Uptake of Three Polychlorinated Biphenyls, DDT, and DDE by the Green Sunfish, *Lepomis Cyanellus* Raf.

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The ubiquitous occurrence of polychlorinated biphenyls (PCB's) in aquatic organisms demonstrates the extreme persistence of these materials and their resistance to be metabolized in fish to water soluble products (HENDERSON *et al.* 1971, KOEMAN *et al.* 1969, ZITKO 1971, RISEBROUGH 1968, JENSEN *et al.* 1969). The complex mixtures of chlorinated biphenyls in commercial polychlorinated biphenyls (PCB's) makes quantitative research on the physiological effects of these compounds and their susceptibility to metabolism very difficult. However, the availability of three ^{14}C biphenyls, 2,2',5-trichlorobiphenyl, 2,5,2',5'-tetrachlorobiphenyl, and 2,4,5,2',5'-pentachlorobiphenyl, provides a partial solution to this problem as these three pure isomers are major components of three important commercial PCB's, namely, 1242, 1248, and 1254 (WEBB and MCCALL 1972). Recently the fate of these pure chlorinated biphenyl isomers has been examined in a model ecosystem and the substantial accumulation of both the tetra- and pentachlorobiphenyl isomers by the mosquito fish, *Gambusia affinis* Baird and Girard, 15.6 and 119.7 ppm (parts per million), respectively, clearly demonstrated the necessity for the examination of the uptake of these materials from water by fish (METCALF *et al.* 1974). This communication reports the uptake of three pure ^{14}C PCB's, DDT, and DDE by the green sunfish, *Lepomis cyanellus* Raf.

MATERIALS AND METHODS

The three pure (>99% by tlc, thin-layer chromatography, and radioautography) chlorinated biphenyls were obtained from Mallinckrodt and had the following specific activities in mCi/mM: 2,2',5-trichlorobiphenyl 9.83, 2,2',5,5'-tetrachlorobiphenyl 9.78, 2,4,5,2',5'-pentachlorobiphenyl 9.78. The ring labeled ^{14}C DDT and DDE were available from previous work and had specific activities of 0.264 and 5.59 mCi/mM, respectively (METCALF *et al.* 1971). Fifteen green sunfish weighing 50-150 mg were placed in 2 liters of

Bulletin of Environmental Contamination & Toxicology,
Vol. 13, No. 2 ©1975 by Springer-Verlag New York Inc.

aged tap water for a 24-hour acclimation period, then two concentrations, approximately one and three ppb of the five compounds in acetone, were added to the jars. Each concentration was run at 22°C in triplicate and the fish were fed *Daphnia magna* Straus, twice a week during the experiment. The experimental design as described in Table I is taken in part from the study on the uptake of DDT and methoxychlor by several species of fish (REINBOLD *et al.* 1971). This type of experiment models the pulsed introduction of pesticides or industrial chemicals into the aquatic environment. Each point on the graphs represents the average of three fish and when the standard deviation for each point was divided by the average for each point an average of 22% was obtained for all of the experiments.

Analysis of concentrations of the various compounds in the fish was accomplished by solubilizing the fish individually in scintillation vials with one ml Protosol® (New England Nuclear) at 60°C for 4 to 6 hours and then adding Aquasol® (New England Nuclear) for scintillation counting. This method assumes that all the radioactivity is parent compound which, except for the trichlorobiphenyl which undergoes substantial metabolism, (see Table III) was an excellent assumption. At the end of the experiment the remaining fish were ground in acetone and the extracts were spotted on tlc plates (silica gel GF-254, Brinkmann, 0.25 mm) and developed in Skellysolve B for metabolite distribution by radioautography (Blue Brand X-ray film, Eastman Kodak) and liquid scintillation counting. The fluid used for counting contained PPO (7 g), POPOP (0.05 g) and napthalene (120 g) in one liter dioxane. Quenching for both the fish and tlc analysis was corrected using an external standard.

RESULTS AND DISCUSSION

Tables I and II show the experimental design of the uptake study and the amounts of ^{14}C compounds added to the environment of the fish. The data in Table III describe the metabolite distribution of the compounds at the end of this uptake investigation. Table IV contains data showing the bioconcentration of the three ^{14}C PCB's, DDT, and DDE. Figures I-V show the time dependent uptake of the five compounds by *Lepomis cyanellus* Raf. Figure VI shows the relationship for the three PCB's and DDE between the unextractable radioactivity in *Gambusia affinis* Baird and Girard, in the 33-day ecosystem

TABLE I

Timetable for experiment on uptake of three PCB's, DDT, and DDE by *Lepomis cyanellus* Raf.

