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Polychlorinated Biphenyls in Extracts of Brain from Manx Shearwaters

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Polychlorinated biphenyls (PCBs) accumulate in the fatty tissues of fish-eating seabirds at the apex of marine food chains by the ingestion of PCB-contaminated lipid components of food. Hence measurements of the concentrations of PCBs that have accumulated can be used to monitor their pollution of the marine environment. In addition, the identification and quantitation of PCB isomers and congeners in the tissues of these birds provides fundamental information on the accumulation of individual PCBs that are present in widely used commercial mixtures of PCBs and which are also highly toxic when tested on laboratory animals. By contrast, the diminished accumulation in tissues of individual PCBs present in commercial mixtures will indicate the ability of fish-eating seabirds to metabolize and presumably excrete individual PCBs that they have ingested *via* marine food chains.

We report here the concentrations of individual PCBs present in the brain tissue of a small sample of manx shearwaters and their enrichment or non-enrichment compared with their proportions in commercial mixtures of PCBs.

MATERIALS AND METHODS

Manx shearwaters (*Puffinus puffinus*) were obtained in 1979 from St. Kilda, an island off the Outer Hebrides, Scotland. This species is partially endangered in the area and consists of a relatively small population with the possible threat of a further decline in numbers. Hence the minimum number of individuals (2 female and 1 male) was caught under special licence as part of a study by the National Environmental Research Councils of Great Britain and stored at -70°C until dissected.

The extraction of total PCBs and 1,1-dichloro-2,2-bis-[4-chlorophenyl] ethylene (p,p'-DDE) from the brain tissue, the separation and quantitation of individual PCB isomers and congeners by high resolution capilliary gas-chromatography and the identification of

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their structures by high resolution capilliary gas chromatography-mass spectroscopy were as described by Borlakoglu (1989c). The IUPAC system of numbering of individual PCB isomers and congeners (see Ballschmitter & Zell 1980) has been used. For convenience, the term 'approximately coplanar' is used to describe non-ortho-substituted PCB congeners.

RESULTS AND DISCUSSION

The mean concentration of total PCBs in the three tissue extracts was 75.5 g/g of wet weight of brain. The only data that appear to be available for comparison are those of Osborn et al. (1987) who reported a 16-fold lower mean concentration of 4.5 g/g of dry weight of adipose tissue in a sample (n = 3) of manx shearwaters obtained from St. Kilda during 1976-77. It is noteworthy that the mean concentration of p,p'-DDE, which is frequently present as a major chemical residue in the fatty tissues of many species, was 0.48 g/g of wet weight of brain in the present study, whereas Osborn et al. (1987) reported a 13-fold higher concentration of 6.34 g/g of dry weight of adipose tissue.

Table 1 details the concentrations of individual PCBs as g/g of wet weight of brain tissue. Since Osborne et al. (1987) did not separate and measure the individual PCBs present in the adipose tissues of manx shearwaters, direct comparisons cannot be made.

Table 1 also gives the enrichment factor that has been calculated (see Borlakoglu et al. 1990a) for each PCB by comparing its abundance in the total PCBs in the extracts of brain tissue with its abundance in one of three commercial mixtures of PCBs (Aroclor 1260, 1248 and 1254) that have been widely used. Hence an enrichment factor of > 1 for an individual PCB suggests that its accumulation in brain tissue exceeds its removal, presumably by metabolism and excretion. By contrast, an enrichment factor of < 1 indicates that its removal, again presumably by metabolism and excretion, exceeds its accumulation in brain tissue.

