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Chronic Toxicity of Pydraul 50E to Lake Trout

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Industrial phosphate esters, both triaryl and alkyl aryl phosphate esters, are used as fire resistant hydraulic fluids and as fire retardant plasticizers (Lapp 1976). Hydraulic fluids probably represent the largest contribution of phosphate ester compounds released into the environment. Lapp (1976) estimated that 65 to 70 percent of all phosphate ester hydraulic fluids were utilized in automotive and steel industries. He also estimated that 80 percent of the annual consumption of hydraulic fluids in 1976 was the result of leaks in industrial hydraulic systems. These data suggest phosphate esters are likely to be constituents of industrial effluents and, consequently, could be in point source discharges.

The toxicity and physiological effects of triaryl phosphate esters have been studied in man, mammals, and birds (Spector 1956, Sax 1968, Gleason et al. 1969, Bondy et al. 1973), but only a limited number of published studies exist concerning their effects on aquatic animals (Mayer et al. 1981, Nevins and Johnson 1978). However, Lombardo and Ergy (1979) reported that total phosphate esters in fish, collected below three industrial effluents, ranged from 0.1 to 0.9 μg/g, with concentrations of individual esters in the range of 0.06 to 0.5 μg/g. The purpose of our study was to determine the chronic toxicity of Pydraul 50E, an alkyl-aryl phosphate ester hydraulic fluid, to lake trout (*Salvelinus namaycush*). The lake trout is an important upper trophic level species inhabiting the Great Lakes where Pydraul fluids have been widely used in the past.

MATERIALS AND METHODS

Testing procedures followed as closely as possible those recommended by the U.S. Environmental Protection Agency (1972). Lake trout eyed-eggs were obtained from the Jackson National Fish Hatchery (Wyoming) and

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held in four, round fiberglass tanks (120 liters each). Each tank received 2 L spring water (pH 7.4, alkalinity 206 mg/L, hardness 162 mg/L) at 3-min intervals through a siphon apparatus equipped with a metering device described by McAllister et al. (1972). Water temperature was maintained at 9°C. Three of the tanks were designated at random to receive one of three concentrations of Pydraul 50E for 120 d. The fourth served as a control and received only the carrier solvent, acetone, at a rate equivalent to the exposure concentrations (0.5 mL/L).

Each of the four tanks contained eight replicates (14 x 22 x 20 cm glass chambers with stainless steel mesh floors) of 25 eggs (embryos) each. Water depth was 15 cm. The incoming flow was split so that each chamber received 250 mL/3 min. By random selection, the eight replicate chambers in each tank were designated as follows: three for survival and growth (75 embryos), four for bioconcentration (100 embryos), and one extra (25 embryos). During hatching and yolk absorption, the tanks received no exposure. Starting at swim-up (24 d after hatching), the exposures were initiated and continued for 120 d.

Mortalities were recorded and removed daily when they occurred. Standard lengths were determined for fry in the growth chambers by the photographic methods of McKim and Benoit (1971) after 15 and 30 days of exposure. Total length and weight were measured at 60, 90, and 120 days by anesthetizing the fish with tricaine methanesulfonate (Schoettger and Julin 1966).

Mortality data were analyzed by binomial chi-square analysis. Growth data were analyzed by analysis of variance and a multiple means comparison test (least significant difference) to compare treatment means (Snedecor and Cochran 1974). The decision level for statistical significance was $p \leq 0.05$.

Pydraul 50E was obtained from Monsanto Company, St. Louis, Missouri, and consisted of the following compounds: triphenyl phosphate (TPP), 36%; nonylphenyl diphenyl phosphate (NPDPP), 40%; cumylphenyl diphenyl phosphate (CPDPP), 22%; and other minor components, 2% (Mayer et al. 1981). The concentrations of the three major components of Pydraul 50E (TPP, NPDPP, and CPDPP) in the test water were determined by thermionic specific gas-chromatography (Gledhill et al. 1980). The Pydraul 50E concentrations were calculated by adding the means for the individual components measured at nine sampling periods (2-wk intervals). The calculated Pydraul 50E concentrations for the three exposures (means for the measured components in parentheses) were: 2.6 µg/L (TPP 0.81, NPDPP 1.2, CPDPP 0.54); 5.3 µg/L (TPP 2.0, NPDPP 2.2, CPDPP 1.1); and 16 µg/L (TPP 6.3, NPDPP 6.0, CPDPP 3.6)--total Pydraul 50E standard deviations were 0.56, 0.43, and 1.1 µg/L, respectively. Measured values

