from the control water was presumably due to volatilization as radioactivity could not be recovered in acetone washes of the containers at the end of treatment time. This means that the curve (Figure 2) for the loss of radioactivity from exposure water represents losses due to volatilization (4% per hour) and uptake by the fish (40% per hour). Radioactivity in the fish was not measured during the treatment time but estimates at the end of that period are shown in Table I. Total recovery of the radioactivity added to water was about 70.9% of which 64.8% was present in fish and 6.1% in water. The balance (29%) was probably lost by volatilization.

Elimination. Figure 3 shows elimination of *cis*-chlordane (^{14}C equivalents) by the exposed fish on transfer to clean water. The fish eliminated approximately 30-μg equivalents of *cis*-chlordane over a period of 20 weeks in a linear fashion. Assuming the fish used in elimination work had absorbed the same amount of the compound as those analyzed at the end of the exposure period (51.9 μg/fish, Table I), the half-life of *cis*-chlordane in cichlids was 17 weeks with the elimination rate of 2.9% per week. Persistence of *cis*-chlordane in cichlids is similar to the persistence of DDT in other fish (Macek, 1971). However, rate of elimination of *cis*-chlordane by cichlids is markedly different from that of *cis*- and *trans*-chlordane (separately or their mixture) in higher vertebrates. Disposition of chlordanes in the rat and rabbit is fast and the major part of the administered dose is excreted within a week or so after the termination of treatment (Poonawalla and Korte, 1964, 1971; Barnett and Dorough, 1974; Tashiro and Matsumura, 1977).

Uptake by and clearance of a xenobiotic from a biological system are complex dynamic processes (Kenaga, 1972; Johnson, 1973; Kerr and Vass, 1973; Hamelink 1977) resulting from the interplay of several parameters which not only include the properties of the compound involved (such as solubility, structure) but also characteristics of the biological system affected (lipid content with organochlorines, age, sex, species etc). The slow elimination of *cis*-chlordane by cichlids may have been partly due to small volume of water (16 L) in relation to body weight (300 g). A larger volume of water would have facilitated more efficient clearance of chlordane despite its highly lipophilic nature (octanol/water partition coefficient, 604; Sanborn et al., 1976). Faster elimination of chlordanes in goldfish observed by Moore et al. (1977) might have been due to this factor. However, in this case, lower capacity of fish to convert the molecule into compounds suitable for elimination appears to be of greater consequence (see section on metabolism).

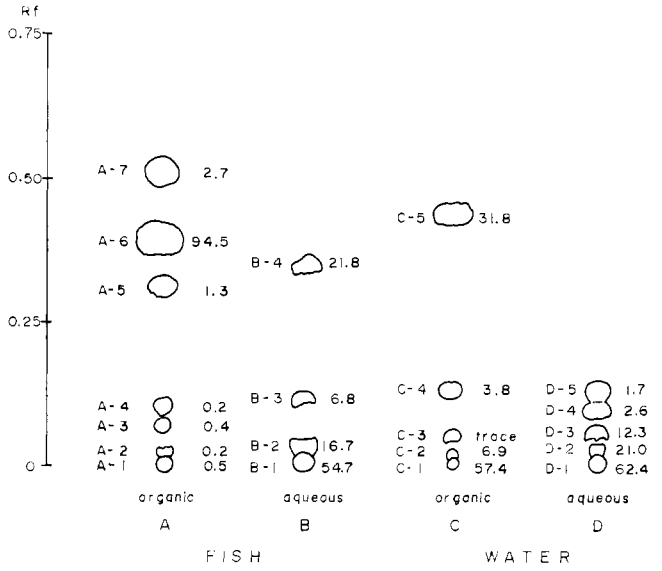

Figure 4. Tracing of X-ray autoradiograms (silica gel G,F-254; hexane–ethyl acetate, 9:1) of organic extract of fish (A), aqueous extract of fish (B), organic extract of exposure water (C), and aqueous fraction of exposure water (D). Numbers on the left side of each tracing represent metabolite designations and numerals on the right show percentage of the radiocarbon recovered in that fraction. The recoveries are given in Table I.

