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Uptake of Sodium Pentachlorophenate (NaPCP) from Water by Rainbow Trout (*Salmo gairdneri*) Exposed to Concentrations in the ng/L Range

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Pentachlorophenol (PCP) is one of the few man-made chemicals that is present in the environment for which a substantial body of information is available on its toxicology. Studies on the sublethal effect of PCP suggests that it acts primarily by uncoupling oxidative phosphorylation (WEINBACH 1957). This action would require an animal to expend a greater effort to maintain its production of adenosine triphosphate (ATP) to meet its energy requirements. This response of increased metabolic activity to PCP exposure have been observed in aquatic organisms (HOLMBERG et al. 1972; CANTELMO et al. 1978).

Approximately 90 million kg of PCP is produced annually worldwide for use primarily as a biocide (DETRICK 1977). The forest products industry is its largest consumer which uses PCP as a wood preservative principally for utility poles, fence posts, and lumber. Approximately 80% of the PCP produced in the U.S. is used in this capacity (CIRELLI 1978). PCP has a solubility of 14 mg/L which is high relative to most other organic contaminants, this is increased to 4000 mg/L at pH 8 when converted to its salt sodium pentachlorophenate (VERSCHUEREN 1977; BEVENUE & BECKMAN 1967). Based on the acid dissociation constant (pKa) at pH 8, more than 99.9% of the soluble PCP should occur as NaPCP. In view of its extensive use and high solubility, PCP have reached levels of concern in some aquatic ecosystems. Concentrations of 0.005-23 ug/L have been monitored in 78 of 85 bulk water samples collected from stream mouths and nearshore areas along the four Canadian Laurentian Great Lakes (FOX 1980). PCP concentrations of 0.1-40 ug/L have been also reported in a Lake Michigan watershed (DELFINO 1979).

The 96 h LC_{50} of PCP for fish generally range from 40-600 ug/L. There is some evidence to suggest that cool water species may be more susceptible to PCP than warm water species. LC_{50} values reported for salmonids generally range from 40-130 ug/L NaPCP (WEBB & BRETT 1973; DAVIS & HOOS 1975; IWAMA & GREER 1977), while values reported for cyprinids and percids range from 200-600 ug/L (CARDWELL et al. 1976; ADELMAN et al. 1976; PRUITT et al. 1977). Deleterious effects have also been observed when fish were exposed to sublethal concentrations, growth rate and food conversion efficiency of young salmon were reduced at 2 ug/L NaPCP (WEBB & BRETT 1973).

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Models which describe the kinetics of the more persistent organic contaminants in fish generally suggest uptake through food is greater than that through water particularly for those species that occupy the higher trophic levels (NIIMI & CHO 1981). It is suggested that uptake through water may be an important pathway for substances with the solubility properties of PCP. Studies on the toxicological effects of PCP have generally been conducted by exposing fish to ug/L concentrations and observed for periods of less than two weeks. This protocol does not allow an assessment of PCP exposure to fish at concentrations in the ng/L range over an extended period which would be the most probable situation in the natural environment. This study examined this aspect of its toxicology.

MATERIALS AND METHODS

Hatchery reared rainbow trout (Salmo gairdneri) were maintained for approximately six months in the laboratory at 14-18 C and fed once daily to satiation on a commercial dry diet. Two weeks before the study commenced, 33 fish averaging 400 g each were transferred to each of three 350 L fiberglass tanks and held at 15±1 C. Each tank was provided with a 5 L/min flow of dechlorinated domestic water which was passed through an activated charcoal filter. The chemical properties of the water have been described (HODSON et al. 1980). Oxygen content of the water was 80-90% of saturation. An external pump was used to circulate the water in each tank to create a current of 8-12 cm/sec. This action reduced aggressive behavior among fish and facilitated removal of waste products from the self-cleaning tanks. The tanks were illumunated by fluorescent lights with a surface intensity of 600 Lux which was regulated on a 16 h L: 8 h D photoperiod. Fish were fed once daily to satiation on the same dry diet (Martin Feed Mills, Elmira, Ont.).

