Uptake of Chloro- and Bromobiphenyls, Hexachloro- and Hexabromobenzene by Fish

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The accumulation of di-, tri-, and tetrabromobiphenyls, the corresponding chlorobiphenyls, and of hexabromo- and hexachlorobenzene by juvenile Atlantic salmon (*Salmo salar*) was examined. The accumulation of bromobiphenyls was similar to that of chlorobiphenyls. In contrast to hexachlorobenzene, hexabromobenzene did not accumulate in the fish.

In comparison to available data on environmental properties of persistent chlorinated hydrocarbons, relatively little is known about the behaviour of their brominated analogues. Polybrominated biphenyls and hexabromobenzene are used as fire retardants in plastics (HUTZINGER et al. 1976). A recent contamination incident involving a commercial hexabromobiphenyl preparation was described (MUMMA and WALLACE 1975). The preparation of individual bromobiphenyls and their gas chromatographic properties were studied by SUNDSTRÖM et al. (1976).

EXPERIMENTAL

Halogenated hydrocarbons. The bromobiphenyls (2,4-, 2,6-, and 3,4-di-, 2,5,4'-tri-, 2,4,2',5'-, and 2,5,3',4'-tetrabromobiphenyl) were prepared by SUNDSTRÖM et al. (1976). The corresponding dichlorobiphenyls originated from our earlier studies (HUTZINGER et al. 1971), and the tetrachlorobiphenyls were obtained from Analabs Inc. (North Haven, Conn.). Hexabromo- and hexachlorobenzene were purchased from Chem Service (Media, Pa.). Two mixtures were prepared (Table 1). 2,5,4'-Tribromobiphenyl was present in both to provide a base for comparison of the accumulation reproducibility.

Exposure of fish from water. Aliquots of the mixtures in 3 mℓ of hexane (Cℓ: 1.717, Br: 1.163 mg) were applied to the bottoms of 4-ℓ Erlenmeyer flasks, swirling the flasks so that the bottoms were completely wetted by the solution, and the solvent was left to evaporate. Dechlorinated tapwater (total hardness 14 mg/ℓ as calcium carbonate), 3 ℓ per flask was added, gently aerated and the flask was placed in a water bath at 15 C. Three

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TABLE 1

Composition of test mixtures

Mixture	Weight (mg)	Composition, %						
		2,6	2,4	3,4	2,5,4'	2,4,2',5'	2,5,3',4'	C_6X_6
Cℓ	5.724	16.2	8.1	31.8	9.2*	27.5	6.1	1.1
Br	3.876	13.5	13.7	13.5	13.7	11.9	14.9	18.8

*tribromobiphenyl

fish, average weight 6.24 g, length 8.41 cm, and hexane-extractable lipid 2.31% were added to each flask (two flasks per mixture). Water samples (100 mℓ) were taken after 24, 48, and 96 h, and analyzed immediately. Fish were sacrificed after 48 and 96 h, and stored frozen until analysis.

Exposure of fish from food. Dry fish food (Purina Trout Chow) was contaminated as described (ZITKO and HUTZINGER 1972) to contain 11.44 and 7.75 µg/g of Cℓ and Br mixtures, respectively, and fed to juvenile Atlantic salmon (average weight 6.77 g, length 8.42 cm, and hexane-extractable lipid 3.54%) over a period of 40 days at 15 C. Twenty fish per mixture were used, sampled (2 fish per sample) after 14, 28, and 40 days, and the samples were stored frozen until analysis.

Analysis. Water samples (100 mℓ) were extracted with 3 mℓ of pesticide-grade hexane in 100-mℓ volumetric flasks, calibrated to 110 mℓ (Canlab No. F4724). The mixture was shaken manually for 3 min, the volume of the hexane phase was noted and several aliquots were analyzed by gas chromatography. The hexane phase and any interphase layer was then removed by a Pasteur pipet and the extraction was repeated with another 3 mℓ of hexane.

Spiked food and whole fish were extracted with hexane. Lipids were removed from aliquots of the extracts by column chromatography on alumina and the cleaned-up solutions were analyzed by gas chromatography. The detailed conditions of the extraction, cleanup, and gas chromatography have been described previously (ZITKO et al. 1974). All reported fish analyses are arithmetic means of concentrations determined by individually analyzing two fish.

The remainder of extracts of fish exposed to the mixture of brominated hydrocarbons was combined and cleaned up on alumina for analysis by GC/MS. Extracts of fish fed food contaminated by the brominated hydrocarbon mixture for 40 days were treated similarly.

