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Uptake of Hexachlorobenzene (HCB) from Feed by Rainbow Trout (*Salmo gairdneri*)

A. J. Niimi[1] and C. Y. Cho[2]

[1]*Canada Centre for Inland Waters, 867 Lakeshore Road, Burlington, Ontario, Canada L7S 1X9, and* [2]*Fish Nutrition Laboratory, University of Guelph, Guelph, Ontario, Canada N1G 2W1*

Hexachlorobenzene, C_6Cl_6, is an industrial chemical that has been used as a fungicide, and more extensively in the manufacture of styrene and rubber products. Approximately 190,000 kg have been used annually for these purposes in the United States (QUINLIVAN et al. 1977). Probably more important is its production as a waste, by-product, or impurity during the manufacture of chlorine, vinyl chloride, and chlorinated solvents. It has been estimated that 3.9 million kg are produced annually in the United States from such sources (QUINLIVAN et al. 1977). In view of the quantity of HCB being used and produced, it is not surprising that this chemically stable and biologically unreactive compound has been monitored at ug/kg concentrations in the aquatic biota from industrialized and urbanized areas such as the Mississippi River drainage (LASKA et al. 1976), and the Great Lakes basin (NORSTROM et al. 1978). Salmonids collected from Lake Ontario average 40-80 ug/kg HCB (NIIMI 1979).

HCB is persistent and does bioaccumulate once it enters the environment. Photodecomposition is minimal (PLIMMER & KLINGEBIEL 1976) and microbial degradation have not been observed (VERSCHUEREN 1977). HCB has a solubility of 6 ug/l in water although environmental concentrations in waters such as Lake Ontario are generally in the low ng/l range. Laboratory studies have demonstrated that HCB in water can be bioaccumulated through the food-chain (METCALF et al. 1973, LU & METCALF 1975). This study examined the uptake of HCB through food. Subadult rainbow trout (*Salmo gairdneri*) were fed prepared diets that contained HCB at concentrations in the low ug/kg range which approximated the levels in biota that occupy the lower trophic levels in waters such as the Lake Ontario environment.

MATERIALS AND METHODS

Groups of eight trout each were placed in twenty-one 30 x 30 x 60 cm polyethylene tanks. Each tank contained 47 liters of water which was vigorously aerated and provided with an inflow of 35 l/h. Water temperature was 15 ± 1 C, and other water quality parameters has been described (HODSON 1976). Illumination was provided by fluorescent lamps, surface light intensity was 2000 Lux. Photoperiod was regulated on a 16 h light: 8 h dark cycle.

Fish were fed on a nutritionally balanced dry, pelleted diet prepared according to CHO et al. (1976). The three batches of feed contained 4 (control), 8, and 780 ug/kg HCB. The 8 and 780 ug/kg

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batches were attained by dissolving the HCB in salmon oil before its addition to the fish meal and other ingredients during mixing of the dry diet. The 4 ug/kg HCB in the control diet could be attributed to impurities contained in the fish oil or fish meal. Contamination of fish feed is not uncommon, levels of 88 ug/kg HCB has been reported in commercial fish feed (LASKA et al. 1976).

After transfer to the experimental tanks, all groups of fish were fed for 14 days on the control diet to ensure that fish would be feeding and become accustomed to the tank cleaning procedure. Fish were fed to satiation at midmorning and late afternoon each day. After each feeding, the excess feed was removed within 10 minutes using a siphon. Following this period, all groups were not fed for 24 h, then weighed. One group of fish was frozen at this time. The remaining twenty groups were divided into five series, each containing four groups. Each series was fed on the twice a day schedule on one of the following diets. The control diet which contained 4 ug/kg HCB, or diets containing 8 or 780 ug/kg HCB, or diets containing 6 or 394 ug/kg HCB derived by mixing equal amounts of one of the two prepared diets with the control diet. One group of fish from each series was sampled 20, 35, 47, and 57 days after feeding on the experimental diets commenced. Fish were not fed for 24 h before sampled to clear the intestional contents, then weighed and frozen until analysis.

To prepare the fish for HCB analysis, residual materials in the intestine was removed, than an individual fish was ground to a homogeneous composition using a Hobart grinder and an Oster blender. A 10 g sample was processed following the extraction and Florisil cleanup procedure recommended by ENVIRONMENT CANADA (1974). During the extraction, a 10 ml aliquot of the 300 ml acetonitrile extract was evaporated to dryness to estimate lipid content. HCB level was determined using a gas chromatograph with an EC detector and 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 carrier gas flow of 45 ml/min. The injection, column, and detector temperatures were 180, 180, and 200 C respectively. This method was also used to measure HCB levels in the feed. The eight fish sampled during each series that were fed the 6-780 ug/kg HCB diets were analyzed individually, while the control fish sampled at the start and at each interval were analyzed as 4 two-fish composites.

