Bull. Environ. Contam. Toxicol. (1994) 53:548–554

Environmental Contamination and Toxicology

PCB Congeners in Tissues of European Otter (*Lutra lutra*)

C. F. Mason, J. R. Ratford

Department of Biology, University of Essex,
Colchester CO4 3SQ, United Kingdom

Received: 30 September 1993/Accepted: 27 March 1994

Otters (Lutra lutra) have declined over much of their European range during the past forty years and are now absent from large areas of the lowlands of Western Europe (Macdonald and Mason in press). The most likely cause of the decline is the effects of bioaccumulating contaminants, organochlorine pesticides and PCBs having been implicated (Mason and Macdonald 1986, Mason 1989). There have been several recent studies of organochlorine residues (pesticides and PCBs) in otter tissues (Mason and O'Sullivan 1992, Mason and Madsen 1993, Mason and Macdonald in press) and scats have been used to monitor residues in otter populations (e.g. Mason et al. 1992, Mason 1993, Mason and Macdonald 1993a). However only from The Netherlands have data on individual PCB congeners in otter tissues (Broekhuizen and de Ruiter-Dijkman 1988) and scats (Hattum et al. 1992) been reported; this Dutch otter population is now extirpated. We report here a survey of PCB congeners in samples of tissues and scats from several populations of otters.

MATERIALS AND METHODS

The otter material analyzed for PCB congeners was as follows:-
1) six livers from Irish animals, a subsample of those used for total PCB analysis by Mason and O'Sullivan (1992), and 2) the brains of five of these animals, 3) five livers from Denmark, a subsample of those presented by Mason and Madsen (1993), 4) six livers from southwest England, a subsample of those presented by Mason and Macdonald (in press), 5) ten scats from western Britain and 6) nine scats from eastern England. Scats were collected from widely dispersed sites. All samples were stored at -20 C.

Samples were homogenized in 7:2 acetone:hexane (approx. 10:1 solvent:tissue, volume:weight). After decanting the solvent the process was repeated with 9:1 hexane:diethyl ether and both reactions were combined. The decanted solvent was filtered through glass wool and mixed with an equal volume of 0.2 M sodium chloride in 0.1 M orthophosphoric acid in a separating

Correspondence to: C. F. Mason

funnel. The non-aqueous phase was collected and evaporated to 1 mL, while a subsample was dried to determine lipid weight. The extract was flushed through a 15 cm column of alumina (7% activated with water) with 50 mL hexane and evaporated to 1 mL. It was then passed through an 8 cm column of silica gel 60 (70-230 mesh, non- activated). The second fraction (2-6 mL) containing PCBs, hexachlorobenzene and p,p-DDE was collected and dried to 1 mL. Standard samples and blanks were extracted after every 5 samples.

Samples were analyzed on a Varian 3300 gas chromatograph with electron capture detector. Good peak separation (notably the closely eluting pair of congeners 149 and 118 and the triplet 163, 138 and 158) was obtained with a BP 5X column (50m, 0.2mm internal diameter, Scientific Glass Engineering). The column temperature program was a) 50 C for 0.5 min, b) 50 C - 200 C at 20 C min^{-1}, c) 280 C for 15 min. The make-up gas was nitrogen, the carrying gas helium at 0.75 mL min^{-1}.

Sample peaks were matched to congener standards. Congener identity was confirmed by using columns of differing separating ability (i.e. BP 5, Scientific Glass Engineering and CP sil 19CB, Chrompack) and by mass spectrometry. Minimum detection limits were 0.5 pg ul^{-1} (equivalent to 0.01 ug g^{-1} lipid).