The GC/MS analysis was performed on a Finnigan 1016D instrument and a Finnigan 6100 Data System. Gas chromatography was carried out in a 4 ft x 1/4 inch glass column filled with 3% OV-1 on HP Chromosorb W 80/100. The samples were injected at 180 C and the column effluent was vented for 30 sec. The column was maintained at 180 C for 3 min after injection and then programmed at 4 C/min to a final temperature of 230 C. The mass range 200-600 a.m.u. was scanned every 5 sec.

RESULTS AND DISCUSSION

Concentration in water. Two extractions were sufficient for quantitative recovery of the compounds, approximately 85-90% being recovered in the first extraction. The concentrations of the chlorinated hydrocarbons decreased linearly between 24 and 96 h, those of the brominated hydrocarbons decreased from 24 to 96 h, but levelled off or even increased between 48 and 96 h (Table 2). On a relative composition basis, both extracted mixtures were enriched in the tri- and tetra- substituted biphenyls as compared to the original mixtures. The relative concentration of hexabromobenzene was about twice that in the original mixture.

TABLE 2

Concentration of halogenated hydrocarbons in water

Time, h	Concentration in water, µg/ℓ						
	2,6	2,4	3,4	2,5,4'	2,4,2',5'	2,5,3',4'	C_6X_6
Cℓ, added	92.7	46.7	182.1	53.0	156.8	34.5	6.60
24	11.6	6.99	28.3	20.6	50.6	12.9	1.24
48	9.21	5.25	21.1	15.0	35.9	9.28	0.95
96	5.85	1.91	2.16	5.95	18.2	3.61	0.22
Br, added	52.3	53.0	52.5	53.0	46.0	57.6	73.2
24	5.20	7.12	8.58	18.7	19.4	29.0	41.8
48	1.61	1.85	5.14	5.42	7.33	8.99	8.76
96	0.91	1.74	2.17	8.18	8.53	2.49	13.1

The aqueous solutions at 24 h were clear with no visible droplets of water-insoluble compounds and it is likely that the halogenated hydrocarbons were dissolved or very finely dispersed rather than suspended in the aqueous phase. At 48 and 96 h the solutions were cloudy due to the presence of excretion products and bacterial activity. It is interesting to note that a high proportion of the compounds, ranging from 10 to 58% of the added amount, was in solution at 24 h, in spite of the fact that the compounds were originally present as a film, deposited on glass.

Uptake from water. Fish accumulated most of the compounds to a concentration of several µg/g on a wet weight basis (Table 3). A notable exception was hexabromobenzene which was not detectable in the fish. The percentages of the chlorinated hydrocarbons were reasonably close to those in the original mixture. On the other hand, 2,6- and 2,4-dibromobiphenyl were relatively somewhat enriched in the fish.

TABLE 3

Uptake of halogenated hydrocarbons from water

Time, h		Concentration in fish, µg/g wet weight						
		2,6	2,4	3,4	2,5,4'	2,4,2',5'	2,5,3',4'	C_6X_6
Cℓ,	48	12.7	7.03	17.2	7.57	20.2	4.89	0.955
	96	6.91	4.14	2.29	6.05	13.65	3.69	0.580
Br,	48	5.32	5.33	0.630	3.95	3.30	1.90	ND*
	96	2.43	3.58	0.270	3.46	3.35	1.40	ND

*Not detectable, <0.004 µg/g

The concentration of the tribromobiphenyl, accumulated from the mixture of the brominated hydrocarbons, was about one half of that accumulated from the mixture of chlorinated hydrocarbons. The reasons for this difference are not clear. It may be that the presence of other brominated hydrocarbons induced a faster metabolism of the tribromobiphenyl, or slowed down the uptake rate.

Mass balance of uptake from water. The amount of the compounds in water and in fish at 48 and 96 h was calculated and expressed as percentage of added amount (Table 4). At 48 h, more than 70% of most compounds was accounted for. The exceptions were hexabromobenzene, 3,4-dichloro- and dibromobiphenyl, and 2,5,3',4'-tetrabromobiphenyl. The amounts found at 96 h were generally lower for all compounds. Evaporation, resulting from the aeration of the solutions, and metabolism, primarily by bacteria, may be the main reasons for this lower recovery.

