Exhibit 3

Running head: DeFoe, Veith, and Carlson: PCB toxicity and minnows

Effects of Aroclor 1248 and 1260 on the fathead minnow (*Pimephales promelas*)

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Fathead minnows were exposed to Aroclor 1248 and 1260 in flow-through bioassays to determine the acute (30-day) and chronic (240-day life cycle) effects on the fry and adult fathead minnows as well as the bioconcentration of the mixtures of PCB's in the fish. Newly hatched young were the most sensitive life stage to the exposure of PCB's. The calculated 30-day TL50 for newly hatched fathead minnows (<8-h-old) was 4.7 µg/liter for Aroclor 1248 and 3.3 µg/liter for Aroclor 1260. PCB's did not measurably affect the ability of fathead minnows to reproduce at concentrations which are acutely toxic to the larvae. With reproduction occurring at and below 3 µg/liter for Aroclor 1248 and at and below 2.1 µg/liter for Aroclor 1260, the 20% reduction in the standing crop in the second generation of minnows at concentrations as low as 0.4 µg/liter is due to the mortality of the fathead larvae soon after hatching.

The bioconcentration factor (concentration in fish tissue/concentration in the water) for PCB's is essentially independent of the PCB concentration in the water. The bioconcentration factor (whole body) for adult male fathead minnows at 25 C is approximately 1.2×10^5 for Aroclor 1248 and 2.7×10^5 for Aroclor 1260. Female fathead minnows accumulated about twice as much PCB's as the male fatheads and the difference is largely due to the greater amount of lipids in the female. The exposed fish placed in untreated Lake Superior water eliminated less than 18% of the body burden after 60 days.

The purpose of this study is the determination of the acute and chronic effects of Aroclor 1248®[1,2] and Aroclor 1260® on the fathead minnow.

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[1]Aroclor is the trade name for mixture of chlorobiphenols (PCB's) manufactured in the United States by Monsanto Company (St. Louis, MO).

[2]Mention of trade names does not constitute endorsement by the U.S. Environmental Protection Agency.

Nebeker and Puglisi (1974) showed that Aroclor 1248 was the most toxic PCB mixture to Daphnia magna under static conditions with a 3-week LC50 of 25 µg/liter. Aroclor 1248 on Gammarus pseudolimnaeus in continuous-flow tests was 20 µg/liter and survival after 60 days (at 5.1 µg/liter Aroclor 1248) was 53%. However, reproduction and survival of young were not affected by 2.2 µg/liter Aroclor 1248. The midge, Tanytarsus dissimilis, in continuous-flow tests, did not emerge (as adults) in abundance above 5.1 µg/liter Aroclor 1248.

The acute oral toxicity of Aroclors 1242, 1248, 1254, and 1260 was greater than 1500 mg/kg in rainbow trout (Stalling and Mayer 1972). The 15-day LC50 to channel catfish was 107 µg/liter for Aroclor 1242, 127 µg/liter for Aroclor 1248 and 741 µg/liter for Aroclor 1254. The 15-day LC50 to bluegills determined by intermittent-flow bioassays was 76 µg/liter for Aroclor 1248. The LC50 decreased significantly with longer exposures and the 20-day LC50 to bluegills was 10 µg/liter for Aroclor 1248, 135 µg/liter for Aroclor 1254, and 245 µg/liter for Aroclor 1260. These data suggest that the toxicity of PCB's decreases with increasing degree of chlorination which is inconsistent with other studies (Nebeker and Puglisi 1974; Nebeker et al. 1974). These data may result from the decrease in

solubility of PCB of greater chlorine content. The solubility of Aroclor 1254 is less than 25 µg/liter and these short-term studies require exposures to PCB/water solutions in which the solubility is greatly exceeded.

Nebeker et al. (1974) showed that the growth of young fatheads was affected above 2.2 µg/liter 1248, and none survived above 5.1 µg/liter after 30 days. Young flagfish did not survive at Aroclor 1248 concentrations above 5.1 µg/liter and did not grow well above 2.2 µg/liter.

Schoettger et al. (1970) showed that the growth of lake trout was retarded by Aroclor 1248 in the diet for 3 months. Concentrations of 1.2, 3.8, and 12 µg/g decreased weight gain by 6, 10, and 28%, respectively, below that of controls. The growth of the high-exposure (12 µg/g in diet) group was only about half that of the controls after 6 months. Conversely, thyroid activity, as indicated by 72-h radioiodine uptake, was stimulated by PCB exposure. Serum cortisol concentrations ranged from 33 to 46% below that of the controls. Depressed growth rates and elevated thyroid activity imply metabolic inefficiency producing a "loading" stress that can adversely affect viability and reproduction.

There is an increasing amount of data indicating that PCB residues bioconcentrate in fish to the extent that the residues pose a threat to consumers even when the fish is not adversely affected. Nebeker et al. (1974) showed that the bioconcentration factor of Aroclor 1254 in fathead minnows ranged from 1.1×10^5 to 2.4×10^5 and that the bioconcentration factor for the PCB's to the fathead minnow (where the tissue is generally less than 10% lipid) at 25°C ranges from about 0.3×10^5 to 2.5×10^5 with the more highly chlorinated PCB's accumulating to a greater extent. Previously, Hansen et al. (1971) showed that Aroclor 1254 is accumulated

in pinfish approximately 2 x 10^4 times that in the water after 35 days of exposure at a salinity of 14-34‰. Södergren and Svensson (1973) show that the nymph Ephemia danica, accumulated Clophen A 50 by an approximate first order process to bioconcentration factors of 3 x 10^3. Also, the bioconcentration factor of Aroclor 1254 in the scud, Gammarus pseudolimnaeus, was approximately 2.2 x 10^4, 4.5 x 10^4 after 48 h in Daphnia magna, and 1.3 x 10^4 in mosquito larvae, Culex tarsalis after only 24 h (Schoettger et al. 1970). After 60 days of exposure to Aroclor 1242 and Aroclor 1248, the maximum bioconcentration factor in Gammarus was found to be approximately 3 x 10^4 and 5 x 10^4, respectively (Nebeker et al. 1974). The bioconcentration factors range from 2 x 10^4 to 6 x 10^4 for bluegills and catfish exposed to Aroclor 1248 and 1254.

Studies resulting from the reproductive failure of mink fed Great Lakes fish (Hartsough 1965; Aulerich et al. 1970; Aulerich and Ringer 1970; Aulerich et al. 1971) led to the conclusion that it was the PCB residues in the fish which caused th adverse affects. Ringer et al. (1972) demonstrated that 10 µg/g of Aroclor 1254 in coho salmon produced 71% mortality in mink and that a mixture of 10 µg/g PCB's and 0.5 µg/gm dieldrin in coho feed produced 100% mortality. No kits were born alive when the diet contained 5 µg/g or greater Aroclor 1254 alone. The "no effect" concentration of PCB's in the mink diet was estimated to be less than 1.0 µg/gm.

Platonov and Karstad (1973) presented similar evidence for mink feed diets enriched with Aroclor 1254. The data show that 0.64 µg/g of the PCB in the rations resulted in only 1 of 12 mink producing 3 kits, all of which died on the first day.

Moreover, research with non-human primates indicates that the toxicity of PCB residues to consumers are not limited to mink and birds. Barsotti and Allen (1975) and Allen et al. (1974) have shown that PCB enriched diets were toxic to primates. Female primates fed 2.5 µg/g Aroclor 1254 in their diet had periorbital edema, acneform lesions on face and neck, and substantially reduced fertility after 2 months of feeding and consumption of 182 mg PCB's.

