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Effects of the Polychlorinated Biphenyl Aroclor® 1254 on Growth, Survival, and Bone Development in Brook Trout (*Salvelinus fontinalis*)

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Eyed eggs of brook trout (*Salvelinus fontinalis*) were exposed to Aroclor® 1254 (0.43–13 μg/L) for 10 d before hatching and the fry for 118 d after hatching. Median hatching time, egg hatchability, and sac fry survival were not affected by Aroclor 1254. At 48 d after hatching, growth was significantly ($P < 0.05$) decreased by Aroclor 1254 concentrations ≥1.5 μg/L, but no significant differences in growth of surviving fry were observed at the end of the 118-d exposure. Mortality occurred in fry exposed to 13 μg/L within 48 d of exposure, and after 118 d of exposure significant mortality occurred in the three highest concentrations. Biochemical constituents in brook trout fry related to growth and development were affected by Aroclor 1254. Hydroxyproline and vitamin C concentrations in sac fry (38 d old) were decreased by ≥3.1 μg/L. Backbone development in fry exposed for 118 d was significantly altered. Collagen was significantly decreased in the backbone as was the phosphorous concentration, while the calcium concentration increased. Hydroxyproline concentration in collagen isolated from the backbone was also decreased. The no-effect exposure concentration on backbone composition was <0.43 μg/L. Whole body residues in fry exposed for 118 d were 40 000 to 47 000 times the concentration in water.

Key words: brook trout, *Salvelinus fontinalis*, Aroclor® 1254, polychlorinated biphenyls, PCB, growth, bone development, vitamin C, collagen

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Des embryons d'omble de fontaine (*Salvelinus fontinalis*), pourvus d'yeux, ont été exposés à l'Aroclor® 1254 (0,43 à 13 μg/L) durant les 10 jours précédant leur éclosion et 118 jours après. L'Aroclor 1254 n'a eu aucun effet sur la médiane de la date d'éclosion, sur le taux d'éclosion ainsi que sur la survie des alevins munis d'une vésicule vitelline. L'Aroclor 1254, à des concentrations de 1,5 μg/L et plus, avait ralenti sensiblement la croissance ($P > 0,05$), 48 jours après l'éclosion, mais à la fin des 118 jours, les survivants ne présentaient aucun retard de croissance appréciable. Au cours des 48 premiers jours, des alevins sont morts à une concentration de 13 μg/L, et après 118 jours, les trois plus fortes concentrations ont été notablement létales. L'Aroclor 1254 a eu des effets sur les constituants biochimiques activant le développement et la croissance. Chez les alevins pourvus d'une vésicule vitelline (de 38 jours), les concentrations de l'hydroxyproline et de la vitamine C ont diminué de <3,1 μg/L. Chez les alevins exposés à l'Aroclor 1254 durant 118 la colonne vertébrale a subi des modification sensibles. Sa teneur en collagène et en phosphore a diminué tandis que celle du calcium a augmenté. Dans le collagène notamment, la concentration d'hydroxyproline a baissé. A la concentration de <0,43 μg/L, l'Aroclor 1254 n'a eu aucun effet sur la colonne vertébrale. Après 118 jours d'exposition, les alevins en contenaient 40 000 à 7 000 fois plus que l'eau.

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POLYCHLORINATED biphenyls (PCBs) are mixtures of chlorinated biphenyl chemicals that have a multitude of industrial uses as dielectric fluids in transformers and capacitors, hydraulic fluids, and plasticizers. During the past 45 yr, about 635 million kg of PCBs were produced in the United States, of which about one third now resides in landfills, air, water, soil, and sediments (USEPA 1976a).

The environmental occurrence of PCBs in aquatic

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and terrestrial habitats and its biological impact were recently reviewed (USEPA 1976b). Various toxicological studies with aquatic organisms showed PCBs to be of relatively low acute toxicity to aquatic invertebrates and fish, to have a high propensity to accumulate in the food chain, to be more toxic to early than to late life stages of fish, and to be highly persistent. However, the chronic sublethal effects of PCBs on growth and development in fish have not been studied adequately. We therefore evaluated the influence of low concentrations of the PCB Aroclor® 1254 on growth, survival, and bone development in brook trout (*Salvelinus fontinalis*).