TABLE 4

Mass balance of uptake from water

Time, h		Amount found as percent of added						
		2,6	2,4	3,4	2,5,4'	2,4,2'5'	2,5,3',4'	C_6X_6
Cℓ,	48	65.2	72.0	49.7	85.9	74.9	84.0	72.7
Br,	48	82.1	81.5	19.1	68.1	71.7	41.3	12.0
Cℓ,	96	47.3	52.8	8.1	74.0	59.5	69.3	52.0
Br,	96	33.6	49.6	7.7	60.2	68.6	38.4	17.9
		Amount in fish as percent of found						
Cℓ,	48	84.8	84.3	76.6	67.1	69.4	68.0	80.6
Br,	48	96.3	95.7	48.8	84.9	77.8	62.1	0
Cℓ,	96	86.6	92.3	85.3	84.8	80.5	84.9	93.2
Br,	96	94.9	93.4	46.3	74.4	72.9	43.4	0

It can be seen from Table 4 that, with only a few exceptions, much larger amounts of the compounds were present in fish than in water.

Uptake from food. On analysis of the spiked food, the determined concentrations of all compounds were >85% of the nominal values, except for hexabromobenzene which had a recovery of 63.5%. The concentration of most of the compounds in fish, fed the contaminated food, ranged from 0.1 to 0.9 µg/g wet weight (Table 5), but 3,4-dichloro- and dibromobiphenyl, and hexabromobenzene did not accumulate. On a relative basis, the tri- and tetra-substituted biphenyls were enriched in fish in comparison to food.

An equilibrium was probably established within 40 days between the accumulation and excretion of 2,6- and 2,4-dichlorobiphenyl. The concentration of the remaining compounds increased steadily throughout the feeding period. In these cases the accumulation could be expressed as a function of time by the empirical equation $c = t/(a + bt)$, and the equilibrium levels ($1/b$) are given in Table 5.

Stability of bromobiphenyls in fish. The carbon-bromine bond is less stable than the carbon-chlorine bond and there is a possibility that, in addition to metabolism by hydroxylation, well established for chlorobiphenyls, a reductive debromination may be a degradation pathway of bromobiphenyls. However, no brominated biphenyls other than those with retention times identical with retention times of the components

TABLE 5

Uptake of halogenated hydrocarbons from food

Time, days	Concentration in fish, μg/g wet weight						
	2,6	2,4	3,4	2,5,4'	2,4,2',5'	2,5,3',4'	C_6X_6
Cℓ, 14	0.154	0.112	ND[a]	0.270	0.485	0.157	0.019
28	0.089	0.090	ND	0.244	0.500	0.159	0.022
40	0.140	0.101	ND	0.425	0.881	0.278	0.027
Equilbrium[b]	0.128	0.101	ND	0.524	1.30	0.405	0.030
Br, 14	0.092	0.094	ND[d]	0.250	0.190	0.200	ND[c]
28	0.106	0.155	ND	0.326	0.271	0.294	ND
40	0.139	0.219	ND	0.390	0.332	0.386	ND
Equilbrium[b]	0.188	0.337	ND	0.476	0.540	0.658	ND

[a,c,d] Not detectable, <0.006, <0.004, and <0.006, respectively

[b] Arithmetic mean for 2,6- and 2,4-dichlorobiphenyl, for other compounds 1/b from equation $c = t/(a + bt)$, where c = concentration in fish, μg/g, t = time, days.

of the administered mixture were detected by GC/MS (Fig. 1).

An indication of the presence of a dibromobiphenylol was obtained from the extract of fish exposed to bromobiphenyls from water (Fig. 1A, a Br_2 cluster at 326 a.m.u.). The formation of bromobiphenylols should be investigated further, since the used cleanup is likely to give only a low recovery of these compounds.

All the background peaks in extracts of fish fed bromobiphenyl-contaminated food were identified as chlorobiphenyls, and DDE was also present (Fig. 1B).

Toxicity of the studied mixtures of halogenated hydrocarbons. No mortality occurred during the 96-h exposure of fish to either mixture. In the feeding experiment after 42 days, both groups of fish were accidentally exposed to a transient low concentration of chlorine due to a failure of a dechlorinator. Fish fed the mixture of chlorinated hydrocarbons survived this additional stress and no mortality occurred for another 30 days. On the other hand, all fish fed the brominated hydrocarbons died during the accident. Consequently, the effects of bromobiphenyls on fish deserve further attention.

