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THE ROLE OF SUSPENDED SEDIMENTS IN THE BIOACCUMULATION OF POLYCHLORINATED BIPHENYLS BY FATHEAD MINNOWS (PIMEPHALES PROMELAS)

By

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ABSTRACT

THE ROLE OF SUSPENDED SEDIMENTS IN THE BIOACCUMULATION OF POLYCHLORINATED BIPHENYLS BY FATHEAD MINNOWS (PIMEPHALES PROMELAS)

By

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A proportional diluter system was used to deliver PCB-contaminated suspended sediment to four flow-through aquaria. Fathead minnows were exposed to suspended sediment in five experiments lasting six to 24 days, using three different soils. Two of the soils were contaminated in the lab with Aroclor 1254 and the other soil contained Aroclor 1242 as a result of environmental contamination. The soils contained 1.0, 5.5, and 12.6% organic matter. Fathead minnows exposed for 14 days to suspended sediment with 5.5% organic matter and contaminated with 100 μg/g of Aroclor 1254 accumulated over twice as much Aroclor 1254 as did fatheads exposed under the same conditions with a suspended sediment of 12.6% organic matter.

Fathead minnows exposed for six days to suspended sediment with 1% organic matter environmentally contaminated with Aroclor 1242 accumulated high levels of Aroclor 1242. Also, the rates of uptake of Aroclor 1242 were high when compared to uptake of Aroclor 1254.

Fish appeared to accumulate the lower chlorinated congeners, however, this phenomenon was not statistically significant.

To my parents, Edith C. and Anderson B. Queener

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INTRODUCTION AND OBJECTIVES

An estimated 353.8 million kg of polychlorinated biphenyls (PCBs) have entered the environment by various routes (Shea, 1973). Much of it has been directly dumped into aquatic environments by industries. It was reported that up to 14 kg a day were at one time being dumped into the Hudson River (Hellman, 1976). Atmospheric fallout, especially rainfall, is thought to be a large source of PCBs now entering aquatic environments (Murphy and Rzeszutko, 1978). A recent study estimates 4,800 kg of PCBs are deposited in Lake Michigan by rainfall in one year (Murphy and Rzeszutko, 1978).

The resulting bioconcentration of these compounds by fish has caused serious problems for sport and commercial fisheries. Fish sampled from Lake Michigan in 1971 contained PCBs similar to Aroclor 1254 and ranging in concentrations from 2.7 μg/g in smelt to 15 μg/g in lake trout. PCB concentrations in fish sampled from Lake Superior on 1972-1973 ranged from 0.12 μg/g in sculpins to a high of 5.6 μg/g in a large lake trout (5.6 kg). However, most of the smaller trout contained less than 5 μg/g which is the tolerance level set by the U. S. Food and Drug Administration for human consumption (Veith, 1975; Veith et al., 1977). As a result of the widespread environmental contamination by PCBs, much research has been conducted on the toxicity, accumulation and fate of PCBs in aquatic environments.

Environmental contamination of fish by PCBs has been most closely associated with highly industrialized areas. Veith (1975), in his survey of Lake Michigan, generally found higher concentrations of PCBs in fish from the

southern half of the lake which is surrounded by highly industrialized areas. Rivers with industrial sites also have a serious contamination problem. In 1972 fish from the Hudson River were averaging 200 µg/g and Ohio River and Allegheny River fish were averaging 100 µg/g (Stalling and Mayer, 1972). Fish, in both lakes and rivers, have been exposed to very high concentrations of PCBs in the water, however, since direct dumping of PCBs is no longer allowed, water concentrations have been greatly reduced. The problem now is the reservoir of PCBs in the sediments leaching into the water providing a continuing source of contamination for aquatic biota. If the sediments are left undisturbed the PCBs would slowly leach from the sediments into the water at low levels (Green, 1974), but little is known about the availability of PCBs from a contaminated sediment which is resuspended in the water column. In such a case the suspended sediments would be in direct contact with fish, possibly increasing the uptake of PCBs.

The object of this study is to investigate the availability of PCBs from resuspended sediments to fish. Specific objectives include: (1) the determination of the availability of PCBs sorbed to different soils to fish, (2) the determination of the effects of organic matter content and other soil parameters on the availability of PCBs to the fish and (3) to test a sediment from an aquatic environment that has already been contaminated with PCBs, to compare with our artificially-contaminated soils.

LITERATURE REVIEW

Toxicity data for PCBs varies a great deal depending on species of fish tested and chlorine content of the biphenyl. It also has been observed that toxicity is inversely related to the percentage of chlorine in the compound (Stalling and Mayer, 1972) and that direct exposure via the water is more toxic than dietary uptake (Stalling and Mayer, 1972; Gruger et al., 1975). Flow through tests yield 30-day LC_{50} values ranging from three to 433 $\mu g/\ell$ in three different species of fish for Aroclors 1242, 1248, 1254 and 1260 (Mayer et al., 1977).

Bioconcentration values range from 10^4 to 10^6 depending on percent chlorine content, the usual pattern being that higher chlorinated PCBs are concentrated to a greater extent than lower chlorinated isomers (Branson et al., 1975; DeFoe et al., 1978; Gruger et al., 1975; Mayer et al., 1977; Metcalf et al., 1975; Sanbord et al., 1975; Veith, 1975; Veith et al., 1977). In a feeding study conducted on juvenile coho salmon (Oncorhynchus kisutch) comparing a tetrachlorobiphenyl with two hexachlorobiphenyls, it was found that after 108 days there was a significantly (90% c.f.) higher accumulation of each hexachlorobiphenyl compared to the tetrachlorobiphenyl (Gruger et al., 1975). In the same study, after a starvation period, the hexachlorobiphenyl concentrations in the juvenile coho increased while the concentration of the tetrachlorobiphenyl decreased. They concluded that there was a mobilization or a transformation of the chlorobiphenyls probably due to metabolization of the lower chlorinated biphenyls or the difference in the partition coefficients of the chlorobiphenyls in

aqueous media. This conclusion is supported by a model ecosystem study suggesting that Gambusia affinis can hydroxylate tetrachlorobiphenyls (Metcalf et al., 1975). Another study indicated that green sunfish (Lepomis cyanellus) were able to hydroxylate trichlorobiphenyls while tetra- and pentachlorobiphenyls were much less susceptible to hydroxylation (Sanborn et al., 1975). Other studies also have indicated a rapid depletion of lower chlorinated biphenyl residues in fish (DeFoe et al., 1978; Mayer et al., 1977).

Many organochlorine pollutants, including PCBs, readily adsorb to suspended particulate matter in the water or become associated with the sediments. Therefore, sediments play a major role in the translocation of PCBs in aquatic environments. This role, however, is not clearly defined, but much depends upon the physical, chemical and biological characteristics of the sediments involved.

Among the characteristics of the sediment that can affect the adsorption and release of chlorinated hydrocarbons, and similar compounds, it is generally considered that organic matter content and particulate matter are the two most important. Other important factors to consider are pH of the soil and water solubility of the compound involved.

The lipophilic nature of PCBs make them easily adsorbed to organic matter. A study with DDT demonstrated a close relationship between organic content and high distribution coefficients, as derived from the Freundlich and Langmuir adsorption equations (Shin, 1970). It has been generally concluded that under field conditions organic matter is the single most important factor in the retention of pesticides in the soil (Weed and Weber, 1974). This conclusion is supported by data obtained from an investigation of southern Lake Michigan sediments in which t-DDT (DDT + DDE + DDD) and dieldrin were found to be related ($r = 0.6$ and 0.66, respectively) to organic carbon content of the

sediments (Leland et al., 1973). Also, it was generally observed that large accumulations of insecticides were associated with sediments containing high amounts of organic matter (Leland et al., 1973).

Bioactivity of chlorinated hydrocarbons is affected by organic content, especially in moist soils (Weber and Weed, 1974). Therefore, the organic content of suspended sediments will play an important role in the bioactivity of adsorbed chlorinated hydrocarbons. Specific studies indicate a similar relationship between PCBs and soil as seen with other chlorinated hydrocarbons. Adsorption studies with 2,4'-dichloro-, 2,2',5,5'-tetrachloro- and 2,2',4,4',5,5'-hexachlorobiphenyls with humic acid, illite clay and Del Monte sand, show the greatest adsorption for hexachlorobiphenyl followed by tetrachloro- and dichlorobiphenyl and total adsorption of the di-, tetra- and hexachlorobiphenyls followed the series humic acid > illite clay > Del Monte sand (Haque and Schmedding, 1976). Therefore, adsorption generally increases with increasing chlorine number and organic content of the soils. Haque and Schmedding (1976) conclude that most of the transport of PCBs in aquatic systems will be by adsorption to particulates and that movement due to solubility will be negligible for most PCBs. In a later study it was concluded that both total organic carbon and particle size distribution were important factors in the partitioning of two tetrachlorobiphenyl isomers, but there was no correlation between organic content and the partitioning of PCB mixtures (Steen et al., 1978). This suggests that the adsorption effects of organic matter are modified by association with various particle sizes, especially clay. In Lake Michigan a correlation ($r = 0.65$) was found between clay material ($< 2\ \mu$) and organic carbon which led to the conclusion that the occurrence of t-DDT and dieldrin in the deep basins of southern Lake Michigan is due to transport by small particles (Leland et al., 1973). In a study on the retention of DDT and methoxychlor on soils treated with

hydrogen peroxide to eliminate organic matter and on the same soils untreated, it was found that in treated and untreated soils the highest retention capabilities were shown by the clay fractions (Richardson and Epstein, 1971).

Chlorinated hydrocarbon compounds have been associated with particulate matter in sediments. It has been determined that pesticides are associated with various particulate components as a function of particle size in water samples from Lake Erie (Pfister et al., 1969).

The high degree of association between clay fractions and PCBs has been demonstrated in studies using percolating water to induce leaching of PCBs from soils (Tucker et al., 1975). It was discovered that the amount of leaching of PCBs was related to the clay content of the soils. When leaching did occur it included only the lower chlorinated, more water-soluble isomers from those soils with a low percent clay content.

The greater water solubility of the lower chlorinated isomers is well documented. A solubility study found that two different batches of Aroclor 1254 had water solubilities of 2 to 3 mg/ℓ in fresh water, the latter having a greater concentration of higher chlorinated isomers (Zitko, 1970). Aroclor 1221, which is predominantly dichlorobiphenyl, was reported to have a water solubility of 5.0 mg in fresh water (Zitko, 1970). Water solubilities of Aroclor 1242 and 1016 in one study are reported at 0.34 mg/ℓ and 0.42 mg/ℓ, respectively (Paris et al., 1978). A value for Aroclor 1016 was also reported at 0.225 to 0.250 mg/ℓ (Tucker et al., 1975). The values cover a wide range and depend on the method used to solubilize the PCBs in water. The suspendion of sediments in the water column can greatly influence the transport of sorbed PCBs. It has been noted that when water is added to soils it displaces sorbed chlorinated hydrocarbon pesticides on soils (Green, 1974). The increased surface area caused by resuspension of sediments increases the competition between water and PCBs for adsorption

sites and increases the amount of PÇBs solubilized in water. In accordance with the solubility values it is the lower chlorinated isomers that are displaced first and become more readily available for direct absorption from the water by aquatic organisms.