Calculation of the enrichment factor of an individual PCB that is abundant in commercial mixtures of PCBs will tend to produce a low value, whereas calculation of the enrichment factor of an individual PCB that is a minor constituent of commercial mixtures will tend to give a large value. Despite these limitations in interpreting enrichment factors, this approach has been found to be a satisfactory and useful way of classifying PCBs into those that are enriched in a tissue and those that are not enriched compared with their abundance in commercial mixtures of PCBs (see Borlakoglu et al. 1989a).
We have found (unpublished work) that samples of brain tissue had low concentrations of the cytochrome P-450 components of enzymes involved in the metabolism of drugs and other xenobiotics and did not metabolize [^{14}C]4-monochlorobiphenyl or[^{14}C]2,2',5,5'-tetrachlorobiphenyl at measurable rates. In addition, the action of lipoprotein lipase in the capilliary endothelial cells of the so called 'blood-brain barrier' will limit or prevent the uptake into brain of plasma lipoprotein-PCB complexes. Hence the rate of uptake

Table 1. PCBs in extracts of brain tissue from manx shearwaters

PCB	isomer or congener[a]	Concentration[b] ug/g	Enrichment factor[c]
	Biphenyl	0.003 ± 0.001	
1	2-	0.01 ± 0.01	0.15
8	2,4'-	0.06 ± 0.05	0.70
10	2,6-	0.06 ± 0.05	0.33
15	4,4'-	0.45 ± 0.24	6.04
24	2,3,6-	1.02 ± 0.16	
26	2,3',5-	0.29 ± 0.02	0.55[d]
28	2,4,4'-	0.19 ± 0.01	1.92
30	2,4,6-	0.23 ± 0.04	
44	2,2',3,5'-	0.30 ± 0.23	0.18[e]
47	2,2',4,4'-	0.36 ± 0.14	5.00
49	2,2',4,5-	0.46 ± 0.14	0.51[d]
50	2,2',4,6-	0.17 ± 0.07	
52	2,2',5,5'-	0.49 ± 0.03	2.29
65	2,3,5,6-	0.72 ± 0.23	
66	2,3',4,4'-	0.48 ± 0.15	2.59
67	2,3',4,5-	0.74 ± 0.29	2.85
77	3,3',4,4'-	1.33 ± 1.00	5.76
82	2,2',3,3',4-	0.34 ± 0.10	1.04
88	2,2',3,4,6-	0.45 ± 0.09	0.25
91	2,2',3,4',6	0.48 ± 0.02	1.87
97	2,2',3',4,5-	0.22 ± 0.09	1.44
100	2,2',4,4',6-	0.31 ± 0.16	
101	2,2',4,5,5'-	0.54 ± 0.35	0.36
110	2,3,3',4,6-	0.61 ± 0.27	0.43
118	2,3',4,4',5-	0.40 ± 0.21	0.58
119	2,3',4,4',6-	1.07 ± 0.02	6.38
138	2,2',3,4,4',5'-	5.20 ± 3.90	1.01
139	2,2',3,4,4',6-	0.31 ± 0.10	1.68
151	2,2',3,5,5',6-	1.92 ± 0.40	0.52
153	2,2',4,4',5,5'-	14.00 ±11.50	3.32
155	2,2',4,4',6,6'-	0.91 ± 0.68	
156	2,2',3',4,4',5-	1.84 ± 0.22	5.71
157	2,3,3',4,4',5'-	1.80 ± 1.04	2.77
168	2,3',4,4',5',6-	0.88 ± 0.40	
170	2,2',3,3',4,4',5-	3.30 ± 2.27	5.44
180	2,2',3,4,4',5,5'-	1.09 ± 0.12	2.89[e]
183	2,2',3,4,4',5',6-	2.49 ± 1.15	4.00[e]
194	2,2',3,3',4,4',5,5'-	4.65 ± 2.90	2.94
206	2,2',3,3',4,4',5,5',6-	1.51 ± 3.20	2.06
209	2,2',3,3',4,4',5,5',6,6'-	0.73 ± 0.73	8.41

[a] IUPAC system of numbering and position of Cl atoms
[b] Values are means ± SD (n = 3 individuals).
[c] By comparison with abundance in Aroclor 1260, [d] in Aroclor 1248 and [e] in Aroclor 1254

of individual PCBs into brain cells is likely to be determined by their rates of diffusion across the plasma membrane of these cells, as discussed by Borlakoglu (1989c). In view of this mode of uptake of PCBs into brain tissue and their subsequent minimal metabolism, enrichment of this tissue with individual PCBs provides a useful indication of the potential of other tissues to accumulate PCBs.