RESULTS AND DISCUSSION

A total of 24 congeners (identified by IUPAC numbers following Ballschmiter and Zell 1980) was found in the samples (Table 1). The table also provides mean concentrations (with ranges) of total PCBs to allow the calculation of concentrations of individual congeners. These means should not be considered representative of summed PCB concentrations in the otter populations from which samples came because samples for congener analysis were selected from a larger tissue bank (see Mason and O'Sullivan 1992, Mason and Madsen 1993 and Mason and Macdonald in press for summaries of total PCBs in otters from Ireland, Denmark and southwest England respectively). These papers reported total PCBs in livers determined against an Aroclor 1260 standard. Total PCBs in livers determined against individual congener standards averaged 59% (range 11-90%) of total PCBs determined from the Aroclor 1260 mixture. Nevertheless the totals determined by the two methods were highly correlated ($r = 0.93$, $n = 17$, $P < 0.001$).

The Kruskal-Wallis one-way analysis of variance was used to compare differences in the proportions of individual congeners in the regional samples. Significance levels for individual comparisons are indicated below by asterisks, using the following convention: * $P<0.05$, ** $P<0.01$, *** $P<0.001$. Livers from Ireland had significantly greater proportions of congeners 194*, 195**, 196**, 201* and 206**, and significantly smaller proportions of 138* and 153*. Samples from Denmark had a significantly greater proportion of congener 118*.

.Table 1. Mean percentage contribution (with percentage range) of 24 PCB congeners and total PCBs ($mgkg^{-1}$ lipid) in otter tissues (nd = limit of detection)

IUPAC no.	Livers: Southwest England	Livers: Denmark	Livers: Ireland	Brains: Ireland
n	6	5	6	5
99	2.3 : 1.3-2.8	2.8 : 1.8-3.7	1.5 :0.2-2.5	2.3 : nd-5.0
101	0.5 : 0.3-1.1	0.6 : 0.2-1.4	0.4 : 0.1-0.8	1.6 : 1.0-2.2
105	1.3 : 0.9-1.7	1.7 : 1.3-2.1	0.9 : 0.3-2.1	2.1 : 1.3-2.7
110	0.6 : 0.3-1.7	0.6 : 0.2-1.4	0.6 : 0.1-1.1	2.2 : 1.3-3.7
118	4.9 : 2.8-5.9	9.5 : 7.2-14.4	4.9 : 1.6-9.1	8.1 : 5.5-11.9
128	2.7 : 2.2-3.4	1.9 : 0.5-2.8	2.0 : 0.3-2.9	2.4 : 0.7-3.3
138	13.6 : 12.0-14.9	14.4 : 10.5-18.0	10.0 : 2.8-13.7	16.5 : 13.9-18.1
149	0.1 : nd-0.2	0.2 : nd-0.4	0.1 : nd-0.2	0.6 : 0.3-0.9
153	16.3 : 11.7-22.4	17.8 : 11.6-25.6	11.8 : 9.4-14.4	22.7 : 20.2-25.7
156	2.8 : 2.2-3.9	3.7 : 2.3-7.7	3.0 : 0.9-5.1	3.5 : 2.7-4.4
157	0.4 : 0.2-0.6	0.5 : nd-0.8	0.3 : 0.1-0.6	0.4 : nd-0.7
163	17.2 : 12.0-19.9	16.3:12.0-24.1	15.1 : 7.2-19.7	6.8 : 5.7-8.8
170	6.6 : 5.0-8.4	7.3 : 5.6-9.5	7.4 : 4.6-10.1	5.7 : 3.4-7.3
177	3.6 : 1.9-5.6	2.1 : 0.6-3.7	2.7 ; 0.5-7.4	0.5 : 0.2-1.6
180	7.9 : 5.8-11.4	8.8 : 6.5-13.0	11.2 : 6.2-23.7	10.8 : 9.5-12.6
183	1.3 : 0.8-1.7	0.9 : 0.6-1.1	1.0 : 0.6-1.6	1.5 : 0.7-2.7
187	9.6 : 4.9-14.5	4.1 : 1.1-6.2	8.2 : 2.2-18.9	3.3 : 1.8-6.7
189	0.1 : nd-0.2	0.5 : 0.1-0.8	0.2 : 0.1-0.4	0.1 : nd-0.2
194	1.4 : 0.6-2.0	1.3 ; 0.8-2.2	3.4 : 1.3-8.2	3.1 : 2.4-5.2
195	1.0 : 0.6-1.5	0.7 : 0.6-0.9	1.5 : 1.1-2.3	0.8 : 0.7-1.1
196	2.5 : 1.4-3.8	1.0 : 0.7-1.5	3.7 : 1.8-5.4	2.2 : 1.5-3.1
201	2.5 : 1.3-3.8	2.2 : 0.5-4.7	7.0 : 2.5-20.0	1.7 : 1.0-2.7
206	0.8 : 0.3-1.2	0.4 : 0.3-0.6	2.4 : 1.0-7.7	1.0 : 0.7-1.6
209	0.1 : nd-0.5	0.7 : 0.5-1.8	0.2 : nd-0.3	0.1 : nd-0.3
Total	50.9 (2.4-190.4)	58.1 (22.4-103.8)	42.8 (18.5-92.1)	4.7(1.2-14.4)

