The Accumulation of Polybrominated Biphenyls by Fish

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The accumulation of commercial polybrominated biphenyl (PBB) preparations, BP-6 (Michigan Chemical Corporation) and OB (The Dow Chemical Company), from water and from food by juvenile Atlantic salmon (*Salmo salar*) was determined and compared with that of Aroclor 1254. Only bromobiphenyls with six or fewer bromine atoms were accumulated from water by the fish. When administered in food, more brominated biphenyls were taken up as well. The accumulation coefficients decreased markedly with increasing number of bromine atoms in the bromobiphenyls. Several partially debrominated biphenyls were detected in the fish.

A review of production, uses, and environmental properties of PBB's was published by MUMMA and WALLACE (1975). The accumulation of PBB's was studied in cattle (FRIES and MARROW 1975; GUTENMANN and LISK 1975), rats (LEE et al. 1975; NORRIS et al. 1973), Japanese quail (BABISH et al. 1975), and hens (FRIES et al. 1976). Fish did not accumulate OB from water (NORRIS et al. 1973). These studies indicate that the accumulation of PBB's is similar to that of PCB's, but that in some instances PBB's penetrated biological membranes slower than PCB's.

EXPERIMENTAL

Commercial halogenated biphenyls were gifts from Michigan Chemical Corporation (BP-6), The Dow Chemical Company (OB), and Monsanto (Aroclor 1254).

Exposure from water. BP-6, OB, and Aroclor 1254 (1.476, 1.470, and 1.500 mg, respectively) were applied in 3 mℓ of hexane to the bottoms of 4-ℓ Erlenmeyer flasks and the solvent was left to evaporate before adding water and 3 fish. Fish, average weight 5.29 g, length 8.06 cm, hexane-extractable lipid 2.43%, were exposed for up to 96 h, as described previously (ZITKO and HUTZINGER 1976).

Exposure from food. Dry fish food (Purina Trout Chow) was contaminated by BP-6 and OB to a concentration of 100 µg/g, respectively. Fish, fed BP-6, had an average weight 16.15 g, length 11.58 cm, and hexane-extractable

Bulletin of Environmental Contamination & Toxicology,
Vol. 17, No. 3 © 1977 by Springer-Verlag New York Inc.

lipid 4.94%. Fish in the OB feeding experiment had an average weight 13.07 g, length 10.23 cm, and hexane-extractable lipid 2.29%. In both experiments a period of feeding not contaminated food followed that of contaminated food.

Analysis. Samples of water containing Aroclor 1254 were analyzed as described (ZITKO and HUTZINGER 1976), those containing BP-6 and OB were extracted with hexane (100 mℓ water, 3 x 3 mℓ hexane), the extracts were combined, adjusted to a suitable volume, and analyzed by gas chromatography. Spiked food and whole fish were extracted with hexane and aliquots of the extracts were cleaned up by column chromatography on alumina (ZITKO et al. 1974).

Gas chromatography of samples containing Aroclor 1254 was performed as described (ZITKO et al. 1974). PBB-containing samples were analyzed on a 2 ft x 4 mm glass column with 3% OV-210 on Chromosorb W 60/80 at 200 C. Flow rate of nitrogen carrier gas was 50 mℓ/min, and the detector (^{3}H) was maintained at 220 C.

Combined samples of fish exposed to BP-6 from water and from food, and to OB from food, were analyzed by GC/MS, (ZITKO and HUTZINGER 1976). Samples were injected at 180 C and the temperature was increased at 6 C/min to 285 C.

RESULTS AND DISCUSSION

Concentration in water. The concentrations of PCB's and BP-6 decreased markedly between 24 and 48 h, whereas the concentration of OB remained quite constant throughout the experiment (Table 1).

The relative proportions of the individual peaks of Aroclor 1254 differed somewhat from those of the original preparation, the relative heights of the first 3 peaks were 70-80%, those of the last 2 peaks 110-130% of the standard. The relative proportions of the peaks in BP-6 showed a similar trend. The composition of the extracted OB was practically identical with that of the standard.