The pH of the suspended sediment-water system can influence the adsorption and release of organic compounds with the soil. Being essentially non-polar, PCBs are not greatly affected, however, there is a general trend of increased sorption with decreased pH due to enhanced hydrogen bonding (Shin, 1970).

METHODS

I. Experimental Plan

As described in the literature review, the dynamics of the relationship between PCBs, soil and water have been examined closely. Halter and Johnson (1977) combined these components with a system containing fish forming a model aquatic system to investigate the "dynamics" of Aroclor 1254. PCB desorption from sediments and accumulation by fish were studied under flow-through and static water conditions with undisturbed bottom sediments contaminated with Aroclor 1254. The experiments by Halter and Johnson were continued in this study using the same model aquatic system to study suspended sediments contaminated with Aroclor 1254 under flow-through water conditions. The purpose of this study was to verify the prediction that PCBs sorbed to sediments would be more available to fish if the sediments were suspended. Also that sediment characteristics, such as percent organic content and particle size distribution, were important factors in the desorption of PCBs from the suspended sediment and the accumulation of PCBs by the fish.

The model aquatic system used throughout this study consisted of a proportional diluter system which continuously delivered contaminated suspended sediment to four aquaria where fish were exposed to four different concentrations of suspended sediment. A fifth aquarium received water from the diluter that contained no suspended sediment or PCBs and acted as a control.

Five experiments were conducted using two different sediments and one soil which were selected for their differences in percent organic content. Two

of the soils were contaminated in the laboratory with PCBs prior to use in the diluter system and the third soil had been contaminated with PCBs in the environment. In all experiments the three components of the system, fish, suspended sediment and water, were sampled and analyzed for PCB content.

The first experiment, lasting 24 days, was conducted primarily to determine if the diluter system could deliver reproducible amounts of suspended sediments to the aquaria and to determine a reasonable time scale for future experiments. Suspended sediment levels (mg/ℓ water) were monitored as well as PCB concentrations in suspended sediment, fish and water. The distribution of the various isomers present in Aroclor 1254 (as represented by different peaks on the chromatograph) was examined in fish, water and suspended sediment and compared to chromatographs of standard Aroclor 1254 solutions. The isomer distribution was monitored to determine if there was a selective retention of isomers by the suspended sediments and/or a selective uptake by the fish.

The time span for the second experiment was shortened to 14 days since it was determined from the first experiment that 14 days was a sufficient amount of time for the fish to concentrate easily detectable levels of PCBs. The distribution of Aroclor 1254 isomers was examined as in the first experiment.

The third experiment was conducted in the same manner as the second experiment, the only difference being the type of soil used. The third experiment utilized a soil with 12.6% organic matter (greenhouse soil) and was used as a direct comparison to the second experiment in which a soil with only 5.5% organic matter was used. Distribution of Aroclor 1254 isomers was examined as in experiments I and II.

Comparison of the second and third experiments was expected to show that PCBs adsorbed to a suspended sediment with a high organic matter content would be less available to fish by direct or indirect absorption than PCBs

adsorbed to a suspended sediment with a low organic matter content. The defintion of direct absorption, as used here, is the absorption of PCBs by fish as a result of contact with PCB-contaminated suspended sediment. In other words, the PCBs are absorbed directly from the suspended sediments by the fish. Indirect absorption is the uptake of PCBs by fish from water resulting from the PCB desorption from soil to water. The exact mechanism of uptake by fish is not known but is probably a combination of both direct and indirect absorption.

A fourth experiment was conducted to monitor the movement of a single PCB isomer, 2,5,3',4'-tetrachlorobiphenyl. The soil used in this experiment contained 12.6% organic matter. The purpose of this experiment was to collect data on tetrachlorobiphenyl uptake by fish where the source of contamination was the suspended sediment. These data were to be compared with literature values of fish uptake of tetrachlorobiphenyl where the source of contamination was water with no suspended sediment present. The nature of the suspended sediment was expected to have a modifying affect on accumulation of tetrachlorobiphenyl by fish because of higher retention of PCBs on the high organic content soil.

In the final experiment a soil was used which had been obtained from a stream that had been contaminated with PCBs. The soil contained only 1% organic matter and the experiment was intended to be used as a direct comparison to the second and third experiments in which soils containing 5.5 and 12.6% organic matter were used. However, a direct comparison of experiments was not possible for the following reasons. First, the soil used in the last experiment contained mostly Aroclor 1242 and in the previous experiments the soils had been dosed with Aroclor 1254. The higher percentage of tetrachlorobiphenyls in Aroclor 1242 increased the desorption from soil to water since tetrachlorobiphenyls are more water soluble than penta- and

hexachlorobiphenyls which are the predominant isomers in Aroclor 1254. Secondly, there was a limited amount of soil available in the last experiment which made it necessary to reduce the experiment to six days, whereas the other experiments that were intended for comparison lasted 14 days. While no direct comparisons could be made with previous experiments, this final experiment was valuable in providing data on the movement of PCBs in the environment under conditions where environmentally-contaminated sediments are redistributed through the water column.

II. Sediments

Three different soils were used in the experiments (Table 1). In experiments I and II, the sediment used was obtained from a pond in Fenner Arboretum, Lansing, MI. This sediment was selected because the arborteum had not used any pesticides for several years, therefore reducing the chance of interfering compounds being present (especially other organo-chlorine compounds). The arboretum soil had a 5.5% total organic conent, a pH of 7.2 and the following percentages of fractions: sand, 85%; silt, 13%; and clay, 2%. Organic content of the fractions was 5.2%, 20.68% and 12.65% for sand, silt and clay, respectively.

The soil used in experiments III and IV was obtained from the Michigan State University Pesticide Research Center greenhouse and showed no PCB-interfering peaks upon analysis. The greenhouse soil had a total organic content of 12.6%, a pH of 7.7,. and sand, silt and clay percentages of 75, 8 and 17, respectively. Percent organic content of the fractions was 9.91, 17.73 and 17.13 for sand, silt and clay, respectively.

The soil used in experiment V was dredged from the south branch of the Shiawassee River, Livingston County, Michigan (approximately 153 m west of

Table 1. Characteristics of Soils and PCB Concentration of the Soil Fractions

Soil	Total Percent Organic	pH	Fractions Type	Percent	Percent Organic	PCB (μg/g)
Arboretum	5.5	7.2	sand[a]	85	5.20	33.78
			silt[b]	13	20.68	40.04
			clay[c]	2	12.65	
Greenhouse	12.6	7.7	sand	75	9.91	100.17
			silt	8	17.73	112.97
			clay	17	17.13	
Shiawassee River	1.0	7.4	sand	89	2.38	19.00
			silt	4	12.97	81.64
			clay	7	25.50	

[a]Sand includes particles with a diameter of 0.0778 mm or larger.
[b]Silt includes particles with a diameter of 0.0201 mm up to 0.0778 mm.
[c]Clay includes particles with a diameter of less than 0.0201 mm.

Highway 59 just north of I-96). An Ekman dredge was used to sample the sediment in approximately one foot of water. This sediment had been contaminated with PCBs, and contained only 1.0% total organics and had a pH of 7.4. The sand fraction accounted for 89% of the soil, silt, 4%, and clay, 7%. Organic content of the fractions was as follows: sand, 2.38%; silt, 12.97% and clay, 25.50%.

The percentages of sand, silt and clay for each soil were determined by the hydrometer method (Bouyoucous, 1927; 1928; 1929).

All soils were filtered in a no. 60 standard sieve (opening size 0.0098 inches, approximately 0.25 mm) to remove large debris and most of the heavy sand to minimize settling in the diluter and aquaria. Soil fractions were separated by sieving with a no. 230 standard sieve (0.0025 inch mesh, approximately 0.06 mm) to remove the sand fraction. The remaining fraction, containing the silt and clay, was suspended in water (using the Bouyoucous method) and allowed to separate by settling out the heavier silt in 30 minutes. The silt fraction was all that had settled out in 30 minutes. The material remaining in suspension at the end of 30 minutes represented the clay-colloid fraction and was separated from water by centrifugation. The three fractions were analyzed for percent organic matter and PCB concentration.

Total percent organic matter of the soil as a whole and percent organic matter for each fraction was determined by combustion in a muffle oven at 500^{o}C for two hours.

For experiments I, II, and III the soils were dosed with Aroclor 1254 at 100 μg/g soil. The Aroclor was dissolved in excess acetone and mixed with the dry soil in a shallow pan. The acetone was allowed to evaporate and then the pans were covered with aluminum foil to minimize volatilization of the PCBs. In experiment IV the soil was dosed at 50 μg/g soil with 2,5,3',4'-

tetrachlorobiphenyl in the same manner. In experiments II, III and IV the dosed soils were prewashed by allowing water to flow over them for 14 days, stopping the flow of water twice, stirring the sediment, allowing it to settle, then resuming the flow of water. This was done to eliminate any excess PCB that was not tightly bound to the soil therefore simulating a sediment contaminated in an aquatic environment.

Before being introduced into the diluter system the soil was mixed with water to form a slurry which was constantly stirred in a reservoir. The slurry was pumped through 0.49 cm i.d. silicone tubing, via a flex pump, to the mixing box of the diluter.

III. Fish

Fathead minnows (Pimephales promelas) were raised in outdoor ponds and were acclimated to well water in indoor holding tanks at least two months prior to each experiment. Aquaria were stocked with 30 fish each, in experiment I, and 20 fish each, in all remaining experiments.

Fish were fed a diet of crushed Purina Trout Chow (residue-free) every day during acclimation and experiment I, but only two or three times during each remaining experiment, generally 25 g every four days.

IV. Water Supply

Water for the fish holding tanks and the diluter sysatem was supplied from a well. It was pumped into large storage tanks and then delivered to each system via PVC piping. The water temperature averaged 13°C. Total hardness was 300 mg/ℓ as $CaCO_3$, pH was 7.4 and dissolved oxygen averaged 8.6 mg/ℓ.

V. Exposure Methods

PCB-contaminated sediment was introduced into the mixing box of the proportional diluter where it was mixed with incoming well water. The suspended-sediment solution was delivered into separate diluter boxes from where it was simultaneously siphoned with well water into four separate aquaria, each receiving a different amount of suspended sediment. The control aquaria in the system received water without suspended sediment. The bottom of each aquaria was screened off (approximately 1 cm mesh) to prevent fish from directly contacting any contaminated soil which had settled out. Aquaria with a 50-liter capacity were used initially, however, due to a high rate of settling of suspended material, smaller aquaria (35 liter) were used. The use of smaller aquaria decreased the total flushing time, therefore decreasing the amount of time the suspended material had to settle out and reducing the amount of sedimentation. Reducing sedimentation was important because non-suspended sediment could still release PCBs into the water. Therefore, by minimizing sedimentation it was assumed that most of the PCBs in the water column were released by the suspended sediment. Also the fish spent much of their time at the bottom of the aquaria where high concentrations of PCBs released from sedimented soil could occur.

