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Biotransformation of the Flame Retardant MC-984 by Goldfish, *Carassius auratus*

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The use of flame retardants in plastics, textiles, hydraulic fluids, etc., in the United States exceeds 4 billion pounds per year (Anonymous 1971). These topically applied compounds may amount to about one-third of the weight of a cotton fabric (Gutenmann and Lisk 1975). Organophosphates (OPs) are extensively used for this purpose, especially in children's sleepware (Bartinger 1972; LeBlane 1973). The washing of fabrics can release up to 10 μg of the flame retardant/inch^2 of the fabric per wash (Gutenmann and Lisk 1975). These flame retardants may reach the aquatic environment by such washings and other sources. Out of various arylphosphates, the halogenated organophosphate flame retardant tris [(2,3-dibromopropyl) phosphate] is very toxic to goldfish at 1 mg/L, compared with nonhalogenated OPs (Gutenmann and Lisk 1975) whose LC_{50} to various freshwater fish lies between 0.32 to 0.78 mg/L (Mayer et al. 1981; Dawson et al. 1977; Huckins et al. 1991) due to factors other than its anticholinesterase activity. Whether this is related to faster absorption and longer retention by fish has not been investigated. The uptake and bioaccumulation of chlorinated hydrocarbons (OCs) by fish and other aquatic organisms has been a subject of great concern (Woodwell et al. 1967; Metcalf and Sanborn 1978; Hamelink and Spacie 1977). Fish accumulate very high levels of insecticidal OCs, such as cyclodienes, DDT, lindane, in their tissues. The resulting concentrations of OCs in fish can be sometimes several thousand times of that in water (Gakstatter and Weiss 1967; Grzenda et al. 1970; Macek 1970; Argyle et al. 1975; Tooby and Durbin 1975). The bioaccumulation of arylphosphate flame retardants has been studied by various workers (Saager et al. 1979; Muir et al. 1982; Gengtsson et al. 1986). The bioaccumulation of OPs by fish has been found not to be higher than about 200-times of their levels in the surrounding water (Kanazawa 1975, 1975; Miyamoto et al. 1979). We have studied the bioaccumulation of the flame retardant, MC-984 [bis(1,3-dichloro-2-propyl)-3-chloro-2,2-dibromomethyl-1-propyl phosphate], by goldfish to compare the results with those obtained in similar investigations with cyclodienes (Khan et al. 1979) and other chlorinated hydrocarbons (Podowski et al. 1991; Frankovic et al., unpublished) as well as with other OP flame retardants.

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MATERIALS AND METHODS

^{14}C-labelled MC-984 (sp. activity 3.3 mCi/mmole, 95% + pure) was synthesized by Pathfinders Laboratories, Columbia, Missouri. This and the non-radioactive compound were provided by Velsicol Chemical Company, Chicago, Illinois.

Goldfish, 1 to 2 g wet wt per fish, were purchased from Auburndale Goldfish Company, Chicago, Illinois. They were kept in aged tap (lake) water in an environmental room set at 21°C and 90% relative humidity and 9-hr light and 15-hr dark photoperiod. Fish were acclimated for at least 6 d. They were fed Tetra-fin Basic Goldfish Food (Tetra Werke, West Germany).

A stock solution of MC-984 in methyl cellusolve (0.30 mg or 948,879 DPM per mL) was prepared. The activity of this solution was verified by counting 0.1-mL aliquots in a mixture of Instagel (Packard Instruments, Downers Grove, Illinois) and water (15:5). Internal standardization of these samples with 6 µL of ^{14}C-toluene (2,949 DPM) indicated a counting efficiency of 87.8%.

One gallon wide-mouth (6 in. dia., screw-capped) pickle jars were filled with 3 L of dechlorinated lake water. 1 mL of the stock solution was added to each jar and stirred vigorously with a glass rod for 1 min. The resulting concentration was 100 ng/g as ascertained by counting several 5-mL aliquots of the water sample in 15 mL of Instagel.

Two methods of exposure (3 fish/3 L) were utilized. In Treatment A, the fish were exposed for 16 d to the same initial concentration (aerated for 5 min each d). Treatment B used an intermittent flow system in which fish were transferred daily to fresh solution of the chemical at the original concentration (100 µg/L, without aeration). At least six fish from each treatment were sampled at 0, 1, 2, 4, 8, 12, and 16 d. 5-mL aliquots of water from each jar were counted (for every experimental period) for radioactivity. The exposure concentrations of all jars were checked just after adding the chemical. Control jars (water without fish) were run in all cases.

Each exposed fish was individually solubilized in Soluene-350 (Packard Instruments, Downers Grove, Illinois) and counted in Dimilume-30 (Packard Instruments, Downers Grove, Illinois), using a Tricarb-3390 Spectrometer equipped with 544A Absolute Activity Analyzer (Packard Instruments, Downers Grove, Illinois), according to Podowski et al. (1991).

