Uptake and Elimination of [^{14}C]Hexachlorobenzene (HCB) by the Green Sunfish, *Lepomis cyanellus* Raf., after Feeding Contaminated Food

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The feeding of [^{14}C]hexachlorobenzene (HCB) contaminated food to green sunfish at 1, 10, and 100 ppm resulted in residues which were related to the concentration in the food. The highest residues were in the alimentary tract of the fish and the lowest residues in the skeletal muscle. The majority of the ^{14}C residue in all tissues consisted of HCB and pentachlorophenol (PCP). The ratio of HCB/PCP in all tissues, except the skeletal muscle, decreased during the course of the experiment. For the skeletal muscle the ratio of HCB/PCP increased to a value of 4.30. The apparent half-life values for elimination of the HCB, PCP, and total ^{14}C between days 14 and 28 after feeding ranged from a low of 8.0 days for elimination of HCB from the stomach–pyloric caeca to 16.6 days for elimination of PCP from the same organ. Several weeks would be required to eliminate residues accumulated from 3-day exposure, but the edible portion would clear most rapidly and contain fewer metabolites.

The presence of chlorinated hydrocarbon pesticide residues in human adipose tissue most often results from consumption of contaminated food, either deliberately treated with chemicals for the control of injurious organisms or incidentally contaminated from environmental or industrial sources. The major contribution to the residues of chemicals such as PCB's in humans is through ingestion of contaminated fish (Kutz, 1975). Other highly halogenated organic compounds should be examined for their environmental disposition and one highly chlorinated aromatic that bears some scrutiny is hexachlorobenzene (HCB).

HCB, a replacement for the mercurial fungicides and by-product of industrial chlorination processes, may be as widespread as the PCB's. It appears to be a contaminant of fish (Holden, 1970; Johnson et al., 1974), wildlife (Vos et al., 1968; Muller and Korte, 1976), poultry products (Stanhope, 1969), milk (Goursaud et al., 1972), other dairy products (Tuinstra and Roos, 1970), and human milk and adipose tissue (Acker and Schulte, 1970a,b; Burns et al., 1974; Burns and Miller, 1975). Because of its hydrophobic nature, significant HCB contamination of fish probably occurs through food webs as well as absorption from water; in addition, HCB is found in commercial fish feeds used in aquaculture (Hansen et al., 1976). We have examined the distribution, elimination, and degradation of [^{14}C]HCB in the green sunfish, *Lepomis cyanellus* Raf., after feeding HCB-contaminated food.

MATERIALS AND METHODS

Green sunfish (*Lepomis cyanellus* Raf.) were field collected and random samples were found to be uncontaminated with HCB residues by electron-capture gas chromatography methods previously reported (Hansen et al., 1977). No HCB could be detected in the control feed (Purina Gamefish chow) at a lower detection limit of 5 ppb. The fish were acclimated in the laboratory for at least 2 weeks prior to the start of the experiment and three fish each were placed into 19-L jars for 24 h prior to the feeding of the treated food. A small pipet aerated the system containing 5 L of aged tap water, and the photoperiod during the experiments was 12/12 h with a laboratory temperature of 23 °C.

In the first experiment, the fish in each of three jars were fed three pellets of food/day (pellet weight ~18 mg)

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treated individually with an acetone solution to contain 1 or 10 ppm (w/w) of [^{14}C]HCB (New England Nuclear; specific activity = 23.36 mCi/mmol). Fish were fed treated pellets for 3 consecutive days. On days 4, 14, and 28, fish were sampled (three per food concentration from three different jars) and the remaining fish were moved to clean water. The fish were weighed and ground in acetone individually for assessment of total ^{14}C residues by liquid scintillation counting in a cocktail of 7 g of PPO, 0.05 g of POPOP, and 120 g of naphthalene in 1 L of dioxane. The samples were quench corrected using an external standard. The acetone-unextracted ^{14}C residue was determined first by dissolving the tissue residue in Protosol (New England Nuclear) for eventual counting in Aquasol (New England Nuclear). Since the variation in the whole fish residue data for the fish fed 1 and 10 ppm (Table I) was large and because higher residues were desirable for tissue comparisons and metabolite identification, another experiment was conducted for the assessment of [^{14}C]HCB residues at 100 ppm HCB in the feed.