VI. Sampling

Fish, water and suspended sediment samples were taken on the same day. Each experiment was sampled four or five times at approximately three-day intervals. Three to five fish were sampled from each tank. Fish from the same tank were wrapped together in aluminum foil and immediately frozen.

Water was sampled with a bent glass siphon tube so that the same depth could be sampled in each tank. Samples were then filtered in buchner funnels using Whatman GF/C glass microfiber paper (effective retention = 1.2 μm)

(Campbell and Elliott, 1975). The filters were pre-extracted in a hexane/acetone solution and dried to a constant weight in an oven at 110°C.

Suspended sediment samples were collected on preweighed filters by filtering 500 or 1000 mℓ of water. Two suspended sediment samples were taken for each tank. One was dried and weighed to determine suspended sediment concentration expressed as mg/ℓ. The other filter containing the sample was placed in a small Erlenmeyer flask and capped for later PCB analysis.

Experiment I lasted 24 days with samples taken on days 2, 4, 8, 12 and 24. Experiments II and III lasted 14 days and the samples were taken on days 3, 6, 8, 11 and 14 in experiment II and days 3, 8, 10, 12 and 14 in experiment III. Experiment IV ran for five days with samples taken on days 1, 2, 3, and 5. Experiment V lasted six days with sampling done on days 1, 3, 5 and 6.

VII. Analytical Methods

Filtered water samples (1 liter) were extracted with three 100 mℓ portions of hexane in a 2000 mℓ separatory funnel. The combined extracts were filtered through anhydrous sodium sulfate then evaporated to approximately 1 mℓ for gas chromatography analysis.

The filters containing the suspended sediment samples were kept moist until extracted four times with 25 mℓ portions of a 1:1 hexane/acetone mixture on a wrist shaker. To increase extraction efficiency dry soil samples were wetted thoroughly by shaking with water before extraction (Bellar and Lichtenburg, 1975; Richardson and Epstein, 1971). The extracts were combined in a separatory funnel and washed with 100 mℓ distilled water. The wash water separated from the extracts was back extracted with 25 mℓ hexane, which was added to the other extracts. The combined extracts were then washed with three 100 mℓ portions of distilled water. The washed extract was then filtered

through anhydrous sodium sulfate in a fritted glass buchner funnel. The filtered extract was evaporated to approximately 1 mℓ. The florisil used for cleanup was prewashed with 50 mℓ hexane then oven dried at 130°C. A 2 cm i.d. column was packed with a glass wool plug, 11.5 cm florisil and 2 cm. anhydrous sodium sulfate. The column was prewashed with 50 mℓ hexane and the sample was added and rinsed with a few milliliters of hexane. The column was eluted with 100 mℓ 6% diethyl ether in hexane. The eluate was evaporated to a suitable volume for gas chromatography analysis. This procedure was slightly modified from that of Goerlitz and Law (1974).

Environmental soil samples were more difficult to extract because of interfering substances. A column extraction technique was used where 50 g of the air-dried soil was placed in a 2 cm. i.d. chromatographic column and eluted with 250 mℓ of a 1:1 hexane/acetone mixture. The eluate was washed with 300 mℓ distilled water which was back-extracted with 20 mℓ of 15% methylene chloride in hexane. The extract was added to the original eluate and the combined extracts were washed twice with 100 mℓ distilled water. The washed extract was filtered through sodium sulfate and evaporated to approximately 1 mℓ. A florisil column as described previously was used for cleanup. The column was eluted with 100 mℓ 6% diethyl ether in hexane and the eluate evaporated to approximately 10 mℓ for gas chromatography analysis (U.S.E.P.A., 1977). Interference due to elemental sulfur was eliminated by treatment with mercury at this point by adding a small drop of mercury to the final solution and mixing well (Goerlitz and Law, 1971).

The fish were thawed, blotted dry and weighed. Whole fish extractions were made in hexane with a tissuemizer. Alternate methods were mortar and pestle grinding with soxhlet extracation in hexane or in a ground-glass tissue grinder with hexane. A total extraction volume of 100 mℓ was used, of which 10

mℓ was placed in pre-weighed pan, air-dried and weighed for percent fat analysis. The remaining extract was evaporated to approximately 5 mℓ for column cleanup. A florisil column, as used for water and soil cleanup, was also used for fish samples and likewise eluted with 100 mℓ 6% diethyl ether in hexane. The eluate was evaporated to between 1 and 10 mℓ for gas chromatography analysis.

Computer-assisted gas chromatographic analysis was used for PCB identification. The glass column was 1.8 m (6 feet) long and packed with 3% SE-30 on 60-80 mesh Gaschrom Q. Detection was by electron capture with a Nickel-63 detector. Column temperature was 180°C. for Aroclor 1254 analysis and 160°C. for Aroclor 1242 analysis. Inlet temperature and detector temperature were 225°C and 350°C., respectively. The detectable limit for Aroclor 1254 was approximately 0.5 mg/ℓ. The total peak area was used as a measure of total PCB. Specific peaks were not included in total peak area due to interference in the samples (DDE interference was confirmed by standard DDE injection on the gas chromatograph). These peaks were also excluded from standard total peak area. Total peak area was calculated as the sum of the individual peak areas as measured by the computer.

The presence of Aroclor 1242 in the Shiawassee River sediment was confirmed by high pressure liquid chromatography using a C-18 reverse phase mode with Octadecyl Silane as stationary phase and methanol as the mobile phase. Flow was 1 cc/min., injection volume was 20 µℓ, R = 0.20, wavelength of 235 nm.

RESULTS

I. Suspended Sediment and Water

In experiments I and II, using Arboretum soil dosed at 100 μg/g with Aroclor 1254, suspended-sediment samples yielded levels of Aroclor 1254 higher than the dosage level. The same phenomenon occurred in experiment III with greenhouse soil dosed at the same level. In the first three experiments Aroclor levels in the suspended sediment varied widely over the course of the experiment (Table 2) but there was no significant difference (Appendix 1, 90% c.f.). It also was noted in each experiment that some suspended material settled to the bottom of the aquaria while smaller, lighter particles remained suspended in the water column. When water samples were siphoned to obtain suspended-sediment samples, it was the smaller particles that made up the sample. Considering this, it was theorized that the smaller particles (i.e., silt and clay), which probably contained most of the organic matter, adsorbed most of the PCBs. This theory was confirmed by analyzing each fraction (sand, silt and clay) for percent organic content and PCB concentration (silt and clay fractions were combined for PCB analysis due to small sample sizes). In each soil sample analyzed, the silt and clay fractions were composed of more organic matter than the sand fraction and correspondingly had higher concentrations of PCB. This pattern was especially evident in the Shiawassee River sediment (Table 1).

In experiment IV the greenhouse soil was dosed with 2,5,3',4'-tetrachlorobiphenyl at 50 μg/g, and the suspended-sediment samples yielded tetrachlorobiphenyl levels near the dosage level. The greenhouse (high organic)

Table 2. PCB Concentrations in Suspended Sediment (μg/g)

Experiment Number	Statistical Value	Aquarium Number 2	3	4	5
I	Mean	125	137	163	159
	Range	91-158	68-208	72-221	81-226
II	Mean	222	215	210	153
	Range	162-341	159-246	194-634	103-184
III	Mean	161	189	293	179
	Range	115-184	103-275	90-450	52-356
IV	Mean	70	63	62	61
	Range	55-84	47-83	50-81	51-81
V	Mean	218	237	346	273
	Range	162-277	114-381	189-555	178-372

soil contained 10% more silt and clay combined than did the Arborteum (low organic) soil. The combined silt and clay fractions of the high organic soil contained a higher level of PCB than did the combined silt and clay fractions of the low organic soil. It was speculated that more of the greenhouse soil would stay in suspension due to the higher percentage of silt and clay, therefore allowing more opportunity for desorption of PCB from soil to water resulting in high PCB concentrations in the water. However, there was no significant difference in PCB concentration in suspended sediment between experiments II and III (Appendix 1, 90% c.f.). Also water concentrations of PCB were compared for experiments II and III and no significant difference (90% c.f.) was found except in aquarium number 3 (corresponding aquaria in each experiment were compared, see Table 3 and Appendix 2). Therefore the higher percentage of silt and clay did not greatly affect the suspended sediment and water concentrations of PCB. In all experiments PCB concentrations in the water fluctuated in response to the total amount of suspended sediment present. As the suspended sediment concentration increased, the PCB concentration in the water increased and decreased when suspended sediment decreased. In experiment III using the high-organic greenhouse soil, the suspended sediment levels were slightly lower than in experiment II using the low-organic, Arboretum soil (Table 4). However, there was no significant difference (90% c.f.) in suspended sediment concentrations between corresponding aquaria in the two experiments (Appendix 3). Correspondingly, the PCB concentrations in the water were lower in experiment III than in experiment II. The small difference between PCB concentrations in the water samples of the two experiments was felt to be due to the difference in suspended sediment concentration but there was no signficant difference (90% c.f.) in the concentrations of PCB in the water samples of the two experiments.

Table 3. PCB Concentrations in the Water* (μg/ℓ)

Experiment Number	Statistical Value	Aquarium Number				
		Control	2	3	4	5
I	Mean	0	1.4	1.4	0.9	1.1
	Range	0	1.1-1.9	0.9-2.1	0.6-1.2	0.4-2.0
II	Mean	0	0.8	0.8	0.6	0.7
	Range	0	0.6-0.9	0.6-1.0	0.5-0.7	0.5-1.1
III	Mean	0.3	0.6	0.5	0.4	0.5
	Range	0.2-0.5	0.4-0.8	0.3-0.6	0.2-0.6	0.2-0.7
IV	Mean	0	0.6	0.3	0.2	0.2
	Range	0	0.3-1.7	0.2-0.4	0.2-0.2	0.1-0.2
V	Mean	1.4	5.2	5.3	2.3	2.3
	Range	0-4.0	3.4-6.7	2.3-10.6	1.2-3.2	1.0-3.5

*Filtered water includes particles < 1.2 μm.

Table 4. Suspended Sediment Concentrations (mg/ℓ)

Experiment Number	Statistical Value	Aquarium Number				
		Control	2	3	4	5
I	Mean	4	100	113	22	23
	Range	2-6	29-173	20-237	15-36	11-48
II	Mean	5	44	24	17	21
	Range	1-8	37-50	17-32	13-21	10-33
III	Mean	0	32	20	12	14
	Range	0	24-41	15-29	9-18	9-22
IV	Mean	1	14	7	5	4
	Range	0-3	12-19	5-9	4-7	3-5
V	Mean	0	103	32	12	10
	Range	0	33-220	18-77	5-17	4-14

The concentration of tetrachlorobiphenyl in water samples of experiment IV, using greenhouse soil dosed at 50 μg/g, was similar to Aroclor concentrations in experiments II and III (Table 3) even though the suspended sediment concentrations in experiment IV were much lower than in the previous two experiments (Table 4). This was probably due to the relatively high water solubility of the tetrachlorobiphenyl compared to the penta- and hexachlorobiphenyls that constitute most of the Aroclor 1254 mixture.