were corrected on the basis of percentage recovery of known concentrations and averaged 58 percent of the nominal exposure concentrations. Percent recovery of the method was determined by spiking water samples (N≥3) with Pydraul 50E at three concentrations (1.0, 5.0, and 10 μg/L. The average percent recoveries ± one standard deviation were: TPP, 96.7 ±7.0%; NPDPP, 72.6 ± 10.2%; and CPDPP, 89.0 ±10.0%.

Fish from the four bioconcentration chambers within each tank were analyzed for Pydraul 50E components on days 15, 30, 60, 90, and 120. The number of fish collected was 25, 25, 15, 10, and 20, respectively. Fish analyses were based on whole-body determinations of the TPP, NPDPP, and CPDPP components. The entire lot of fish from each sample date was pooled, and one determination was made for each sample day. Fish samples were ground and extracted with diethylether/petroleum ether, and lipids were removed by gel permeation chromotagrophy. Pydraul 50E tissue residues were analyzed in the same manner as water samples. Fish tissue was spiked at four levels ranging from 0.05 μg/g to 0.5 μg/g and one sample was analyzed at each level. The average percent recoveries were: TPP, 80%; NPDPP, 81%; and CPDPP, 94%. Tissue concentrations were not corrected for recovery.

RESULTS AND DISCUSSION

Survival was significantly reduced for trout exposed to 16 μg/L Pydraul 50E as compared with controls at 90 days, and there were no survivors at 120 days (Table 1). No significant difference in fish survival existed between controls and the other two Pydraul 50E concentrations. Lake trout growth was affected earlier and at lower concentrations than survival. Lake trout growth at 60, 90, and 120 days was significantly less in the 5.3 and 16 μg/L Pydraul 50E exposures than it was in the control. Survival and growth were not affected at a concentration of 2.6 μg/L.

Pathological changes (cataracts) first appeared after 90 days exposure to 5.3 μg/L Pydraul 50E. After 120 days, fish in the 5.3 μg/L concentration were observed to have a high incidence (66%) of light skin coloration and cataracts in one or both eyes (92%). No gross pathological changes were present in any of the fish from the control or 2.6 μg/L exposures during the 120-day study. Adverse effects on lake trout (survival, growth, and cataracts) occurred at the same concentration range of Pydraul 50E that affected rainbow trout in a previous study (Mayer et al. 1981).

As the exposure concentrations of Pydraul 50E in water increased, both the uptake in lake trout and the bioconcentration factor increased (Table 2). After 15 days of exposure, fry in the 2.6 μg/L concentration contained 1.2 μg/g Pydraul 50E (460 times the water concentration), and fry in the 16 μg/L concentration contained 42 μg/g Pydraul 50E (2,600 times the water

Table 1. Survival and growth (mean ± SD) of lake trout during 120-d continuous exposures to Pydraul 50E in water. Values represent the mean of 3 observations for survival and 8 to 75 observations for growth.

Exposure time and parameter measured	Pydraul 50E in water (µg/L) Control	2.6	5.3	16
15 days				
Survival (%)	100	100	100	100
Length (mm)	25 ± 1.5	26 ± 1.3	25 ± 1.3	26 ± 1.4
n[a]	74	74	75	74
30 days				
Survival (%)	99 ± 2.3	100	100	100
Length (mm)	29 ± 1.8	30 ± 2.3	29 ± 1.7	28 ± 1.5
n	75	75	75	75
60 days				
Survival (%)	99 ± 2.3	100	100	95 ± 4.6
Length (mm)	45 ± 2.9	46 ± 2.9	40 ± 2.4*	30 ± 1.1*
Weight (g)	0.66 ± 0.13	0.71 ± 0.14	0.46 ± 0.13*	0.22 ± 0.04*
n	73	75	75	71
90 days				
Survival (%)	99 ± 2.3	100	97 ± 2.5	14 ± 6.9*
Length (mm)	55 ± 3.2	56 ± 3.1	48 ± 6.8*	31 ± 1.3*
Weight (g)	1.3 ± 0.3	1.3 ± 0.2	0.88 ± 0.36*	0.21 ± 0.03*
n	61	40	65	8
120 days				
Survival (%)	99 ± 2.3	100	91 ± 8.3	0*
Length (mm)	66 ± 4.8	67 ± 4.1	60 ± 8.4*	-
Weight (g)	2.1 ± 0.5	2.2 ± 0.5	1.7 ± 0.7	-
n	61	37	61	0

