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Chlorinated Biphenyls in Fish, Mussels and Birds from the River Rhine and the Netherlands Coastal Area

by

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Polychlorinated biphenyls are widely used in industry, and they are now making an appearance in the world's wildlife. Besides being a hazard in their own right, they interfere with the detection of organochlorine insecticides such as DDT.

At the end of 1966 a note in the *New Scientist*[1] mentioned a group of compounds which appear as unknown peaks in gas chromatograms of the tissues of fish and wildlife. In Sweden it was found that these peaks corresponded to polychlorinated biphenyls (PCBs).

PCBs are used in industry as lubricants, heat transfer media and insulators. They are compatible with paints, synthetic resins, varnishes and waxes, and improve such properties of these products as their chemical resistance, water resistance, adhesiveness and flexibility[2,3].

In 1967, PCBs were reported present in fish and wildlife in Great Britain and the Netherlands[4–6]. In these studies the identification was primarily based on the comparison of the gas–liquid chromatography retention times in the sample extracts with those in solutions of technical PCB mixtures supplied by the industry. No information was given about the identity of the individual peaks.

We describe here the occurrence and distribution of PCBs in the River Rhine and the coastal area in the Netherlands. The identification was carried out by gas chromatography and mass spectrometry. For this purpose a CH 4 mass spectrometer (Varian Mat Gmbh) was coupled to a Varian Aerograph Oven type 1700 P. Particular attention was paid to the design of the interface between the two instruments in order to obtain the best operating conditions[7]. The relative retention times (r_x) were estimated with a 204-*1B* Varian Aerograph gas chromatograph with electron capture detection. Pyrex glass columns (6 feet × 1/8 inch), filled with 10 per cent *DC* 200 on Gaschrom *Q* (80–100 mesh), were used in both gas chromatographs, and the columns were operated at 200° C with helium or nitrogen as carrier gas.

The tissues of the indicator organisms used throughout this study were extracted with petroleum ether in a Soxhlet extraction apparatus after drying with anhydrous sodium sulphate. The samples were cleaned up by di-

Table 1. MASS NUMBERS AND NUMBERS OF CHLORINE ATOMS/MOLECULE (IN PARENTHESES) OF PEAKS WITH RELATIVE RETENTION TIMES (RELATIVE TO HEPTACHLOR = 1) LONGER THAN 0·80 AND SHORTER THAN 1·00

Sampling area	Type of sample	0·25	0·32	0·35	0·51	0·60	r_x 0·63	0·67	0·81	0·86	0·71	0·72	0·83	0·95
1	Mussels (*Anodonta cygnea*) total body extracts 1967, 1968	HCB 292 (5)	326 (5)	326 (5)		326 (5) 290 (4)		290 (4)	290 (4)		290 (4) 370 (6)		324 (5)	324 (5)
1	Grass-frog (*Rana esculenta*) total body extracts 1968	+	+	+		+	+		+		+		+	+
2	Mussel (*Mytilus edulis*) 1969	+			+	+				+			+	
2	Eel-pout (*Lycodes vahlii*) total body extracts 1968	+		+		+	+	+	+		+			+
3	Sandpiper (*Calidris canutus*) tissue and egg extracts 1968, 1969	HCB 292 (5)				326 (5)	290 (4)	290 (4)	Tok-drin 400 (6)		290 (4) 370 (6)		324 (5)	
3	Eider (*Somateria mollissima*) tissue and egg extracts 1968, 1967	HCB 292 (5)				326 (5)			Telodrin 400 (6)		290 (4) 370 (6)		324 (5)	
	Phenarsine DP 6											326 (5)	326 (5)	
	Ancobior 1270											326 (5)	326 (5)	
	Cioplen A 60											324 (5)	324 (5)	

With the exception of HCB, telodrin and compound 370 (?) all compounds identified are conformable to PCBs. + At the given retention time a peak is present in the relevant mass-no. A 10 per cent *DC* 200 on Gaschrom *Q* (80–100 mesh) column was used. (Courtesy C. Cooper, Tox. Sc. Lab., Wadsworth Hospital Center, Los Angeles, Cal. 90025.) The sampling places are indicated in Fig. 2. HCB = hexachlorobenzene; telodrin = 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-endo-5,8-dimethanonaphthalene.

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methylformamide, partition[4] and column chromatography *over activated Florisil*. Column chromatography was performed in two steps. First the apolar compounds including the PCBs were eluted with hexane and then a 10 per cent diethyl ether in hexane solution was used to elute dieldrin and endrin. Only the results of the first fraction analyses are reported here.