Experimental Procedures

Physical Test System

Identical flow-through exposure systems for each Aroclor study were constructed and maintained similar to those procedures described in "Recommended Bioassay Procedure for Fathead Minnow Chronic Test 1971 and 1972" (National Water Quality Laboratory 1971). In an effort to eliminate varying concentrations of the toxicant dispersant (acetone) and to supply a control exposure with only the dispersant, the conventional diluter (Mount and Brungs 1967) was modified with mechanical syringe injectors (DeFoe 1975). The water temperature is maintained at 25 $\pm$ 1 C, and the continuous recording of temperature shows that the test tanks vary no more than 0.3 C from each other. The mean dissolved oxygen concentration was 80-95% saturation and was never allowed to fall below 50% saturation. The dissolved oxygen among tanks with acetone present never varied more than 1 ppm of each other.

The test animal was the fathead minnow (Pimephales promelas Rafinesque) which was obtained from the culture at the National Water Quality Laboratory, Duluth, Minnesota. Throughout the testing period the test animals were fed once daily a constant mixture of three different commercial dry fish foods. A second feeding, daily, consisted of live brine shrimp. Once weekly, adults were fed live Daphnia magna from laboratory cultures.

Acute (30-day) tests were started with fathead larvae less than 8-h-old, which were randomly selected from three individual spawns. Each test tank received 30 larvae. After 30 days the larvae were counted, weighed, and measured for total length. The larvae were frozen and held for residue analysis.

The full life-cycle chronic studies were conducted, for the most part, within the guidelines described in "Recommended Bioassay Procedures for Fathead Minnow Chronic Test 1971 and 1972" (National Water Quality Laboratory 1971). From 5 individual spawns, 65-75 fathead embryos were selected and randomly placed in incubation cups oscillating in the adult chambers. Fifty fry were released in each tank and measured (standard length) as juvenile fish at 66 days after hatching using a photographic method similar to that described by McKim and Benoit (1971). The juvenile fish were thinned to 15 per adult tank and a sample of the excess fish removed were saved for immediate 66 day residue analysis. The remaining excess fish were placed in a clean flow-through system where they were sampled bimonthly to determine residue elimination.

The spawning substrates were 6 to 8 cm longitudinal domed sections of brown glass quartz beverage bottles. Whenever all 5 substrates were occupied by males, one was classified as excess and was removed. Excess males were weighed, measured (total length), frozen, and saved for residue determination. Removal of excess males was initiated at approximately the same time spawning began (4 month). Excess male removal lasted over a 2 month period. Spawning duration was continued for approximately 4 months. The spawning was monitored each morning and embryos were removed from spawning substrates by using the finger-roll method (Gast and Brungs 1973).

Embryo incubation cups were constructed from 4.2 cm O.D. glass jars with bottoms sawed off and replaced with stainless steel screen (18 mesh/cm). The egg cups oscillated 3 cm in test water by means of a motor driven rocker arm at a rate of 2 oscillations per minute. The determination of embryo hatchability was performed by impartially selecting 25-50 viable

eggs and placing them in an oscillating egg cup. The embryos were not chemically treated for disease. Infertile eggs and dead embryos were removed and recorded daily and embryo cup screens were sprayed clean with a jet of water from a neoprene squeeze bottle. Tests measuring the importance of acclimation on hatchability, which involved transferring embryos between acetone control, raw lake water control tanks and tanks with high toxicant concentrations were run in the same manner. Adult fish were killed after approximately 8.5 months of exposure. Survival, weight, length (total), and deformities, if any, were determined and recorded. Sex was determined by outward physical characteristics and by autopsy. Adult fish were also frozen and retained for residue analysis.

Chemical Methods

The polychlorinated biphenyls (PCB's) tested were Aroclor® 1248 and Aroclor® 1260, manufactured by the Monsanto Chemical Company. The standard stock solutions were prepared by dissolving 1 mg PCB/ml acetone from which working stock solutions for the injector syringes were prepared every 3 months by diluting with acetone. The nominal acetone concentrations in chronic studies were 4 µl/liter for the first 110 days and 2.2 µl/liter for the remainder of the test because of the suppression of the dissolved oxygen concentration. One-hundred ml of test water were taken daily for 5 days and the composite samples (500 ml) were held in a 1-gal glass vessel containing 100 ml of petroleum ether and were poured into separatory funnels containing 50 ml of petroleum ether.

A duplicate of either the acetone control or the lake water control was spiked with a known quantity of toxicant and two or three drops of phosphoric acid was added to each sample. The samples were placed on an Eberbach

shaker for 20 min after which they were allowed to stand until the phrases separated. The aqueous phase was drained off into a cleaned Erlenmeyer flask and saved for a second extraction with 10% isocatane/hexane (v/v). The organic phase was drained through a anhydrous sodium sulfate funnel which had been solvent-washed and placed atop a Kuderna-Danish condensing apparatus. A 3-ball Schneider column was affixed to the Kuderna and the solvent extract evaporated over a steam bath until approximately 5-6 ml remained in the evaporator receiver. The mean measured recovery was 76% for Aroclor 1248 and 88% for Aroclor 1260. The concentrations were corrected for recovery and the mean measured concentrations were used in the presentation of results.

The fish were sampled for residue analysis by removing the desired number of fish, wrapping them individually in aluminum foil, and storing them in a freezer until the tissue could be analyzed. The whole fish samples were homogenized with anhydrous Na_2SO_4 using approximately 3 g of the dessicant to each grain of wet tissue. The mixture was extracted by blending with two 50 ml portions of 6% ethyl ether in petroleum ether and the resulting extract was filtered with glass fiber mats to remove particulate material. A 10% aliquot was removed for the lipid determination (by weight).

The remaining extract was transferred to a 20 column of activated florisil and eluted with 200 ml of petroleum ether (Veith and Lee 1971a). The cleaned-up extract was adjusted to an appropriate volume for analysis by electron capture gas chromatography. The recovery of standard additions of the PCB mixtures indicated the recovery of this procedure was approximately 90%.

A Tracor Model 550 Gas Chromatograph with two 6-ft 1/4-inch O.D. glass columns packed with 3% OV-1 on chromosorb W, 60-80 mesh, was used for analysis of PCB's. The column temperature was maintained at 190 C for Aroclor 1248 and 210 C for Aroclor 1260. The Ni^{63} detectors were held at 280-290 C. Purified nitrogen carrier gas was maintained between 50 to 70 ml/min.

In the acute tests with Aroclor 1248 the mean concentration ranged from 0.1 µg/liter to approximately 8.5 µg/liter. In the chronic tests, the mean concentration of Aroclor 1248 ranged from 0.1 µg/liter to 3.0 µg/liter. The standard errors of the mean were generally less than 20% of the mean. The PCB content (as Aroclor 1254) of the Lake Superior water supply at the National Water Quality Laboratory is approximately 3 ng/liter (Veith et al. 1975). The PCB's in the acute tests with Aroclor 1260 ranged from 0.5 to 11.5 µg/liter whereas the mean concentrations in the chronic tests ranged from 0.1 to 2.1 µg/liter. The variability of the measurements of PCB's was greatest during the first two months of the chronic exposure and the concentration of the PCB's in both studies varied less than 15% after the diluter reached a steady-state operation.

Untreated Lake Superior water used in these studies was monitored weekly for pH, alkalinity, acidity, and dissolved oxygen using standard methods (American Public Health Association 1965). The test tanks were sampled occasionally to confirm uniformity of test tanks; however, test tanks were analyzed weekly for dissolved oxygen because of the variability observed in amount between individual test chambers with that of raw test water. The Lake Superior water used in the tests had a mean hardness of 43.6 mg/liter (as $CaCO_3$) and a mean alkalinity of 41.1 mg/liter (as $CaCO_3$). The pH varied between 7.10 and 7.85 during the test and the arithmetic mean

of the pH was 7.52 ± 0.02. The dissolved oxygen varied from 4.6 to 8.8 mg/liter with a mean concentration of 7.0 ± 0.07.