RESULTS AND DISCUSSION

Analyses of water for radioactivity, 10 to 15 min after adding the chemical to obtain required concentration (100 µg/L MC-984), showed that almost all of the added MC-984 remained in water. The recoveries of added MC-984 from water and fish (Treatment A) ranged from 91 to 99.6% up to 12 d of experimentation. This was seen in both Treatment A, in which exposure was made to an initial concentration of 0.300 mg/3 L of water, and Treatment B, in which cumulative concentration during the 16-d exposure was 4.8 mg/48 L/3 fish (Table 1).

In Treatment A, the fish seem to absorb maximum amount of MC-984 (19.8 μg/g) during the first 2 d of exposure (Table 2). Similar maximum absorption in a static system has been reported for photodieldrin (Khan and Khan 1974) and chlordanes (Khan et al. 1979) by this species. The body levels of radioactivity decline after this period — first slowly in following 2 d and then rapidly up to d 12 (1 μg/g in fish). Steady-state equilibrium is seen during the initial phase of absorption, i.e., around d 2, then during d 12 to d 16. Similar elimination has been reported for hexachlorocyclopentadiene in goldfish (Podowski et al. 1991), hexachlorobenzene in steelhead trout (Frankovic et al., unpublished), and of diazinon by topmouth gudgeon (Kanazawa 1978). It has been observed that after the rapid initial loss of the absorbed chemical by fish, the remaining low levels of residues are eliminated very slowly over a long period of time (Gakstatter and Weiss 1967; Grzenda et al. 1970; Macek et al. 1970; Tooby and Durbin 1975; Khan et al. 1979; Podowski et al. 1991; Frankovic et al., unpublished).

In Treatment B, the fish absorbed the maximum amount of radioactivity, i.e., about 13% of the dose, in 24 hr (Table 2) and the subsequent absorption kept decreasing on transfer of the pre-exposed fish to fresh concentration (100 μg/L) of MC-984. However, the total amount of radioactivity in fish showed a steady-state equilibrium on d 12 when the fish had retained about 66 μg/g of MC-984. Further exposure beyond this period did not increase the body levels of radioactivity (Table 2). Similar results have been reported with other chemicals (Veith et al. 1979; Podowski et al. 1991; Frankovic et al., unpublished).

The differences in the levels of radioactivity in fish seen between Treatments A and B may indicate that in the latter case the parent compound may be converted to more hydrophilic compound(s) by chemical and/or biological degradation. These hydrophilic products are not retained or reabsorbed either prior to or after their excretion from the body. On the other hand, in Treatment B, fresh concentrations of MC-984 are available to the fish and, therefore, the fish keep accumulating radioactivity. The attainment of equilibrium around d 12 in Treatment B as a response to this dosage of MC-984 is a typical phenomenon dependent on the dosage and nature of the chemical, animal species, and the route of entry (Hamelink and Spacie 1977; Veith et al. 1979).

The bioaccumulation ratio, i.e., concentration of the chemical in fish ÷ concentration in water, was calculated from the data presented above. In Treatment A, 3 L of water contained .300 mg of MC-984 and 3 fish, and thus, each fish was exposed to a total amount of 0.100 mg. In Treatment B, the concentration in water maintained at a constant level resulted in the exposure of each fish to 1.6 mg (cumulative) of MC-984 during the 16-d exposure.

In Treatment A, the maximum bioaccumulation occurred on d 2 (Table 2), and the ratio started declining thereafter, and by d 16 the value was only 11 (the total radioactivity in water was equivalent to about 87 μg/L of the parent chemical). Similarly, the minnows (*Psedorasbora parva*) exposed to 1 mg/L of insecticidal organophosphates showed (in their bodies) 211 mg/L of diazinon after 3 d, 162 mg/L of fenitrothion after 4 d, and 2.4 mg/L of malathion after 1 d (Kanazawa 1978). After 30 d fish contained 17, 4.9, and 0.01 μg/g, respectively, of diazinon, fenitrothion, and malathion. These static systems represent an

Table 1. Recovery of MC-984 from water and fish.

Exposure Time days	DPM* x 10^3							
	added to water		recovered from water		recovered from fish		% accounted for in water + fish	
	Treatment A**	Treatment B	Treatment A**	Treatment B	Treatment A	Treatment B	Treatment A	Treatment B
1	964 ± 2	952 ± 3	538 ± 15	537 ± 30	351.00 ± 16	325 ± 23	91.72 ± 0.44	94.56 ± 1.51
2	966 ± 15	1927 ± 51	529 ± 15	1166 ± 31	370.00 ± 10	660 ± 20	93.15 ± 2.50	95.17 ± 1.52
4	956 ± 16	3787 ± 3	677 ± 25	2849 ± 80	237.00 ± 30	878 ± 80	95.42 ± 1.40	99.10 ± 1.25
8	980 ± 17	7598 ± 12	915 ± 10	6601 ± 90	45.00 ± 10	831 ± 35	97.42 ± 1.81	98.20 ± 2.12
12	967 ± 4	10906 ± 31	929 ± 48	9943 ± 30	21.40 ± 0.10	1218 ± 13	92.81 ± .19	97.27 ± .15
16	972 ± 12	15288 ± 32	827 ± 11	13906 ± 112	18.45 ± 2.41	999 ± 121	87.50 ± 2.01	97.22 ± .12

*3163.262 DPM = 1 microgram; each of the 2 jars contained 3 L of water and 3 fish.