Tissue Distribution. Tissue samples taken from the treated fish contained varying amounts of radioactivity. The tissue values (ppm) are arranged in decreasing order in Table II. The highest concentration was present in adipose tissue (2.8 ppm). Relatively high concentrations in bile (1.2 ppm) and gall bladder (1.1 ppm) suggest that *cis*-chlordane (or its metabolic products) may be excreted in bile. Low concentrations were present in gills, spinal cord, nerves, ovaries, kidney, and brain. In general, the distribution of *cis*-chlordane in cichlids is similar to the distribution of other organochlorine insecticides in other piscian species studied by Gackstatter (1966).

Metabolism. Figure 4 shows extractable metabolic products from fish and water, along with their relative abundance. TLC and GLC characteristics of reference standards are listed in Table III. The nature of the compounds in each fraction is discussed below with reference to the spots in Figure 4. Table IV summarizes the results.

Organic Extract of Fish (Fraction A). Radioactivity extractable with organic solvents from the whole fish homogenates constituted the major fraction (Table I). Analysis of the fraction on thin-layer chromatoplates revealed seven products (Figure 4, A-1 to A-7).

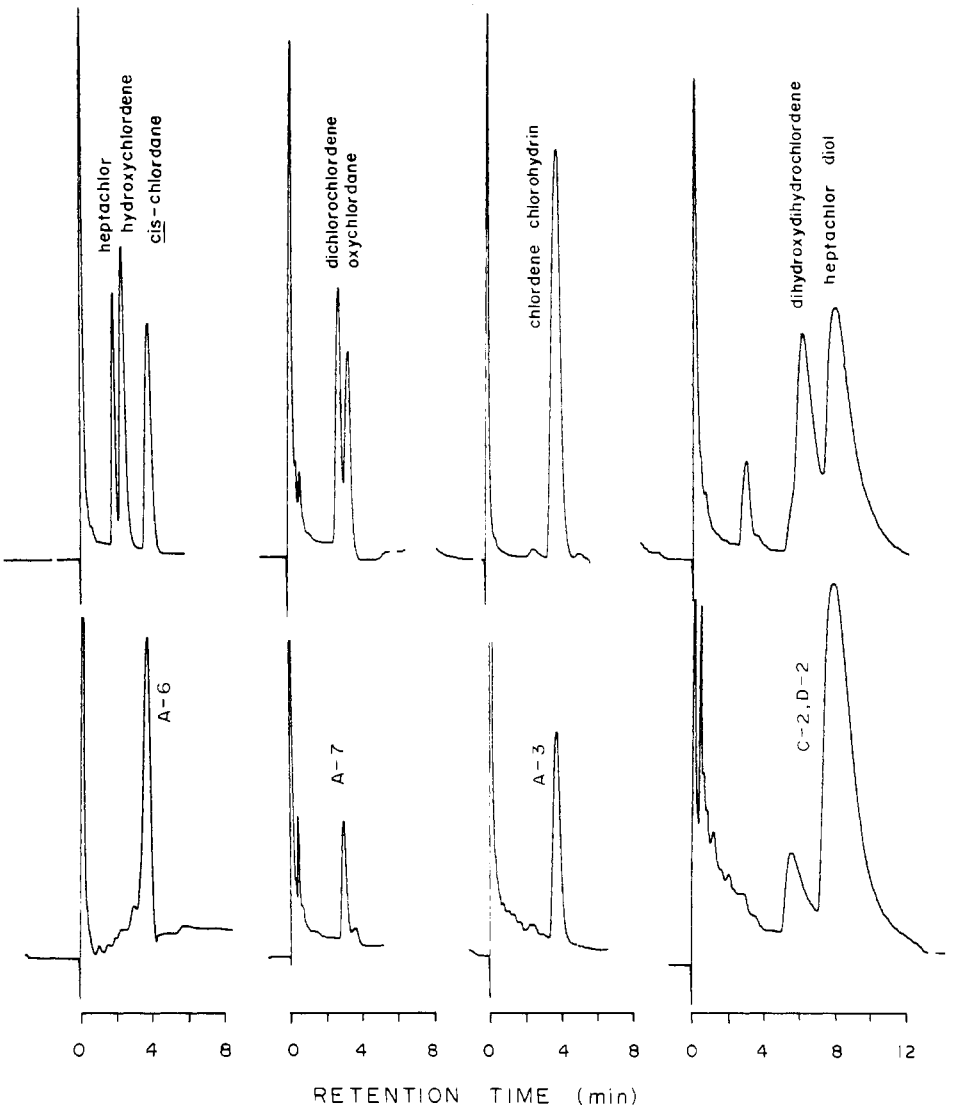

Figure 5. Gas chromatograms of some of the reference standards (upper row) and metabolites (lower row) isolated from fish and exposure water treated with *cis*-chlordane. Metabolite designations according to Figure 4. (Column: SE-30; conditions stated in text.)