II. Isomer Distribution Analysis

Statistical comparison of average percent peak areas between each of the parameters (fish, water suspended sediment) and standard chromatograms revealed a pattern of distribution between the three parameters that can be seen generally in Figure 1. Peaks 1 and 2 are mixtures of tetrachlorobiphenyls, 3, 4, 5 and 6 are mixtures of pentachlorobiphenyls, and 7, 8 and 9 are mixtures of hexachlorobiphenyls. Peaks 1, 2, 3, 4, 6, 7, 8 and 9 were used for comparison when no interference was present.

Chromatograms of samples in experiment I after 24 days were selected randomly and compared with an equal number of standard chromatograms (usually from the same day that the samples were analyzed on the G.C. because G.C. response varied from day to day). Table 5 gives the results of the calculated 'student t' values compared with the 'critical t' value at the 90% confidence level. In the suspended sediment, peaks 2 and 3 (tetra- and pentachlorobiphenyl) were significantly lower in percent area than standard peaks 2 and 3 and percent area of peak 8 (hexachlorobiphenyl) was higher than in the standards. For peaks 1, 4, 6, 7 and 9, there was no significant difference in percent peak area when compared with standard peak area.

Figure 1. Sample Chromatographs of PCBs in Fish, Water, Soil and a Standard Aroclor 1254 Chromatograph

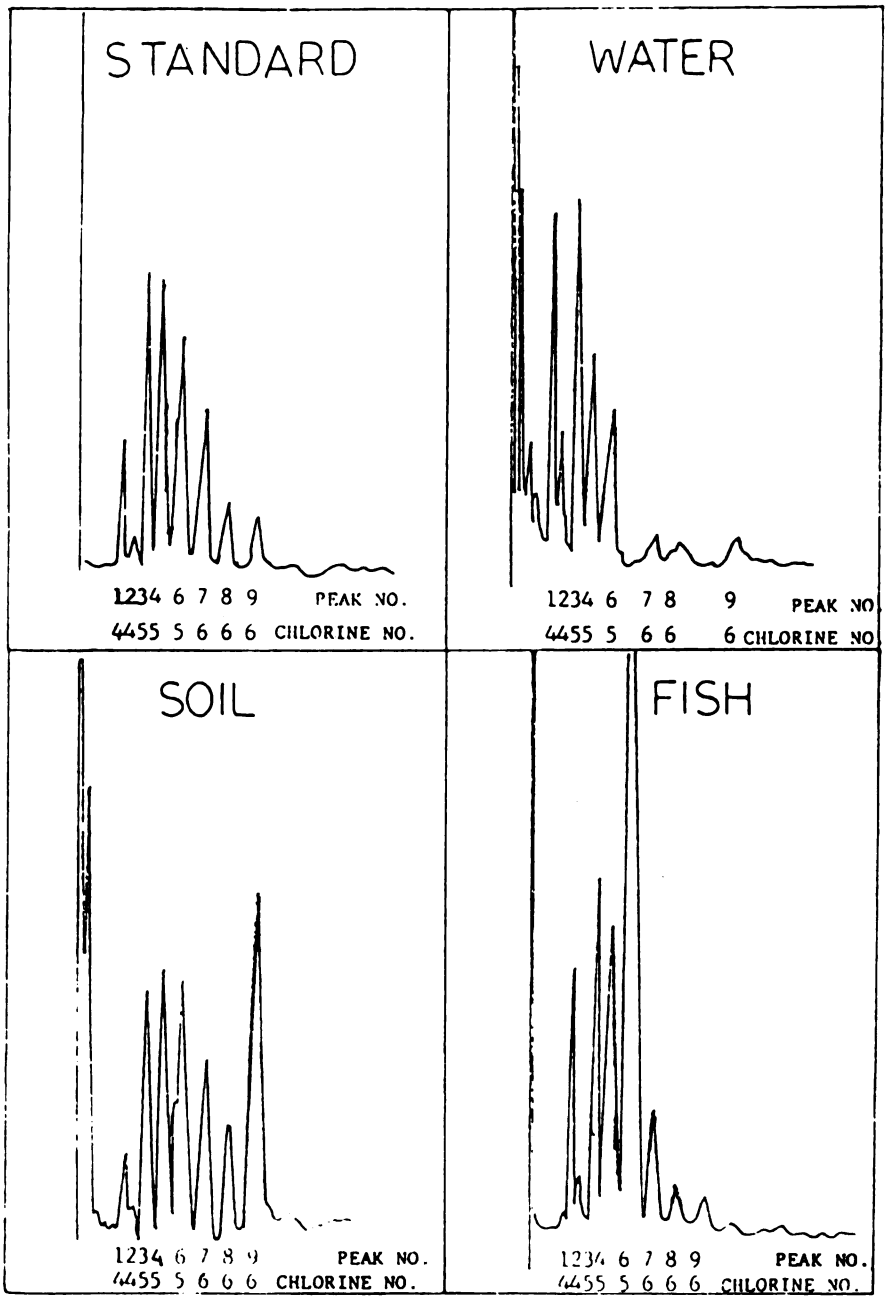

STANDARD
1234 6 7 8 9 PEAK NO.
4455 5 6 6 6 CHLORINE NO.
WATER
1234 6 7 8 9 PEAK NO.
4455 5 6 6 6 CHLORINE NO.
SOIL
1234 6 7 8 9 PEAK NO.
4455 5 6 6 6 CHLORINE NO.
FISH
1234 6 7 8 9 PEAK NO.
4455 5 6 6 6 CHLORINE NO.

Table 5. Comparison of Percent Peak Areas by Student's t test. Critical value $t_{\alpha/2, 8}$ = df for α=0.10: 1.860

Sample Type	Statistical Value	Peak Number							
		1[a]	2[a]	3[b]	4[b]	6[b]	7[c]	8[c]	9[c]
Suspended Sediment	Mean	3.71	0.12	10.74	18.21	26.78	15.36	11.02	14.00
	Standard Deviation	2.47	0.15	1.83	3.51	4.02	1.66	1.43	6.72
Standards	Mean	4.04	1.20	15.92	22.33	28.02	13.70	8.10	6.69
	Standard Deviation	0.58	0.29	2.16	1.63	1.02	1.29	1.18	0.97
	t value	0.18	4.68	2.59	1.51	0.42	1.12	2.23	1.52
Water	Mean	19.92	2.57	18.74	15.54	16.39	5.01	2.33	13.26
	Standard Deviation	3.78	2.97	2.26	0.46	6.19	0.51	1.62	10.54
Standards	Mean	4.13	0.44	15.58	21.98	27.76	14.20	8.40	7.22
	Standard Deviation	1.04	0.76	1.18	1.05	1.19	1.04	0.35	1.34
	t value	5.70	0.98	1.75	7.94	2.55	11.22	5.18	0.80
Fish	Mean	4.35	0.88	16.05	23.68	30.06	12.10	6.14	5.82
	Standard Deviation	1.42	1.02	0.93	0.71	0.91	1.44	1.79	2.34
Standards	Mean	4.40	1.50	15.22	22.15	28.68	13.84	7.91	5.96
	Standard Deviation	0.20	0.10	1.26	1.49	0.47	1.22	0.13	0.58
	t value	0.05	0.86	0.75	1.31	1.91	1.30	1.40	0.08

[a] tetrachlorobiphenyls
[b] pentachlorobiphenyls
[c] hexachlorobiphenyls

In water samples peak 1 (tetrachlorobiphenyl) represented a significantly higher percent area, while peaks 4, 6, 7 and 8 (penta- and hexachlorobiphenyls) were significantly lower in percent peak area compared to standards. Peaks 2, 3 and 9 (tetra-, penta-and hexachlorobiphenyls respectively) showed no significant difference.

In fish samples the only significant difference was found in peak 6 (pentachlorobiphenyl) which was slightly higher in percent peak area than the corresponding standards. No difference was found for peaks 1, 2, 3, 4, 7, 8 and 9.

III. PCB Accumulation by Fish

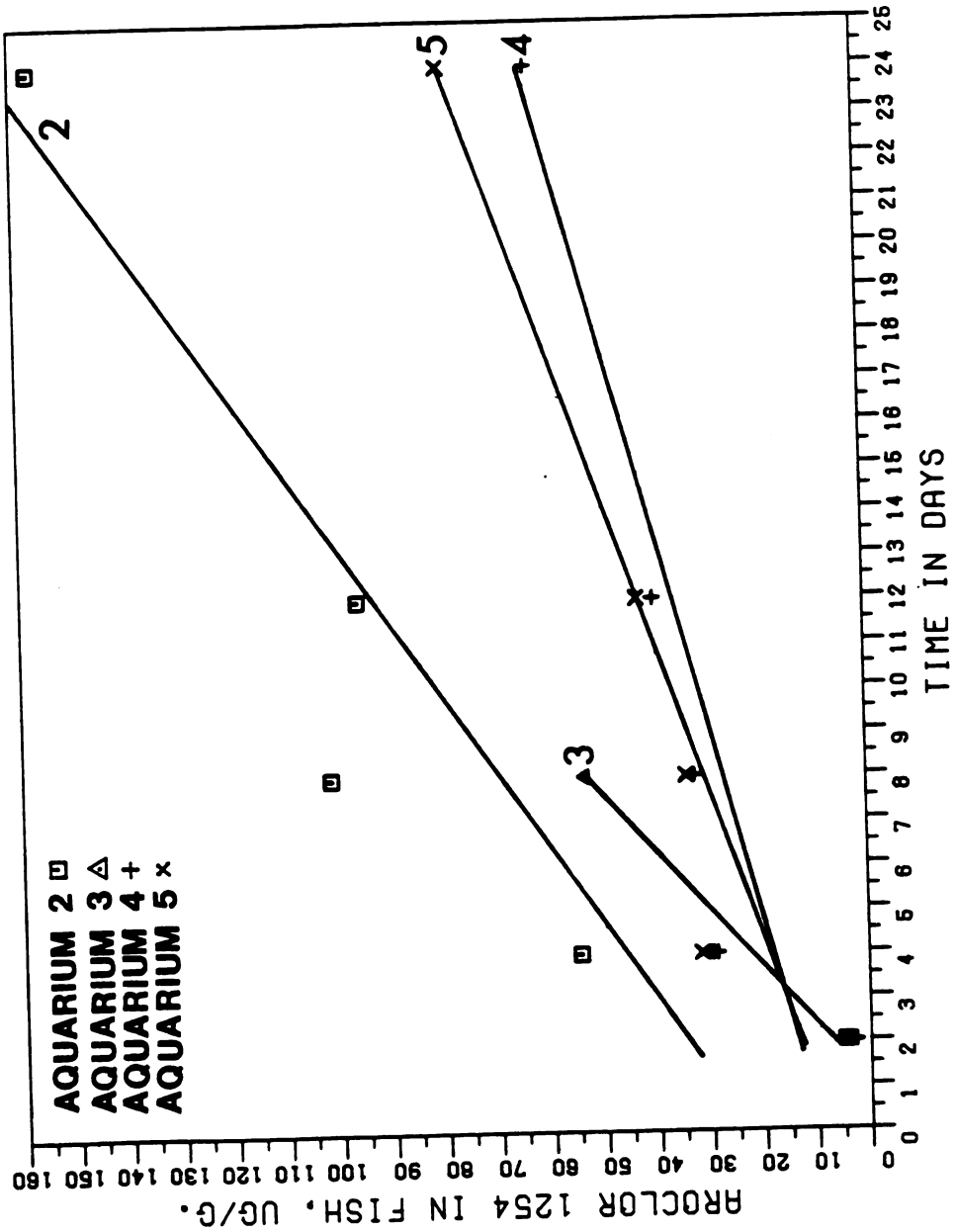
As expected the highest suspended sediment loads resulted in the highest PCB concentration in the fish. The highest suspended sediment loads were received by aquarium 2 followed by aquaria 3, 5 and 4. In experiments IV and V the order was reversed between aquaria 4 and 5, but in all experiments the two aquaria (4 and 5) were very similar (Table 4). Likewise, the concentration of PCBs in fish were highest in fish from aquarium 2 followed, in decreasing concentration, by fish in aquaria 3, 4 and 5 (Table 6). The PCB concentration values in fish were compared using the last sample day values.

