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Patterns of PCB Accumulation by Fry of Lake Trout*

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The accumulation of PCBs by fish has been the subject of many recent laboratory studies (HATTULA & KARLOG 1973; SUGUIRA et al. 1978; BRANSON et al. 1975; ZITKO & HUTZINGER 1976). Researchers have estimated bioconcentration factors, partition coefficients, and uptake rate constants, both for individual PCB isomers and for Aroclor mixtures. In addition, the physiological processes that occur in fish must be examined to understand the dynamics of PCB residues. The redistribution and elimination of "2,5,2',5' -tetrachlorobiphenyl" by rainbow trout (Salmo gairdneri) during gonadal maturation and spawning exemplifies such a process (GUINEY et al. 1979).

In a previous study, BERLIN et al. (1981) observed that one-day-old sac fry of lake trout (Salvelinus namaycush) containing 3.8 µg/g PCBs had decreased in wet weight concentration of PCBs, even though they had been continuously exposed to 50 ng/L for 176 days. These results, coupled with the fact that earliest life stages of fry appear to be most sensitive to the effects of organic contaminants (DEFOE et al. 1978; MAUCK et al. 1978), motivated us to design a study for examining the accumulation pattern of PCBs by lake trout fry exposed through the period of yolk absorption and the onset of feeding. To more adequately assess the dynamics of PCBs in the fry, we examined changes in concentration based on wet weight, dry weight, and body burden.

METHODS AND MATERIALS

We examined the PCB uptake pattern in lake trout sac fry by exposing them to a nominal concentration of 50 ng/L PCB (Aroclor 1254) for 48 days. Fish were sampled and analyzed for PCB concentration seven times during the exposure.

We put 1000 newly hatched lake trout fry into each of four 190-L fiberglass tanks. Fry in two tanks received PCBs in their food and water, and fry in two tanks served as controls. A

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volume-dependent diluter provided a pulsed flow averaging 2 L/min at 9°C to each tank. A chemical description of the laboratory well water was given by BERLIN et al. (1981).

Exposure System. PCBs were dissolved in water by percolating water through a column of glass beads coated with PCBs (MAC & SEELYE 1981). Control tanks received processed water at identical flow rates.

Fish. Eggs and milt were obtained from spawning lake trout gillnetted off Saugatuck, Michigan, in southeastern Lake Michigan on October 31, 1977. The eggs were fertilized on site and incubated at the Great Lakes Fishery Laboratory. Hatching started on January 16 and fry 1-4 days old were moved to test tanks on January 20.

Feeding with Silver Cup[1] salmon starter began when 10-20% of the fry were at swim-up (day 25). When most of the fry were observed to be feeding (day 39), the ration was set at 10% of body weight/day based on the number and average weight of fry in the tank. Fry were fed 3 or 4 times each day. Fish exposed to PCBs in the water received food with PCBs added; the controls received unaltered food. We prepared spiked food by adding 80 mL of acetone containing 5 mg Aroclor 1254 to 5000 g of Silver Cup feed. The solution was added dropwise while the food was being mixed in a Hobart mixer. Mixing continued several hours until the feed appeared dry. Analysis of the food for PCBs revealed levels of 0.72 ± 0.06 µg/g ($\bar{x}$±SE) in spiked food samples and 0.06 ± 0.0005 µg/g in unaltered food.

Analytical Techniques. We analyzed for PCBs in water in each tank twice weekly throughout the 48-day exposure. PCBs were extracted from water with petroleum ether and quantified by gas chromatography (MAC & SEELYE 1981).

Fish samples were prepared for analysis by first compositing 25 fry per sample. Ten samples taken on day 0, before fry were distributed to the tank, provided pre-exposure PCB concentrations for all treatments. Five samples of 25 fry each were collected from each tank on days 4, 7, 10, 17, 32, and 48. Fry samples were freeze-dried in 20-mL scintillation vials and saponified; PCBs were quantified by gas chromatography (MAC & SEELYE 1981). We measured wet weight and freeze-dried weight of each sample and determined water content of fry from the difference between freeze-dried weight and the wet weight of 25 fry blotted on a paper towel. This procedure enabled us to report contaminant concentrations based on wet as well as dry weight. Wet weight concentration (µg/g) was multiplied by wet sample weight to obtain body burden (µg PCB/sample). We also measured total lengths of 25 fry from each

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[1] Use of trade names does not imply U.S. Government endorsement of commercial products.

tank on each sampling day, recorded mortality daily, and measured total length of dead fry. Lipid was determined gravimetrically by using a hexane extract and expressed as a percentage of the fish's wet weight.