These differences may relate to patterns of usage of the various PCB mixtures in the countries of origin of the samples.

Overall the most abundant congeners in the samples were, in order of proportion, 163, 153, 138 and 170, each making up 10% or more of the total. The only comparative data for otters are those of Broekhuizen and de Ruiter-Dijkman (1988), who analysed livers from four otters from The Netherlands. They did not determine congener 163 but congeners 153, 138 and 170, as well as 180, made up the greatest proportion of the total.

From five of the Irish otters samples of both livers and brains were analyzed. Overall the sum of PCBs in brains was only 11% (range 3-31%) of that in livers. It is considered that PCBs concentrate less in phospholipids and cholesterol, which dominate the lipid composition of the brain, than in triglycerides, which contribute most to liver lipids (Aguilar 1985, Kawai et al. 1988). Brain samples

Table 2. Mean percentage contribution (with percentage range) of 24 PCB congeners and total PCBs ($mgkg^{-1}$ lipid) in otter scats (nd = limit of detection)

IUPAC No. n	Scats: Western Britain 10	Scats: Eastern England 9	IUPAC No. n	Scats: Western Britain 10	Scats: Eastern England 9
99	4.1 : 2.5-5.7	4.9 : 2.5-8.7	170	4.1 : 2.8-5.4	3.9 : 3.4-5.4
101	3.6 : 1.7-5.0	2.4 : 1.2-3.9	177	0.6 : 0.2-1.7	1.0 : 0.4-3.3
105	3.1 : 2.1-4.8	5.0 : 2.9-6.8	180	6.6 : 3.4-8.9	5.7 : 4.0-7.8
110	9.3 : 4.6-17.9	5.7 : 2.1-13.1	183	1.1 : 0.6-2.2	1.4 : 0.6-4.8
118	9.2 : 6.7-13.4	12.7 : 9.2-13.4	187	2.9 : 1.2-9.8	2.3 : 1.8-3.9
128	2.9 : 1.0-4.1	3.1 : 1.3-4.0	189	0.1 : nd-0.2	0.1 : nd-0.3
138	14.7 : 9.2-18.3	16.1 : 13.0-18.5	194	1.3 : nd-3.4	1.3 : 0.5-2.7
149	3.1 : 1.4-7.8	1.8 : 0.8-3.8	195	0.3 : nd-0.6	0.6 : nd-2.5
153	19.1 : 12.9-23.4	18.3 : 14.9-21.6	196	2.6 : 0.5-9.7	0.9 : 0.4-2.2
156	3.0 : 1.7-3.9	3.0 : 2.2-4.4	201	1.2 : 0.4-3.1	1.3 : 0.9-3.1
157	0.9 : 0.6-1.7	1.0 : 0.9-1.6	206	0.6 : 0.2-1.4	0.7 : 0.1-2.8
163	5.5 : 3.6-7.1	6.7 : 6.4-8.3	209	0.2 : nd-0.6	0.1 : nd-0.4
			Total	5.8 (0.8-13.3)	15.7 (3.7-38.2)