Concentration in fish. PCB's were accumulated from water to a much larger extent than PBB's (Table 2). The accumulation of the latter decreased significantly with increasing molecular weight. In the case of BP-6, only a pentabromobiphenyl and 2,2',4,4',5,5'-hexabromobiphenyl were detected in the fish. The third major component of BP-6, a heptabromobiphenyl, was not detectable. OB as such was not taken up by the fish, but a hexabromobiphenyl was present in a low concentration. This unidentified hexabromobiphenyl was quantitated by using the BP-6 detector response.

TABLE 1

Concentration of PCB's and PBB's in water

		µg/ℓ		
		Aroclor 1254	BP-6	OB
Nominal concentration		500	492	490
Time, h	24	210	23.3	46.0
	48	83.0	12.9	47.3
	96	82.0	18.0	30.4

TABLE 2

Accumulation of PCB's and PBB's from water

	Concentration in fish, µg/g wet weight			
Time, h	Aroclor 1254	BP-6	OB	
			as such	Br_6[a]
48	23.4	0.615	ND[b]	0.082
96	16.8	1.15	ND	0.042

[a]An unidentified hexabromobiphenyl, not present in OB

[b]Not detectable, <0.01 µg/g

Mass balance of uptake from water. The fractions of halogenated biphenyls accounted for in water and in fish were much lower for PBB's than for Aroclor 1254 (Table 3). In comparison, the recoveries of some di-, tri-, and tetrahalobiphenyls in identical experiments ranged from 65 to 86% for the chlorinated, and from 41 to 82% for the brominated biphenyls (ZITKO and HUTZINGER 1976).

TABLE 3

Mass balance of the accumulation from water

Time, h	Amount found (water + fish) as percent of added		
	Aroclor 1254	BP-6	OB
48	51.0	3.3	9.6
96	36.0	4.5	6.2
	Amount in fish as percent of found		
48	67.5	20.4	0
96	54.6	19.4	0

Accumulation from food. The recovery of BP-6 and OB from contaminated food was 81 and 71%, respectively, and the peak patterns were practically identical with those of the standards. In contrast to the accumulation from water, fish accumulated both PBB preparations from food.

The relative proportions of the 3 major peaks of BP-6, recovered from fish, were somewhat different from those of the original preparation. The percentage of penta-, hexa-, and heptabromobiphenyl on a peak-height basis was 9, 76, and 15%, respectively, in the original preparation, and 22, 73, 5% in fish extracts after 42 days of feeding.

PBB's recovered from fish, fed OB-contaminated food, contained only a small proportion of the OB bromobiphenyls. The major portion consisted of several partially debrominated biphenyls, and the main component was a hexabromobiphenyl with a retention time similar to that of 2,2',4,4',5,5'-hexabromobiphenyl. Only the original OB bromobiphenyls and this hexabromobiphenyl were quantitated (Table 4).

Data on the accumulation of Aroclor 1254 from food are presented in Table 4 for comparison. A part of these data was published previously (ZITKO and HUTZINGER 1972).

An equilibrium between uptake and excretion of halogenated biphenyls was reached for OB and Aroclor 1254, but not for BP-6. The extrapolated equilibrium values for all three preparations are given in Table 4.

The excretion of halogenated biphenyls during the period of not-contaminated food feeding was very slow. The decrease of Aroclor 1254 concentration was due primarily to growth dilution with the total body burden remaining practically constant.

No mortality in excess of that of control fish was observed in any of the feeding experiments. The PBB feeding experiments were not conducted long enough to determine any effects of these compounds on the growth of the fish.

Debromination of PBB's in fish. Fish, exposed to BP-6 from water, contained several mono- to pentabromobiphenyls, not present in BP-6. Several additional pentabromobiphenyls were detected in fish fed BP-6-contaminated food.

Fish fed OB-contaminated food contained, in addition to the OB bromobiphenyls, unidentified penta-, hexa-, and heptabromobiphenyls (Fig. 1).