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Results and Discussion

Biological Effects

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Table 1 presents the results of the acute tests with Aroclor 1248. Total mortality occurred when fathead larvae (<8-h-old) were exposed for 30 days to 8.5 µg/liter 1248, Forty-four percent of the larvae died at 4.4 µg/liter and no effect on survival was evident at 2.2 µg/liter. The calculated 30-day LC50 for Aroclor 1248 from these data was 4.7 µg/liter. The weight, length, and standing crop of the young fish increased slightly when exposed to 1.1 µg/liter but decreased with increasing PCB concentration. When exposed to 4.4 µg/liter Aroclor 1248 the mean larvae weight was reduced 66% the mean length (total) was 30% less, and mean standing crop was only 20% of that of the controls. The mean standing crop in the 2.2 µg/liter exposure was reduced to 79% of the control, due largely to a 22% reduction in mean weight.

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The results of the acute exposure of fish to Aroclor 1260 are presented in Table 2. All of the fish died during the 30-day exposure to 9.0 µg/liter Aroclor 1260. Sixty-nine percent of the larvae died at 4.0 µg/liter and no effect on survival was evident at 1.3 µg/liter. The calculated 30-day LC50 for Aroclor 1260 from these data was 3.3 µg/liter. The weight, length, and standing crop of the young fish exposed to 4.0 µg/liter were reduced by 30, 11, and 78%, respectively. The standing crop of the fish exposed to 1.3 µg/liter was 79% of that in the control.

The results of this study of the effects of PCB's on the fathead minnow agree well with results of previous studies. Nebeker et al. (1974), found that fathead minnows died rapidly when exposed to 18 µg/liter of Aroclor 1248 and that 25% mortality occurred when exposed to 5.1 µg/liter. This study has shown that after 30 days there was total mortality in fathead larvae when exposed to 8.5 µg/liter, 43% mortality at 4.4 µg/liter, and no

apparent effect at 2.2 µg/liter. A calculated 30-day LC50 for Aroclor 1248 is estimated to be 4.7 µg/liter. Nebeker et al. (1974), also found that the weight of the fish exposed to 2.2 µg/liter Aroclor 1248 was approximately one-half that of the control. This study has shown that the mean larvae weight was reduced 66% when exposed to 4.4 µg/liter and that the weight of the adult male minnows exposed to 1.1 µg/liter for 239 days was 22% below that of the control fish.

The survival of juvenile fathead minnows exposed to 3.0 µg/liter of Aroclor 1248 for 66 days, was 89% of the controls when indicating much of the observed mortality occurs within the first 30 days. Moreover, the adult male minnows exposed to 1.1 µg/liter for 239 days experienced no deaths but a 22% reduction in weight compared to the control fish. The adult fish exposed to 3.0 µg/liter all lived but the weight of the males and females was depressed 24 and 15%, respectively.

Reproduction occurred at all concentrations of Aroclor 1248 up to 3.0 µg/liter (Table 3); however, the variability in reproduction of the fish in this test was great enough to preclude the disclosure of definite trends at these exposure concentrations. Also, the hatching of embryos was equally successful at all test concentrations of 3.0 µg/liter and below. Embryos transferred from Lake Superior control water to 3.0 µg/liter of Aroclor 1248 hatched with equal success.

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The reproductive success of fathead minnows exposed to Aroclor 1260 is summarized in Table 4. The egg production per female was approximately 1000 for all exposure conditions up to 2.1 µg/liter Aroclor 1260. There was no discernable trend in reproduction below 2.1 µg/liter of this PCB. Likewise, the hatching success of the embryo produced ranged from 126% of

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the controls at 0.1 µg/liter PCB to 90% of the control at 2.1 µg/liter of Aroclor 1260. A consistent trend was not evident in these data. The mean hatching success of 3 batches of embryos transferred from the control tanks to 2.1 µg/liter Aroclor 1260 was 45 ± 13% which is 27% below the hatch success of the control.

Nebeker et al. (1974), has shown that reproduction in the fathead minnow occurs at and below 1.8 µg/liter of Aroclor 1254 and at and below 5.4 µg/liter Aroclor 1242. Since reproduction occurred at and below 3 µg/liter in this study and the 30-day LC50 is 4.7 µg/liter, the toxicity of Aroclor 1248 appears to be greater than that of Aroclor 1242 and less than that of Aroclor 1254.

The 30-day LC50 for Aroclor 1260 with fathead minnow larvae was 3.3 µg/liter indicating that the toxicity of Aroclor 1260 (on the acute basis) is slightly greater than that of Aroclor 1248. Although 4 µg/liter of Aroclor 1260 reduced the weight, length, and standing crop of the young fish after 30 days, 2.1 µg/liter of Aroclor 1260 was found to reduce the length of the 66-day-old fish by 8% (significant at P=0.05) that of the control, and no effect on weight or length of the fish was observed after 237 days of 2.1 µg/liter. As with Aroclor 1248, this study using Aroclor 1260 has shown no discernable effect on the reproductive success of fathead minnows exposed to concentrations of Aroclor 1260 which approach the 30-day LC50. The data indicates that the PCB's do not produce major effects on reproduction at concentrations up to the 30-day LC50 and that the reduced standing crop in a multi-generation study is largely due to the larvae mortality. This is consistent with Nebeker et al. (1974) who concluded that fathead minnow larvae was the most sensitive stage in the chronic bioassay of PCB's.

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Table 5 presents the data concerning the 30-day survival and growth of the F_2 generation larvae in the chronic exposure. As was observed with the first generation larvae, exposure to 0.1 µg/liter Aroclor 1248 did not affect survival, weight, or length of the fish after 30 days. However, at greater concentrations of this PCB mixture the length, weight, and standing crop decreased with increasing concentrations. At 3.0 µg/liter a 33% reduction in weight resulted in a 30% reduction in standing crop.

Table 6 near here

Table 6 presents a summary of the survival and growth of the F_2 generation after 30 days in water containing Aroclor 1260. The data show that the larvae exposed to 2.1 µg/liter Aroclor 1260 weighed 84% that of the control fish and that there exists a trend of decreasing weight with increasing concentration of toxicant. However, effects on length or standing crop were not observed in this chronic test and the mean standing crop of the larvae in 2.1 µg/liter Aroclor 1260 was 98% of the control fish.

The 30-day study of the survival and growth of the F_2 generation of fathead larvae indicated there was a 30% decrease in the mean standing crop with the fish exposed to 3 µg/liter, a 16% decrease at 1.1 µg/liter, and a 13% decrease at 0.4 µg/liter of Aroclor 1248. Thus, the mixture of PCB's found in Aroclor 1248 is acutely toxic to fathead minnow larvae at concentrations less than 10 µg/liter with a 30-day LC50 of 4.7 µg/liter. Aroclor 1248 produces chronic effects in the form of growth retardation in adult minnows at concentrations greater than 1 µg/liter and slight reductions in the weight of second generation larvae at concentrations above 0.4 µg/liter.

PCB Residues in Exposed Fathead Minnows

The composition of the PCB mixtures observed as residues in fathead minnows exposed during the course of the bioassay were similar to the composition of the toxicant used to dose the bioassay system. Fig. 1(a) presents a chromatogram of Aroclor 1248 used in the exposure. The major peaks and shoulders of the chromatogram are numbered 1-16. Fig. 1(b) shows a chromatogram of the Aroclor 1248 residue observed in fish after approximately 200 days of exposure. The composition of this residue is similar to the standard Aroclor 1248 in that the major components labeled 3-16 have essentially the same relative percentages of the residue. However, subtle changes appear to be taking place in the fish. For example, components number 1 and 2 are absent in the chromotogram of the fish.