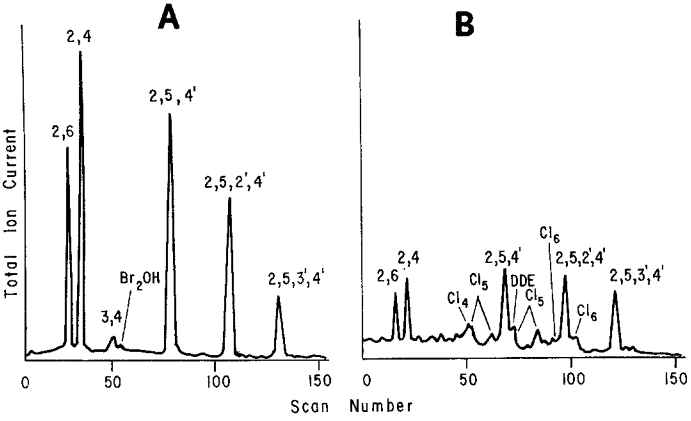

Fig. 1. Reconstructed gas chromatograms of extracts of fish exposed from water (A) and from food (B) to the mixture of brominated hydrocarbons.

Br_2OH = tentatively identified dibromobiphenylol,

$C\ell_4$,$C\ell_5$,$C\ell_6$ = tetra-, penta-, and hexachlorobiphenyls, respectively.

Accumulation coefficients. For a better comparison of the accumulation of the studied chlorinated and brominated hydrocarbons by fish, the accumulation coefficients from water (μg/g wet weight/μg per mℓ) and from food (μg/g wet weight/μg per g food) are summarized in Table 6. The coefficients were calculated on the basis of accumulation from water after a 48-h exposure, and of the extrapolated equilibrium levels in fish fed contaminated food.

It can be seen from Table 6 that di- and tetrachloro- and bromobiphenyls accumulate from water to an approximately equal degree. An exception is 3,4-dibromobiphenyl which has a much lower accumulation coefficient than its chlorinated analogue. For both chlorinated and brominated biphenyls the accumulation from water coefficients decrease with increasing molecular weight, although the substitution pattern affects the coefficients as well.

The accumulation from food coefficients are higher for brominated than for chlorinated biphenyls

TABLE 6

Accumulation coefficients (concentration in fish µg/g wet weight, divided by concentration in water, µg/mℓ, or in food, µg/g)

Uptake from	Accumulation coefficients						
	2,6	2,4	3,4	2,5,4'	2,4,2',5'	2,5,3',4'	C_6X_6
Water							
Cℓ	919	894	358	385	389	389	690
Br	1267	1343	63	425	314	111	0
Food							
Cℓ	0.137	0.054	0	0.494	0.414	0.586	0.174
Br	0.179	0.318	0	0.449	0.587	0.571	0

in most cases. It is interesting to note that, in contrast to accumulation from water, in accumulation from food the accumulation coefficients increase with increasing molecular weight. The 3,4- substituted biphenyl does not accumulate from food.

Hexabromobenzene is not accumulated either from water or from food. Its molecular weight (546), highest among the investigated compounds, suggests that there may be an upper limit, above which little or no uptake takes place. A study of the uptake of high molecular weight chlorinated paraffins (ZITKO 1974) concluded that this limiting molecular weight may be approximately 600. On the other hand, it is possible that hexabromobenzene is taken up but converted into a non-extractable form in the fish. Experiments with a labelled hexabromobenzene are required to determine its fate. In any case, the difference between hexachloro- and hexabromobenzene indicates that great care must be exercised in predicting the properties of structurally analogous compounds.

CONCLUSIONS

No major differences between the accumulation of di- and tetra-, bromo- and chlorobiphenyls by fish are apparent. In contrast to hexachlorobenzene, hexabromobenzene is not accumulated either from water or from food.

The concentration of both chloro- and bromo-substituted compounds in water was of the same order of magnitude, and in both cases fish accumulated higher concentrations from water than from food. In both groups of compounds, the accumulation coefficients generally decreased with increasing degree of substitution during the uptake from water, and increased with increasing degree of substitution when taken up from food. Of the dihalobiphenyls, the 3,4-isomer accumulated from water much less than 2,6- and 2,4-dihalobiphenyl, and did not accumulate from food.

Partially debrominated compounds were not detectable in fish exposed either from water or from food, but a dibromobiphenylol was tentatively detected in the former. Mortality after exposure to chlorine occurred among fish fed the mixture of brominated hydrocarbons and their toxicity should be further studied.

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