[a]Number of fish measured for length or weighed
*Statistically significant from controls ($p \leq 0.05$)

concentration). The accumulation of Pydraul 50E in lake trout exposed to 2.6 µg/L Pydraul 50E appeared to reach a maximum at 30 days (2.3 µg/g) and then declined until 120 days, when the whole-body residue was equal to the 15-day value (1.2 µg/g). In the 5.3 µg/L exposure, residues of Pydraul 50E in lake trout appeared to reach equilibrium after 30 days (15 to 20 µg/g). However, fish exposed to 16 µg/L continued to accumulate additional Pydraul 50E through 90 days of exposure when, whole-body residues were 88 µg/g. No survivors were available from the 16 µg/L concentration for residue analysis at 120 days.

Table 2. Whole-body residues of Pydraul 50E components in lake trout during continuous exposures to Pydraul 50E in water. Bioconcentration factor (BCF) = total Pydraul 50E in fish/water concentration. Pydraul 50E or its components were not detected (<0.1 μg/g) in any of the control fish.

Exposure:time and Pydraul 50E (μg/L)	Pydraul 50E components in fish (μg/g)				BCF
	TPP	NPDPP	CPDPP	Total	
15 days					
2.6	0.23	0.58	0.35	1.2	462
5.3	2.0	4.4	7.7	14	2,642
16	10	7.0	25	42	2,625
30 days					
2.6	0.73	0.84	0.76	2.3	885
5.3	1.4	6.9	13	21	3,962
16	4.7	12	46	63	3,938
60 days					
2.6	0.11	0.76	0.51	1.4	538
5.3	1.3	6.9	10	18	3,396
16	4.0	17	50	71	4,438
90 days					
2.6[a]					
5.3	1.6	4.7	8.5	15	2,830
16	6.8	26	55	88	5,500
120 days					
2.6	0.16	0.55	0.48	1.2	462
5.3	2.5	5.3	12	20	3,774
16[b]					

[a] Not measured, [b] 100% mortality

The CPDPP component was more frequently accumulated readily in fish than the TPP or NPDPP component. While Pydraul 50E and the exposure water had roughly the same proportions of TPP, NPDPP, and CPDPP components (36%, 40%, and 22%, respectively), the concentration of CPDPP was usually over 50% of the total Pydraul 50E concentration in fish exposed to 5.3 and 16 μg/L. Based on octanol-water partition coefficients (TPP = 4.2×10^4, NPDPP = 8.6×10^5, CPDPP = 1.2×10^6) fish bioconcentration factors of 420, 2,100, and 2,500 would be expected for TPP, NPDPP, and CPDPP, respectively (Mayer et al. 1981). Laboratory experiments with lake trout support the octanol-water bioconcentration estimates for TPP and NPDPP; however, the observed bioconcentration factor for CPDPP was as much as six times greater than that predicted for fish exposed to chronically lethal concentrations.

The advent of phosphate esters as replacements for PCB's has been a good first step in reducing the hazard of PCB's to the environment. The chronic toxicity for salmonids is very close to that of PCB's, but the phosphate esters are more environmentally acceptable because they degrade much more rapidly (Mayer et al. 1981). Also, the toxicity differences are of less concern with warmwater species because the margin of safety for those species may be as much as three orders of magnitude more than that for coldwater species (Mayer et al. 1981). Regardless of the safety of the chemical, improved management of hydraulic fluid use is recommended to reduce discharges to the environment and to protect our natural resources.

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