Figure 2 shows the general pattern of PCB accumulation by fish in the four different suspended sediment loads in experiment I. By day 2 they had all accumulated approximately the same amount of PCB. By day 4 fish from aquaria 3, 4 and 5 were similar in PCB concentration but fish in aquarium 2 were beginning to accumulate higher loads of PCB. By the end of the experiment fish in aquaria 4 and 5 were still increasing their concentrations of PCBs but there was not much difference between the two groups. Fish from aquarium 3 were not sampled after day 8 because of fish mortality due to entanglement in the siphon tube. Fish in aquarium 2 had accumulated approximately twice as much

Table 6. PCB Concentrations in Fish Samples from the Final Sample in Each Experiment (μg/g)

Experiment Number	Statistical Value	Aquarium Number				
		Control	2	3	4	5
I	Mean	0	156	53	62	79
	Range	0	93-240	48-59	33-83	57-111
II	Mean	1.4	55	32	18	15
	Range	0-2.2	30-80	26-44	11-25	9-20
III	Mean	0.4	7.6	6.5	4.6	3.8
	Range	0.3-0.4	6.8-8.5	4.8-7.5	3.8-5.6	3.6-3.9
IV	Mean	0	6.2	2.9	2.9	2.4
	Range	0	2.8-8.7	1.7-4.3	2.0-4.0	1.6-3.5
V	Mean	0	30	19	9.0	7.1
	Range	0	13-44	14-22	6.4-11	1.3-11

Figure 2. Aroclor 1254 Uptake by Fathead Minnows (Pimephales promelas) in Four Aquaria in Experiment I


AQUARIUM 2 □
AQUARIUM 3 △
AQUARIUM 4 +
AQUARIUM 5 ×
2
3
4
×5
AROCLOR 1254 IN FISH, UG/G.
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
TIME IN DAYS

PCB by the end of the experiment as compared to fish in aquaria 4 and 5 even though suspended sediment loads averaged only 1.5 times higher in aquarium 2. This suggests that even a small increase in suspended sediment load over a long period of time can result in a high PCB concentration in fish.

IV. Effects on Accumulation by Organic Matter

Direct comparison between experiments II and III shows the effects of low organic and high organic suspended sediments on PCB accumulation by fish. Statistically, conditions were the same in both experiments. That is, there was no significant difference (90% c.f.) in suspended sediment load, PCB concentration in the suspended sediment, or water concentrations of PCB (Appendices 1, 2 and 3). Corresponding aquaria were compared between the two experiments. If all conditions were similar then any difference in PCB accumulation by fish should be due to suspended sediment type, i.e., low or high organic matter content.

A comparison of corresponding aquaria in each experiment shows the large difference in rate and amount of PCB uptake by fish in the two experiments (see Figures 3, 4, 5 and 6). A comparison of regression slopes of Figures 3, 4, 5 and 6 was made using an 'approximate t' test assuming heterogenous variance (Appendix 4). The results indicated a significant difference between the slopes on each graph at the 95% confidence level (Table 7).

The 'student t' test was used to compare accumulation of PCB by fish at the end of experiments II and III (Table 6 and Appendix 5). The results showed no significant difference in PCB uptake between fish in corresponding aquaria in experiments II and III at the 90% confidence level. In conclusion, there was no significant difference in PCB exposure between the two experiments, however,

there was a significantly higher accumulation of PCB by fish exposed to low organic suspended sediment.

V. Bioconcentration

Even though no statistical difference (90% c.f.) in the concentration of PCB in water existed between experiments II and III, experiment III levels averaged slightly lower and may have been responsible for less accumulation of PCB by the fish in experiment III. Therefore bioconcentration values were compared between the two experiments to determine the relative uptake of PCB by fish due to PCB concentration in the water (Table 8). Bioconcentration factors were calculated by dividing the average PCB concentration in the water into the average PCB concentration in the fish on the last day of the experiment. This was calculated for fish in each aquaria in experiments II, III and IV.

If PCB accumulation by fish is due only to PCB concentrations in the water then the bioconcentration factors should be similar given the same amount of time for accumulation. This was not the case in experiments II and III. Bioconcentration values were two to five times higher in experiment II than in experiment III. Even between aquaria in experiment II there is a wide range of bioconcentration values indicating that another factor is involved in the accumulation of PCB by fish.

VI. Transfer Coefficients

Another way of comparing PCB accumulation in fish is by transfer coefficients (Halter and Johnson, 1977). As described by Halter and Johnson (1977), the water-to-fish transfer coefficients are calculated by " ... dividing the slope of the uptake regression line by the exposure concentration and represent time-independent expressions of the bioconcentration factor."

Figure 3. Aroclor 1254 Uptake by Fathead Minnows (Pimephales promelas) in Aquarium No. 2 in Experiments II (Low Organic Soil) and III (High Organic Soil)

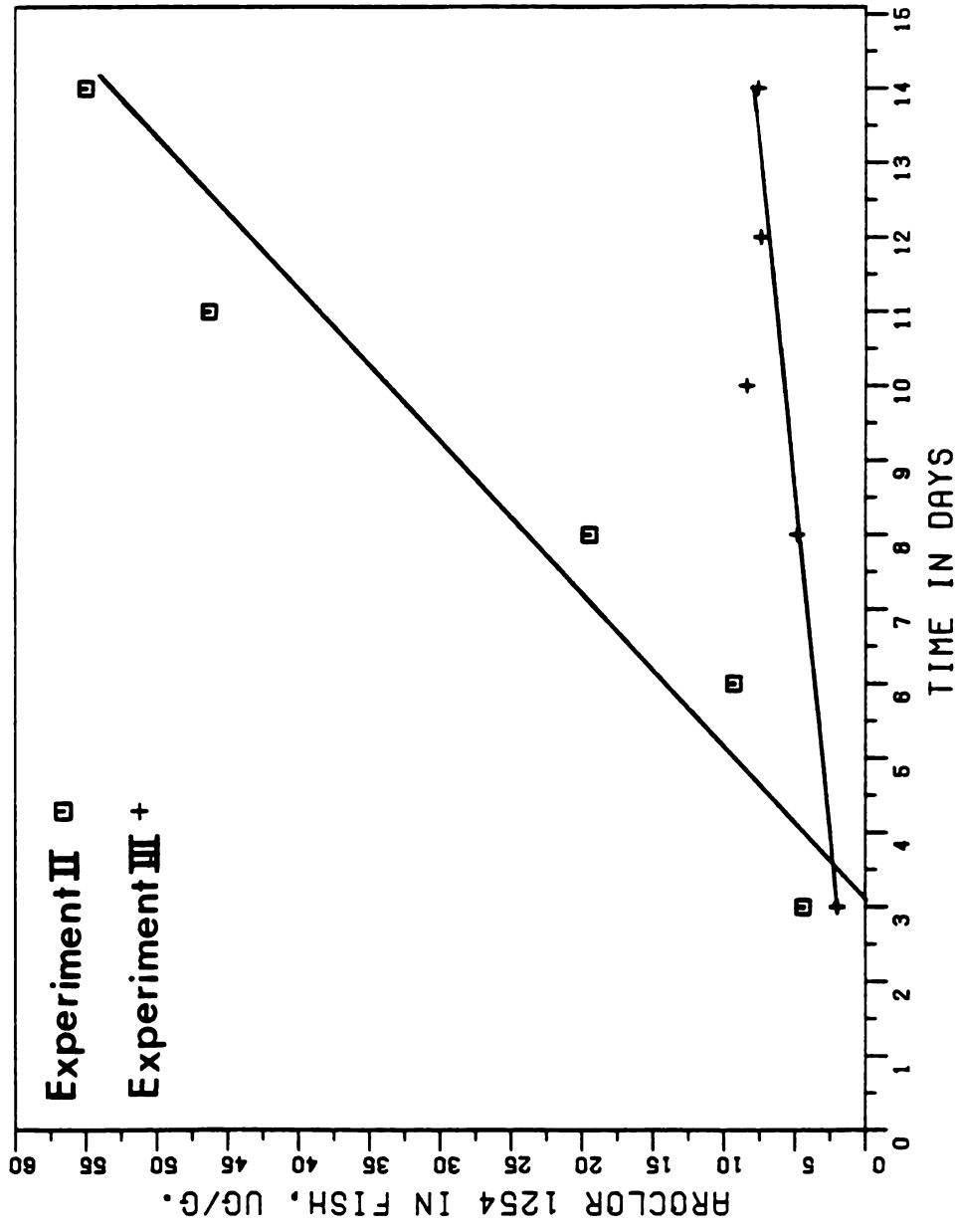

AROCLOR 1254 IN FISH, UG/G.
0
5
10
15
20
25
30
35
40
45
50
55
60
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
TIME IN DAYS
Experiment II
Experiment III

Figure 4. Aroclor 1254 Uptake by Fathead Minnows (Pimephales promelas) in Aquarium No. 3 in Experiments II (Low Organic Soil) and III (High Organic Soil)