Table II. Tissue Distribution of Radioactivity in Cichlids Exposed to 5 ppb *cis*-[^{14}C]Chlordane for 72 h

tissue	mean wt of tissue, g ± SE[a]	ppm ± SE
fat	1.397 ± 0.036	2.776 ± 0.057
bile		1.251 ± 0.092
gall bladder	0.244 ± 0.064	1.131 ± 0.176
arachnoid fluid		0.927 ± 0.491
spleen	0.129 ± 0.006	0.885 ± 0.091
gills	4.223 ± 0.129	0.544 ± 0.129
spinal cord		0.488 ± 0.072
liver	3.100 ± 0.633	0.422 ± 0.030
nerves		0.405 ± 0.059
eyeball	1.030 ± 0.197	0.342 ± 0.122
gut	1.092 ± 0.149	0.295 ± 0.047
ovary	0.851 ± 0.218	0.289 ± 0.026
kidney	0.220 ± 0.008	0.222 ± 0.013
gut contents	0.267 ± 0.056	0.135 ± 0.016
skin		0.115 ± 0.012
air sac	0.531 ± 0.025	0.094 ± 0.005
heart	0.472 ± 0.028	0.087 ± 0.052
brain	0.206 ± 0.006	0.078 ± 0.004
muscle		0.061 ± 0.019
lens	0.088[b]	0.012 ± 0.002

[a] Sample size three in all cases except gut and heart where it was two. [b] Mean only.

A-1 stayed at the origin in all solvent systems which showed its extremely polar nature, and judging from its behavior, it was either a trihydroxy derivative of chlordane or a conjugate. R_f values of the product A-2 in various solvent systems and GLC retention times correspond to those of dihydroxyheptachlor reference standard (Tables III and IV). The compound recovered from the fish as well as dihydroxyheptachlor reference standard slowly degraded into two other minor components of unknown structure. A-3 was identified as chlordene chlorohydrin because of its complete matching with the reference standard (Tables III and IV, Figure 5). A-4 was not comparable to any of the reference standards used but its behavior on TLC plates close to chlordene chlorohydrin and different GLC retention times of its trimethylsilyl ether suggest its monohydroxylated nature. A-5 behaved as a single compound on TLC plates but its GLC analysis showed multiple peaks, indicating that either the compound was not pure or it was heat labile. A-6 constituted 95% of the fraction (Figure 4) and TLC and GLC analyses showed it to be unchanged *cis*-chlordane (Tables III and IV). A-7 consisted mainly of dichlorochlordene along with a trace of oxychlordane (Tables III and IV).

Aqueous Fraction of Fish (Fraction B). Extracts of acid hydrolysate of aqueous fraction comprised only 0.6% of the radioactivity applied (Table I). TLC analyses of the fraction showed four spots (Figure 4, B-1 to B-4). B-1, most likely a polyhydroxylated derivative of chlordane (R_f

Table III. Thin-Layer and Gas-Liquid Chromatographic Behavior of Reference Standards

compound	TLC (R_f values)[a]				GLC (t_R, min)[b]			
					SE-30		QF-1	
	I	II	III	IV	U	D	U	D
heptachlor	0.42	0.55	0.59	0.69	1.77	1.77	0.59	0.59
dichlorochlordene	0.43	0.55	0.59	0.69	2.95	2.95	0.68	0.68
oxychlordane	0.27	0.49	0.55	0.69	3.49	3.49	1.08	1.08
cis-chlordane	0.30	0.42	0.53	0.68	3.74	3.74	1.18	1.18
heptachlor epoxide	0.11	0.39	0.49	0.62	3.49	3.49	0.98	0.98
chlordene chlorohydrin	0.00	0.08	0.25	0.55	3.93	4.33	1.67	1.27
hydroxychlordene	0.00	0.12	0.25	0.50	2.26	2.55	0.93	*c*
dihydroxyheptachlor	0.00	0.005	0.09	0.36	8.70	10.04	3.00	3.54
dihydroxydihydrochlordene	0.00	0.00	0.04	0.21	6.72	7.8	ND	1.97

