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Effect of a Polychlorinated Biphenyl Metabolite on Early Life Stage Survival of Two Species of Trout

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PCBs (polychlorinated biphenyls) are a common class of environmental contaminants known to reduce hatching success and larval survival in fish. Exposure via contaminated water (DeFoe et al. 1978; Hansen et al. 1975; Nebeker et al. 1974), sediment (Benson et al. 1994), and maternal transfer of PCBs to eggs (Nimmi 1983) have resulted in toxicity to early life stages of fish. Adverse effects associated with bioaccumulation of PCBs have included reduced viable hatch in Baltic flounder (Von Westernhagen et al. 1981), egg mortality in charr (Monod 1985), reduced larval size in winter flounder (Black et al. 1988), and reduced egg hatchability in lake trout (Mac and Swartz 1992). Recent studies have linked exposure to environmental contaminants such as PCBs with depressed estradiol levels, which could inhibit spawning (Johnson et al. 1989). Even after hormone treatments, fish from PCB contaminated areas were less likely to spawn, took longer to spawn, and produced a higher proportion of abnormal larvae than fish from less contaminated areas (Casillas et al. 1991).

PCBs can be broken down through photolysis, microbial degradation, or metabolism (Safe 1994). Oxidative metabolism is a major pathway for detoxification and elimination of contaminants within organisms. PCB congeners with few chlorines can be readily metabolized through the addition of a hydroxyl group (Bergman et al. 1994; Safe 1994), which may increase their solubility and biological activity. Therefore, metabolites may not be less toxic than parent compounds.

The objective of this study was to determine whether eggs that are exposed to and accumulate a specific hydroxylated PCB (2',4',6'-trichloro-4-biphenylol) show reduced hatch success and survival of larvae through the yolk resorption stage in rainbow trout (*Oncorhynchus mykiss*) or westslope cutthroat trout *(O. clarki lewisi).* The toxicity of PCB metabolites to fish has not yet been reported in the literature. The metabolite investigated in this study was reported by Korach et al. (1988) to have affinity for estrogen receptors greater than that of other PCBs tested, and exposure to mice for a short period altered uterine development (Korach et al. 1988).

MATERIALS AND METHODS

Rainbow trout gametes were obtained from the Spring Valley Trout Farm in Langley, British Columbia, Canada. Westslope cutthroat eggs were obtained

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from the Washoe Park Fish Hatchery in Anaconda, Montana. For each species, a composite of eggs from 10 or more females was fertilized with a composite of milt from 3 or more males. Rainbow trout eggs were fertilized after they arrived in Seattle, while cutthroat eggs were fertilized before shipping from the hatchery.

To determine whether there is a relationship between concentration of the PCB and any observed responses, three exposure concentrations were evaluated. The PCB metabolite was obtained from ULTRA Scientific (Kingstown, RI), dissolved in dimethyl sulfoxide (DMSO) and mixed with dechlorinated city water to obtain target concentrations and a final DMSO concentration of 0.65%. Exposure concentrations included 5, 45, and 95 mg/L. Controls included DMSO in water and water only. Three replicates were created of each exposure to evaluate variability in laboratory conditions.

Each replicate exposure contained approximately 60 apparently fertile eggs. Fertility was verified at the end of the experiment, when all dead and infertile eggs had been removed and preserved. Eggs were examined using 40 power magnification to verify fertilization and cell division.

All groups were held in flowing dechlorinated city water at 11°C for the duration of the test. Temperature, pH, and dissolved oxygen were monitored several times each week.

Eggs were immersed in PCB solutions for 24 hours beginning 36 hours post-fertilization. One subset of eggs from each exposure was chemically analyzed to determine actual concentrations accumulated at the end of the immersion period. This subset was selected and treated in same way as other replicates. Chemical analysis was conducted by Analytical Resources, Inc. (Seattle, WA). PCB analyses were conducted using dual capillary column gas chromatography with electron capture detection. A portion of the PCB metabolite was provided to the chemical analysis laboratory for use as an analytical standard.

Data collected from the treatments included hatching success (relative to the number of fertile eggs) and survival of viable larvae to yolk resorption as determined by the following formula:

$$\% \text{ Viable Larvae} = \frac{(\#\text{live larvae})-(\#\text{deformed larvae})}{\#\text{ fertile eggs}} \times 100$$

This value provides an indication of the number or proportion of larvae which may be expected to survive after hatching. Larvae with obvious spinal or other severe deformities were considered non-viable. Dead eggs and larvae were counted, removed, and preserved in stockards solution or 10% formalin daily.