In the second experiment, nine groups of three fish each were used as previously described, except that the fish were fed pellets containing 100 ppm [^{14}C]HCB. On days 4, 14, and 28, the nine fish were removed (one per jar), and the following organs or tissues were removed: liver, stomach–pyloric caeca, intestine, skeletal muscle, and carcass (carcass is defined as that portion of the fish remaining after removal of sampled organs and a small amount of skeletal muscle). The organs or tissues were randomly pooled from three jars, weighed, and extracted with acetone. Aliquots of the acetone extracts were counted as previously described and separate aliquots spotted on thin-layer chromatography plates (0.50 mm silica gel GF-254 Brinkman) for development in a solvent system of benzene:*n*-hexane:acetic acid 90:5:5 (v/v). Radioautograms were prepared (Bluebrand x-ray film, Eastman Kodak) to facilitate the location of HCB and its degradation products. To obtain the quantitative data in Tables II–IV, describing the metabolite distribution of HCB and its degradation products in the fish tissue, the radioactive spots on the thin-layer chromatographic plates were scraped into scintillation vials for liquid scintillation counting in the previously mentioned cocktail. Unlabeled standards (HCB and pentachlorophenol, PCP) were cochromatographed with the radioactive extracts to aid in the identification of the degradation products.

RESULTS AND DISCUSSION

Table I reveals that an expected dose–residue response was found in the order of feeding of the contaminated food,

Table I. Radioactivity in Fish Fed [¹⁴C]Hexachlorobenzene Contaminated Food for 3 Consecutive Days and then Sampled for Residues on Days 4, 14, and 28

Food level, ppm		Concentration, ppm		
		Day 4	Day 14	Day 28
1	extractable	0.0319 ± 0.00294[a]	0.0137 ± 0.00222	0.00713 ± 0.00059
	unextractable	0.0221 ± 0.0108	0.0172 ± 0.0000788	0.000446 ± 0.0000461
10	extractable	0.239 ± 0.0450	0.209 ± 0.0539	0.0473 ± 0.0226
	unextractable	0.0378 ± 0.00480	0.009806 ± 0.00472	0.00539 ± 0.000839

[a] Standard error of the mean.

Table II. Distribution of [¹⁴C]Hexachlorobenzene and Its Degradation Products in *Lepomis cyanellus* Raf. Sacrificed on Day 4

	R_f[a]	Concentration, ppm									
		Carcass		Liver		Stomach		Intestine		Muscle	
		Mean	SEM[b]	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Extractable ¹⁴C		11.86	0.49	10.99	0.79	56.48	0.99	49.55	0.80	2.79	0.10
HCB[c]	0.95	5.25	0.76	4.99	0.61	27.16	3.55	26.82	1.74	1.53	0.058
PCP[d]	0.66	5.63	h	2.96	0.30	19.14	3.62	15.94	1.85	0.71	h
I[e]	0.34	0.63	0.074	1.29	0.039	4.05	0.12	2.67	0.14	0.39	0.015
II	0.06	f		0.35	g			0.74	g		
III	0.03	0.0034	g	0.23	g	1.09	g			0.0032	g
Origin	0.00	0.35	0.057	1.17	0.053	5.04	0.64	3.38	0.39	0.16	0.024
Unextractable ¹⁴C		0.33	0.052	0.028	0.0043	1.12	0.041	0.17	0.050	0.020	0.091
Grand total ¹⁴C		12.19	0.54	11.02	0.79	57.60	1.03	49.72	0.84	2.81	0.10

[a] Silica gel GF-254, 0.50 mm; benzene:n-hexane:acetic acid 90:5:5 (v/v). [b] Standard error of the mean. [c] Hexachlorobenzene. [d] Pentachlorophenol. [e] Roman numerals, chemical structure unknown. [f] Degradation product not present. [g] Degradation product found in one group of three fish. [h] Degradation product found in two groups of three fish.

Table III. Distribution of [¹⁴C]Hexachlorobenzene and Its Degradation Products in *Lepomis cyanellus* Raf. Sacrificed on Day 14