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The changes in the composition of the PCB isomers are more pronounced in fish which were exposed for approximately 66 days and then placed into Lake Superior water for 34 days. Fig. 1(c) illustrates a chromotogram of these fish residues and although the components labeled 3, 5, 6, 8, 11, 12, and 13 are clearly the major components in the residues, the relative composition of each of these components in the mixture has changed strikingly. Most noticeable are the absence of peaks 1, 2, and 4, the change in the doublet labeled peaks 6 and 7 which now look like a single, unresolved component. A similar effect with peaks 8 and 9 and the relative increase in peaks 11, 12, and 13 relative to the Aroclor 1248 standard.

Unlike the Aroclor 1248 exposure, a comparison of chromatograms of the Aroclor 1260 residue in fish with reference Aroclor 1260 on a component by component basis showed that the composition of the PCB mixtures were essentially identical. No preferential elimination of Aroclor 1260 components were observed in the detoxification study.

The fish were sampled at 30 days, 60 days, and at the termination as well as excess males being thinned from the tanks during the course of the bioassay, the residues of these fish were plotted as indicated in Fig. 2 to show the uptake of Aroclor 1248 in the residue of the fish. Fig. 2 shows the variation of the concentration of Aroclor 1248 on a wet weight basis in the fish tissue with the numbers of days of exposure from the egg stage. The data at 30 days are composite samples of the 30-day fry and the circles represent the residues in the male fathead minnows, whereas the squares show the residues in the females. As is shown in Fig. 2, the residues in the fish reached an apparent steady-state after approximately 100 days of exposure and growth, and that the uptake could be approximated as indicated in the figure by a first order uptake mechanism. Fig. 2 also shows that the concentration of Aroclor 1248 in the female is twice as large as that in the males and that the variation in the female fish on a wet weight basis was much more variable than that of the male, presumably due to a variable lipid content of the female at the termination of the test.

Similar results were obtained in looking at the residue uptake of Aroclor 1260 as presented in Fig. 3. These data also indicate that the PCB residues in the fish reached an apparent steady state after approximately 100 days, and that the female fish again contained approximately twice that in the male and were much more variable. Although the apparent mechanism for the uptake in 1260 and 1248 appear to be similar, greater body burdens resulted from the scales of the Aroclor 1260 exposure. For example, the males in the Aroclor 1248 exposure contained approximately 190 µg/g of Aroclor 1248 after being exposed to 3 µg/liter for 250 days.

This corresponds to an apparent bioconcentration factor of approximately 60,000. The male fish in the Aroclor 1260 exposure contained approximately 350 µg/g of Aroclor 1260 after being exposed to 2.1 µg/liter of Aroclor 1260. This corresponds to an apparent bioconcentration factor of 160,000. The data in Fig. 2 and 3 clearly show that the more highly chlorinated PCB mixtures are bioconcentrated to a greater extent in the lipids of the fish.

The residue of PCB's in the tissue of the fish is directly proportional to the concentration of the toxicant in the water. Fig. 4 shows that the wet weight residue of Aroclor 1248 in the fathead minnows is directly proportional to the concentration of Aroclor 1248 in the water and that the slopes of the uptake curves are about twice as great for the female fish as the male fish, the slope of the line indicating the bioconcentration factor. Fig. 5 presents similar data for the 1248 exposure, with the exception that the data has been normalized with respect to the lipid concentration in the fish. This plot demonstrates that the concentration of Aroclor 1248 is relatively the same in the fish on a lipid basis and is directly proportional to the concentration of PCB's in the water. Fig. 6 presents the data for the uptake of Aroclor 1260 in which the residues, when normalized for lipid concentration, are approximately the same in the female and the male fish.

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Fig. 6 near here

Some evidence exists that the bioconcentration factors measured in the laboratory are essentially the same as that found in the river environment. Veith and Lee (1971a) and Veith and Lee (1971b) report that goldfish in the lower Milwaukee River accumulated Aroclor 1248 approximately 0.7×10^5 to 2×10^5 times (depending on the lipid content) the estimated PCB concentration in the river water. The maximum concentration of Aroclor

1254 in Saginaw River shad was 165 µg/gm whereas typical water concentrations averaged 1.1 µg/liter with a maximum of 2.9 µg/liter (Michigan 1973). This indicates a bioconcentration factor of 0.6×10^5 to 1.5×10^5, in good agreement with other estimates.

During the 66-day thinning, the fish exposed to the highest concentrations of Aroclor 1260 and 1248 were placed in a diluter system containing raw Lake Superior water. Groups of five fish were sampled at 2-week intervals to determine if the concentration of PCB's decreased with time in clean water. The data for the analysis of these composite 5 fish are presented in Fig. 7. The decrease in the concentration of Aroclor 1248 and Aroclor 1260 (after the 66 days of exposure) was first order with respect to time, and the rate of decrease for the two PCB mixtures was essentially the same. The apparent "half-life" for the decrease in concentration of PCB's in the fish tissue was approximately 45 days.

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However, the data presented in Fig. 7 does not indicate a true elimination of PCB's from these contaminated fish because the 66-day-old fish were still growing quite rapidly during this part of the study and the fish approximately doubled in weight during this phase of the study. Therefore, the apparent decrease in concentration is due to the growth of the fish as well as possible elimination from the fish tissue. For example, the fish exposed to Aroclor 1248 contained a body burden of 176 g of Aroclor 1248 per 5 fish at the initiation of the elimination study. At the end of 14 days, the 5 fish sampled contained 147 µg and after 28 days the 5 fish sampled contained 143 µg. Therefore, only 18% of the original PCB burden had been eliminated in the 28-day elimination study. The five fish composite which were exposed to Aroclor 1260 contained

approximately 100 µg per fish and this value decreased to 85 µg per fish, or 15%, after 42 days of elimination. However, because of the large variation in the PCB concentrations in fathead minnows from the same tank, the 10 to 20% decline in total body burdens is insufficient to indicate PCB elimination.

Lieb et al. (1974) have concluded that Aroclor 1254 was not eliminated from rainbow trout at a measurable rate. These data together with the results of the Aroclor 1248 and Aroclor 1260 studies show that the rates of elimination of PCB's containing more than 4 or 5 chlorine atoms are extremely slow.

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Allen, J. R., L. A. Carstens, and D. A. Barsotti. 1974. Residual effects of short-term, low-level exposure of nonhuman primates to polychlorinated biphenyls. Toxicol. Appl. Pharmacol. 30: 1-12.

American Public Health Association. 1965. Standard Methods for the Examination of Water and Wastewater. 12th ed. New York. 769 p.

Aulerich, R. J., and R. K. Ringer. 1970. Some effects of chlorinated hydrocarbon pesticides on mink. Amer. Fur. Breeder. 43: 10-11.

Aulerich, R. J., R. K. Ringer, P. J. Schaible, and H. L. Seagran. 1970. An evaluation of processed Great Lakes fishery products for feeding mink. Feedstuffs. 42: 48-49.

Aulerich, R. J., R. K. Ringer, H. L. Seagran, and W. G. Youatt. 1971. Effects of feeding coho salmon and other Great Lakes fish on mink reproduction. Can. J. Zoology. 49: 611-616.

Barsotti, D. A., and J. R. Allen. 1975. Effects of polychlorinated biphenyls on reproduction in the primate. Meeting Abstract.. 1975 American Society for Experimental Pathology.

DeFoe, D. L. 1975. Multichannel toxicant injection system for flow-through bioassays. J. Fish. Res. Board Can. 32: 544-546.

Gast, M. H., and W. A. Brungs. 1973. A procedure for separating eggs of the fathead minnow. Prog. Fish-Cult. 35: 54.