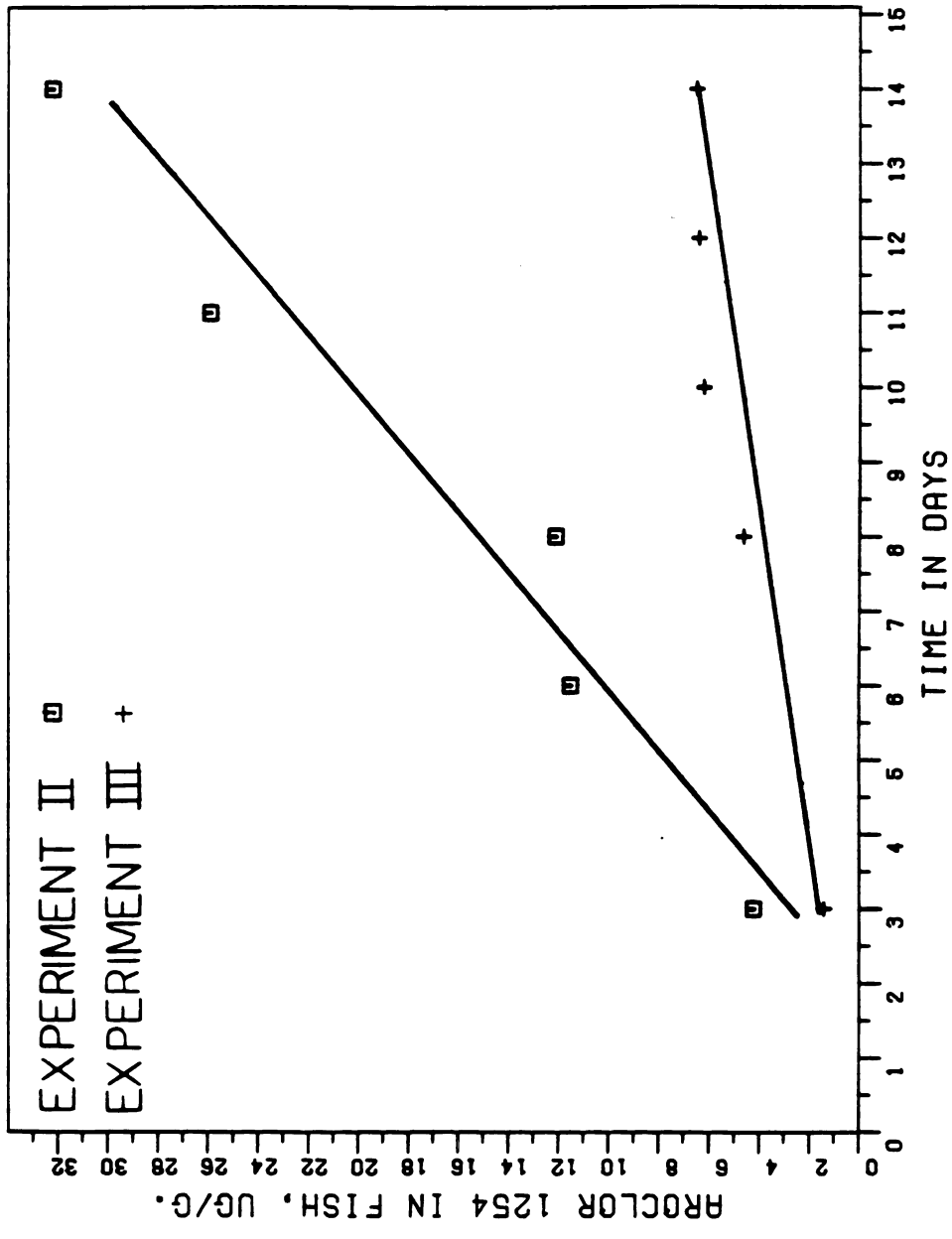

EXPERIMENT II
EXPERIMENT III
AROCLOR 1254 IN FISH, UG/G.
TIME IN DAYS
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
1
3
5
7
9
11
13
15

Figure 5. Aroclor 1254 Uptake by Fathead Minnows (Pimephales promelas) in Aquarium No. 4 in Experiments II (Low Organic Soil) and III (High Organic Soil)

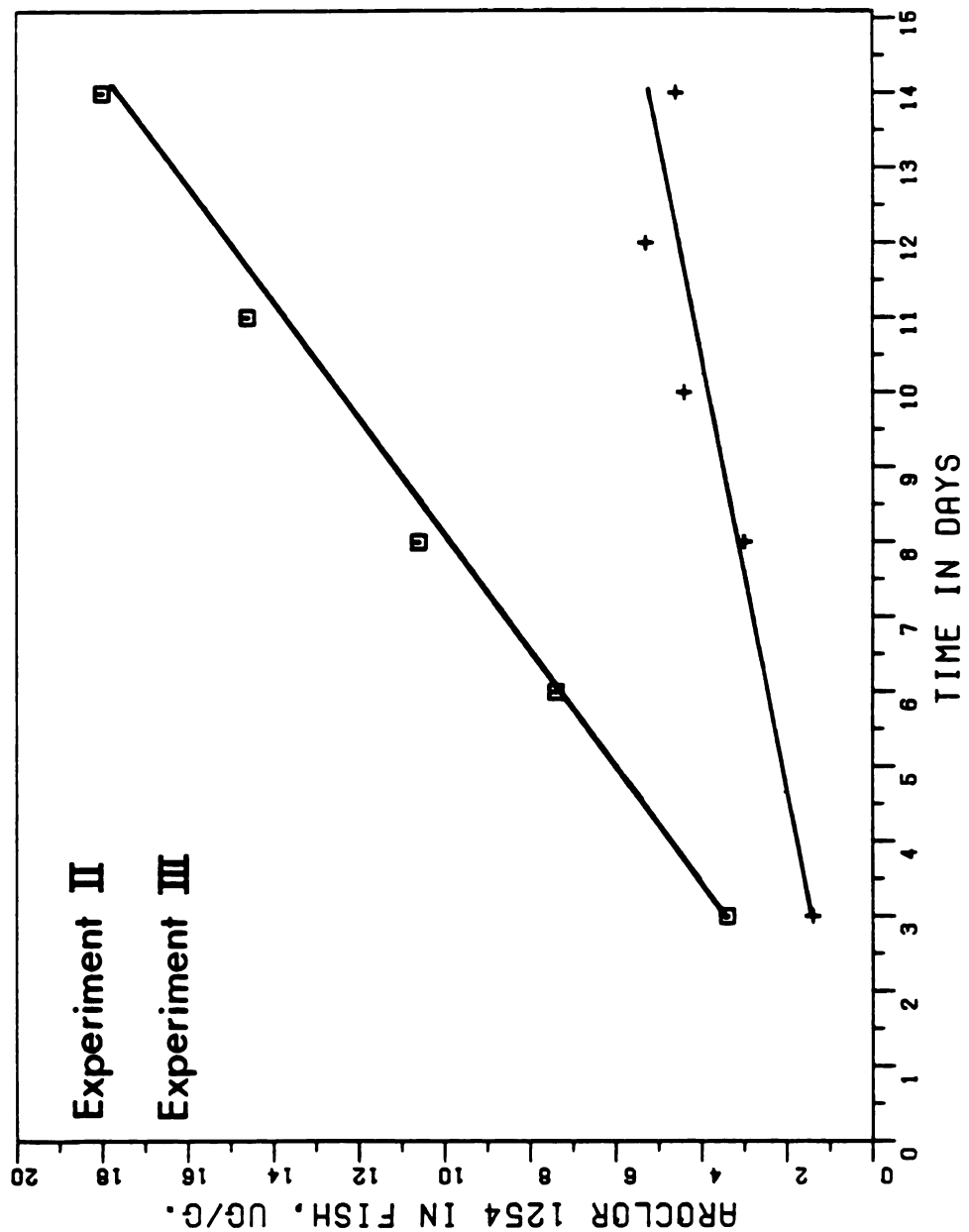

AROCLOR 1254 IN FISH, UG/G.
TIME IN DAYS
Experiment II
Experiment III
0
2
4
6
8
10
12
14
16
18
20
1
3
5
7
9
11
13
15

Figure 6. Aroclor 1254 Uptake by Fathead Minnows (Pimephales promelas) in Aquarium No. 5 in Experiments II (Low Organic Soil) and III (High Organic Soil)

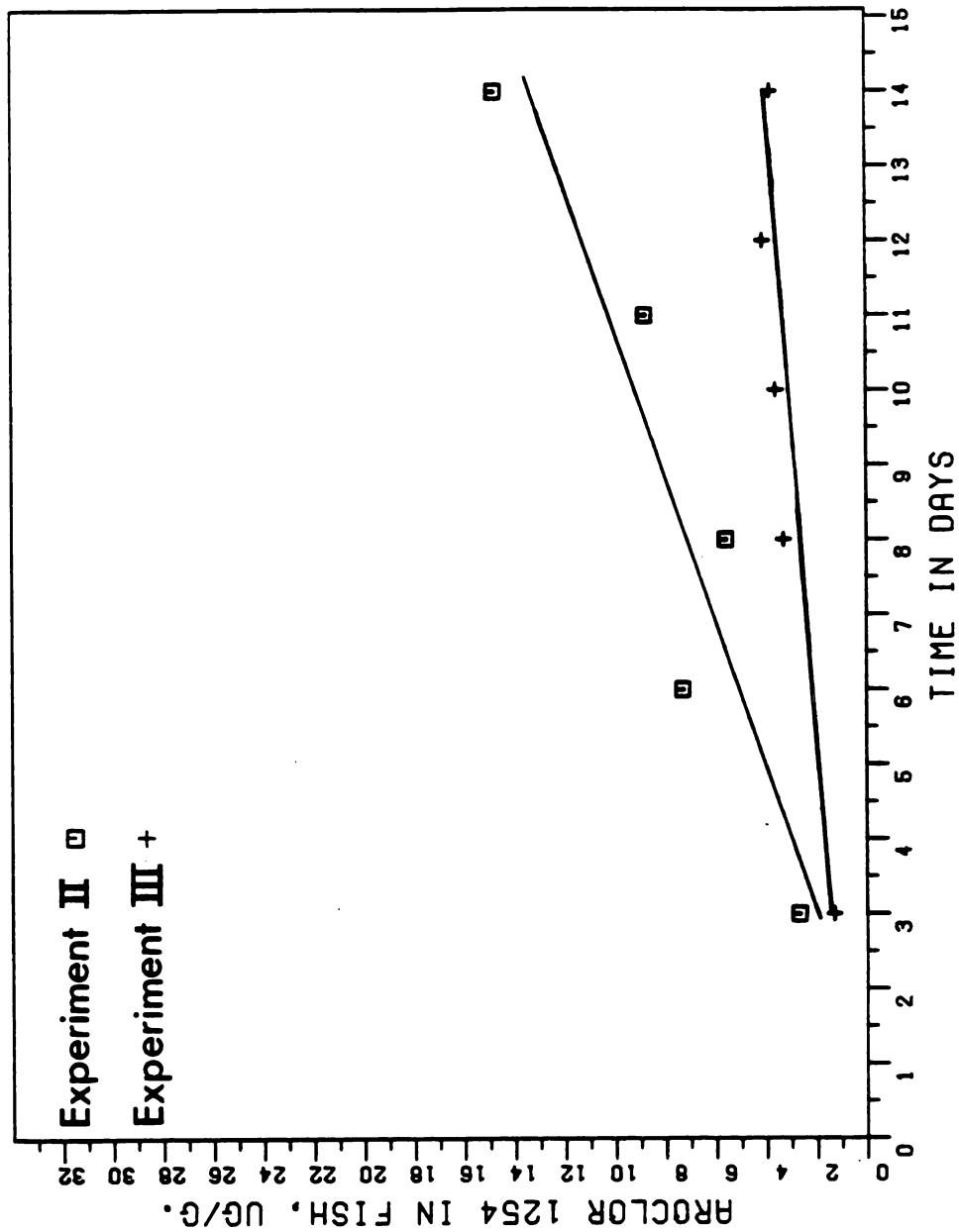

AROCLOR 1254 IN FISH, UG/G.
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
TIME IN DAYS
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Experiment II □
Experiment III +

Table 7. Results of Approximate t' Test on Slopes of Uptake Regression Lines from Experiments II and III

Experiment Number	Aquarium Number	Slope	t'	Critical t'	$\hat{V}$
II	2	5.12	8.555	3.182	2.89
III	2	0.56			
II	3	2.63	6.544	2.776	3.47
III	3	0.49			
II	4	1.34	3.623	2.776	3.53
III	4	0.34			
II	5	0.98	3.071	2.776	3.84
III	5	0.24			

$t_{\alpha/2,\hat{V}}$ α =0.05TABLE 7

Table 8. Comparison of Bioconcentration Values from Experiments II, III, IV and V

Aquarium Number	Soil Type: Arboretum Low Organic (Experiment Number II)	Soil Type: Greenhouse High Organic (Experiment Number III)	Soil Type: Greenhouse High Organic (Experiment Number IV)	Soil Type: Shiawassee River Low Organic (Experiment Number V)
2	69,000	13,000	10,000	5,800
3	40,000	13,000	10,000	3,600
4	30,000	11,500	14,500	3,900
5	21,000	8,000	12,000	3,100

Comparison of the transfer coefficients in Table 9 shows transfer coefficient values in experiment II, two to six times higher than in experiment III. Again, the data indicate that the difference in PCB accumulation by fish is due to more than just PCB concentrations in the water medium.