Waterborne concentrations of <10 (control), 35, and 660 ng/L NaPCP were achieved by preparing stock solutions of 0, 1, and 17 mg/L reagent grade PCP dissolved in a 0.04 M solution of sodium hydroxide. Each stock solution was contiouously infused into the inflowing water using a multichannel peristaltic pump.

To estimate NaPCP uptake, samples of two fish each were taken from the treated tanks and one from the control tank before exposure. Five fish were then sampled from each tank after 20, 40, 65, 90, and 115 days exposure. All fish were wrapped in aluminum foil and frozen until analysis. Water samples were taken at approximately 10 day intervals and immediately analyzed for NaPCP.

Analysis for PCP was done according to the following procedure. The intestinal content of each fish was discarded then the liver and gall bladder were removed. The remaining fish, herein described as tissue, was ground to a uniform consistency using a Hobart grinder and an Oster blender. The liver and gall bladder, henceforth referred to as organs, were macerated to the same consistency using a spatula. Analyses on both portions of the same fish were

done according to BOGGS (1980). Using a 3-4 g sample, recovery rate was 80%, and detection limit was 0.8 ug/kg. Analysis for PCP in the dry diet was also done using the same method. Measurements of PCP concentrations in water were done following Environment Canada (1979), detection limit was 0.01 ug/L PCP using a 1 L sample. Analyses of the fish and feed required the methylation of PCP with diazomethane, while water samples required acetylation, for gas chomatographic analyses.

PCP levels were determined using a GC with an electron capture detector equipped with a 2 mm ID x 183 cm long glass column packed with 5% OV-101 on 100/120 mesh Chromosorb W(HP), and a 5% methane: 95% argon mixture carrier gas flow of 40 ml/min. The injector, column, and detector temperatures were 190, 200, and 300 C respectively.

The mathematical model $Y=AX^b$, commonly expressed as Log Y= LogA + bLogX, was used to establish the relationship between PCP exposure and its accumulation. The relationship between days fed and growth was expressed as $Y=A_e{}^{bX}$.

RESULTS

Fish exposed to waterborne NaPCP averaging 35 ± 6 (mean$\pm$SD) and 660 ± 220 ng/L accumulated levels of PCP that were related to the concentration and duration of exposure (TABLE 1). NaPCP concentration in water provided to the control group was below the detection limit of 10 ng/L. Fish weight also increased approximately two-fold over the 115 day period. To distinguish between the relative changes in PCP levels where concentrations are influenced by "growth dilution," and absolute increments which are independent of growth, values were expressed as ug/kg and as ug PCP per fish.

PCP levels in tissue, organs, and the percent of total PCP in the organs remained relatively uniform among the control fish over the 115 day period. Fish exposed to 35 ng/L contained slightly higher PCP levels with a higher percentage found in the organs. The highests levels of PCP occurred in fish exposed to 660 ng/L where concentrations in the organs averaged 2200 ug/kg after 115 days.

PCP levels increased significantly with time of exposure ($P<0.01$) at the two concentrations (TABLE 2). An examination of the information presented would suggest the relationship reporting absolute levels would be easier to interpret than those reporting relative concentrations. Regression analysis of the information reported in TABLE 1 suggests fish exposed to PCP levels of 35 ng/L accumulated an average of 9 ug PCP, while those exposed to 660 ng/L accumulated 115 ug PCP after 115 days. A significant relationship ($P<0.01$) was also suggested for the control group although PCP accumulation was less than 1 ug after 115 days.

TABLE 1. PCP levels in tissue and organs of rainbow trout, expressed as ug/kg and ug PCP per fish, exposed to waterborne concentrations of <10 (control), 35, and 660 ng/L NaPCP for up to 115 days. The values shown represent the mean, standard deviation, and range of five fish.