Hansen, D. J., P. R. Parrish, J. I. Lowe, A. J. Wilson, and P. D. Wilson. 1971. Chronic toxicity, uptake, and retention of Aroclor 1254 in two estuarine fishes. Bull. Environ. Contam. Toxicol. 6: 113-119.

Hartsough, G. R. 1965. Great Lakes fish now suspect as mink food. Amer. Fur Breeder. 38: 25-27.

Lieb, A. J., D. D. Bills, and R. D. Sinnhuber. 1974. Accumulation of dietary polychlorinated biphenyls (Aroclor 1254) by rainbow trout. J. Agr. Food. Chem. 22: 638-642.

McKim, J. M., and D. A. Benoit. 1971. Effects of long-term exposure to copper on survival, growth, and reproduction of brook trout (Salvelinus fontinalis). J. Fish. Res. Board Can. 28: 655-662.

Michigan. 1973. Monitoring for polychlorinated biphenyls in the aquatic environment. Report to the Lake Michigan Toxic Substances Committee, May. Michigan Water Resources Commission, Bureau of Water Management, East Lansing. 26 p.

Mount, D. I., and W. A. Brungs. 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1: 21-28.

National Water Quality Laboratory. 1971. Recommended Bioassay Procedure for Fathead Minnow Pimephales promelas Rafinesque Chronic Tests. (Revised January, 1972). 13 p.

Nebeker, A. V., and F. A. Puglisi. 1974. Effect of polychlorinated biphenyls (PCB's) on survival and reproduction of Daphnia, Gammarus, and Tanytarsus. Trans. Am. Fish. Soc. 103: 722-728.

Nebeker, A. V., F. A. Puglisi, and D. L. DeFoe. 1974. Effect of polychlorinated biphenyl compounds on survival and reproduction of the fathead minnow and flagfish. Trans. Am. Fish. Soc. 103: 562-568.

Platonow, N. S., and L. H. Karstad. 1973. Dietary effects of polychlorinated biphenyls on mink. Can. J. Comp. Med. 37: 391-400.

Ringer, R. K., R. J. Aulerich, and M. Zabick. 1972. Effect of dietary polychlorinated biphenyls on growth and reproduction of mink. Presented at the American Chemical Society Meeting, Division of Air, Water, and Waste. New York, New York. pp. 149-154.

Schoettger, R. A., B. Grant, H. D. Kennedy, F. L. Mayer, H. O. Sanders, and D. Swedberg. 1970. Special report on polychlorinated biphenyls (PCB's), p. 1-23. In Progress in Sport Fishery Research. Fish-Pesticide Research Laboratory. Division of Fisheries Research, Bureau of Sport Fishery and Wildlife, Washington, D.C.

MONS 047607

Södergren, A., and B. Svensson. 1973. Uptake and accumulation of DDT and PCB by Ephemera danica in continuous-flow systems. Bull. Environ. Contam. Toxicol. 9: 345-354.

Stalling, D. L., and F. L. Mayer, Jr. 1972. Toxicities of PCB's to fish and environmental residues. Environmental Health Perspectives. 1: 159-164.

Veith, G. D., G. E. Glass, D. W. Kuehl, F. A. Puglisi, and J. G. Eaton. 1975. PCB's and DDT in fish from western Lake Superior. National Water Quality Laboratory Report. Duluth, Minnesota.

Veith, G. D., and G. F. Lee. 1971a. PCB's in fish from the Milwaukee River. Proc. 14th Conf. Great Lakes Res. pp. 157-169.

Veith, G. D., and G. F. Lee. 1971b. Chlorobiphenyls (PCB's) in the Milwaukee River. Water. Res. 5: 1107-1115.

MONS 047608

Table 1. Fathead larval survival, growth, and standing crop after 30 day acute exposure to Aroclor 1248.

Mean measured concentration (ug/liter)	Replicate	Fry survival (percent)	Percent of control	Weight (mg) $\bar{X} \pm$ standard error	Percent of control	Length (total) mm $\bar{X} \pm$ standard error	Percent of control	Mean standing crop (mg)	Percent of control
8.5	A	0	0	0	0	0	0	0	0
	B	0		0		0		0	
4.4	A	23	56[a]	27.4±9.8	34[a]	13.3±1.4	70[a]	192	20[a]
	B	70		37.7±4.1		15.9±0.4		792	
	C	53		33.5±3.8		15.0±0.6		536	
	D	53		46.1±4.1		16.7±0.6		738	
2.2	A	93	98	94.7±6.3	78	21.0±0.5	93	2652	79
	B	80		73.4±5.5		19.4±0.4		1762	
1.1	A	90	102	106.7±7.9	102	22.2±0.5	102	2881	105
	B	90		112.4±8.0		22.2±0.5		3035	
0.0 (Control)	A	87	100	93.2±9.1	100	20.3±0.7	100	2423	100
	B	90		122.1±8.4		23.2±0.3		3297	

Values showing significant decrease P = 0.05 from controls using analysis of variance and Dunnett's test.

MONS 047609

Table 2. Fathead larval survival, growth, and standing crop after 30 days acute exposure to Aroclor 1260.

Mean measured concentration (µg/liter)	Replicate	Fry survival (percent)	Percent of control	Weight (mg) $\bar{X} \pm$ standard error	Percent of control	Length (total) mm $\bar{X} \pm$ standard error	Percent of control	Mean standing crop (mg)	Percent of control
9.0	A	0	0	0	0	0	0	0	0
	B	0		0		0		0	0
4.0	A	33	31[a]	59.6±12.8	70[a]	18.0±1.2	89[a]	596[a]	22[a]
	B	23		62.0±14.3		17.9±1.2[a]		434[a]	
1.3	A	87	98	68.8±8.2	82	18.4±0.7[a]	92[a]	1789[a]	79[a]
	B	87		72.5±6.8		19.0±0.7[a]		1885[a]	
0.0 (Control)	A	83		70.8±8.4		19.3±0.7		1770	
	B	97		100.4±6.9		21.2±0.4		2912	
0.0 (Control)	C	87	100	91.5±8.3	100	20.6±0.6	100	2379	100
	D	87		84.3±8.8		20.0±0.6		2192	

[a]Values showing significant decrease P = 0.05 from controls using analysis of variance and Dunnett's test.

Table 3. Spawning and egg production of the fathead minnow exposed to Aroclor 1248.

Mean measured concentration (µg/liter)	Duplicate	Sex ratio at termination (male/female)	Spawning duration in days	Percent of control	Number of spawnings	Number of spawnings/ female	Total eggs produced	Number of eggs/ spawning	Number of eggs/ female
3.0	A	7/3	71	85	28	9.0	2224	79	741
	B	6/5	73		18	3.6	923	51	185
1.1	A	5/8	87	83	59	7.4	5183	88	648
	B	5/7	53		24	3.4	1247	52	178
0.4	A	4/9	99	110	75	8.3	8017	107	891
	B	5/6	88		38	6.3	3753	99	626
0.1	A	4/4	78	98	25	6.2	904	36	226
	B	4/7	87		36	5.1	3344	93	478
0.0 (Control)	A	4/9	93	100	86	8.8	9948	116	1105
	B	3/7	77		55	7.7	5226	95	747

MONS 047611

Table 4. Spawning and egg production of the fathead minnow exposed to Aroclor 1260.

