Bull. Environm. Contam. Toxicol. 23,064-069 (1979)

Fate of ^{14}C-*cis*-chlordane in Goldfish, *Carassius auratus (L.)*

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Main application of chlordanes has been for agricultural and domestic purposes but surveys from different parts of the U.S.A. and Canada report their wide occurrence in aquatic environments especially sediments (BEVENUE et al. 1972, BLUS et al. 1977, BUGG et al. 1967, BURNS et al. 1975, LICHTENBERG et al. 1970, MATTRAW 1975, MILES and HARRIS 1973, TANITA et al. 1976). Residues of chlordanes have been found in various fish (MILES and HARRIS 1973) and trophic transfer of the residues has also been indicated in natural environments (BLUS et al. 1977). SANBORN et al. (1976) studying behavior of chlordanes in a model ecosystem found very high (8258x) bioconcentration ratio for the mosquito fish, Gambusia affinis.

Whereas several reports on the metabolism of chlordane in mammals are available (BARNETT and DOROUGH 1974, BRIMFIELD et al. 1978, POONAWALLA and KORTE 1971, STREET and BLAU 1972, TASHIRO and MATSUMURA 1977), the information on its fate in fish is scanty. This communication reports metabolism of cis-chlordane in goldfish, Carassius auratus.

EXPERIMENTAL

^{14}C-cis-chlordane (1-exo, 2-exo, 4, 5, 6, 7, 8, 8-octachloro-3a, 4, 7, 7a-tetrahydro-4,7-methanoindane); specific activity 10.9 mCi/mmol, was a gift from the Velsicol Chemical Corporation, Chicago, IL. The compound used was free from impurities. Reference compounds for cochromatography were supplied by the Velsicol Chemical Corporation. Solvents used were of reagent or analytical grade.

A group of nine acclimated goldfish, Carassius auratus weighing about 16 gm each (Table 1), bought from the Midwest Aquarium, Bensenville, IL, were treated with 25.94 ppb ^{14}C-cis-chlordane in 18 liters of dechlorinated tap (lake) water (total amount in tank 466.92 µg). The insecticide was delivered in 4 ml of 3:1 mixture of methyl cellusolve and acetone. After thorough stirring with a glass rod, the fish were immediately transferred to the exposure water. The container was covered with aluminum foil and left for 24 hr without bubbling air. During the treatment time, radioactivity in the exposure water was monitored by

0007-4861/79/0023-0064 $01.20

withdrawing 5 ml samples at 0, 3, 6, 9 and 24 hr.

One of the fish died during the treatment time. It was removed and frozen. The remaining 8 fish were transferred to clean water (18 liters). Two more fish died, one on day 1 and the other on day 4, after transfer to clean water. They were also removed and frozen. Of the surviving 6 fish, 3 were withdrawn on day 10 and frozen. Other 3 were given a change of water (18 liters) and carried through day 25 when the experiment was terminated. Air was constantly bubbled in the tank during the post-treatment period and the fish were fed beef kidney twice a week. Whole study was carried out at room temperature, 21°C. Radioactivity in water was estimated on day 10 and day 25. It may be mentioned that pH of the water had dropped from 7.5 to about 5 both on day 10 and day 25.

For analysis fish were individually processed. They were cut into small pieces, mixed with anhydrous sodium sulfate (3-4 x weight of fish), pulverized in pestle-mortar to a dry powder and extracted by vigorous shaking with 200 ml (4 x 50 ml) diethyl ether, 200 ml (4 x 50 ml) acetone and 100 ml (2 x 50 ml) methanol, in that order. Pooled extract was concentrated by evaporation under dry nitrogen. The residue was cleaned on Florisil column (Velsicol Chemical Corporation, 1972) by using diethyl ether and methanol as elutants. Further cleaning, isolation and identification of metabolites was done by TLC and GLC (FEROZ and KHAN, 1979). Confirmation of hydroxylated products was carried out by on-column derivatization (ESPOSITO 1968) with Tri-Sil 'Z' (Pierce Chemical Co., Rockford, IL). Quantitation was performed by radiometry on liquid scintillation counter as described by FEROZ and KHAN (1979).

RESULTS AND DISCUSSION

Estimates of radioactivity in water during the 24-hr treatment time are shown in Figure 1 which shows rapid absorption of the compound by the fish. About 92% of the radioactivity added to water was lost during the initial 6 hr with first order kinetics. This was followed by a slow phase and by the end of the treatment time only 1.8% of the added radioactivity was present in water.

Table 1 lists the results of preliminary analyses of fish extracts. Almost all radioactivity in fish was extractable with organic solvents. Only small amounts were present in water both on day-10 and day-25 samples. This indicates that the compound is stored in the fish showing little change in body residue levels over the period of study.

Amounts of radioactivity in water (Table 1) were not sufficient to work with for meaningful analyses. Extracts of day-10 sample of fish exhibited 5 compounds (A, B, C, D, E) on TLC plates (Figure 2). Only 0.13% of the radioactivity was present as conversion products while the remainder was unchanged *cis*-chlordane. Metabolic picture in the day-25 sample was essentially similar except that *cis*-chlordane transformations amounted to 0.61%.