Days	Body wt. g	Organs wt. g	PCP in organs		PCP in tissue		Total PCP in fish		Percent PCP in organs
			ug/kg	ug	ug/kg	ug	ug/kg	ug	
					Control				
0	402±46	4.4±1.2	2.3±1.7	0.009±0.005	1.1±0.5	0.4±0.2	1.1±0.4	0.5±0.2	2.1±1.1
	358-465	3.1-5.5	0.7-5.1	0.004-0.016	0.8-1.8	0.3-0.6	0.8-1.7	0.3-0.7	0.8-3.6
20	372±66	5.5±1.2	1.2±0.3	0.006±0.002	1.5±0.4	0.6±0.2	1.5±0.4	0.6±0.2	1.2±0.4
	297-426	4.0-6.9	0.8-1.6	0.005-0.009	1.3-2.2	0.4-0.9	1.3-2.2	0.4-0.9	0.5-1.6
40	545±123	7.2±1.8	1.5±0.7	0.010±0.003	1.8±0.7	0.9±0.2	1.8±0.6	0.9±0.2	1.0±0.3
	418-677	5.6-10.1	0.9-2.6	0.007-0.015	1.3-3.0	0.8-1.2	1.3-2.9	0.8-1.2	0.7-1.3
65	603±181	7.8±3.1	3.9±2.7	0.023±0.002	3.7±2.3	2.3±1.9	3.6±2.3	2.3±1.9	1.5±0.6
	327-760	2.8-11.1	2.2-8.7	0.021-0.025	1.9-7.7	1.2-5.7	1.8-7.5	1.2-5.7	0.4-2.0
90	625±71	8.9±1.0	1.2±0.3	0.011±0.002	0.8±0.4	0.5±0.3	0.8±0.4	0.5±0.4	2.8±1.4
	533-712	7.6-10.1	0.8-1.5	0.007-0.013	0.4-1.5	0.2-1.0	0.4-1.5	0.2-1.0	1.0-4.6
115	771±134	11.8±4.4	3.1±5.0	0.023±0.020	1.9±0.6	1.5±0.7	1.9±0.5	1.5±0.7	1.9±2.4
	621-985	5.6-15.9	0.6-12.0	0.009-0.068	1.2-2.7	0.7-2.6	1.3-2.6	0.8-2.6	0.5-6.2

TABLE 1. Continued.

				35 ng/L NaPCP					
20	429±82 342-521	7.2±2.4 3.7-9.3	28±21 12-64	0.2±0.1 0.1-0.4	7±2 4-10	3±1 2-4	7±2 6-10	3±1 2-4	7.0±6.8 2.8-18.9
40	504±97 367-613	7.2±1.6 4.5-8.8	145±147 40-402	1.0±1.1 0.3-3.0	25±5 19-32	12±3 10-16	27±6 20-31	13±3 10-17	7.5±7.4 2.3-20.6
65	650±110 521-785	9.4±2.9 7.2-14.3	135±79 67-259	1.3±1.0 0.6-2.4	20±6 15-29	13±5 9-22	21±6 16-31	14±6 10-24	9.0±4.9 5.4-17.2
90	794±168 633-1061	10.7±5.6 5.6-20.2	54±48 14-132	0.5±0.4 0.1-1.2	13±3 8-16	10±2 8-12	13±3 8-17	10±2 9-12	4.7±3.5 1.5-10.3
115	1035±188 754-1170	16.8±4.4 11.1-21.8	63±29 26-101	1.0±0.5 0.6-1.8	6±2 4-9	6±1 4-7	7±2 5-9	7±1 5-8	14.8±6.6 8.0-22.4
				660 ng/L NaPCP					
20	462±66 377-539	8.7±3.2 6.0-12.9	674±621 331-1770	5±4 2-12	77±28 33-100	34±14 17-49	86±32 39-119	39±15 21-53	14.0±9.0 4.6-23.9
40	585±113 467-770	9.4±2.0 7.1-11.5	372±159 174-556	3±1 2-4	50±23 25-75	29±13 13-41	60±29 32-97	32±13 17-43	12.3±7.4 4.6-22.9
65	630±59 564-712	6.6±0.8 5.5-7.8	1984±843 1146-3240	13±4 9-18	135±41 82-186	84±26 48-113	153±42 101-209	96±27 60-128	14.0±6.0 7.6-20.6
90	619±166 450-872	8.7±1.7 6.6-10.7	891±403 253-1320	8±4 2-11	163±36 111-204	97±19 65-115	173±38 122-220	104±22 67-126	7.2±2.9 3.1-9.6
115	798±123 597-923	12.9±4.0 7.0-17.0	2204±549 1730-2860	27±5 20-31	128±43 72-178	99±32 60-148	160±44 100-209	126±34 84-177	22.2±5.5 16.6-28.7