Table 3. Percentage of total PCB congeners in four priority groups of environmental concern, following McFarland and Clarke (1989)

	Group 1	Group 2	Group 3	Group 4
Livers, Southwest England	31.9	29.7	15.7	0.5
Livers, Denmark	38.5	32.2	8.4	1.0
Livers, Ireland	28.2	29.3	17.9	0.5
Brains, Ireland	35.7	42.0	5.5	0.4
Scats, Western Britain	36.8	35.8	4.7	1.0
Scats, Eastern England	43.8	33.8	4.6	1.1

of otters contained significantly greater proportions of congeners 101***, 105*, 110***, 138***, 149***, 153*** and 180* than liver samples, and significantlysmaller proportions of congeners 163***, 170*, 177*, 187*, 195***, 196* and 201*. Thus penta- and hexa- chlorobiphenyls were generally over-represented in brain samples and hepta- and octa- chlorobiphenyls under-represented relative to livers. Similarly, Duinker et al. (1989) found a larger contribution of early eluting congeners in the brains of striped dolphins (Stenella coeruleoalba).

Contaminants in scats (Table 2) are considered to derive largely from the unassimilated proportion in the diet (Mason et al. 1992), which in otters is predominantly fish (Mason and Macdonald 1986). Scat samples tended to have a

greater proportion, relative to liver, of penta-chlorobiphenyls and a smaller proportion of hepta-chlorobiphenyls. Smit and de Jongh (1991) have shown a greater proportion of the higher chlorinated congeners in Dutch otters compared to their major prey items, eels (Anguilla anguilla) and this shift to a higher proportion of more persistent congeners at the top of the food chain is likely to be a general phenomenon (Tanabe and Tatsukawa 1991). Scats from eastern England contained a significantly higher proportion of congeners 105***, 118***, 157* and 163*** and a significantly lower proportion of congeners 101*, 110* and 149*. Overall concentrations were also much higher in scats from eastern England, which has been found to be generally the case with total PCBs in much larger samples (Mason and Macdonald 1993b, c).

McFarland and Clarke (1989) classified PCB congeners into four groups of highest concern as environmental contaminants based on their potential toxicity, frequency of occurrence and abundance. The percentage of summed PCBs in the current samples in these four categories is given in Table 3. Group 1 (only mixed-type inducers in the present study) made up 28-44% of summed PCBs and included congeners 105, 118, 128, 138, 156 and 170. Group 2 (PB- type MFO inducers) made up a further 30-42% of samples (congeners 99, 101, 153, 180, 183, 194). Group 3 are weak or non-inducers while Group 4 are infrequent in biological samples. Thus the most environmentally threatening PCBs (Groups 1 and 2) made up 58-78% of the summed PCBs in the sample.

There have been no experimental studies of the effects of either PCB mixtures or individual congeners on the physiology of otters but the closely related mink is known to be highly sensitive (e.g. Kihlström et al, 1992). In view of the endangered status of many otter populations in Europe, the presence of total PCBs in many individual otters at concentrations known to cause physiological damage in mink (Mason 1989) and the high proportion, in tissues, of the more toxic congeners reported here, there is clearly a need for more detailed congener-specific studies of both otters and their prey.

Acknowledgements. We are grateful for the financial support of the National Westminster Bank, through sponsorship with WWF UK. Tissue samples were provided by S.M. Macdonald, A.B. Madsen, W.M. O'Sullivan and V. Simpson. C. Allchin (Burnham on Crouch Laboratory, Ministry of Agriculture, Fisheries and Food) kindly assisted in confirmations and provided valuable discussion.