Materials and Methods

The study was conducted according to procedures for partial chronic tests recommended by the USEPA (1972). Brook trout eggs obtained from the Nashua (N.H.) National Fish Hatchery were exposed continuously to Aroclor 1254 in a proportional diluter system (Mount and Brungs 1967) for 10 d before hatching, and the resulting fry for 118 d. Aroclor 1254 concentrations in water were determined at 2-wk intervals by gas–liquid chromatography (Hesselberg and Johnson 1972); the measured concentrations (standard deviation in parentheses) were 0.43 (0.11), 0.69 (0.12), 1.5 (0.2), 3.1 (0.4), 6.2 (1.2), and 13 (2) μg/L. An experimental-use sample of Aroclor 1254 from Monsanto Chemical Co., St. Louis, Mo., was used throughout the study. Acetone, used as the carrier solvent, did not exceed 0.30 mL/L in any concentration. Dechlorinated municipal water from La Crosse, Wis. (pH 7.7, alkalinity 260 mg/L, and hardness 305 mg/L), was delivered to each exposure tank at 333 mL/min. The water temperature was maintained at 12°C.

Median hatching time was 8–9 d after the start of the study, and median yolk absorption was completed 38 d later. The study continued for 80 d after the median yolk absorption date.

Each glass exposure container was divided into two 24-L compartments by a glass partition. Flow-splitting chambers were mounted above each container to divide the flow equally between the two compartments. One hundred eyed brook trout eggs were placed in one compartment and 150 in the other. From the 100-egg lots we determined egg mortality and percentage hatch, sac fry mortality, and fry growth and mortality. After yolk absorption, 25 fry were randomly selected for growth determinations for the remainder of the exposure. These were fed brine shrimp for several days and then fed ad libitum a modification (Mehrle et al. 1977) of the Oregon Test Diet (National Academy of Science 1973).

The 150-egg lots were used for biochemical and residue determinations. Eight pooled samples of six 38-day-old fry each were collected after the yolk was absorbed and before they were fed, for determinations of whole body vitamin C (Hubmann et al. 1969), hydroxyproline (Woessner 1961), and protein (Lowry et al. 1951). Backbones were dissected from six to eight 118-day-old fry from each concentration except the highest, in which mortality was 100%. The vertebrae were dried at 110°C for 2 h in a forced air oven, split into two fractions, and weighted. Collagen was isolated from one fraction by the method of Flanagen and Nichols (1962). The isolated collagen was weighed and subjected to hydrolysis at 115°C in 5 mL of 6 N HCl for 16 h. Hydroxyproline was determined in a 2-mL sample. The other bone fraction was subjected to hydrolysis at 115°C in 3 mL of 6 N HCl for 16 h, and calcium in the hydrolysate was determined by atomic absorption spectrophotometry and phosphorus by the Fiske and Subbarow (1925) method. Pooled samples of fry were collected and analyzed for Aroclor 1254 concentrations (Stalling and Huckins 1971) at 7, 34, 48, and 118 d after hatching. Minimum detectable limits for Aroclor 1254 were 0.1 μg/g in fish tissue and 0.1 μg/L in water, respectively.

We analyzed growth and biochemical data by analysis of variance to determine whether significant differences existed, and used a multiple means comparison test (least significant difference) to compare treatment means. Mortality data were analyzed by binomial chi-square analysis (Snedecor 1965).

TABLE 1. Median hatch time, egg hatchability, and mortality of brook trout sac fry and fry continuously exposed to Aroclor 1254 at 12°C.

Aroclor 1254 concn (μg/L)	Median hatch time (days)	Egg hatch (%)	Sac-fry mortality (%)	Fry mortality (%) after 48 d	59 d	71 d	118 d
0	9	92	0	0	0	0	4
0.43	9	94	0	0	0	2	2
0.69	9	91	4	0	0	2	5
1.5	9	97	0	0	0	0	5
3.1	9	94	4	0	0	10	21[a]
6.2	8	90	4	0	8	50[a]	50[a]
13	8	88	0	68[a]	96[a]	96[a]	100[a]

[a]Significantly different from the control ($P < 0.05$).

Results

Median hatching time, egg hatchability, and sac-fry mortality were not affected by exposure to Aroclor 1254 (Table 1). In addition, the relative rate of yolk absorption was not noticeably different between the control and treated fish. Mortality of the fry at 48 d was significantly greater than that in the control ($P < 0.05$) in only the highest concentration (13 μg/L). Mortality of fry continued to increase throughout the study and at 118 d was significantly higher for fish held at the three highest concentrations than for the controls (Table 1). Growth of the brook trout fry as indicated by weight was decreased significantly ($P < 0.05$) after 48 d of exposure to Aroclor 1254 concentrations of 1.5, 3.1, 6.2, and 13 μg/L (Tables 2 and 3). However, no significant differences in surviving fry weights were noted at the end of the 118-d exposure.