TABLE 2. Relationships among PCP levels in tissue and organs of rainbow trout exposed to waterborne concentrations of <10 (control), 35, and 660 ng/L NaPCP for up to 115 days. A relationship between days fed and fish weight is also shown. Each equation was calculated from a sample of 30 fish and statistical significance of the t-value is noted by a single ($P<0.05$) or double ($P<0.01$) asterisks.

Exposure ng/L PCP	Equation	r^2	t	Equation	r^2	t
	Days fed (X) vs body weight in g (Y)			Days exposed (X) vs percent of total PCP in organs (Y)		
Control	$Y = 378.8_e^{0.006X}$	0.58	6.17**	$LogY = Log0.197 - 0.036LogX$	0.02	0.71
35	$Y = 372.4_e^{0.008X}$	0.80	10.50**	$LogY = Log0.463 + 0.205LogX$	0.34	3.79**
660	$Y = 421.2_e^{0.005X}$	0.61	6.57**	$LogY = Log0.574 + 0.282LogX$	0.56	6.01**
	Days exposed (X) vs PCP in organs			(Y_1 in ug/kg, Y_2 in ug)		
Control	$LogY_1 = Log0.233 - 0.021LogX$	0.01	0.39	$LogY_2 = Log-2.187 + 0.154LogX$	0.29	3.42**
35	$LogY_1 = Log0.834 + 0.529LogX$	0.71	8.20**	$LogY_2 = Log-1.436 + 0.669LogX$	0.82	11.21**
660	$LogY_1 = Log1.264 + 0.963LogX$	0.92	17.93**	$LogY_2 = Log-1.116 + 1.147LogX$	0.95	21.95**
	Days exposed (X) vs PCP in tissue			(Y_1 in ug/kg, Y_2 in ug)		
Control	$LogY_1 = Log0.158 + 0.001LogX$	0.04	1.12	$LogY_2 = Log-0.267 + 0.120LogX$	0.17	2.37**
35	$LogY_1 = Log0.638 + 0.011LogX$	0.64	7.11**	$LogY_2 = Log0.084 + 0.449LogX$	0.82	11.22**
660	$LogY_1 = Log0.886 + 0.032LogX$	0.93	19.13**	$LogY_2 = Log0.405 + 0.774LogX$	0.95	23.08**
	Days exposed (X) vs total PCP in fish			(Y_1 in ug/kg, Y_2 in ug)		
Control	$LogY_1 = Log0.105 + 0.053LogX$	0.05	1.16	$LogY_2 = Log-0.259 + 0.121LogX$	0.17	2.43**
35	$LogY_1 = Log0.452 + 0.366LogX$	0.68	7.69**	$LogY_2 = Log0.103 + 0.462LogX$	0.84	11.98**
660	$LogY_1 = Log0.779 + 0.722LogX$	0.94	21.32**	$LogY_2 = Log0.434 + 0.794LogX$	0.96	25.31**

The percentage of PCP in organs also increased significantly with exposure ($P<0.01$). Fish exposed to 35 ng/L retained 5-15% of the total PCP in the organs while those exposed to 660 ng/L retained 7-22% of that accumulated after 115 days (TABLE 1). There were variations of 0.8-2.4% in the weight of the liver and gall bladder relative to body weight among the fish. These differences were due mainly to the amount of bile in the gall bladder, therefore the relationship between organ weight and body weight weight was not examined. There were differences in growth rates among the three groups, growth after 115 days was significantly higher ($P<0.01$) for fish exposed to 35 ng/L than those exposed to 660 ng/L or the control group. No fish mortality was incurred over the 115 day study, therefore the one remaining fish in the treated tanks, and the two control fish were not analyzed.