[a] On silica gel GF-254 using solvent systems: (I) cyclohexane/chloroform (9:1), (II) hexane/ethyl acetate (9:1), (III) hexane/methyl ethyl ketone (4:1), (IV) benzene/ethyl acetate (1:1). [b] U = underivatized or without Tri-Sil "Z" treatment, D = derivatized or Tri-Sil "Z" treated. ND = not detectable. [c] Buried in solvent.

values 0.00 in all solvent systems), was the most abundant of the products. B-2 and B-3 were, respectively, chromatographically identical with dihydroxyheptachlor and chlordene chlorohydrin reference standards. B-4 was determined to be *cis*-chlordane. The presence of *cis*-chlordane in the acid hydrolysate of aqueous fraction may be due to either the incomplete extraction of the fish homogenates by organic solvents or binding to an unspecific component(s).

Organic Extract of Exposure Water (Fraction C). Analysis of this fraction by TLC showed five compounds. The most abundant compound was the polyhydroxylated derivative of chlordane or a conjugate (Figure 4, Table IV, C-1). C-2 consisted mainly of heptachlor diol with a small amount (about 20%) of dihydroxydihydrochlordene (Tables III and IV, Figure 5). C-3 was present in trace amounts only and could not be analyzed further. Chromatographic behavior of C-4 was identical with that of chlordene chlorohydrin (Tables III and IV). C-5 was identified as unchanged *cis*-chlordane.

Aqueous Extract of Exposure Water (Fraction D). TLC of organic extracts of acid-hydrolyzed aqueous fraction of exposure water indicated five compounds (Figure 4, D-1 to D-5). D-1 represented major percentage of the radioactivity in the fraction and TLC behavior (R_f values 0.00 in all solvent systems, Table IV) suggested polyhydroxylated nature of the product. D-2 was a mixture of dihydroxyheptachlor and dihydroxydihydrochlordene in approximately the same ratios as in C-2 (Figure 5). TLC and GLC characteristics of D-3 suggest that it was a monohydroxylated product (Table IV) but it was not comparable to any of the reference standards. D-4 was chlordene chlorohydrin, while D-5 appeared to be monohydroxylated product identical with A-4.

Leaving aside the unextractables of unknown composition, 12.5% of the radioactivity recovered from the treated fish was in the form of metabolites (Table I and Figure 4) and the remainder (87.5%) as the parent compound. The metabolic products included polyhydroxy derivatives/conjugates (A-1, B-1, C-1, D-1), dihydroxyheptachlor (A-2, B-2, C-2, D-2), dihydroxydihydrochlordene (C-2, D-2), chlordene chlorohydrin (A-3, B-3, C-4, D-4), two monohydroxy derivatives (one: A-4, D-5; the other: D-3), an unknown (A-5), dichlorochlordene (A-7), and oxychlordane (A-7). A-6, B-4, and C-5 were the parent compound, *cis*-chlordane (Table IV). Qualitatively, the range of transformation products of chlordane in cichlids is similar to that observed in mammals but quantitatively mammalian species are far more active than the fish (Poonawalla and Korte, 1964, 1971; Barnett and Dorough, 1974; Tashiro and Matsumura, 1977; Brimfield et al., 1978). Slow elimination rate of chlordane in cichlids may be attributed to their less efficient metabolic activity toward the molecule.