Data Analysis. Comparisons of wet and dry weight concentrations and body burden were made by analysis of variance. Because we found no differences in concentration between replicate exposure tanks, the results were combined and compared across all sampling days in each treatment. Where significant differences occurred, least significant difference (LSD) at $P = 0.05$ was used for mean contrast. The LSD statistic was used to facilitate graphical determination of differences in average PCB concentrations over time. We used a nested analysis of variance to evaluate the effects of PCBs on fry growth (dry weight) and water content. Mortality data were adjusted for sampling (see BERLIN et al. 1981) and examined over 10-day intervals as well as for the total 48-day period. We used chi-square to test the effects of PCB on mortality and length frequency distributions after combining data from replicate tanks.

RESULTS AND DISCUSSION

Mean concentrations (ng/L) of PCBs in water in the two exposure tanks were 38 ± 3 ($\bar{x}$±SE) and 45 ± 4. These concentrations were within one standard deviation of the desired 50 ng/L and were not significantly different ($P > 0.05$) from each other. Trace amounts of PCBs (<10 ng/L) were found in the two control tanks.

Patterns of PCB accumulation (Fig. 1) in the lake trout fry appeared similar, regardless of how the data were expressed (wet weight concentration, dry weight concentration, or body burden): PCBs in exposed fry increased slowly, peaked at day 32 (just before yolk absorption was complete) and decreased by day 48. However, though these patterns appeared similar, important differences in the relative changes in PCB levels of exposed fry became evident when we compared the different ways of expressing uptake data.

Results of residue analysis on the fry indicated a dramatic decrease in the wet weight concentration of PCBs in exposed fry shortly after complete yolk absorption, near days 35-40 (Table 1, Fig. 1). Though these results confirmed our earlier observation, examination of the data expressed as both dry weight concentrations and body burdens provided a better assessment of the dynamics of PCBs in the fry. For example, dry weight concentration of PCBs in exposed fry was significantly higher on day 17 than on day 0 (Fig. 1). Conversely, both the PCB concentration based on wet weight and the body burden of PCBs were not different between days 0 and 17 (Fig. 1). Differences in the dynamics of PCBs, depending on how the residues are expressed, were even more marked on the final sampling day: on day 48, PCB concentrations based on wet weight of fry were not significantly different from those on day 0 (Fig. 1); however, both body burdens of PCBs (Fig.