RESULTS AND DISCUSSION

HCB levels in trout, expressed as ug/kg, increased in relation to the HCB content in the diet, and the number of days fed (Table 1). Moderate increases were suggested for those groups fed the control, 6, and 8 ug/kg HCB diets. Over the 57 day period, HCB levels increased 0.02-0.09 ug/kg per day. Groups fed the 394 and 780 ug/kg diets showed the greatest increase during the first 20 day interval, incerases averaged 9 and 15 ug/kg HCB per day for the respective diets. These rates of accumulation gradually decreased over 57 days, daily increases averaged 0.8 and 8.5 ug/kg HCB for the respective diets over the last 10 day interval.

TABLE 1. Influence of feeding duration on HCB levels in rainbow trout fed diets containing 4-780 ug/kg HCB. The HCB (ug/kg), body weight (g), and lipid (mg/g) values represents the mean ± standard deviation, and range of eight replicates.

Days fed			HCB content of diet, ug/kg				
			Control(4)	6	8	394	780
0	HCB,	mean	3[a]				
		range	2-3				
	Wt.,	mean	84±16				
		range	65-105				
	Lipid,	mean	95[a]				
		range	88-105				
20	HCB,	mean	3[a]	4±1	5±1	175±21	305±57
		range	3-4	3-5	4-6	134-205	182-359
	Wt.,	mean	117±17	112±16	125±26	114±39	129±22
		range	98-146	78-130	84-161	55-178	96-167
	Lipid,	mean	88[a]	88±9	90±6	91±8	89±7
		range	81-108	76-104	80-100	75-103	77-98
35	HCB,	mean	6[a]	6±1	9±2	228±41	419±100
		range	2-7	4-8	6-12	199-322	270-590
	Wt.,	mean	191±27	166±26	167±29	166±40	150±26
		range	156-223	139-215	144-235	116-249	129-196
	Lipid,	mean	92[a]	97±3	93±8	102±7	88±4
		range	84-99	92-100	84-107	94-115	82-95
47	HCB,	mean	4[a]	10±3	9±1	268±30	550±88
		range	3-4	4-14	7-11	203-310	390-640
	Wt.,	mean	192±16	195±50	210±44	214±46	188±24
		range	164-219	143-277	152-286	152-294	151 233
	Lipid,	mean	92[a]	87±10	91±7	94±7	89±11
		range	84-112	75-100	83-104	83-106	74-110
57	HCB,	mean	4[a]	6±2	8±1	276±50	635±135
		range	3-5	5-12	6-9	194-358	445-910
	Wt.,	mean	268±54	232±59	235±50	208±76	246±37
		range	202-358	149-327	142-289	129-341	197-318
	Lipid,	mean	95[a]	89±10	86±6	83±5	88±8
		range	88-105	76-104	77-96	76-91	80-100

[a] Values represent the mean of 4 two-fish composite samples.

Body weight increased nearly two-fold over 57 days (Table 1). There were no significant differences in body weight among the five series at each sample interval. Body lipid levels were relatively uniform for all groups over 57 days, mean value for most groups was 88-92 mg/g lipid.

To further examine the relationship between the intake of HCB and its accumulation, the mean tissue concentrations, expressed as

ug/kg, were adjusted using mean body weight to estimate body burden which was expressed as ug of HCB per fish (Table 2). Again, HCB levels in trout increased in relation to dietary content and the number of days fed. An examination of Table 1 suggested the rate of HCB uptake per day was highest during the first 20 days, and lowest the last 10 days, in contrast, when the HCB content was expressed on a ug HCB per fish basis levels generally increased as feeding progressed (Table 2). Uptake rates averaged 1.0, 1.3, 1.5, and 0.3 ug HCB per day for fish fed the 394 ug/kg HCB diet, and 1.9, 1.7, 3.3, and 5.6 ug HCB per day for those fed the 780 ug/kg diet over the four sample intervals. The increase of 0.3 ug HCB over the last sample interval was due in part to the low mean weight of 208 g per fish at the end of the experiment.