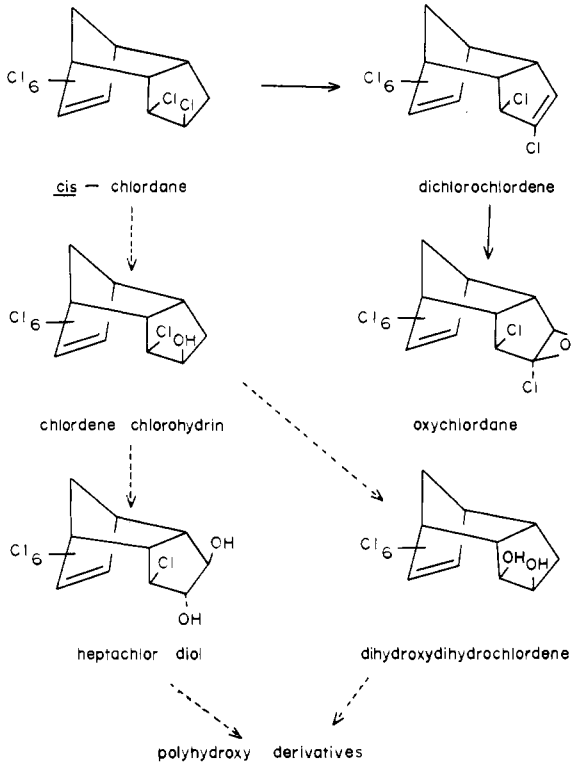

Figure 6. Proposed metabolic pathways for *cis*-chlordane in cichlids. Solid lines indicate major and broken lines show minor conversions.

Tashiro and Matsumura (1977) and Brimfield et al. (1978) have discussed the metabolic pathways of chlordanes on the basis of the present knowledge. These molecules can undergo a variety of conversions in biological systems involving oxidative and reductive processes. The well-documented route is the desaturation of the molecule by formation of a double bond between C_2 and C_3 to yield dichlorochlordene which can be further epoxidized to oxychlordane. The presence of both these compounds in cichlids indicates that the route is operative in this species (Figure 6). (It may be mentioned that no dichlorochlordene or oxychlordane was detected in goldfish and bluegills treated with *cis*-chlordane; this laboratory, unpublished.)

The hydroxylated products of chlordane can be viewed as successive hydroxylations on the cyclopentane ring of the molecule as shown in Figure 5, although the possibility of other pathways of their origin exists. These molecules

Table IV. Chromatographic Characteristics of the Products in Different Fractions of Treated Fish and Exposure Water

meta-molite[a]	identity[b]	TLC (R_f values)[c]				GLC (t_R, min)[d]			
						SE-30		QF-1	
		I	II	III	IV	U	D	U	D
						Fraction A			
A-7	dichlorochlordene, oxychlordane	0.43[e] (0.26)	0.55	0.60	0.66	2.95[e] (3.49)	2.95[e] (3.49)	0.68[e] (1.08)	0.68[e] (1.08)
A-6	*cis*-chlordane	0.32	0.42	0.55	0.66	3.74	3.74	1.18	1.18
A-5	unknown	0.00	0.32	0.25	0.64	*f*		*f*	
A-4	unknown (monohydroxy derivative)	0.00	0.10	0.25	0.54	3.34	3.64	1.57	1.18
A-3	chlordene chlorohydrin	0.00	0.08	0.22	0.54	3.93	4.33	1.67	1.27
A-2	dihydroxyheptachlor	0.00	0.01	0.10	0.31	8.07	10.04	3.00	3.54
A-1	unknown (conjugate/polyhydroxy derivative)	0.00	0.00	0.00	0.00	ND		ND	
						Fraction B			
B-4	*cis*-chlordane	0.35	0.40	0.57	0.65	3.74	3.74	1.18	1.18
B-3	chlordene chlorohydrin	0.00	0.12	0.23	0.51	3.93	4.33	1.67	1.27
B-2	dihydroxyheptachlor	0.00	0.01	0.11	0.35	8.07	10.04	3.00	3.54
B-1	unknown (polyhydroxy derivative)	0.00	0.00	0.00	0.00	ND		ND	
						Fraction C			
C-5	*cis*-chlordane	0.31	0.46	0.55	0.67	3.74	3.74	1.18	1.18
C-4	chlordene chlorohydrin	0.00	0.13	0.25	0.52	3.93	4.33	1.67	1.27
C-3	unknown	0.00	0.07	0.22	0.53				
C-2	dihydroxyheptachlor, dihydroxydihydrochlordene	0.00	0.01	0.08	0.35[g] (0.21)	8.07[g] (5.72)	10.04[g] (7.48)	3.00	3.54[g] (1.97)
C-1	unknown (polyhydroxy derivative)	0.00	0.00	0.00	0.00	ND		ND	
						Fraction D			
D-5	unknown (monohydroxy derivative)	0.00	0.12	0.25	0.52	3.34	3.64	1.57	1.18
D-4	chlordene chlorohydrin	0.00	0.12	0.25	0.50	3.93	4.33	1.67	1.27
D-3	unknown (monohydroxy derivative)	0.00	0.07	0.22	0.50	2.67	3.14	1.08	0.98
D-2	dihydroxyheptachlor, dihydroxydihydrochlordene	0.00	0.01	0.10	0.33[g] (0.20)	8.07[g] (5.72)	10.04[g] (7.48)	3.00	3.54[g] (1.97)
D-1	unknown (polyhydroxy derivative)	0.00	0.00	0.00	0.00	ND		ND	