	R_f[a]	Concentration, ppm									
		Carcass		Liver		Stomach		Intestine		Muscle	
		Mean	SEM[b]	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Extractable ¹⁴C		6.68	0.17	6.66	0.17	33.49	4.06	43.38	2.52	3.09	0.49
HCB[c]	0.91	2.77	0.42	2.16	0.18	12.70	2.45	16.26	1.71	1.76	0.20
PCP[d]	0.63	3.34	0.62	2.31	0.041	17.51	2.50	20.27	3.16	0.68	0.17
I[e]	0.37	0.28	0.026	0.95	0.051	1.49	0.15	2.98	0.23	0.44	0.11
II	0.06	0.13	h	0.51	0.048	0.92	0.042	2.00	0.095	f	
III	0.03	0.024	h	0.21	h	0.27	h	0.22	g		
Origin	0.00	0.14	0.023	0.52	0.02	0.60	0.015	1.65	0.088	0.21	0.025
Unextractable ¹⁴C		0.28	0.016	0.039	0.0094	0.023	0.023	0.072	0.044	0.0097	0.0029
Grand total ¹⁴C		6.96	0.17	6.70	0.17	33.56	4.09	43.45	2.53	3.10	0.49

[a] Silica gel GF-254, 0.50 mm; benzene:n-hexane:acetic acid 90:5:5 (v/v). [b] Standard error of the mean. [c] Hexachlorobenzene. [d] Pentachlorophenol. [e] Roman numerals, chemical structure unknown. [f] Degradation product not present. [g] Degradation product found in one group of three fish. [h] Degradation product found in two groups of three fish.

Table IV. Distribution of [¹⁴C]Hexachlorobenzene and Its Degradation Products in *Lepomis cyanellus* Raf. Sacrificed on Day 28

	R_f[a]	Concentration, ppm									
		Carcass		Liver		Stomach		Intestine		Muscle	
		Mean	SEM[b]	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Extractable ¹⁴C		2.42	0.13	3.18	0.20	12.22	1.01	12.16	1.75	0.47	0.015
HCB[c]	0.91	0.83	0.11	1.39	0.050	1.64	0.14	2.18	0.38	0.31	0.010
PCP[d]	0.66	1.47	0.12	1.00	0.084	10.13	1.13	9.25	1.81	0.072	0.0023
I[e]	0.38	0.081	0.0069	0.47	0.052	0.28	0.021	0.45q	0.060	0.055	0.0081
II	0.07	0.0092	h	0.057	h					0.0012	g
III	0.04	0.0057	h	0.020	h	0.066	h	0.071	g	0.0049	h
Origin	0.00	0.027	0.0037	0.24	0.033	0.11	0.015	0.21	0.025	0.026	0.0046
Unextractable ¹⁴C		0.10	0.030	0.019	0.0049	0.021	0.0034	0.040	0.011	0.0060	0.00058
Grand total ¹⁴C		2.52	0.16	3.20	0.19	12.24	1.01	12.20	1.74	0.48	0.014

[a] Silica gel GF-254, 0.50 mm; benzene:n-hexane:acetic acid 90:5:5 (v/v). [b] Standard error of the mean. [c] Hexachlorobenzene. [d] Pentachlorophenol. [e] Roman numerals, chemical structure unknown. [f] Degradation product not present. [g] Degradation product found in one group of three fish. [h] Degradation product found in two groups of three fish.

but the residues were not adequate for tissue subsamples and quantitation of metabolites.

Characterization of the residues in the various organs and tissues of the fish fed 100 ppm contaminated food (Tables II–IV) demonstrates that the stomach–pyloric caeca and intestine contained the highest total radioactivity on all three sampling days. The fish obtained the HCB residue in the food primarily by ingestion which

Table V. Comparison of $t_{1/2}$ Values for Removal of Total ^{14}C, HCB and PCB from Various Tissues of *Lepomis Cyanellus* Raf.

	$t_{1/2}$, days[a]		
Tissue	Total ^{14}C	HCB	PCP
Carcass	11.0	10.0	12.5
Liver	13.4	19.6	12.4
Stomach and pyloric caeca	10.9	8.0	16.6
Intestine	9.7	8.1	12.9
Muscle	8.2	8.5	8.9

[a] Based on data for days 14 and 28.

might account for the highest titer or residue in these three elements of the alimentary canal. On the other hand, it has been shown that PCB's redistribute to the offal, other than liver and kidney, during periods of elimination in channel catfish (Hansen et al., 1976). During the shorter elimination period in this study, intestine and muscle clearance lagged behind the other tissues (Table III) and this is strongly suggestive of biliary excretion with enterohepatic recirculation as has been found with HCB in the dog (Sundlof et al., 1976). The more rapid elimination of ^{14}C from the muscle between days 14 and 28 may indicate an early phase of redistribution into the offal.