Vitamin C and hydroxyproline concentrations were significantly decreased in sac fry by Aroclor 1254 concentrations of 1.5, 3.1, 6.2, and 13 μg/L (Table 2). After 118 d of exposure, fry backbone development was impaired by Aroclor 1254 exposure (Table 3). Vertebral collagen concentrations were decreased significantly in fish exposed to 0.69, 3.1, and 6.2 μg/L; calcium concentrations were increased significantly in fish exposed to 0.69, 1.5, 3.1, and 6.2 μg/L; and phosphorus concentrations and hydroxyproline concentrations in the isolated collagen were decreased significantly by all Aroclor 1254 concentrations. The reason for collagen concentration not being affected by 1.5

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TABLE 2. Effects of Aroclor 1254 on hydroxyproline and vitamin C concentrations in brook trout sac fry continuously exposed for 38 d and on weight of fry exposed for 48 d. Values represent the mean of 19–50 observations for weight and 8 pooled samples of 6 sac fry each for hydroxyproline and vitamin C determinations.

Aroclor 1254 concn (μg/L)	Fish wt (g)	Hydroxyproline (mg/g)	Vitamin C (μg/g)
0	0.065	1.71	139
0.43	0.062	1.59	144
0.69	0.068	1.50	150
1.5	0.050[a]	1.40[a]	105[a]
3.1	0.049[a]	1.16[a]	101[a]
6.2	0.049[a]	1.29[a]	100[a]
13	0.042[a]	1.29[a]	100[a]

[a]Significantly different from the controls ($P < 0.05$).

TABLE 3. Weight and backbone composition of brook trout fry continuously exposed to Aroclor 1254 for 118 d after hatching. Backbone components expressed as milligrams per gram of dried backbone, except hydroxyproline, which is expressed as milligrams per gram dried collagen. Values represent the mean of 6–20 observations for growth and 4–8 observations for bone analysis.

Aroclor 1254 concn (μg/L)	Fish wt (g)	Backbone composition (mg/g)			
		Collagen	Hydroxy-proline	Calcium	Phos-phorus
0	0.68	454	29	80	3.9
0.43	0.64	437	23[a]	77	2.4[a]
0.69	0.65	351[a]	21[a]	162[a]	2.5[a]
1.5	0.64	435	23[a]	159[a]	1.9[a]
3.1	0.52	386[a]	23[a]	215[a]	1.8[a]
6.2	0.74	397[a]	25[a]	297[a]	2.2[a]

[a]Significantly different from the controls ($P < 0.05$).

μg/L is not completely understood, but the decrease in hydroxyproline in this group indicates that collagen metabolism was altered.

One week after hatching, fry had accumulated Aroclor 1254 residues from 3700 times (1.6 μg/g) that in the lowest water concentration to 1100 times (14.0 μg/g) that in the highest water concentration (Table 4). At the time when significant fry mortality occurred among fry exposed to the highest concentration, or 48 d after hatching, whole body residues were 419 μg/g or 32 000 times the water concentration. At that time, fry with residues ≤182 μg/g were surviving. After 118 d of exposure, Aroclor 1254 had accumulated in the surviving fry from 40 000 to 48 000 times the water concentration and 50% mortality had occurred in fry having 284 μg/g whole body residues.

Discussion

Chronic toxicity studies with eggs and alevins of coho salmon (*Oncorhynchus kisutch*) have shown that water concentrations of 56 μg/L of Aroclor 1254 significantly reduced percentage hatch, and exposure to 15 μg/L for 6 wk reduced length and weight of coho salmon (Halter and Johnson 1974). Nebeker et al. (1974) reported that the no-effect concentration for mortality in fathead minnow (*Pimephales promelas*) fry and reproduction in adults was between 1.8 and 4.6 μg/L, which is similar to the no-effect concentration for mortality and growth of brook trout fry in the present study. Our results agree with those from other studies of chronic effects in that the earliest life stages of fish are the most susceptible to Aroclor 1254. Snarski and Puglisi (1976) reported that Aroclor 1254 at concentrations ≤0.94 μg/L did not affect growth, reproduction, or survival in adult brook trout exposed for 71 wk and did not influence growth or survival in offspring exposed for 90 d after hatch. Results from these studies concur with our findings that Aroclor 1254 concentrations ≤1 μg/L have no effect on growth of brook trout; however, our results show that bone development was affected.