[a] Metabolite designation according to spots in Figure 4. [b] Identities based on cochromatography. Possible nature is indicated in parentheses where a matching reference standard was not available. [c] Thin-layer plates and solvent systems same as in Table III. [d] Same as in *b* of Table III. [e] Figures in parentheses for the minor component oxychlordane. [f] The compound showed multiple peaks which may be due to unknown extraneous material or thermal lability. [g] Figures in parentheses for the minor component dihydroxydihydrochlordene.

can undergo conjugation.

LITERATURE CITED

Barnett, J. R.; Dorough, H. W. *J. Agric. Food Chem.* **1974**, *22*, 612.
Bevenue, A.; Hylin, J. W.; Kawano, Y.; Kelley, T. W. *Pestic. Monit. J.* **1972**, *6*, 56.
Blus, L. J.; Neely, B. S.; Lamont, T. G.; Mulhern, B. *Pestic. Monit. J.* **1977**, *11*, 41.
Bugg, J. C., Jr.; Higgins, J. E.; Robertson, E. A., Jr. *Pestic. Monit. J.* **1967**, *1*, 9.
Burns, B. G.; Peach, M. E.; Stiles, D. A. *Pestic. Monit. J.* **1975**, *9*, 35.
Brimfield, A. A.; Street, J. C.; Futrell, J.; Chatfield, D. A. *Pestic. Biochem. Physiol.* **1978**, *9*, 84.
Brooks, G. T. "Chlorinated Insecticides", Vol. I and II; CRC Press: Cleveland, OH, 1974.
Casper, V. L. *Pestic. Monit. J.* **1967**, *1*, 13.
Feroz, M.; Khan, M. A. Q. *J. Agric. Food Chem.* **1979a**, *27*, 95.
Feroz, M.; Khan, M. A. Q. *J. Agric. Food Chem.* **1979b**, *27*, 108.
Feroz, M.; Khan, M. A. Q. *Arch. Environ. Contam. Toxicol.* **1979c**, *8*, 519.
Gackstatter, J. H. Ph.D. Thesis, University of North Carolina at Chapel Hill, Chapel Hill, NC, 1966.
Glooschenko, V.; Lott, J. N. A. *Can. J. Bot.* **1977**, *55*, 2866.
Godsil, P. J.; Johnson, W. C. *Pestic. Monit. J.* **1968**, *1*, 21.
Hamelink, J. L. *Ann. Rev. Pharmacol. Toxicol.* **1977**, *17*, 167.
Johnson, D. W. In "Environmental Pollution by Pesticides", Edwards, C. A., Ed.; Plenum Press: New York, 1973; Chapter 5.
Kenaga, E. E. In "Environmental Toxicology of Pesticides"; Matsumura, F., Boush, G. M., Misato, T., Ed.; Academic Press: New York, 1972.
Kerr, S. R.; Vass, W. P. In "Environmental Pollution by Pesticides", Edwards, C. A., Ed.; Plenum Press: New York, 1973; Chapter 4.
Law, M. L.; Goerlitz, D. F. *Pestic. Monit. J.* **1974**, *8*, 33.
Lawrence, J. H.; Barrow, R. P.; Chen, J. Y.; Lombardo, P.; Benson, W. R. *J. Assoc. Off. Anal. Chem.* **1970**, *53*, 261.
Lichtenberg, J. J.; Eichelberger, J. W.; Dressman, R. C.; Longbottom, J. E. *Pestic. Monit. J.* **1970**, *4*, 71.
Macek, K. J. In "The Biological Impact of Pesticides in the Environment", Gillete, J. W., Ed.; Oregon State University Press, 1971; pp 17–21.
Mattraw, H. C., Jr. *Pestic. Monit. J.* **1975**, *9*, 106.
Metcalf, R. L. In "Pesticides in Aquatic Environments", Khan, M. A. Q., Ed.; Plenum Press: New York, 1977; pp 127–144.
Miles, J. R. W.; Harris, C. R. *Pestic. Monit. J.* **1973**, *6*, 363.
Moore, R.; Toro, E.; Stanton, M.; Khan, M. A. Q. *Arch. Environ. Contam. Toxicol.* **1977**, *6*, 411.
Podowski, A. A.; Banerjee, B. C.; Feroz, M.; Dudek, M. A.; Willey, R. L.; Khan, M. A. Q. *Arch. Environ. Contam. Toxicol.* **1979**, *8*, 509.
Polen, P. B.; Hester, M.; Benziger, J. *Bull. Environ. Contam. Toxicol.* **1971**, *5*, 521.
Poonawalla, N. H.; Korte, F. *Life Sci.* **1964**, *3*, 1497.
Poonawalla, N. H.; Korte, F. *J. Agric. Food Chem.* **1971**, *19*, 467.
Saha, J. G.; Lee, Y. W. *Bull. Environ. Contam. Toxicol.* **1969**, *4*, 285.
Sanborn, J. R.; Metcalf, R. L.; Bruce, W. N.; Lu, P. Y. *Environ. Entomol.* **1976**, *5*, 533.
Schwemmer, B.; Cochrane, W. P.; Polen, P. B. *Science* **1970**, *169*, 1087.
Street, J. C.; Blau, S. E. *J. Agric. Food Chem.* **1972**, *20*, 395.
Tanita, R.; Johnson, J. M.; Chun, M.; Maciolek, J. *Pestic. Monit. J.* **1976**, *10*, 24.
Tashiro, S.; Matsumura, F. *J. Agric. Food Chem.* **1977**, *25*, 872.
Velsicol Chemical Corporation, "Methods of Analysis of Residues from Technical Chlordane", 1972.