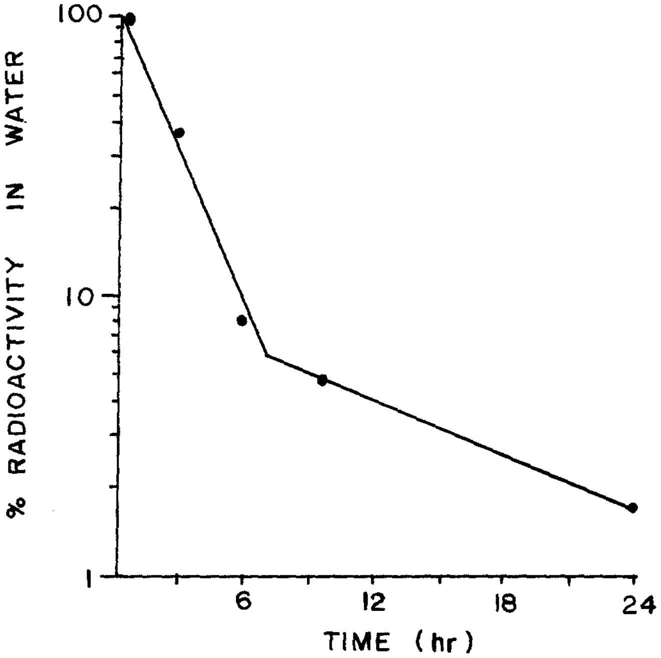

FIGURE 1: Radioactivity in water containing goldfish, during the 24 hour treatment time.

TABLE 1
Residues and bioconcentration ratios in different samples of goldfish treated with cis-chlordane.

COMPONENT	Sample [a]		
	Dead	10-day	25-day
Fish			
Weight (gm)	16.10± 0.44	15.40±2.65	17.26±0.61
Extractables(μg)[b]	21.34±12.58	44.48±9.26	36.20±4.23
Unextractables(μg)[b]	0.51± 0.19	0.16±0.05	0.27±0.04
Total (μg)[b]	21.85±12.73	44.64±9.21	36.47±4.21
Concentration (ppb)	1357	2898	2113
Water			
Concentration (ppb)	--[c]	1.27	1.16
Bioconcentration ratio	--[c]	2282	1821

[a] where applicable values indicate mean ± S.E.,sample size 3 in all cases.[b] equivalent of chlordane. Amount initially applied was 51.88 μg/fish.[c] the fish died on different days.

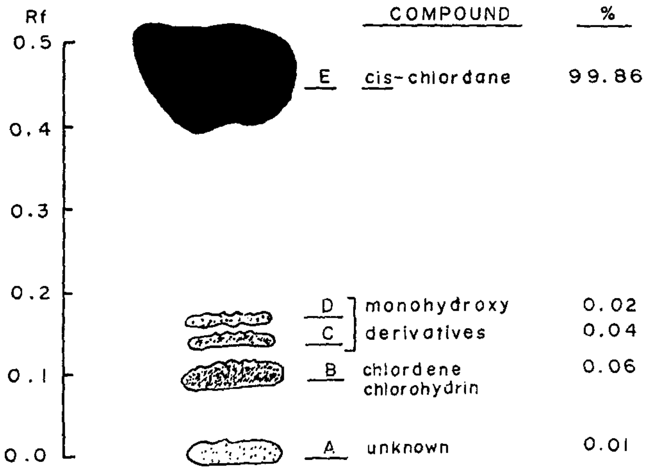

Figure 2: Tracing of an x-ray autoradiogram (Silica gel G F-254; hexane-ethyl acetate, 9:1) showing compounds in organic extract of ^{14}C-*cis*-chlordane-treated goldfish (day-10), their possible nature and their relative amounts.

Identification of the metabolites shown in Figure 2 was carried out by cochromatographic techniques. The compound A was more polar than dihydroxydihydrochlordene and dihydroxyheptachlor (FEROZ and KHAN 1979) and though its exact nature could not be ascertained, it appeared to be a trihydroxy product of chlordane or a conjugate from TLC behavior. TLC and GLC characteristics of the most abundant product B were identical with chlordene chlorohydrin reference standard. The derivatives C and D were less polar than dihydroxy derivatives of chlordane on thin layer chromatoplates and showed different retention times as trimethylsilyl ethers indicating that both were monohydroxy compounds. The compound E constituting over 99% in day-10 and day-25 samples was unchanged *cis*-chlordane.

The most important observation on the fate of *cis*-chlordane in this species was extremely low amounts of biotransformation products. The other noticeable feature was the total absence of apolar products such as 1,2-dichlorochlordene and oxychlordane described in mammals (STREET and BLAU 1972, BARNETT and DOROUGH

1974, TASHIRO and MATSUMURA 1977) and a tropical fish (FEROZ and KHAN 1977). Both of these features may be related to the isomer of chlordane used in the study. Reports on toxicity of pure trans and cis isomers of chlordane show that the latter is more toxic to mammals, fish and invertebrates (BROOKS 1974, PODOWSKI *et al.* 1978) which may be due to greater stability of cis-chlordane. Other studies also support this. For example, SANBORN *et al.* (1976) introduced cis- and trans-chlordane in a laboratory model ecosystem in a 3.04:1 ratio but recoveries at the conclusion of the study showed (cis:trans 1) 4.02 in water, 3.08 in alga, 5.39 in snail and 6.98 in fish. Also trans isomer is a more favored substrate for conversion to 1,2-dichlorochlordene with further epoxidation to oxychlordane in rat (STREET and BLAU 1972). Thus absence of these products from cis isomer in goldfish with little metabolic activity toward the compound is not surprising.

CONCLUSIONS

1. Absorption of ^{14}C-cis-chlordane from water by goldfish is very rapid.

2. Over 99% of the radioactivity recovered from the fish on day-10 and day-25 post-treatment times was unchanged cis-chlordane indicating its inert storage in body tissues.

ACKNOWLEDGEMENTS

Financial support by a USPHS Grant #ES-01479 from the National Institute of Health and Environmental Sciences is acknowledged.

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