**Represent the cumulative values for each of the 6 samples; DPM in fresh water following daily transfer of fish were always about 960,000.

Table 2. Absorption of MC-984 by goldfish.

Exposure Time days	fish wt.** gm	MC-984* ng/L in water	MC-984* µg/g in fish**	MC-984* % absorbed by*** each fish	MC-984* bioaccumulation ratio**
Treatment A					
1	2.194 ± .155	56.6	16.843 ± .625	13.09 (5.97)	298 ± 14
	1.995 ± .152	56.4	19.210 ± .790	13.49 (6.76)	342 ± 10
2	1.990 ± .102	55.7	19.805 ± .816	13.73 (6.90)	356 ± 15
4	1.851 ± .191	71.4	14.020 ± 1.887	8.65 (4.67)	196 ± 25
8	1.950 ± .149	95.7	2.601 ± .607	1.58 (0.800)	32 ± 9
12	2.051 ± .208	92.5	1.130 ± .097	.79 (0.385)	12.21 ± 1.05
16	1.986 ± .130	87.1	0.989 ± .066	.75 (0.340)	11.35 ± .53
Treatment B					
0	2.153 ± .300	100.0			
1	1.995 ± .152	56.4	19.21 ± 0.29	13.49 (6.76)	342 ± 10
2	2.194 ± .133	68.9	32.17 ± 2.20	16.79 (7.65)	469 ± 38
4	1.966 ± .099	89.2	48.16 ± 5.07	17.07 (8.68)	551 ± 75
8	2.131 ± .176	97.8	41.31 ± 2.18	15.74 (7.39)	422 ± 18
12	1.970 ± .353	92.4	66.32 ± 8.14	19.39 (9.84)	730 ± 95
16	1.825 ± .204	81.7	57.62 ± 4.05	19.02 (10.42)	737 ± 96

*Each of the two jars contained 3 L water, 3 fish and 100 µg/L MC-984. **Mean ± SEM (of 6 fish). ***Calculated from total micrograms actually present in water and fish; values in parentheses corrected to the percent of this total on per gm fish wt.

environmental situation where a body of water can become accidentally polluted in one or more discharges. In such a case equilibrium seems to be in favor of outflow of the absorbed chemicals from the fish (Kanazawa 1978; Khan et al. 1979) because of their degradation and excretion (Miyamoto et al. 1979) but irrespective of their water solubility and concentration in water.

In Treatment B, the highest bioaccumulation ratio, about 730, was seen from d 12 on (Table 2). In similar studies, underyearling trout, yearling trout (*Salmo gairdneri*) and southern topmouthed minnow exposed to 0.1 mg/L of fenitrothion showed maximum absorption within 3 to 6 d, which did not decrease up to 16 d, with bioaccumulation ratios of 250, 230, and 200, respectively (Miyamoto et al. 1979). The bioaccumulation value for MC-984 is higher than 206 reported for topmouth gudgeon exposed for 7 d to 50 µg/L diazinon (Kanazawa 1978) or 10 and 80 for *Fundulus heteroclitus* exposed to 0.32 µg/L diazinon or 0.2 mg/L parathion, respectively (Miller et al. 1966). Treatment A simulates an aquatic environment continuously receiving discharges from the pollution source. The water solubility of MC-984 is much higher than that of most cyclodienes. However, the residues of MC-984 in fish show levels as high as 66 µg/g. As compared with OCs, which are volatilized from water surface (at concentrations above their solubilities) rather rapidly (Podowski et al. 1991; Frankovic et al., unpublished), MC-984 levels in water did not change under similar conditions. In spite of this the bioaccumulation value of MC-984 is comparable with those of cyclodienes. It is about twice as much as that of *cis*- and *trans*-chlordanes and photodieldrin (Khan et al. 1979), is comparable with that of heptachlor (in a continuous flow system) for bluegills (Cope 1966) and lower than those reported: for heptachlor (Sanborn et al. 1976) and DDT (Meikle et al. 1972) in mosquitofish (Sanborn et al. 1976), as well as for dieldrin in trout (Holden 1966) and minnow (Mount and Putnicki 1966). This flame retardant, therefore, can pose ecological problems if released in aquatic environments.

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