Day	Treatment
0	Add ^{14}C in acetone
2	Take out one fish, weigh and count ^{14}C
3	Take out one fish, weigh and count ^{14}C
9	Change water and add ^{14}C in acetone
10	Take one fish, weigh and count ^{14}C
13	Take one fish, weigh and count ^{14}C
16	Take one fish out, weigh and count ^{14}C, grind remaining fish up in acetone, spot acetone extracts on tlc for metabolite distribution

TABLE II

Amounts of three PCB's, DDT, and DDE added to environment of *Lepomis cyanellus* Raf.

	First	Second		First	Second
Trichlorobiphenyl	ppb	ppb	DDT	ppb	ppb
Low	0.94	2.80	Low	0.11	0.11
High	2.80	5.61	High	0.33	0.33
Tetrachlorobiphenyl			DDE		
Low	2.50	1.57	Low	1.13	2.25
High	7.50	5.00	High	3.40	6.75
Pentachlorobiphenyl					
Low	1.77	1.77			
High	5.31	5.31			

TABLE III

Percent distribution of ^{14}C labeled PCB's, DDT, and DDE at end of experiment in *Lepomis Cyanellus* Raf.

	Parent	Polar*
Trichlorobiphenyl	18.39	81.61
Tetrachlorobiphenyl	98.84	1.16
Pentachlorobiphenyl	99.41	0.69
DDE	99.00	1.00
DDT	97.23	2.27

*Rf = 0 Skellysolve B

TABLE IV

Bioconcentration of ^{14}C labeled PCB's, DDT, and DDE in *Lepomis cyanellus* Raf. after 15 days.

Trichlorobiphenyl	54
Tetrachlorobiphenyl	460
Pentachlorobiphenyl	1,510
DDE	890
DDT	17,500