It is concluded from the enrichment factors listed in Table 1 that the brain tissue was enriched with the approximately coplanar PCB congeners 15 and 77, which together accounted for 2.77% of the concentration of total PCBs present, as well as with the mono-ortho-substituted congeners 156 and 157 which constituted 4.04% of the concentration of total PCBs in the tissue. The concentration of the mono-ortho-substituted congener 118 in the tissue and its enrichment factor were surprisingly low since it is usually enriched in extracts of many biological materials (Safe 1980). The presence of these approximately coplanar congeners and mono-ortho-substituted congeners is significant since they are very effective inducers of microsomal cytochrome P-450-dependent monooxygenases and are known to produce toxic effects such as loss of body weight and atrophy of the thymus. They also induce the gene expression of drug-metabolizing enzymes that are involved in the metabolism of precarcinogens to carcinogens such as benzo[a]pyrene.

Of the 40 individual PCBs identified, nine congeners (119, 138, 153, 170, 180, 183, 194, 206 and 209) accounted for 60% of the total concentration of PCBs in the tissue. Apart from congener 138, these mainly di-ortho-substituted congeners were greatly enriched compared with their abundance in Aroclor 1260 and Aroclor 1254. This strongly suggests that their accumulation far exceeded their metabolism and presumably excretion.

A group of congeners (1, 8, 10, 26, 44, 49, 88, 101, 110, and 151) was identified that had enrichment factors of < 1, indicating that their metabolism exceeded their accumulation. Despite differences in molecular mass, molecular size, degree of steric hindrance (effects of ortho-chlorine substitution) and intramolecular distance, these non-persistent congeners share the common molecular feature of at least one pair of adjacent unsubstituted meta-para carbon atoms in the biphenyl rings. These results support strongly the 'molecular rule' that we have proposed (Borlakoglu et al. 1988a, 1989b) based on the enrichment or non-enrichment of individual PCBs in the adipose tissue of five species (razorbills, puffins, guillemots, shags and cormorants) of fish-eating seabirds. That is, the presence of meta-para adjacent H-atoms on at least one of the biphenyl rings appears to be a critical structural feature for the metabolism of PCB congeners. This structure presumably allows the correct presentation of carbon atoms to the active site of the cytochrome P-450 component of microsomal monooxygenases to enable either the insertion of an oxygen atom to form arene oxides and for further metabolism to occur, or to abstract a hydrogen atom to permit a direct hydroxylation reaction.

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REFERENCES

Ballschmitter K, Zell M (1980) Analysis of polychlorinated biphenyls (PCBs) by glass capilliary chromography. Composition of technical Aroclor and Clophen PCB mixtures. Z Analyt Chem 302:20-31

Borlakoglu JT, Wilkins JPG, Walker CH, (1988a) Polychlorinated biphenyls in fish-eating seabirds - Molecular features and metabolic interpretations. Mar Environ Res 24 : 15-19

Borlakoglu JT, Walker CH (1989b) Comparative aspects of congener specific PCB-metabolism. Eur J Drug Metab Pharmacokinet 24 (2) 127-131

Borlakoglu JT (1989c) Comparative studies on the biochemical toxicology of polychlorinated biphenyls (PCBs) Ph.D-Thesis, University of Reading, England

Osborne D, Harris MP, Young WJ (1987) Relationships between tissue contaminant concentrations in a small sample of seabirds. Comp Biochem Physiol 87C : 415-420

Safe S (1980) Metabolism uptake, storage and bioaccumulation. In : Kimbrough (ed) Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products, chapter 4, Elsevier/North Holland Biomedical Press, Amsterdam

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