Fish feed used in this study contained 3 ug/kg PCP although its contribution to the total PCP uptake is suggested to be minimal. Based on a feed to weight gain ratio of 1.3:1, it is estimated that uptake from food contributed no more than 2 ug PCP over the 115 day period.

DISCUSSION

The results of this study indicate that uptake from water is an important pathway for the accumulation of PCP by fish. It is suggested that environmental contaminants with similar properties that have a low affinity for adsorption on to particulate materials would demonstrate a comparable response. Contaminant models which describe the kinetics of the more persistent substances in fish such as DDT and HCB generally suggest uptake from food is substantially greater than that from water when levels approaching environmental concentrations are taken into consideration (MACEK & KORN; NIIMI & CHO 1981). Concentrations of these and related substances such as PCB and mirex in aquatic ecosystems like the Great Lakes range from less than 10 ng/L in water to 0.1-10 mg/kg in fish (NORSTROM et al. 1978; NIIMI 1979; GLWQB 1980). Bioconcentration factors (BCF) of these persistent contaminants can range from 8400-61000 (KENAGA & GORING 1980). In contrast, substances like PCP may not bioaccumulate as readily, but environmental concentrations in the ug/L range can occur. KENAGA & GORING (1980) reported a BCF of 16 for PCP while the results of this study, which perhaps better approximates environmental conditions, suggests BCF's of 200 and 240 at the low and high exposure levels respectively.

Fish exposed to 660 ng/L NaPCP grew less than those exposed to 35 ng/L. This would be consistent with the observations on the pharmacological effects of PCP on growth and energetics, however, a corresponding response was not demonstrated for the control group whose growth was lower than those exposed to 35 ng/L, but similar to those exposed to 660 ng/L. One plausible explanation for the observed growth response among the groups is that the 35 ng/L exposure level may not have been sufficient to impose growth restrictions on fish of this size because fish were fed to

satiation each day. It is suggested that other unidentified factors influenced this growth response.

Estimates on the clearance rate of PCP from tissues and organs of fish indicate T 1/2 values can be less than one day (KOBAYASHI & AKITAKE 1975; GLICKMAN et al. 1977), although intervals of 4-17 days have been reported (PRUITT et al. 1977; HOLMBERG et al. 1972). These estimates were derived by placing exposed fish in PCP-free water and monitoring levels. This study demonstrated that rainbow trout will accumulate PCP when exposed to waterborne concentrations as low as 35 ng/L over prolonged periods. Although its net accumulation has been determined, its kinetics cannot be established because estimates on the efficiency of extraction and volume of water respired, and the depuration rate for fish of similar size are not yet available.

Interspecific differences suggest rainbow trout may be less efficient in eliminating PCP than other species. Studies on the detoxification mechanism of PCP indicate goldfish (Carassius auratus) can eliminate PCP as a sulfate conjugate through branchial excretion, and as a glucuronide conjugate through biliary excretion (KOBAYASHI 1978) while rainbow trout appears to excrete PCP principally as a glucuronide conjugate (GLICKMAN et al. 1977). These observations would be consistent with the differences in the 96 h LC_{50} levels suggested for the respective species.

The toxicological implications on the accumulation of PCP from prolonged exposure to fish can only be surmised from the information that is available. Exposure to sublethal concentrations of 100 ug/L for prolonged periods periods may induce cytological changes in the liver (OWEN & ROSSO 1981). LC_{50} studies indicate PCP can be lethal at 40-600 ug/L and growth is impaired by prolonged exposure to concentrations of less than 10 ug/L, but studies of this nature generally do not measure PCP levels in the fish. Measurements on fish surviving in heavily contaminated waters indicate tissue levels of approximately 700 ug/kg PCP is not acutely toxic (PIERCE et al. 1977). Results from acute toxicity studies on goldfish suggest mortality occurs when PCP concentrations in tissues approach 100 mg/kg (KOBAYASHI & AKITAKE 1975; KOBAYASHI & KISHINO 1980). In view of these observations and the results of this study, it is suggested that chronic exposure of rainbow trout to waterborne NaPCP at concentrations up to 660 ng/L would not likely to be lethal with prolonged exposure, but adverse effects at the sublethal level particularly among young fish is probable.

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