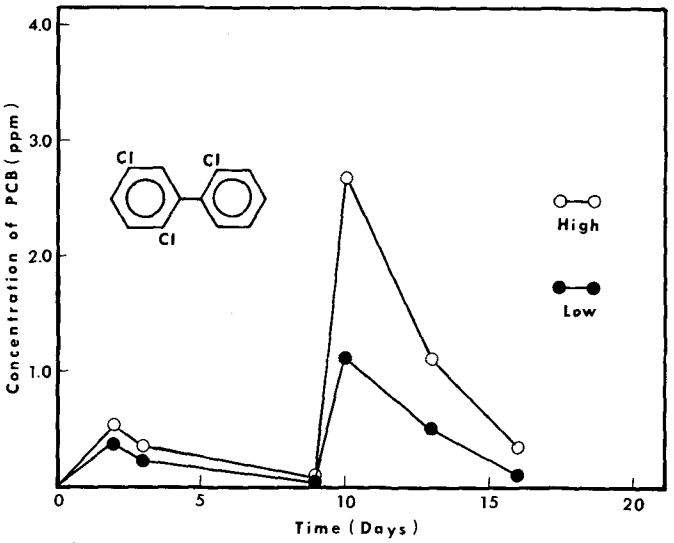

Figure I

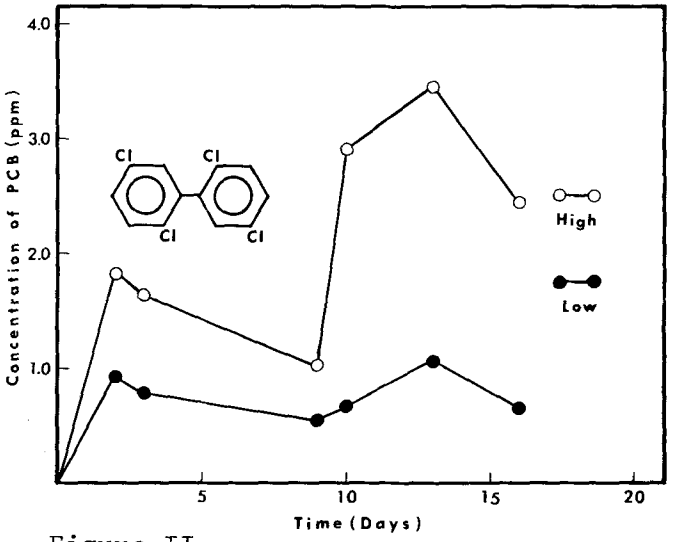

Figure II

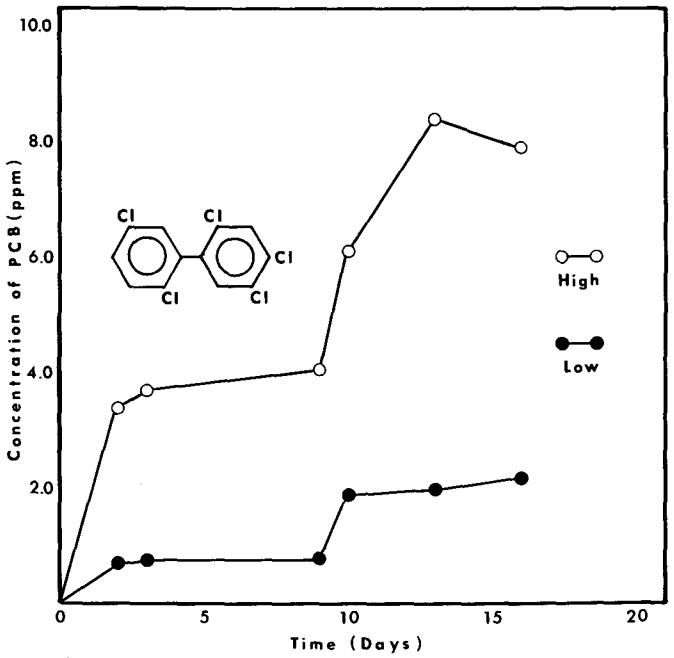

Figure III

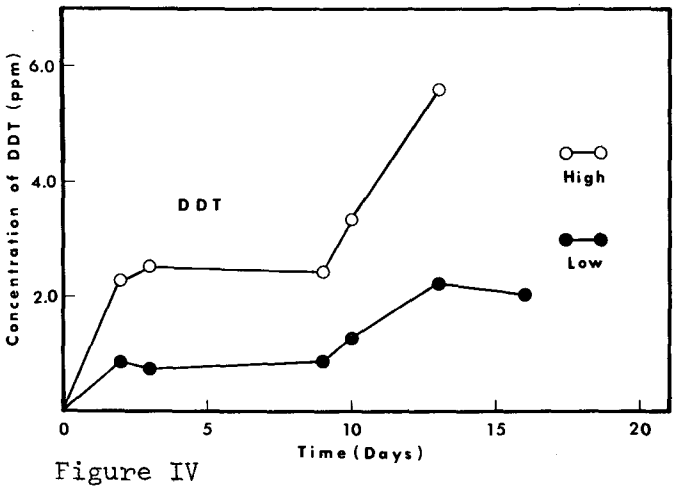

Figure IV

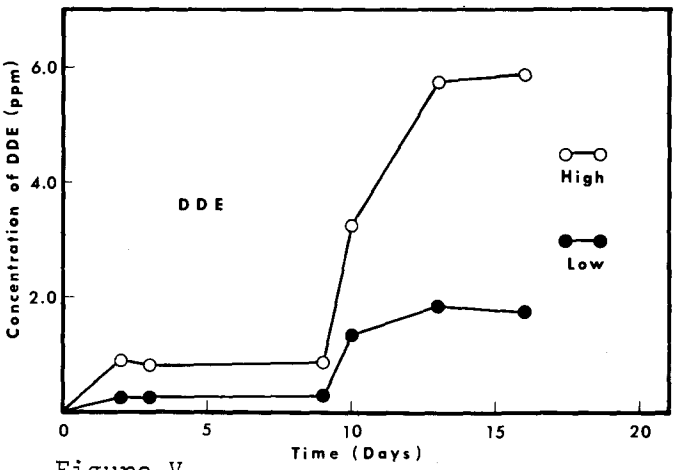

Figure V

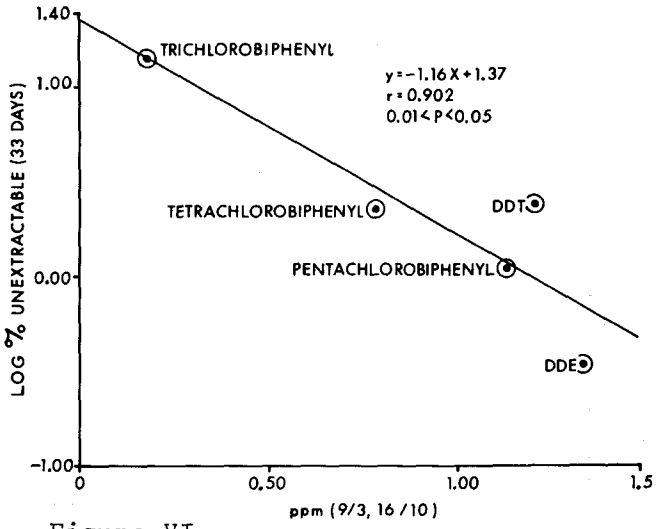

Figure VI

(METCALF *et al.* 1974) and the average change in concentration of radioactivity from day three to day nine (ppm day 9/ppm day 3) and day ten over day sixteen (ppm day 16/ppm day 10). Each point on Figure VI on the abscissa is an average of four values.