It is not known whether the partially debrominated biphenyls are generated by the fish, or by the associated microflora. Partially debrominated biphenyls were not detectable in fish exposed to di-, tri-, and tetrabromobiphenyls (ZITKO and HUTZINGER 1976).

TABLE 4

Accumulation of halogenated biphenyls from food (concentration in food 100 μg/g)

Time, days	Concentration in fish, μg/g wet weight				
	Aroclor 1254	BP-6	OB		
			as such	Br_6[a]	Total
14		16.0			
26	13.9		1.64	7.80	9.44
28		25.7			
42		36.9			
48			2.66	5.80	8.46
59			2.16	7.93	10.1
68	22.6		2.11	9.29	11.4
74			4.20	14.9	19.1
90	20.9				
120	27.9				
Equilibrium[b]	35.8	100	2.32		11.4
Not contaminated food[c]					
14		36.5			
22			1.17	10.0	11.2
28		27.7			
141	7.74				

[a] An unidentified hexabromobiphenyl, not present in the original OB preparation. Additional unidentified bromobiphenyls are also present, but not quantitated. See Figure 1.

[b] Extrapolated values, 1/b, from the equation $c = t/(a + bt)$, where c = concentration in fish, t = time, days.

[c] Feeding of contaminated food was terminated after 224, 42, and 90 days in the case of Aroclor 1254, BP-6, and OB, respectively. Fish fed Aroclor 1254-contaminated food were further analyzed after 160 and 222 days. The concentration of Aroclor 1254 in the fish was 28.2, and 32.1 μg/g, respectively.

Accumulation coefficients. The accumulation coefficient of PBB's is much lower than that of PCB's in the uptake from water, but the situation is reversed for uptake from food in the case of BP-6. For OB, only some of the accumulated bromobiphenyls were quantitated, and the total accumulation coefficient may be 2-3 times higher than listed (Table 5).