Hatching success and survival between treatments were compared using nonparametric analysis of variance. Percent values were arcsin square-root transformed before analysis. Significance values of 0.05 or less were considered to be statistically significant .

RESULTS AND DISCUSSION

The eggs and larvae of rainbow trout accumulated up to 570 µg/g of 2',4',6'-trichloro-4-biphenylol after the 24 hour exposure period (Table 1). Uptake in

Table 1. Accumulation of PCB metabolite in rainbow trout eggs µg/g wet weight) after 24 hour exposure.

Exposure Concentration (mg/L)	Tissue Concentration µg/g wet weight)
5	35
45	120
95	570

Table 2. Survival of rainbow trout after 24-hour exposure to PCB metabolite as newly fertilized eggs. *p=0.05

Treatment	N Fertile at Start of Test	N Surviving through Yolk Resorption	Mean Percent Viable	Standard Deviation Percent Viable
Water Control				
Rep 1	20	18		
Rep 2	4	0		
Rep 3	15	10		
Solvent Control				
Rep 1	11	6		
Rep 2	6	3		
Rep 3	20	14		
Combined Controls			55.2	30.5
Low			65.2	6.3
Rep 1	19	11		
Rep 2	35	24		
Rep 3	42	29		
Medium			26.0	22.7
Rep 1	5	0		
Rep 2	12	5		
Rep 3	11	4		
High			11.1*	19.3
Rep 1	6	0		
Rep 2	6	0		
Rep 3	15	5		

cutthroat trout was not verified due to insufficient numbers of eggs being available from the hatchery at the last moment. Recovery from fortified samples ranged between 57.5 and 84.6%. PCBs were not detected in method blanks.

Hatch success and percent viable larvae did not differ ($p>0.49$) between water and DMSO controls for both species. Therefore, the control treatments were combined for further comparisons.

Table 3. Survival of westslope cutthroat after 24-hour exposure to PCB metabolite as newly fertilized eggs. *p<0.00l

Treatment	N Fertile at Start of Test	N Surviving through Yolk Resorption	Mean Percent Viable	Standard Deviation Percent Viable
Water Control				
Rep 1	36	32		
Rep 2	32	27		
Rep 3	34	26		
Solvent Control				
Rep 1	32	29		
Rep 2	33	24		
Rep 3 .	34	22		
Combined Controls			80.0	10.1
Low			63.6	6.1
Rep 1	30	20		
Rep 2	37	25		
Rep 3	30	17		
Medium			28.9*	13.2
Rep 1	31	7		
Rep 2	35	7		
Rep 3	25	11		
High			30.7*	7.4
Rep 1	26	7		
Rep 2	27	7		
Rep 3	23	9		

The PCB metabolite was significantly toxic to fish eggs. The highest exposure concentration of the PCB metabolite significantly reduced the percentage of viable rainbow trout larvae when compared to controls (p=0.05; Table 2).

Although the intermediate concentration (45 mg/L) was not significantly different from the controls, it did result in a 50% mean reduction in the percentage of viable larvae. Both the high and intermediate concentrations of the PCB metabolite reduced the percentages of viable cutthroat trout larvae when compared to controls (p<0.0001; Table 3). There were no significant differences between mean hatching success in any of the treatments (p>0.24). Because hatching success was not affected at the concentrations tested, the effects of the PCB metabolite were manifested after hatch and before yolk resorption.

The PCB metabolite tested in this study may be present in the environment and biota as a result of hydroxylation of the parent compounds 2,4,4',6 tetrachlorobiphenyl (IUPAC #76) or 2,4,6 trichlorobiphenyl(#30). Congener #30 is present in Aroclor 1254 and 1260 and has been detected in the tissues of piscivorous birds (Borlakoglu et al. 1990). Congener #76 is among the most prevalent congeners in Wisconsin fish, contributing up to 9.7% of the total PCB concentration (Maack and Sonzogni 1988). It has been detected in Aroclor 1242,

1248, and 1254 (Swartz and Stalling 1991). Although the metabolites would be expected to be more soluble than parent compounds, hydroxylated PCB metabolites were found to be retained in the blood of seals from Sweden (Bergman et al. 1994).

The results of this study indicate that the PCB metabolite, 2',4',6'-trichloro-4-biphenylol, which is not routinely monitored in the environment, can be toxic to fish embryos and larvae, and could reduce larval survival.

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