Mean measured concentration (ug/liter)	Duplicate	Sex ratio at termination (male/female)	Spawning duration in days	Percent of control	Number of spawnings	Number of spawnings/ female	Total eggs produced	Number of eggs/ spawning	Number of eggs/ female
2.1	A	4/9	84[a]	84[a]	55	6.1	6811	124	757
	B	4/8	88[a]		64	8.0	10701	167	1338
0.7	A	4/7	87[a]	84[a]	48	6.9	8193	171	1170
	B	5/7	85[a]		42	6.0	5227	125	747
0.4	A	4/10	107	101	67	6.7	6803	102	680
	B	6/8	102		69	9.9	9908	144	1239
0.1	A	4/3	72[a]	77[a]	31	10.3	1969	64	656
	B	5/8	87[a]		66	8.3	7472	113	934
0.0 (Control)	A	6/4	108	100	52	13.0	3836	74	959
	B	6/10	99		67	6.7	9667	144	957

[a]Values showing significant decrease P = 0.05 from control using analysis of variance and Dunnett's test.

MONS 047917

Table 5. Survival and growth of second generation (F_2) fathead fry after 30 days exposure to Aroclor 1248.

Mean measured concentration (µg/liter)	Replicate	Survival percent	Mean survival $\bar{X}$ ± standard error	Percent of control	Weight (mg) $\bar{X}$ ± standard error	Mean weight (mg) $\bar{X}$ ± standard error	Percent of control	Mean length (mm) $\bar{X}$ ± standard error	Mean length (mm) $\bar{X}$ ± standard error	Percent of control	Standing crop survival x weight (mg)	Mean standing crop $\bar{X}$ ± standard error	Percent of control
	1	50			60.9±20.4			16.3±1.5			609		
	2	70			107.8±20.3			21.0±1.6			1509		
	3	85			107.9±15.1			20.0±1.2			1834		
3.0	4	100	78±7.0	104	49.3±4.5	98.2[a] ±21.1	67[a]	16.2±0.5	19.6[a] ±1.3	84[a]	986	1504 ±322.5	70
	5	85			71.8±7.8			19.8±0.7			1220		
	6	75			191.2±17.3			24.5±0.8			2858		
	1	90			136.0±6.3			23.0±0.3			2448		
	2	100			92.7±11.1			20.3±0.8			1854		
	3	85			65.5±6.0			19.5±0.5			1114		
1.1	4	70	86±3.5	115	114.7±7.7	105.6[a] ±9.5	72[a]	22.0±0.5	21.4 ±0.6	92	1603	1807 ±176.2	84
	5	85			86.1±6.9			19.7±0.5			1464		
	6	80			116.3±6.9			22.4±0.4			1661		
	7	90			128.1±7.7			23.1±0.3			2306		
	1	95			123.9±13.7			21.5±0.9			2354		
	2	65			99.8±10.8			20.4±0.7			1297		
	3	90			111.2±14.4			21.3±1.0			2002		
	4	80			135.4±7.3			22.8±0.4			2166		
0.4	5	95	78±6.5	103	171.1±8.8	117.9[a] ±6.9	80[a]	25.8±0.4	21.7 ±0.5	93	3251	1866 ±218.2	87
	6	85			109.1±10.4			21.8±0.7			1855		
	7	100			107.2±8.7			21.7±0.5			2144		
	8	40			105.0±20.6			19.5±1.2			840		
	9	80			99.2±9.5			20.8±1.0			1587		
	10	50			116.6±15.1			20.9±1.8			1166		
	1	75			139.3±14.7			22.8±1.0			2547		
	2	70			149.1±19.7			22.3±1.4			1938		
0.1	3	90	81±3.5	109	166.4±19.9	138.5 ±11.0	94	24.1±1.3	22.2 ±0.6	93	2995	2334 ±214.0	104
	4	85			138.2±12.3			21.1±1.0			2211		
	5	85			99.3±7.3			20.5±0.6			1877		
	1	50			176.9±16.7			24.9±0.8			1769		
	2	80			159.1±11.2			24.1±0.6			2546		
	3	45			179.6±13.5			25.8±0.5			1616		
	4	90			98.8±6.2			21.5±0.4			1778		
0.0 (Control)	5	75	75±6.0	100	150.3±7.8	146.7 ±10.8	100	24.5±0.4	23.3 ±5.6	100	2555	2155 ±168.7	100
	6	85			168.2±13.9			21.8±1.2			2859		
	7	80			109.4±13.3			21.0±0.8			1754		
	8	90			131.2±10.8			22.8±0.5			2362		

[a]Values showing significant decrease P = 0.05 from control using analysis of variance and Dunnett's test.

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Table 6. Survival and growth of second generation (F_2) fathead fry after 30 days exposure to Aroclor 1260.

Mean measured concentration (ug/liter)	Replicate	Survival (percent)	Mean survival $\bar{X}$ ± standard error	Percent of control	Weight (mg) $\bar{X}$ ± standard error	Mean weight (mg) $\bar{X}$ ± standard error	Percent of control	Mean length (mm) $\bar{X}$ ± standard error	Mean length (mm) $\bar{X}$ ± standard error	Percent of control	Standing crop survival x weight (mg)	Mean standing crop $\bar{X}$ ± standard error	Percent of control
	1	100			101.0±6.0			21.6±0.3			1020		
	2	90			135.6±5.9			23.5±0.4			2430		
	3	85			130.8±5.1			23.7±0.3			2224		
	4	90			157.7±14.0			24.7±0.6			2839		
2.1	5	90	89±3.5[a]	120[a]	179.6±12.4	141.2 ±7.9	84	25.0±0.6	23.7 ±0.4	96	3229	2498 ±144.0	98
	6	90			144.7±12.3			23.7±0.6			2605		
	7	90			142.7±6.0			24.6±0.3			2569		
	8	75			137.8±13.7			22.5±0.9			2066		
	1	80			153.9±17.8			23.4±1.0			2462		
	2	90			139.1±8.0			23.4±0.5			2504		
	3	90			147.5±13.5			24.1±0.5			2655		
	4	100			191.1±7.5			24.9±0.3			3834		
	5	90			139.7±7.5			24.4±0.4			2515		
0.7	6	100	93±2.0[a]	125[a]	204.0±14.6	130.0 ±9.5	89	26.5±0.6	24.2 ±0.3	98	4080	2793 ±220.6	99
	7	90			118.2±6.9			23.2±0.5			2128		
	8	100			144.2±7.8			24.8±0.3			2884		
	9	95			158.0±15.2			24.3±0.9			3002		
	10	90			103.7±8.0			22.8±0.5			1867		
	1	95			138.3±16.5			22.2±0.9			2628		
	2	95			104.3±15.6			20.1±0.9			1982		
	3	100			145.1±7.9			22.5±1.1			2902		
	4	90			183.8±12.3			25.2±0.5			3308		
0.4	5	100	92±3.0[a]	124[a]	141.0±8.3	152.3 ±10.1	89	24.3±0.3	23.4 ±0.6	94	2820	2770 ±160.0	108
	6	85			122.4±9.8			21.8±0.7			2081		
	7	70			199.2±10.2			25.6±0.4			2789		
	8	95			175.4±7.6			25.8±0.3			3333		
	9	95			162.4±16.1			23.4±1.2			3086		
	1	100			181.6±11.4			26.2±0.6			3632		
	2	95			138.6±5.9			24.6±0.4			2633		
	3	100			139.5±9.1			24.3±0.6			2790		
0.1	4	95	97±1.0[a]	131[a]	182.0±13.7	170.1 ±8.2	104	25.0±0.6	25.3 ±0.7	102	3458	3303 ±158.2	129
	5	100			178.9±7.4			24.8±0.3			3578		
	6	95			177.5±9.5			24.9±0.4			3373		
	7	95			192.5±6.1			27.4±0.3			3658		
	1	45			136.9±27.3			22.6±1.8			1232		
	2	85			120.2±10.6			22.0±0.8			2043		
	3	45			140.0±11.0			24.1±0.6			1260		
	4	85			199.2±10.5			26.7±0.5			3386		
0.0 (Control)	5	80	74±6.5	100	171.6±9.9	169.4 ±13.6	100	25.3±0.4	24.8 ±0.7	100	2778	2558 ±349.6	100
	6	85			236.7±11.0			27.8±0.4			4024		
	7	85			158.8±11.3			24.9±0.6			2700		
	8	80			190.0±12.7			25.3±0.6			3040		

[a]Values showing significant increase P = 0.05 from control using analysis of variance and Dunnett's test.