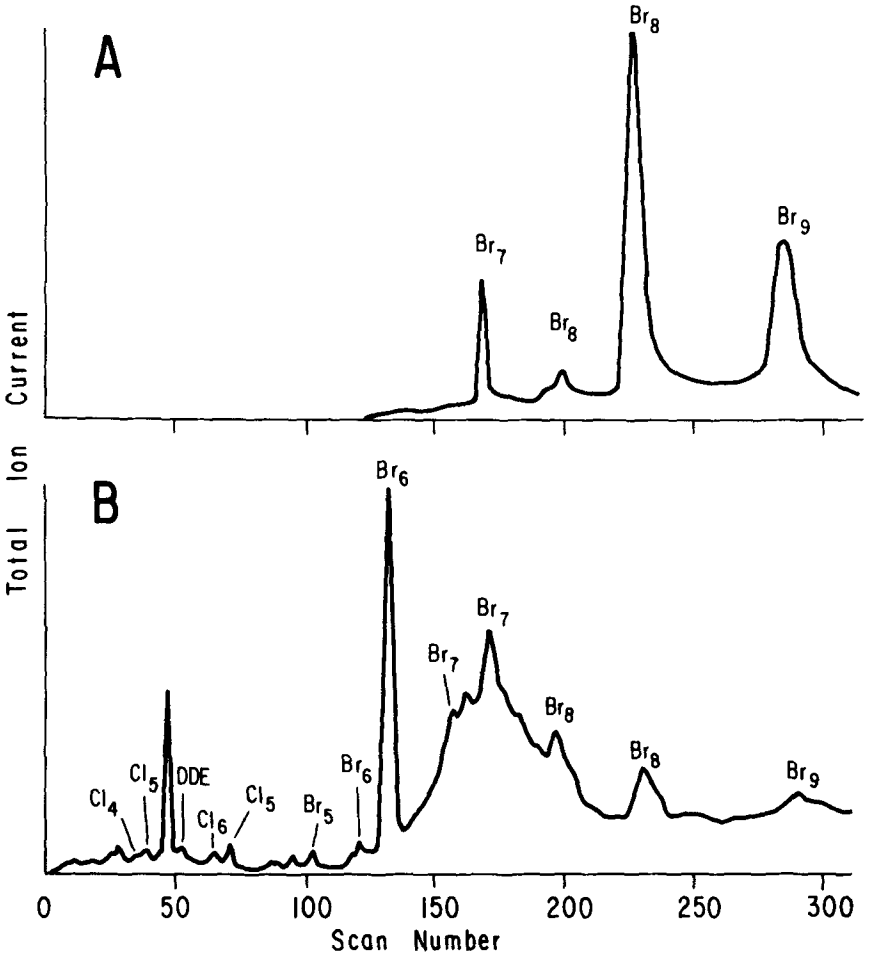

Fig. 1. Reconstructed gas chromatograms of OB (A) and extract of fish fed OB-contaminated food (B).

$Br_n, C\ell_n$ = biphenyls with n bromine or chlorine atoms, respectively.

Chlorobiphenyls and DDE are background contaminants in the fish.

TABLE 5

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

Uptake from	Aroclor 1254	BP-6	OB	
			as such	Br_6[a]
Water, 48 h	282	48	0	1.73
Food, equilibrium	0.358	1.0	0.023	0.114

[a]An unidentified hexabromobiphenyl, not present in OB

CONCLUSIONS

In contrast to bromobiphenyls with up to 4 bromine atoms, which resemble quite closely the corresponding chlorobiphenyls, the more substituted bromobiphenyls are less accumulated by fish from water than the corresponding PCB's, represented by Aroclor 1254. On the other hand, the accumulation of these bromobiphenyls from food is higher or equal to that of Aroclor 1254.

The highly substituted bromobiphenyls are debrominated in the fish. This reaction may have toxicological consequences not encountered with PCB's.

ACKNOWLEDGMENTS

I thank Mr. W.G. Carson for technical assistance, Mrs. Madelyn M. Irwin for typing the manuscript, and Messrs. P.W.G. McMullon and F.B. Cunningham for drawing the figure. Drs. D.W. McLeese and D.J. Wildish commented on the manuscript.

REFERENCES

BABISH, J.G., W.H. GUTENMANN, and G.S. STOEWSAND. J. Agric. Food Chem. 23, 879 (1975).

FRIES, G.F., and G.S. MARROW. J. Dairy Sci. 58, 947 (1975).

FRIES, G.F., H.C. CECIL, J. BITMAN, and R.J. LILLIE. Bull. Environ. Contam. Toxicol. 15, 278 (1976).

GUTENMANN, W.H., and D.J. LISK. J. Agric. Food Chem. 23, 1005 (1975).

LEE, K.P., R.R. HERBERT, H. SHERMAN, J.G. AFTOSMIS, and R.S. WARITZ. Toxicol. Appl. Pharmacol. 34, 115 (1975).

MUMMA, C.E., and D.D. WALLACE. Survey of industrial processing data. Task II. Pollution potential of polybrominated biphenyls. EPA-560/3-75-004, Washington 1975.

NORRIS, J.M., J.W. EHRMANTRAUT, C.L. GIBBONS, R.J. KOCIBA, B.A. SCHWETZ, J.Q. ROSE, C.G. HUMISTON, G.L. JEWETT, W.B. CRUMMETT, P.J. GEHRING, J.B. TIRSELL, and J.S. BROSIER. Appl. Polymer Symp. No. 22, 195 (1973).

SUNDSTRÖM, G., O. HUTZINGER, and S. SAFE. Chemosphere 5, 11 (1976).

ZITKO, V., and O. HUTZINGER. ACS Div. Water, Air, and Waste Chem. 12, 157 (1972).

ZITKO, V., and O. HUTZINGER. Bull. Environ. Contam. Toxicol. in press (1976).

ZITKO, V., P.M.K. CHOI, D.J. WILDISH, C.F. MONAGHAN, and N.A. LISTER. Pestic. Monit. J. 8, 105 (1974).