VII. Experiment IV

In this experiment the high organic greenhouse soil was dosed with 2,5,3',4'-tetrachlorobiphenyl at 50 μg/g. Results show that suspended sediment levels were lower than in any of the other experiments (Table 4). Water concentrations of the tetrachlorobiphenyl were also lower than the PCB concentration in the water of other experiments (Table 3). Subsequently, PCB uptake by fish was the lowest of all the experiments (Table 6 and Figure 7). Comparing bioconcentration values for experiments III and IV in Table 8 (both experiments with high organic soil), show that they are similar, even though experiment IV ran less than half the time of experiment III. A better comparison is with transfer coefficients which are time independent and take into account the rate of uptake. Transfer coefficients in experiment IV are much higher than those in experiment III (approximately two to four times, see Table 9) and more closely resemble transfer coefficients in experiment II (low organic soil). So even though overall concentrations were lower in experiment IV the fish accumulated relatively high levels of tetrachlorobiphenyls.

VIII. Experiment V

There was no statistically significant difference (90% c.f.) between Aroclor 1242 concentrations in the Shiawassee River suspended sediment used in experiment V and Aroclor 1254 concentrations in suspended sediments used in experiments II and III (Appendix 1). The Aroclor 1242 concentrations did average

Figure 7. 2,5,3',4'-Tetrachlorobiphenyl Uptake by Fathead Minnows (Pimephales promelas) in Four Aquaria in Experiment IV

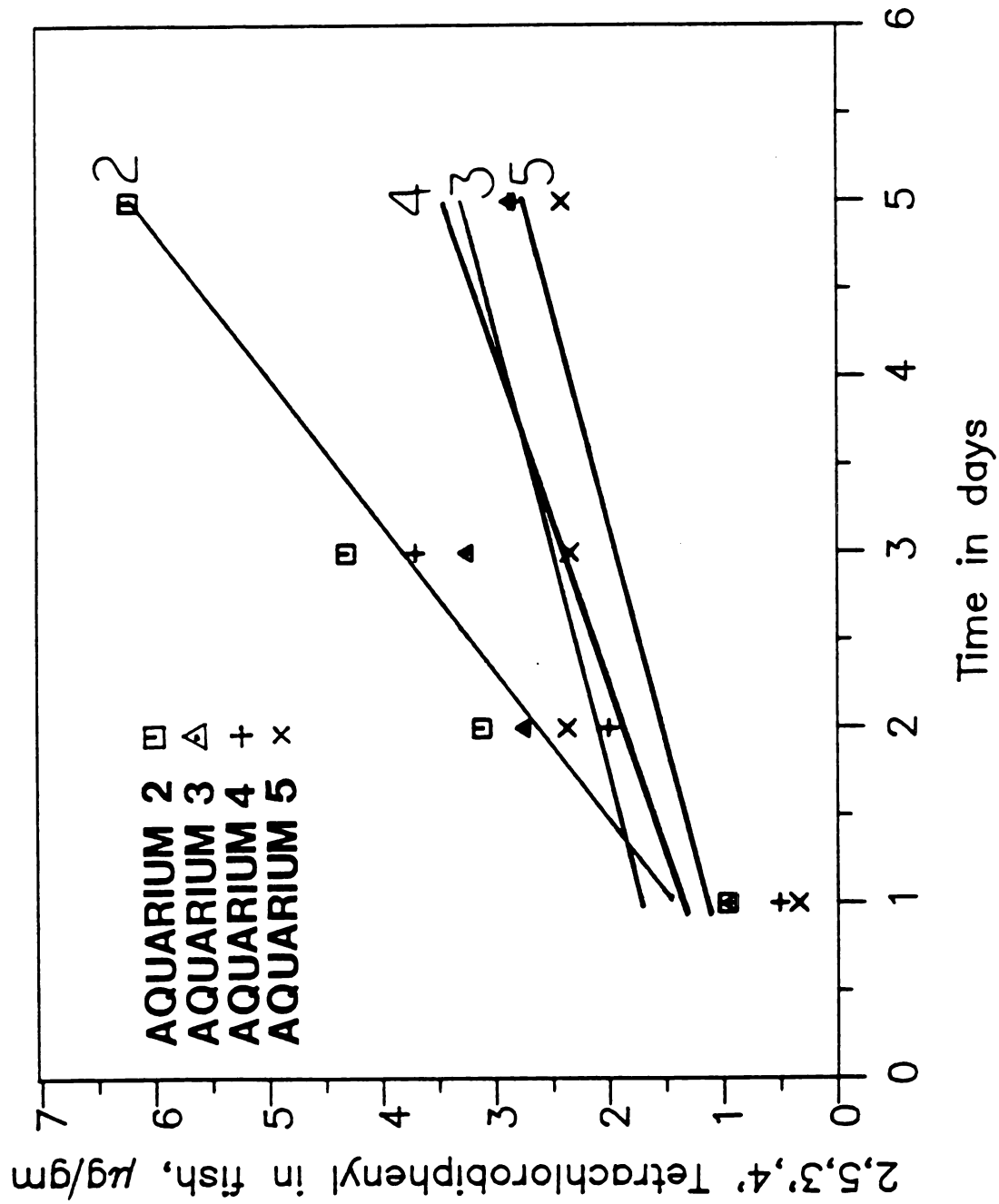

2,5,3',4' Tetrachlorobiphenyl in fish, µg/gm
Time in days
AQUARIUM 2
AQUARIUM 3
AQUARIUM 4
AQUARIUM 5
0
1
2
3
4
5
6
7

Table 9. Comparison of Transfer Coefficients for Experiments II, III, IV and V

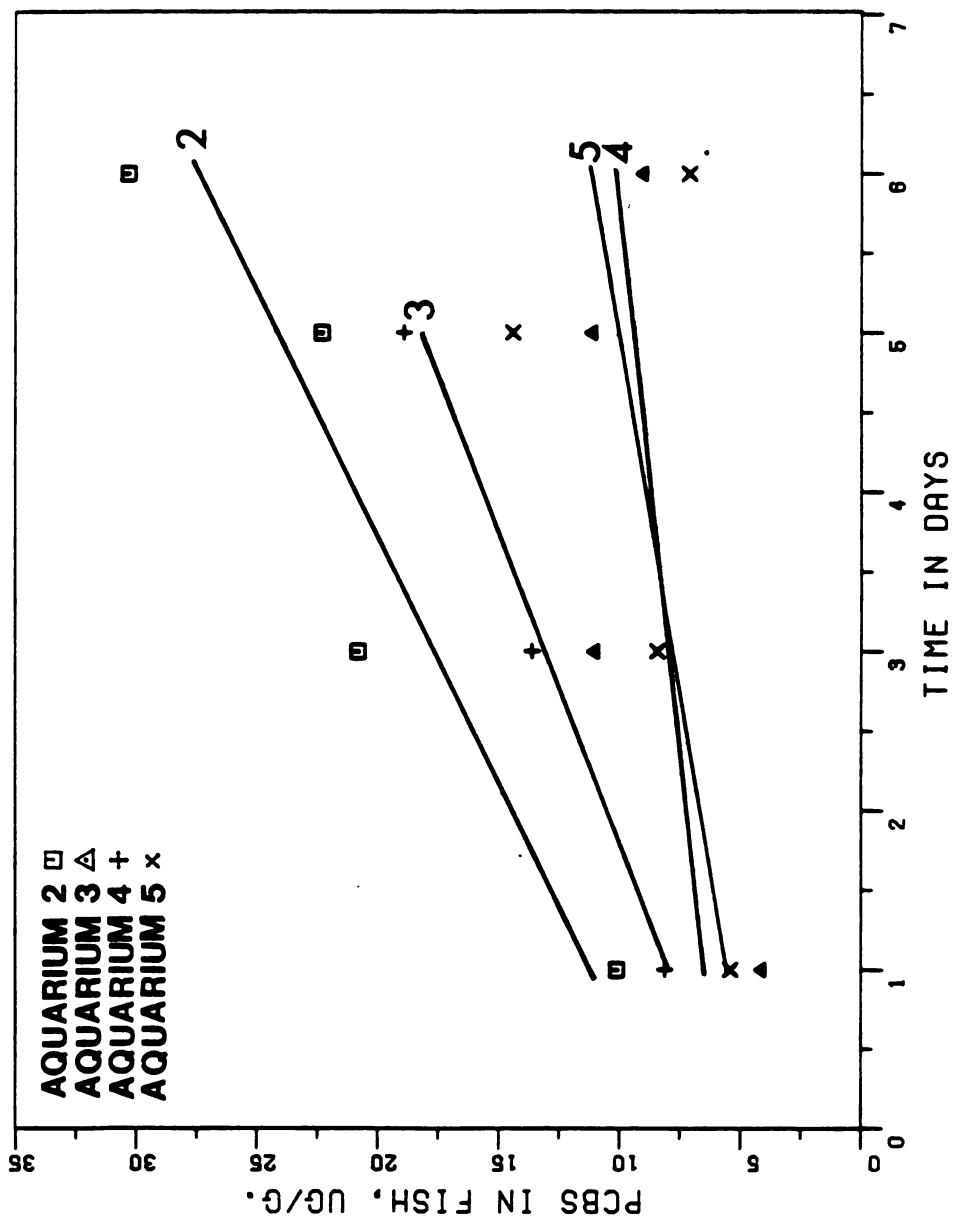
Experiment Number	Aquarium Number	Slope of Regression Uptake	PCB Concentration in Water (μg/ℓ)	Transfer Coefficient
II	2	5.12	0.8	6400
	3	2.63	0.8	3287
	4	1.34	0.6	2233
	5	0.98	0.7	1400
III	2	0.56	0.6	933
	3	0.49	0.5	980
	4	0.34	0.4	850
	5	0.24	0.5	480
IV	2	1.26	0.6	2100
	3	0.41	0.3	1267
	4	0.57	0.2	2850
	5	0.42	0.2	2100
V	2	3.57	5.2	686
	3	2.70	5.3	509
	4	0.99	2.3	430
	5	0.87	2.3	378

higher than the Aroclor 1254 concentrations but there was a great deal of fluctuation (Table 2).