The next highest residues throughout the experiment were found in the carcass and liver, which, on the 28th day of the experiment, were about four–five times lower than the residues observed in the elements of the alimentary canal. Finally, the lowest residues isolated from the fish as measured by total ^{14}C were found in the skeletal muscle which, in a larger fish, would be the edible portion of the fish. The muscle residues were about six times lower than the residues in the carcass and liver. The ordering of these residues previously discussed is reasonable and is related to the proximity of the tissues to the initial three-day feeding of contaminated HCB food. Distribution of HCB in various organs and tissues after feeding results in the edible portion, which is the most distal tissue from the initial contamination of the fish, containing the least amount of residue. Elimination is also more rapid from the muscle, further reducing residues in the edible portion.

The data in Table V permits the estimation of the time necessary for the fish to eliminate one-half $(t_{1/2})$ the residues from the various tissues and organs following absorption and distribution. The residue values on days 14 and 28 were utilized to calculate the $t_{1/2}$. Since each point on days 14 and 28 is a composite of nine fish, the $t_{1/2}$ values in this table are considered to be a good estimate for elimination of total ^{14}C, HCB, and PCP from the various tissues and organs; however, since the slopes are determined from only two points, it should be emphasized that these are estimates only and may be considerably skewed if elimination is multiphasic. For removal of the

total ^{14}C, the liver has the longest $t_{1/2}$ and the skeletal muscle has the shortest. For elimination of HCB, the liver again has the longest $t_{1/2}$, more than twice the values for the stomach–pyloric caeca, intestine, and muscle. Finally, for elimination of PCP, the stomach–pyloric caeca had the longest $t_{1/2}$ and the muscle, again, the shortest. In general, the skeletal muscle or edible portion of the fish has the greatest capacity to purge itself of residues of HCB and its degradation products. For the three contaminants (total ^{14}C, HCB, and PCP) listed in Table V, the $t_{1/2}$ averaged 8.55 days.

HCN/PCP Ratio. The previous discussion considered the relative rates of elimination of total ^{14}C, HCB, and PCP from selected tissues and organs. The ratio of HCB to PCP changes during the experiment in the various organs and tissues of fish fed 100 ppm HCB contaminated food. For all tissues, with the exception of the muscle, the ratio of HCB to PCP decreases from day 4 to 28 as HCB is metabolized to PCP. Values range from near unity for the liver to approximately 0.20 for the stomach–pyloric caeca. However, the skeletal muscle has an increasing HCB/PCP ratio changing from ~2.15 on day 4, ~2.60 on day 14, and ~4.30 on day 28 which is nearly 22 times greater than that of the stomach, the tissue with the lowest HCB/PCP ratio.

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LITERATURE CITED

Acker, L., Schulte, E., *Dtsch. Lebensm.-Rundsch.* **66**, 385 (1970a).
Acker, L., Schulte, E., *Naturwissenschaften* **57**, 497 (1970b).
Burns, J. E., Miller, F. M., Gomes, E. D., Albert, R. A., *Arch. Environ. Health* **29**, 192 (1974).
Burns, J. E., Miller, F. M., *Arch Environ. Health* **30**, 44 (1975).
Goursaud, J., Luguet, F. M., Boudier, J. F., Casalis, J., *Ind. Aliment. Agric.* **89**, 31 (1972).
Hansen, L. G., Wiekhorst, W. B., Simon, J., *J. Fish Res. Board Can.* **33**, 1343 (1976).
Hansen, L. G., Wilson, D. W., Byerly, C. S., Sundlof, S. F., Dorn, S. B., *J. Toxicol. Environ. Health,* **2**, 557 (1977).
Holden, A. V., *Pestic. Monit. J.* **4**, 117 (1970).
Johnson, J. L., Stalling, D. L., Hogan, J. W., *Bull. Environ. Contam. Toxicol.* **11**, 393 (1974).
Kutz, F. R., National Conference on Polychlorinated Biphenyls, November 19–21, 1975, Chicago, Ill.
Müller, W., Korte, F., *Chemosphere* **5**, 95 (1976).
Stanhope, W., *J. Agric. (Victoria, Aust.)* **67**, 245 (1969).
Sundlof, S. F., Hansen, L. G., Welborn, M. E., *Pharmacologist* **18**, 149 (1976).
Tuinstra, L. G. M. Th., Jr., Roos, J. B., *Ned Melk-Zuiveltijdschr.* **24**, 65 (1970).
Vos, J. G., Breeman, H. A., Benschop, H., *Landbouwet. Gent.* **33**, 1263 (1968).