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and Carlson, M.

Captions for Illustrations

Fig. 1 Comparison of GLC chromatograms of A) Reference Aroclor 1248 and B) Aroclor 1248 residues in fish after 200 days exposure and C) Fish residue after 34 days in clean water.

Fig. 2 PCB residues in fish exposed to 3.0 µg/liter Aroclor 1248.

Fig. 3 PCB residues in fish exposed to 2.1 µg/liter Aroclor 1260.

Fig. 4 Variation of Aroclor 1248 residues (wet weight) with concentrations in water.

Fig. 5 Variation of Aroclor 1248 residues (lipid basis) with concentrations in water.

Fig. 6 Variation of Aroclor 1260 residues (lipid basis) with concentrations in water.

Fig. 7 Variation of PCB concentrations in exposed fish in uncontaminated water.

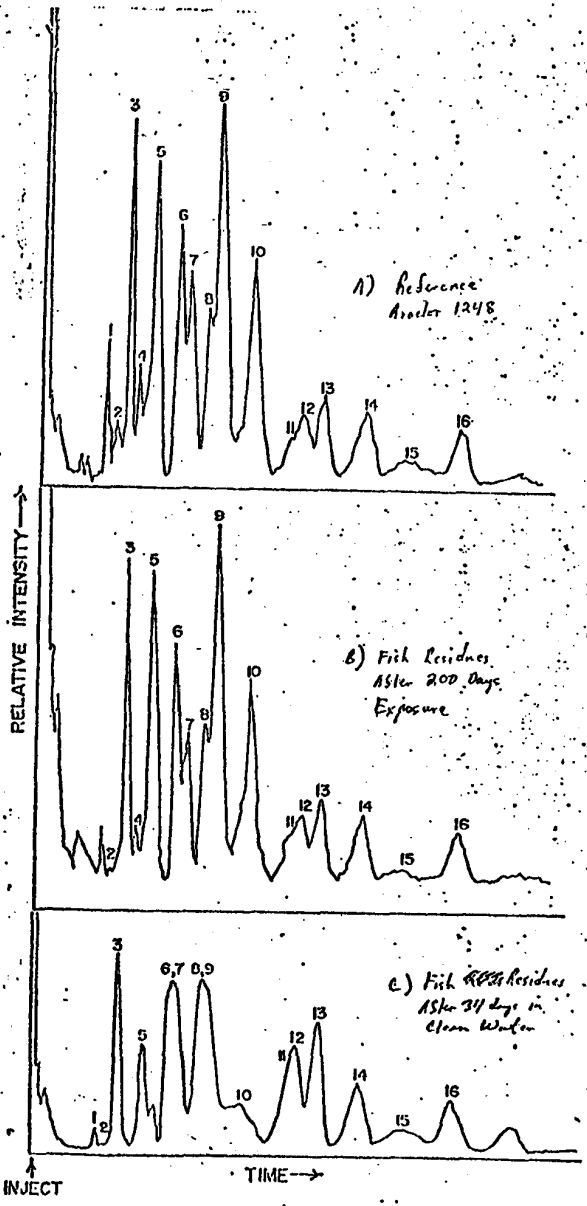


Fig. 1
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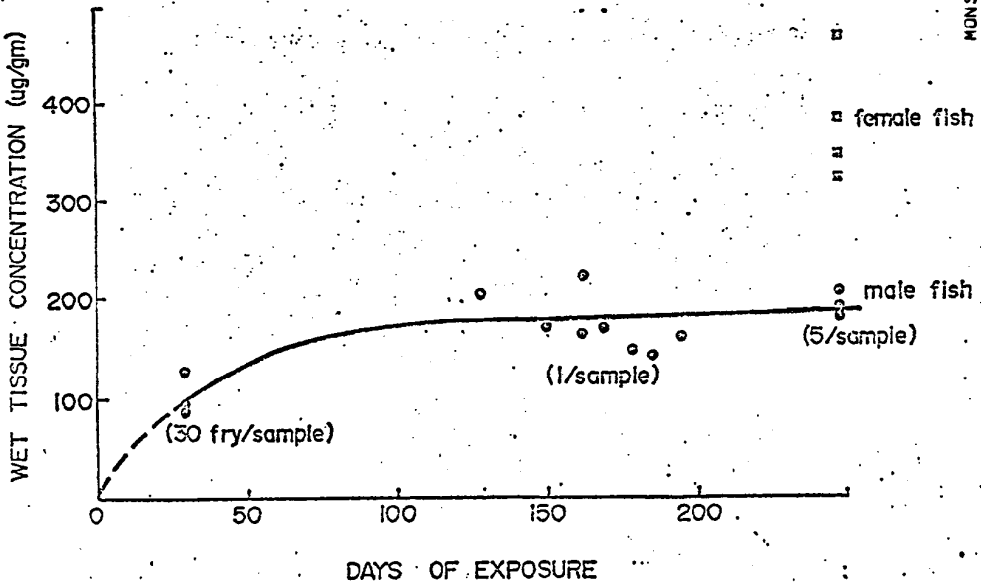