The effects of Aroclor 1254 on growth, bone composition, and vitamin C metabolism in brook trout are similar to effects induced by the organochlorine insecticide toxaphene in brook trout (Mayer et al. 1975) and fathead minnows and channel catfish (*Ictalurus punctatus*) (Mayer et al. 1977a; Mehrle and Mayer 1975). Chronic exposure of these species to toxaphene resulted in reduced growth, decreased collagen concentrations, and increased calcium concentrations in the backbone. Mayer et al. (1978) reported that the increased use of vitamin C by the fish for liver detoxica-

TABLE 4. Whole body residues (micrograms per gram) of Aroclor 1254 in brook trout fry during continuous exposure to Aroclor 1254. Values in parentheses are accumulation factors. ND = not determined.

Aroclor 1254 concn (μg/L)	Duration of fry exposure after hatch at			
	7 d	34 d	48 d	118 d
0	0.1	0.5	0.3	0.2
0.43	1.6 (3720)[a]	6.2 (14418)	8.9 (20697)	17 (39534)
0.69	1.8 (2608)	15 (21735)	ND	31 (44919)
1.5	3.2 (2133)	ND	ND	71 (47333)
3.1	7.5 (2419)	ND	ND	125 (40322)
6.2	13 (2096)	122 (19677)	182 (29354)	284 (45806)
13	14 (1076)	119 (9153)	419 (32230)	—[b]

[a]Accumulation factor = (fry residue ÷ water concentration) × 1000.
[b]100% mortality.

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tion of toxaphene appears to cause a functional deficiency of vitamin C in the backbone and reduced collagen synthesis in fingerling channel catfish. Since fish are unable to synthetize vitamin C (Chatterjee 1973; Wilson and Poe 1973), only finite quantities are available from yolk during the sac-fry stage and from the diet after yolk absorption, for detoxication, collagen synthesis, and other metabolic processes.

In the present study, the decrease in growth and in hydroxyproline and vitamin C concentrations in sac fry exposed to Aroclor 1254, and the later increased mortality in fry 10 d after yolk absorption, suggest that the PCB exposure induced competition between developmental and detoxication processes for the use of vitamin C similar to that reported for other chemicals by Mayer et al. (1977b). After 118 d of exposure, fish weights were not significantly different, but bone development was impaired by Aroclor 1254. The continued mortality probably negated the earlier differences in growth by eliminating the more susceptible fish, as was reported by Mayer et al. (1977a) in channel catfish exposed to toxaphene. Although the more obvious effects of Aroclor 1254, such as increased mortality and decreased growth, were less apparent in the older fish, the effects of Aroclor 1254 on bone development may cause the backbones to be more susceptible to fracture during periods of stress. This condition may adversely influence the ability of trout to adapt to various environmental challenges (Mayer et al. 1977b).

An application factor of 0.01 has been recommended in establishing water quality criteria for organochlorine insecticides (Committee on Water Quality Criteria 1973). This factor is derived by dividing the maximum acceptable toxicant concentration (MATC; the highest continuous toxicant concentration that has no adverse effect on growth, reproduction, and mortality) by the 96-h LC50 value (Mount and Stephen 1967). The 96-h LC50 of Aroclor 1254 for brook trout fry was >100 μg/L. On the basis of an application factor of 0.01, the MATC should be in the range of ≥1 μg/L. The no-effect concentration of Aroclor 1254 for brook trout fry growth and mortality was 0.7 and 1.5 μg/L, respectively. However, phosphorus and hydroxyproline content in backbone collagen was decreased significantly in fish exposed to all concentrations, indicating that the application factor should be <0.01 for Aroclor 1254.

Residues of PCBs are ubiquitous in the aquatic environment, but their biological significance in fish is not known. Aroclor 1254 residues reported in various species of fish from across the United States ranged from 1.4 to 42 μg/g (Walker 1976). Total PCB residues ranged from 3.5 to 47 μg/g in lake trout (*Salvelinus namaycush*) from Lake Michigan (Willford et al. 1976) and from 0.10 to 5.1 μg/g in various fish species in Canada (Graham 1976). In the present study, whole body residues of Aroclor 1254 in fry with impaired bone development ranged from 17 μg/g in the lowest exposure to 284 μg/g in fish exposed to 6.2 μg/L. The Aroclor 1254 residues in brook trout fry from the two lowest exposure concentrations (0.43 and 0.69 μg/L) were within the range of residues found in fish in the natural environment. This suggests that the effects on bone development are realistic indications of the adverse influence of Aroclor 1254 in brook trout, and perhaps in other species of fish, in the aquatic environment. However, studies of longer duration than the present one are needed if the significance of Aroclor 1254-induced alterations in growth and bone development and the residue dynamics of PCBs are to be fully understood.

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