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Delayed Neurotoxicity of *O*-Alkyl *O*-Aryl Phenylphosphonothioate Analogues Related to Leptophos Administered Orally to the Hen

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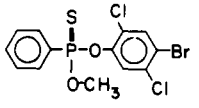
Analogues of leptophos [*O*-(4-bromo-2,5-dichlorophenyl) *O*-methyl phenylphosphonothioate] were examined for acute toxicity to mice and houseflies and for delayed neurotoxic activity in adult hens following adminstration of a single oral dose. Development of ataxia after recovery from acute poisoning was the criterion for delayed neurotoxic acitivity. All mono- and dichlorophenyl analogues were delayed neurotoxic, the 2,5-dichlorophenyl analogue being the most potent delayed neurotoxic compound tested. Substitution for the methyl by an ethyl group abolished delayed neurotoxic activity in both leptophos and the 2,5-dichlorophenyl analogue at doses of 1000 mg/kg. The propyl and butyl analogues of the latter were also nondelayed neurotoxic at 500 and 333 mg/kg, respectively. Substitution of methyl for ethyl in EPN [*O*-*p*-nitrophenyl *O*-ethyl phenylphosphonothioate] did not alter its delayed neurotoxicity.

The discovery of the potential of some organophosphorus compounds to cause an insidious neuropathic anomaly, termed delayed neurotoxicity, has necessitated careful screening of potential organophosphorus pesticides for such delayed neurotoxic activity (Johnson, 1975). A recent incidence of delayed neurotoxicity involved the use of leptophos [Phosvel or *O*-(4-bromo-2,5-dichlorophenyl) *O*-methyl phenylphosphonothioate] for control of cotton



pests in Egypt. In this instance, some 1300 water buffalo were paralyzed and later six people were discovered to have

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