Fig. 2
DeFoe, Veith, and Carlson

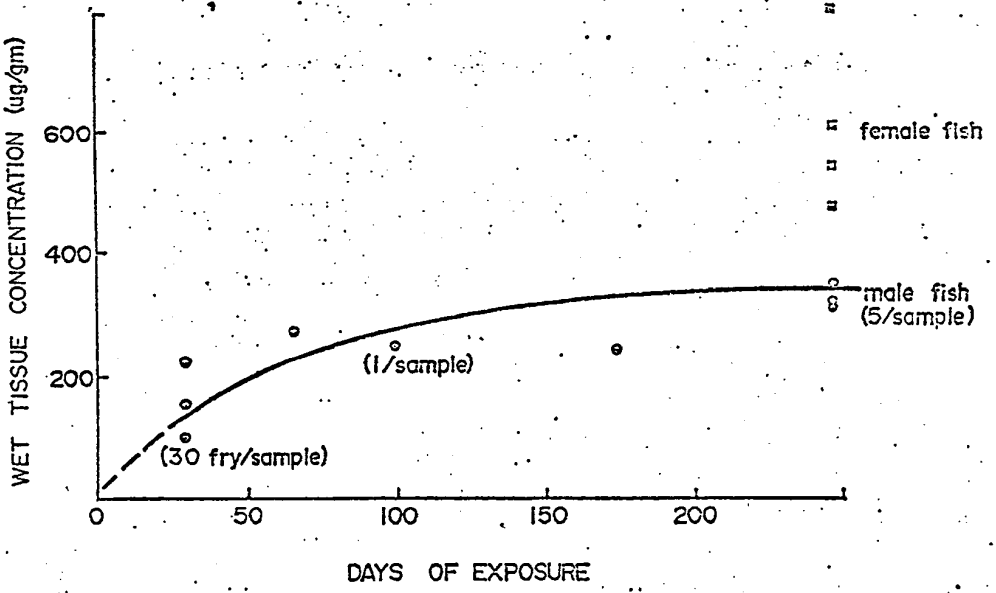


Fig. 3

Defoe, Veith, and Carlson

MONS 047618

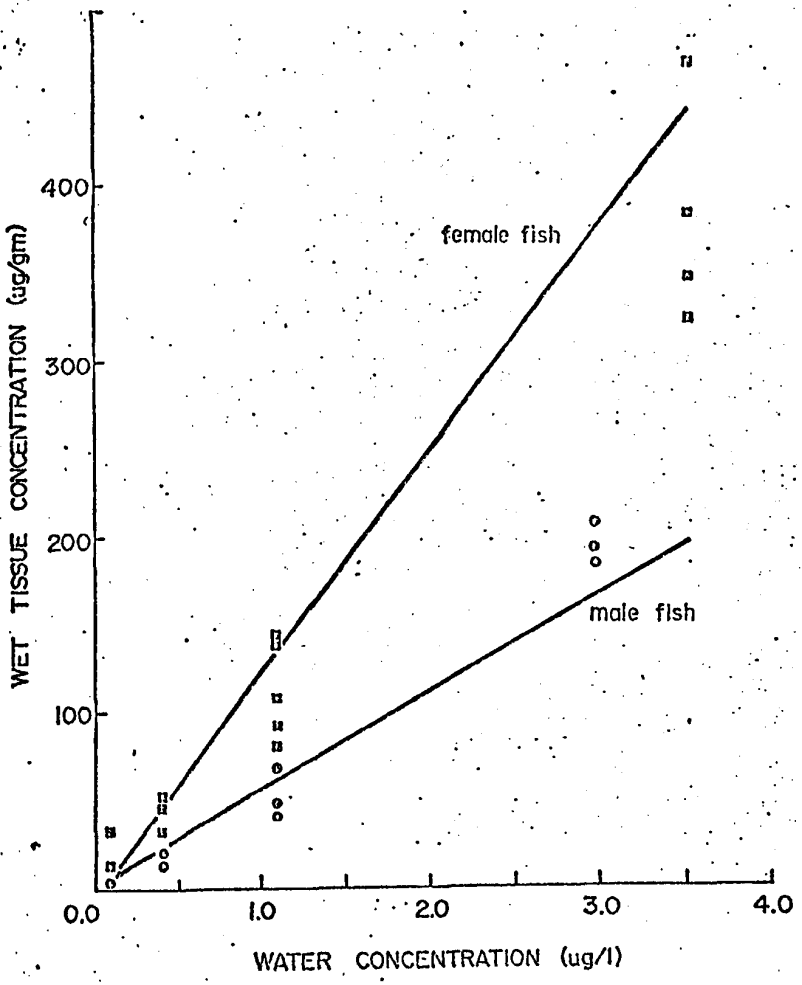


Fig. 4.

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and Carlson

MONS 047619

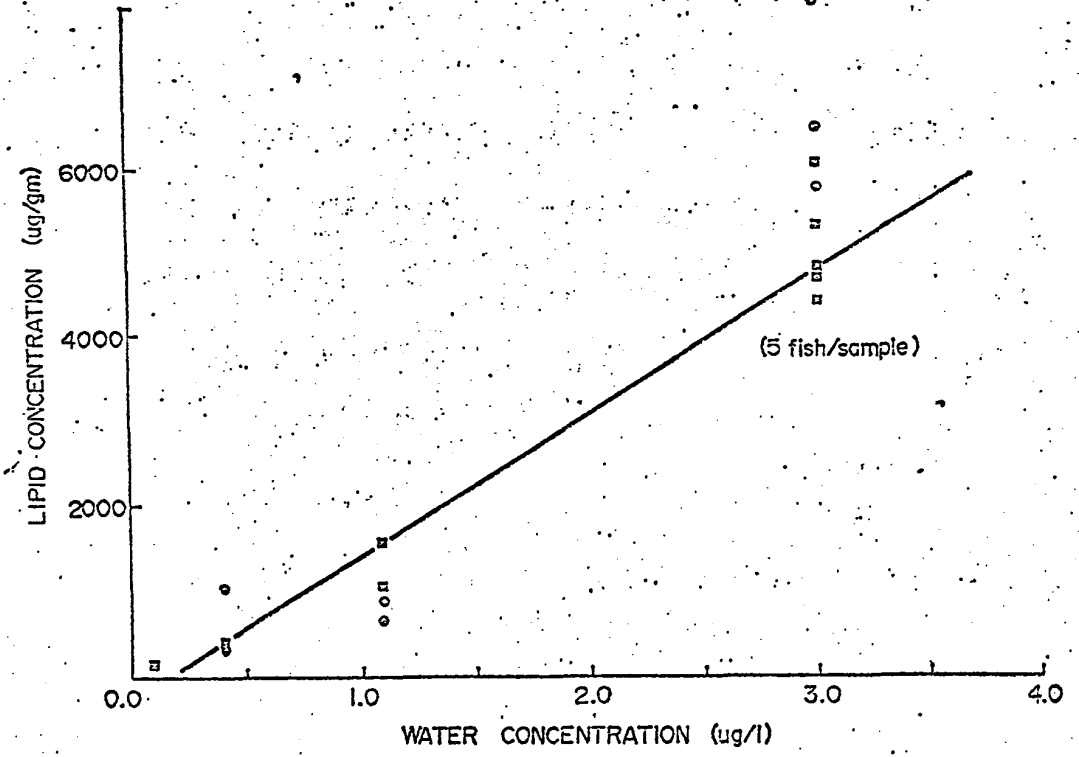


Fig. 5

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MONS 047620

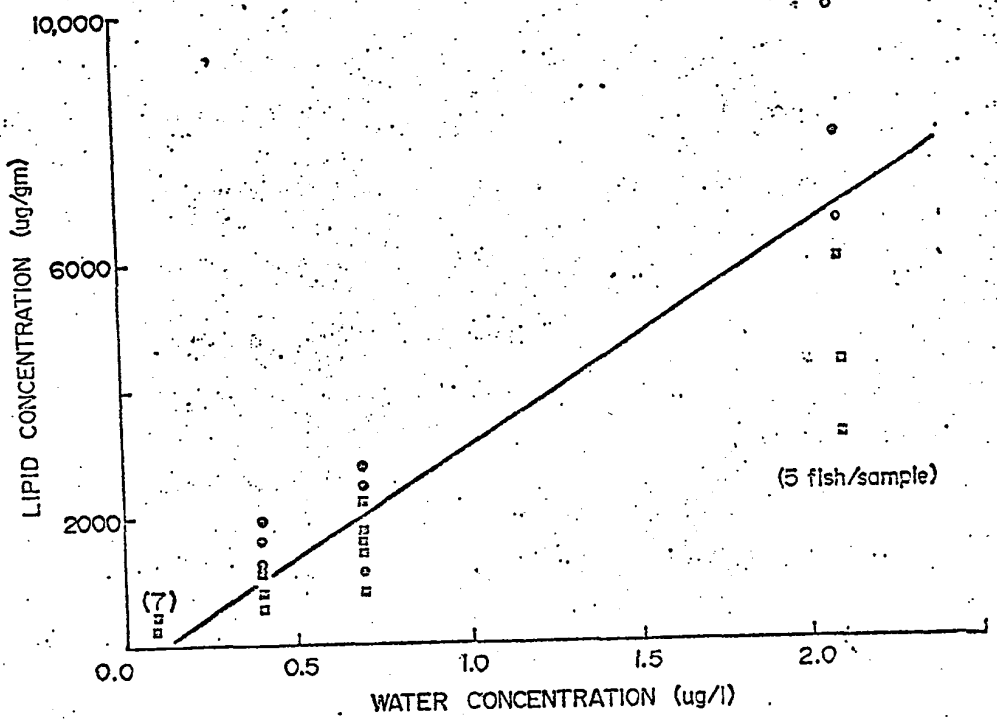


Fig. 6
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MONS 047621

Fig. 7
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and Carlson

MONS 047622