REFERENCES

Aguilar A (1985) Compartmentation and reliability of sampling procedures in organochlorine pollution surveys of cetaceans. Residue Rev. 95: 91-114

Ballschmiter K, Zell M (1980) Analysis of polychlorinated biphenyls (PCB) by glass capillary gas-chromatography. Composition of technical Aroclor and Clophen-PCB mixtures. Z. Anal. Chem. 302: 20-31

Broekhuizen S, de Ruiter-Dijkman EM (1988) Otters Lutra lutra met PCB's : de zeehondjes van het zoete water? Lutra 31: 68-78

Duinker JC, Hillebrand MTJ, Zeinstra T, Boon JP (1989) Individual chlorinated biphenyls and pesticides in tissues of some cetacean species from the North Sea and the Atlantic Ocean; tissue distribution and biotransformation. Aquat. Mammals 15: 95-124

Hattum B van, Korthals G, Leonards P, Smit M, de Jongh A (1992) Biologische monitoring van PCB's in een voormalige otterbiotop, De Oude Venen (Friesland). Institute for Environmental Studies, Free University of Amsterdam

Kawai S, Fukushima M, Miyazaki N, Tatsukawa R (1988) Relationship between lipid composition and organochlorine levels in the tissues of striped dolphin. Mar. Pollut. Bull. 19: 129-133

Kihlström JE, Olsson M, Jensen S, Johansson A, Ahlbon J, Bergman A (1992) Effects of PCB and different fractions of PCB on the reproduction of the mink (Mustela vison). Ambio 21: 563-569

Macdonald SM, Mason CF (in press) Status and conservation needs of the otter (Lutra lutra) in the Western Palearctic. Council of Europe, Nature and Environment Series, Strasbourg

Mason CF (1989) Water pollution and otter distribution : a review. Lutra 32: 97-131

Mason CF (1993) Regional trends in PCB and pesticide contamination in Northern Britain as determined in otter (Lutra lutra) scats. Chemosphere 26: 941-944

Mason CF, Macdonald SM (1986) Otters, ecology and conservation. Cambridge University Press, Cambridge, 236pp

Mason CF, Macdonald SM (1993a) PCBs and organochlorine pesticide residues in otter (Lutra lutra) spraints from Welsh catchments and their significance to otter conservation strategies. Aquat. Conserv. 3: 43-51

Mason CF, Macdonald SM (1993b) Impact of organochlorine pesticide residues and PCBs on otters (Lutra lutra): a study from Western Britain. Sci. Total Environ.

Mason CF, Macdonald SM (1993c) Impact of organochlorine pesticide residues and PCBs on otters (Lutra lutra) in eastern England. Sci. Total Environ.

Mason CF, Macdonald SM (in press) PCBs and organochlorine pesticide residues in otters (Lutra lutra) and in otter spraints from southwest England and their likely impact on populations. Sci. Total Environ.

Mason CF, Macdonald SM, Bland HC, Ratford J (1992) Organochlorine pesticide and PCB contents in otter (Lutra lutra) scats from Western Scotland. Water, Air, Soil Pollut. 64: 617-626

Mason CF, Madsen AB (1993) Organochlorine pesticide residues and PCBs in Danish otters (Lutra lutra). Sci. Total Environ. 133: 73-81

Mason CF, O'Sullivan WM (1992) Organochlorine pesticide residues and PCBs in otters (Lutra lutra) from Ireland. Bull. Environ. Contam. Toxicol. 48: 387-393

McFarland VA, Clarke JU (1989) Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners : considerations for a congener-specific analysis. Environ. Health Perspect. 81: 225-239

Smit MD, de Jong AWJJ (1991) PCB contamination of otter areas in the Netherlands. In: Reuther C and Rochert R (eds) Proceedings of the Vth International Otter Colloquium, Habitat 6: 229-234

Tanabe S, Tatsukawa R (1991) Persistent organochlorines in marine mammals. In: Jones KC (ed) Organic contaminants in the environment, Elsevier, London, p275.