Suspended sediment concentrations were similar in experiment V to those in experiments II and III with the exception of aquaria 2 which received very high loads of suspended sediment due to a mechanical failure in the diluter system (Table 4). Other than that, suspended sediment conditions and PCB concentrations in the sediment were simlar between experiments II and III (using Aroclor 1254) and experiment V (Aroclor 1242).

Water concentrations of PCB were much higher in experiment V than in experiments II and III (three to ten times, see Table 3). A higher concentration of PCBs in the water was expected due to the low organic content of the Shiawassee River sediment (1.0%) and the higher content of tetrachlorobiphenyls in Aroclor 1242 making it more water soluble. Therefore it was assumed that fish would be able to accumulate more PCBs, however, this was not the case (Figure 8). Bioconcentration values were lower in experiment V (Table 8) than in any of the other experiments. This was not too surprising because of the shorter duration of experiment V. Transfer coefficients, on the other hand, take into account the time factor but these too are lower in experiment V than in any of the other experiments (Table 9).

Figure 8. PCB (as Aroclor 1242) Uptake by Fathead Minnows (*Pimephales promelas*) in Four Aquaria in Experiment V (Shiawassee River Sediment)


AQUARIUM 2 □
AQUARIUM 3 △
AQUARIUM 4 +
AQUARIUM 5 ×
PCBS IN FISH, UG/G.
TIME IN DAYS
0
5
10
15
20
25
30
35
1
2
3
4
5
6
7
2
3
4
5

DISCUSSION

I. Distribution of Isomers

In the suspended sediment the higher chlorinated isomers were retained while the lower chlorinated isomers were released. This corresponds with the isomer distribution in the water where there were high concentrations of tetrachlorobiphenyls and low concentrations of penta- and hexachlorobiphenyls. This was expected because of the higher water solubility of the tetrachlorobiphenyls.

The only significant difference in isomer distribution in fish was a higher accumulation of pentachlorobiphenyls as represented by peak 6. Although no statistically significant differences (90% c.f.) were seen in most of the isomers of fish samples it was observed that the general trend was to accumulate the lower chlorinated isomers. Figure 1 is a good illustration of what was usually seen. The lack of statistical difference was probably due to low sample numbers and a high degree of variation among the samples.

Under similar experimental conditions Halter and Johnson (1977) found a similar isomer uptake pattern in fish (i.e., accumulation of the lower chlorinated isomers). However, other studies indicate an opposite trend (DeFoe et al., 1978; Mayer et al., 1977).

II. Effects on Accumulation by Organic Matter

These experiments suggest that fish exposed to PCBs sorbed on a high organic soil will accumulate less PCB and at a slower rate than fish exposed

under the same conditions with a low organic soil. Comparison of regression slopes, bioconcentration factors and transfer coefficients are fairly conclusive in pointing out the difference in PCB accumulation by fish in the two treatments. The difference in bioconcentration factors and transfer coefficients indicates that the source of PCBs for the fish is not just PCB concentrations in the water, since levels were essentially the same in both treatments. The fish may be directly absorbing PCBs from the suspended sediment by direct contact with gill and/or body surfaces and/or by intake of suspended particles with food. The fish treated with low organic suspended sediment accumulated more PCBs because the compounds were not as tightly bound as in the high organic suspended sediment. Also, within each treatment, the higher chlorinated isomers are going to be more tightly bound than the lower chlorinated isomers explaining, in part, Figure 1, where the peak profile of the fish sample shows accumulation of the tetra-and pentachlorobiphenyls and the peak profile of the soil sample indicates retention of the hexachlorobiphenyls.

III. Experiment IV

The organic content of soils appears to have little affect on accumulation of tetrachlorobiphenyls by fish as is evident in experiment IV. The transfer coefficients (Table 9) were similar to those in experiment II where a low organic soil was used indicating the rate of uptake by fish was not greatly reduced by the presence of a high organic sediment. This is consistent with the results of the isomer retention study that indicated the tetrachlorobiphenyl concentrations were significantly reduced in the suspended sediment. This is due to the relatively high water solubility of the tetrachlorobiphenyls compared to the penta- and hexachlorobiphenyls (Paris et al., 1978; Tucker et al., 1975; Zitko, 1970).

Comparison of the bioconcentration factors of tetrachlorobiphenyl obtained in experiment IV with those in the literature shows very little difference between direct uptake from water (literature values) and contamination from suspended sediment. Static and flow-through bioassays yielded bioconcentration values ranging from 460 to approximately 12,000 (Branson et al., 1975; Metcalf et al., 1975; Sanbord et al., 1975). My experimental results yielded bioconcentration values ranging from 10,000 to 14,500. No conclusion could be drawn as to the effects of suspended sediment on tetrachlorobiphenyl uptake by fish.

IV. Experiment V

Comparing the bioconcentration and transfer coefficient data of experiment V (Shiawassee River sediment) to experiments II, III and IV indicates that the fish in experiment V accumulated much less PCB than in any of the other experiments. This is the opposite of what would have been predicted for a system with a suspended sediment containing only 1% organic matter and contamined with a high percentage of tetrachlorobiphenyls. The difference may be due to the high PCB concentration in the water which, when divided into the uptake regression slopes and concentration of PCB in the fish, produces artificially low results. High levels of PCBs in the water may be due to PCB adsorbed to particles < 1.2 μm which are not filtered out with the suspended sediment and become part of the water sample. Comparison of the uptake regression slopes indicates that the rate of uptake in experiment V more closely resembles the rate of uptake in experiment I when a low organic sediment dosed with Aroclor 1254 is used, and is two to six times higher than uptake rates in experiment IV when only tetrachlorobiphenyl was used. The results from the environmentally contaminated sediment are not conclusive but suggest the same

as the results from the other experiments because of the high rate of uptake. A longer study time with environmentally contaminated sediments is needed to determine if fish uptake of PCBs will be significantly accelerated by suspension of these sediments in the water column.

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APPENDICES

APPENDIX I

Statistical Analysis to Detect a Difference in PCB Concentration in Suspended Sediment between Experiments (μ g/g)

Experiment #	I	II	III	V
	91.68	161.83	161.88	276.77
	104.79	209.96	115.20	381.14
	124.89	194.98	183.90	554.69
	146.22	201.97	176.50	190.55
	157.92	159.07	163.85	113.66
	96.94	245.67	190.54	350.00
	125.21	237.65	160.24	372.09
	208.04	213.58	211.67	162.46
	186.57	219.47	275.00	150.56
	163.83	194.27	242.59	188.55
	221.11	231.75	300.00	178.02
	194.52	204.76	134.46	240.44
	81.25	176.00	135.23	303.76
	181.82	184.16	219.05	291.33
	149.12	103.59	356.14	268.79
$\bar{X}$	148.91	195.91	201.75	268.19
S.D.	44.10	36.12	67.19	114.76
Variance	1814.76	1217.48	4213.01	12,291.27
n	15	15	15	15

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{(S.D._1)^2 + (S.D._2)^2}{2}}}$$

Critical $t = \pm t\,\alpha/2, (n+n) - 2$

for α: 0.10

$\pm t = 1.701$

t II, III	=	0.108
t I, II	=	1.166
t II, V	=	0.850
t III, V	=	0.710

APPENDIX II

Statistical Analysis to Detect a Difference in PCB Concentration in Water Samples from Experiments II and III (μg/ℓ)

Experiment #	II				III			
Aquaria #	2	3	4	5	2	3	4	5
$\bar{X}$	0.77	0.80	0.62	0.66	0.55	0.47	0.40	0.46
S.D.	0.13	0.20	0.11	0.28	0.17	0.12	0.15	0.21
n	4	5	5	5	5	5	5	5

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{(S.D._1)^2 + (S.D._2)^2}{2}}}$$

Critical $t = \pm t_{\alpha/2, (n+n)-2}$

for α: 0.10

$\pm t = 1.860$

Aquaria II, 2 & III, 2 — t = 1.467
Aquaria II, 3 & III, 3 — t = 2.000
Aquaria II, 4 & III, 4 — t = 1.667
Aquaria II, 5 & III, 5 — t = 0.806

APPENDIX III

Statistical Analysis to Detect a Difference in Suspended Sediment Concentration in Experiments II and III (mg/ℓ)

Experiment #	II				III			
Aquaria #	2	3	4	5	2	3	4	5
$\bar{X}$	44.44	24.82	17.06	21.00	31.65	20.40	11.76	13.70
S.D.	5.41	5.55	3.24	8.49	8.13	5.93	3.76	4.90
n	5	5	5	5	5	5	5	5

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{(S.D._1)^2 + (S.D._2)^2}{2}}}$$

Critical $t = \pm t\,\alpha\,/2, (n+n) - 2$

for α: 0.10

$\pm t = 1.860$

Aquaria II, 2 & III, 2	t = 1.850
Aquaria II, 3 & III, 3	t = 0.666
Aquaria II, 4 & III, 4	t = 1.510
Aquaria II, 5 & III, 5	t = 1.050

APPENDIX IV

Formula for the Statistical Comparison of Regression Slopes Assuming Heterogenous Variance

Approximate t $$t' = \frac{b_{II} - b_{III}}{\sqrt{V(b_{II}) + V(b_{III})}}$$

b = Slope
x = Days
y = PCB Concentration in Fish µg/g

$$V(b) = \frac{[(SS_y) - b(SP_{xy})] / (n-2)}{(SS_x)}$$

$$(SP_{xy}) = \sum^{n}(x_i y_i) - \frac{(\Sigma x_i)(\Sigma y_i)}{n}$$

$$(SS_y) \text{ or } (SS_x) = \sum^{n}(y_i^2) \text{ or } (x_i^2) - \frac{\Sigma(y_i)^2 \text{ or } (x_i)^2}{n}$$

$$g = V(b_{II})/V(b_{III})$$

Approximate D.F. $$\hat{v} = \frac{(1 + g)^2}{g^2/(n_{II}-2) + 1/(n_{III}-2)}$$

$$\pm t\alpha/2, \hat{v}$$

APPENDIX V

Statistical Analysis to Detect a Difference in PCB Concentration in Fish (μ g/g)

Experiment #	II				III			
Aquaria #	2	3	4	5	2	3	4	5
$\bar{X}$	54.89	32.21	17.95	14.75	7.64	6.54	4.58	3.77
S.D.	25.36	10.02	6.97	5.44	0.85	1.45	0.90	0.19
n	3	3	3	3	3	3	3	3

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{(S.D._1)^2 + (S.D._2)^2}{2}}}$$

Critical $t = \pm t_{\alpha/2, (n+n)-2}$

for α: 0.10

$\pm t = 2.132$

Aquaria II, 2 & III, 2 $t = 2.633$
Aquaria II, 3 & III, 3 $t = 3.586$
Aquaria II, 4 & III, 4 $t = 2.690$
Aquaria II, 5 & III, 5 $t = 2.853$