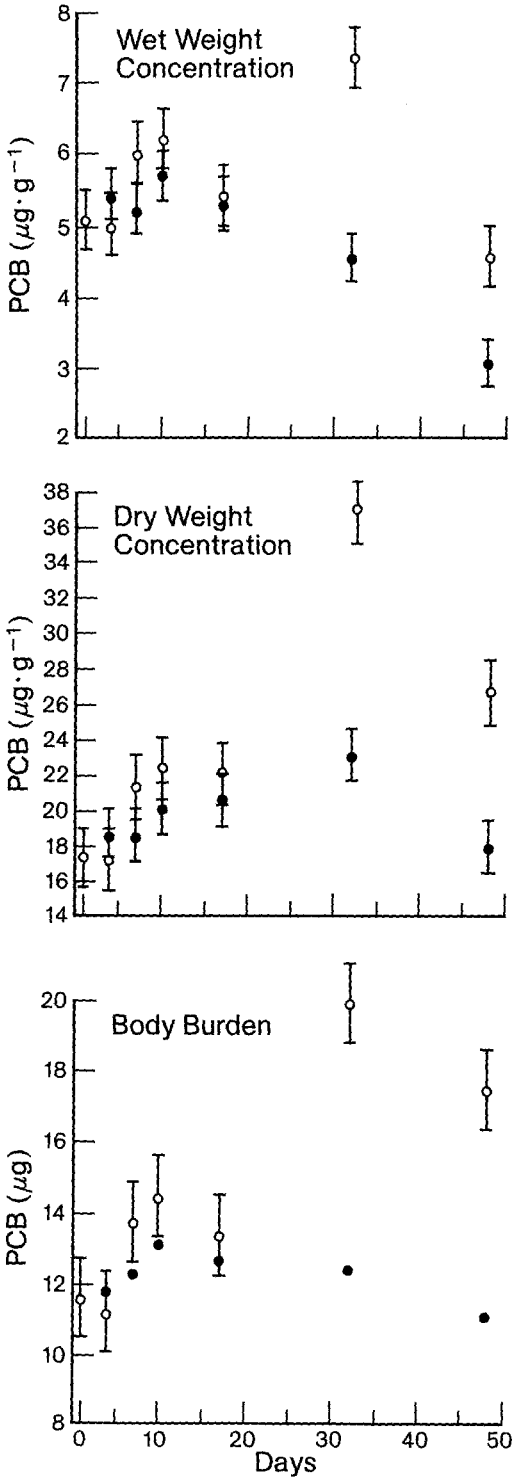

Figure 1. PCB concentrations (μg/g) based on wet weight, dry weight, and body burden of exposed fry (open circles) and control fry (solid circles). Vertical lines represent ± 0.5 LSD.

1) and concentrations based on dry weight were significantly higher on day 48 than on day 0.

TABLE 1. PCB concentrations in lake trout fry exposed to PCBs after various days of exposure. Means are reported with standard errors in parentheses. Values are based on wet weight concentration (µg/g), dry weight concentration (µg/g), and body burden (µg/sample). N = 10 for all samples.

	Concentration (µg/g) by wt.				Body burden (µg/sample)	
	Wet wt.		Dry wt.			
Days	Exposure	Control	Exposure	Control	Exposed	Control
0	5.1 (0.23)	5.1 (0.23)	17.5 (0.92)	17.5 (0.92)	11.6 (0.68)	11.6 (0.68)
4	5.0 (0.26)	5.4 (0.17)	17.2 (1.22)	18.6 (1.30)	11.2 (0.71)	11.8 (0.38)
7	6.0 (0.22)	5.2 (0.26)	21.4 (0.83)	18.5 (0.77)	13.7 (0.58)	12.3 (0.66)
10	6.2 (0.18)	5.2 (0.26)	21.4 (0.83)	20.2 (0.97)	14.4 (0.46)	13.1 (0.76)
17	5.3 (0.24)	5.4 (0.29)	22.2 (0.90)	20.7 (0.98)	13.3 (0.68)	12.6 (0.67)
32	7.3 (0.38)	4.5 (0.23)	37.0 (1.86)	23.0 (1.24)	19.8 (1.13)	12.3 (0.63)
48	4.5 (0.26)	3.0 (0.15)	26.7 (1.54)	17.8 (0.82)	17.3 (0.90)	10.9 (0.45)

Differences in the clearance of PCBs from fry held in control tanks also varied, depending on how the data were expressed. Dry weight concentration of PCBs was significantly greater on day 32 than on day 0, whereas neither wet weight concentration nor body burden of PCBs differed between these two sample days (Fig. 1). Comparison of initial and final concentrations of PCBs in control fry revealed a significant decrease based on wet weight, but no change in dry weight concentration (Fig. 1). The body burden of PCBs in control fry did not change significantly during the entire 48 day experiment (Fig. 1).

Physiological changes in the fry through yolk absorption and initial feeding were responsible for the differences between PCB levels based on wet weight, dry weight, and body burden. Water content in the fry changed drastically from 71% on day 0 to 83% on day 48 (Table 2). This represents a change in dry tissue weight from 30% of the total wet weight to 17%--a 43% reduction. The increasing water content accounts for a loss in dry weight and an increase in wet weight of fry between days 0 and 32 (Table 2). Similar patterns in wet and dry weight of fry have been reported in steelhead fry, Salmo gairdneri (HAYES et al. 1973), and are

TABLE 2. Length (mm), wet and dry weights (mg), and water content (%) for exposed and control lake trout fry after various exposure times. Means are reported with standard errors in parentheses.