In retrospect, the accumulation of HCB, or any other chemical, would be better illustrated using body burden than relative tissue concentrations because of the minimal influence due to body weight. Where the uptake rate of a chemical is greater than its losses due to depuration and biodegradation, body burden would increase, the rate of increase being dependent on the concentration in the food or water and the intake rate by the animal. Furthermore, increases in chemical levels may not be apparent when the concentrations are expressed on a relative basis. Residue trends of a chemical may increase, decrease, or remain constant, depending on the relationship between the rate of accumulation of the chemical and the rate of gain in body weight by the animal.

Table 2. Estimated HCB ingested and percent retained from feed by rainbow trout fed diets containing 4-780 ug/kg HCB for up to 57 days calculated from the ug HCB body burden per fish.

Days fed	Mn. wt. fish g.	Feed g.	HCB ug	HCB content of diet, ug/kg				
				Control(4)	6	8	394	780
0	90	0		Control fish estimated to contain 0.3 ug HCB initially				
20	120	56	Fish	0.2	0.3	0.3	20	38
			Ingest	0.4	0.3	0.4	22	44
			Retain	50%	100%	75%	91%	86%
35	170	106	Fish	0.5	0.7	1.1	39	64
			Ingest	0.4	0.6	0.8	42	83
			Retain	125%	117%	140%	93%	77%
47	200	158	Fish	0.5	0.9	1.5	57	104
			Ingest	0.6	0.9	1.3	62	123
			Retain	83%	100%	115%	92%	85%
57	240	205	Fish	0.7	1.1	1.6	60	160
			Ingest	0.8	1.4	1.6	81	160
			Retain	88%	79%	100%	74%	100%

The feeding of fish to satiation on a dry diet and subsequent removal of excess feed precluded any measurements on the amount of food consumed. To estimate feed consumption, daily increases in body weight was calculated from the relationship $W = 87.44^{0.0178X}$ where X is the number of days fed (R^2=0.99). Daily food intake was estimated using a graduated feeding rate of 3% body weight per day for fish of 90 g to 2% weight per day for fish of 240 g. Over the 57 day period, each fish consumed approximately 205 g feed (Table 2). This estimate is consistent with the feed: gain ratio (FGR) of 1.4 for fish fed under these conditions (CHO et al. 1976).

To examine the retention of HCB from feed by trout, the amount of HCB ingested for each diet was calculated over the four sample intervals (Table 2). Estimates for fish fed the 394 and 780 ug/kg HCB diets suggests approximately 80-90% of the HCB ingested was retained. Two contributing factors may account for this retention rate, the large body burden of HCB would suggest a low rate of depuration , and biodegradation could be minimal. While estimates of HCB depuration by fish are not readily available for comparative purposes, a half-life of 8-20 days for different tissues in sunfish (*Lepomis cyanellus*) has been estimated (SANBORN et al. 1977). This estimate may be conservative when compared with other observations reported for similar persistent compounds. A recalculation of the data of BRANSON et al. (1975) suggested a half-life of 55-60 days for 2,2',4,4' tetrachlorobiphenyl for rainbow trout, while LIEB et al. (1974) reported no clearance of Aroclor 1254 by rainbow trout over a 32 week period. Estimated half-life of HCB for mammals is in the order of 2-4 months for rats, and 2.5-3 years for monkeys (MORITA & OISHI 1975, MULLER et al. 1978). In view of the chemical and stoichiometric configuration of HCB, its biodegradation is suggested to be minimal particularly in lower vertebrates. LU & METCALF (1975) observed some metabolites of HCB after mosquitofish (*Gambusia affinis*) were exposed for 24 h, in contrast, LASKA et al. (1978) reported no breakdown of HCB by largemouth bass (*Micropterus salmoides*) after 24-48 h ingestion.

The 80-90% HCB retained by trout would also be consistent with the 83% retention by rats followed for 7 days after exposure where 16% was excreted in feces and 1% was eliminated through the urine (MEHENDALE et al. 1975). Similarly, 80% of the HCB mixed in oil and administered orally was absorbed by rats (KOSS & KORANSKY 1975). These rates are also in general agreement with those reported for other persistent chemicals. Approximately 68% of the Aroclor 1254 fed to rainbow trout over a 32 week period was retained (LIEB et al. 1974).

The bioaccumulation of HCB is consistent with that observed for other persistent chemicals such as PCB's, DDT, and dieldrin. Like these compounds, HCB has been monitored globally in countries such as Japan (CURLEY et al. 1973), Australia (SIYALI 1972), Italy (LEONI & D'ARCA 1976), and Norway (OFSTAD et al. 1978). Furthermore, the bioaccumulation of HCB is not limited to the aquatic environment, food-chain related increments have been suggested for mammals (BEST 1973, KOSS & MANZ 1976).

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