It is quite clear from examination of Figures I-V that three of the compounds, namely the pentachlorobiphenyl, DDT, and DDE have similar uptake curves in that the fish retain the radiolabeled compound after it is added to the environment of the fish. The point for the last day for DDT is not shown as the

fish died before final value could be measured. The tetrachlorobiphenyl is retained somewhat less than pentachlorobiphenyl, DDT, and DDE in that a few days after a peak concentration is reached after addition of labeled biphenyl the curve begins to drop. The trichlorobiphenyl differs dramatically from the preceding four compounds in that after the peak concentration the curve drops off sharply.

This type of metabolic susceptibility for chlorinated biphenyls, namely that the lower chlorinated biphenyls undergo metabolism more readily than the higher chlorinated PCB's, has been shown for the complex commercial mixtures using rats (GRANT *et al.* 1971) and Japanese quail (BAILEY and BUNYAN 1972). However, the literature appears to be lacking in metabolism studies on pure isomers of the PCB's except for some work on 4-chlorobiphenyl in the rabbit (BLOCK and CORNISH 1959, HUTZINGER *et al.* 1972). A comparative metabolism study with pigeons, rats, and trout and 4-chlorobiphenyl, 4,4'-dichlorobiphenyl, 2,5,2', 5'-tetrachlorobiphenyl, and 2,4,5,2',4',5'-hexachlorobiphenyl has been reported (HUTZINGER *et al.* 1972). These authors found that the trout could not hydroxylate any of these compounds while the pigeon and rat were able to hydroxylate all but the hexachlorobiphenyl. Recently an elimination study with the rhesus monkey and two pure ^{14}C biphenyls, 2,4'-dichlorobiphenyl and 2,5,2'-trichlorobiphenyl, has been reported but no metabolite structure or distribution was reported (GREB *et al.* 1973). The first hydroxylated metabolite from a higher chlorinated biphenyl has been reported in a study of the excretion products of 2,4, 3',4'-tetrachlorobiphenyl in rats (YOSHIMURA and YAMMAMOTO 1973). These authors identified two hydroxylated species as the 2- and 5-hydroxy derivatives of the tetrachlorobiphenyl. Further the 5-hydroxy biphenyl was determined to be about four times more toxic to the rat than the tetrachlorobiphenyl.

The data reported in this paper corroborate quite well the information already available, except for the trout, on the metabolism of PCB's by organisms. In the present work the data indicate rather convincingly that the green sunfish is able to transform the trichlorobiphenyl into polar species as only 18% (Table III) of the radioactivity in the fish was parent material at the end of the experiment. Perhaps species differences and temperature optimum of detoxifying enzymes account for the discrepancy between the relative abilities of the trout and green sunfish to metabolize chlorinated biphenyls. Further, previous investigations have shown that trout liver microsomes as compared to mouse liver microsomes are very inefficient at

introducing a hydroxyl group into an aromatic nucleus such as biphenyl (CREAVEN *et al.* 1965) and therefore the reported inability of trout to hydroxylate any chlorinated biphenyl is not surprising.

Finally, Figure VI clearly demonstrates an interesting relationship between the dynamics of uptake in the present study, that is the change in concentration of the biphenyls in the fish after labeled compounds are added to the water of the fish, and the percent unextractable radioactivity in *Gambusia affinis* Baird and Girard, in the 33-day model ecosystem experiment. This is a logical relationship as it would be expected the greater the metabolic susceptibility the larger the amount of incorporation of ^{14}C radioactivity into unextractable polar compounds.

This study emphasizes the need for further research and information on uptake and metabolism of polychlorinated biphenyls by aquatic organisms. It has been established that 2,5,2'-trichlorobiphenyl is quite susceptible to metabolism by the green sunfish, and 2,5,2',5'-tetrachlorobiphenyl and 2,4,5,2',5'-pentachlorobiphenyl are much less susceptible. These latter two chlorinated biphenyls, especially the pentachloro isomer, resemble the relatively metabolic inert chlorinated hydrocarbons DDT and DDE. Much of the previous work, and this paper, deal with the qualitative assessment of metabolic susceptibility as a function of number of chloro substituents. This type of information is useful as it provides the background data for work that desperately needs to be done on the effect of chlorine isomer distribution on metabolic susceptibility, and, even more important, the further structural elucidation of the hydroxylated chlorinated biphenyls.

ACKNOWLEDGMENTS

The authors want to thank Dr. Ching-Chieh Yu, Janet Matusumoto, and M. Kathryn McClendon for their skillful assistance during this work. This investigation was supported in part by the Illinois Natural History Survey, the Illinois Agricultural Experiment Station, Regional Project NC 96, U.S. Environmental Protection Agency Grant No. EPA 800736, U.S. Department of the Interior Grant No. 14-31-000-3879 and a grant to RLM from U.S. Department of the Interior No. 14-31-0001-3273, Project No. B-050-ILL.

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