	Length (mm)		Wet wt. (mg)		Dry wt. (mg)		Water (%)	
Days	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
0	17.3	17.3	90.7	90.7	26.5	26.5	71	71
	(0.16)	(0.16)	(1.51)	(1.51)	(0.66)	(0.66)	(0.7)	(0.7)
4	18.0	18.0	87.2	88.5	26.3	26.3	70	70
	(0.14)	(0.13)	(1.10)	(1.86)	(1.53)	(1.21)	(1.7)	(1.2)
7	18.6	18.8	94.0	91.4	26.5	25.7	72	72
	(0.13)	(0.14)	(1.60)	(2.11)	(0.37)	(0.45)	(0.5)	(0.4)
10	19.6	19.6	91.4	93.3	25.7	25.7	72	72
	(0.11)	(0.15)	(0.95)	(1.32)	(0.53)	(0.40)	(0.5)	(0.4)
17	21.1	21.5	92.4	99.4	24.3	23.9	74	76
	(0.17)	(0.19)	(1.19)	(1.74)	(0.30)	(0.48)	(0.4)	(0.4)
32	24.3	24.8	109.8	108.8	21.5	21.5	80	80
	(0.17)	(0.20)	(1.66)	(1.58)	(0.34)	(0.34)	(0.4)	(0.1)
48	28.9	29.5	144.6	153.4	24.6	26.0	83	83
	(0.30)	(0.30)	(2.78)	(3.75)	(0.54)	(0.60)	(0.1)	(0.1)

responsible for the differences observed between wet and dry weight concentration in both exposed and control fry (Fig. 1).

Body burden measurements, which are not affected by changes in water content, gave a more accurate representation of the dynamics of PCBs in the fry. The peak level of PCBs (Fig. 1) of 20 µg/sample in exposed fry on day 32 coincided with data expressed as wet or dry weight (Fig. 1); however, the significant drop to 17.3 µg/sample on day 48 was unexpected. At this point, the fry were feeding on contaminated food, which although at 0.72 µg PCB/g would dilute the concentration of PCBs in the fry, should still add to the body burden (µg PCB/sample) of PCBs. Near day 32, the fry may have reached a steady-state level with PCBs in their environment and this level may have decreased because lipid content decreased. Lipid content (wet weight basis) in control fry was 2.5% on day 19, 2.6% on day 27, and 1.9% on day 41. HAYES et al. (1973) reported a similar decrease in total lipid content in developing steelhead fry. They found that little of the lipid in the yolk was incorporated into the fry toward the end of yolk absorption.

TABLE 3. Percent mortality of lake trout fry during 48 day exposure to PCBs.

Time period (days)	Control	Exposed
0-10[a]	1.2	2.5
11-20	4.5	4.0
21-30	1.0	0.7
31-40	2.6	3.4
41-48[a]	3.7	7.0
0-48[a]	12.5	16.6

[a] Denotes significant difference between control and exposed fry as determined by chi-square.

Cumulative mortality of lake trout fry after 48 days was 12.5% for control fry and 16.6% for exposed fry (Table 3)--a highly significant difference ($P < 0.001$). Examination of the mortality data over 10-day periods revealed that significant differences in mortality rate between control and exposed fry occurred in the first 10 days ($P < 0.004$) and between days 41 and 48 ($P < 0.001$). The higher mortality observed between days 41 and 48 occurred just after the peak concentration of PCBs in the fry and therefore may have been contaminant related.

Statistical tests revealed no significant differences ($P > 0.05$) between treatments in either mean weight or length of fry on any sampling day (Table 2). Although in a concurrent study, we reported growth enhancement in lake trout fry due to PCB exposure, those fry were of hatchery origin (MAC & SEELYE 1981), as opposed to the fry in this study, which were of lake origin. Both growth and mortality of hatchery origin fry in that study differed from that of fry of lake origin from this study. Unexposed hatchery fry attained a dry weight of 29.1 mg after 52 days, while fry of lake origin weighed only 24.6 mg after 48 days. Mortality of hatchery fry was only 4.9%, while that of lake origin fry was 12.5%. Because one basic difference between these two groups of fry is their contaminant concentrations at hatching (SEELYE & MAC 1981), it is not surprising that they would respond differently to PCB exposure.

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