In Tables 1 and 2 the relative retention times (relative to dieldrin as 1·00), the mass numbers and the numbers of chlorine atoms/molecule are given. Mass spectra were taken from all peaks present in the total ion current chromatogram obtained with the double ion source of the CH 4 mass spectrometer[4]. In Fig. 1A and 1B gas chromatograms are given of an eider extract and a technical PCB mixture (Phenochlor DP 6). There is a strong resemblance between the electron capture chromatograms and the ion current chromatograms.

Both from the mass numbers and the numbers of chlorine atoms/molecule we conclude that most of the compounds present in the extracts can be identified as PCBs. The retention times increase in proportion to the increase in the number of chlorine atoms/molecule. A remarkable resemblance was found in the chromatograms of Phenochlor DP 6, Clophen A 60 and Aroclor 1260.

Other chlorinated compounds found are the fungicide hexachlorobenzene (HCB), *p,p'*-DDE and the insecticide taledrin. HCB occurs in all places where indicator organisms were collected, and has also been detected in scavenging and predatory birds in the Netherlands[5]. It does not seem likely that the widespread distribution in the aquatic environment is caused merely by the small scale use of the compound in seed dressing practice. On the other hand, it must be emphasized that HCB is a very inert compound which shows a strong tendency to accumulation in animal tissues. Of the DDT derived type of compounds only *DDE* was detected, although one PCB interfered with its determination. In a former study DDT and DDD were detected in seals from the Wadden Sea[6]. Taledrin was only found in sea birds, and from other studies we can conclude that this insecticide must have originated from a pesticide producing plant near the mouth of the river[5,6]. Both in the roach and the birds a compound with $r_x = 0{\cdot}71$, mass number 376 and 8 Cl was detected. Further studies concerning the identity of this compound are in progress. Tetrachlorobenzene and pentachlorobenzene were detected in the roach by the mass spectrometer at relative retention times shorter than 0·25.

There is a marked resemblance between the three sampling areas in the identity and pattern of the PCBs with relative retention times longer than 0·71. It seems likely therefore that most of the PCB material present in the fish and sea birds living in the Wadden Sea originated in the River Rhine. The coastal water chiefly flows northwards. It has been observed that dumpings of chemicals are diluted to a low degree in the littoral current.

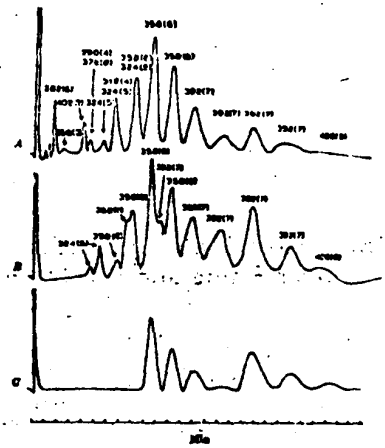

Fig. 1. Gas chromatograms of *A*, an eider extract, *B*, a technical PCB mixture (phenochlor DP 6) and *C*, a quail liver and brain extract (for an explanation, see the text).

In 1965 a clandestine dumping of copper sulphate was displaced over a range of 100 km in a northerly direction within a fortnight. It was diluted by a factor of 10[10].

As can be seen in the tables, the lower chlorinated PCBs occur more frequently in the tissues of birds than in the sea birds. This may indicate that the lower chlorinated PCBs are less persistent, and this has been confirmed by an experiment with Japanese quail which were fed a diet containing 2,000 p.p.m. of phenochlor DP 6 of which the chromatogram is given in Fig. 1*B*. All birds ($n = 20$) died between 6 and 55 days after dosing started. A chromatogram representing the residue in the tissues (liver and brain) of these birds is given in Fig. 1*C*. It can be seen that a number of the compounds present in the original mixture were metabolized, particularly the lower chlorinated ones.

Residues in the brains and livers of the quail were measured on a semiquantitative scale by peak height comparison of peak $r_x = 1{\cdot}45$ using the Phenochlor DP 6 mixture as a standard. Residue levels calculated in this way were about twenty times higher in the quail tissues than in the livers and brains of the eiders. The quail

Table 2. MASS NUMBERS AND NUMBERS OF CHLORINE ATOMS/MOLECULE (IN PARENTHESES) OF PEAKS WITH RELATIVE RETENTION TIMES (RELATIVE TO DIELDRIN = 1) OF PEAKS SMALLER THAN 1·00

Sampling area	Type of sample	1·01	1·17	1·22	1·45	1·57	1·69	1·80	1·87	1·97	2·20	2·56
1	Roach (*Leuciscus rutilus*) total body extracts 1967, 1968	DDE 318 (4) 316 (5)	316 (5)	316 (5)	316 (5)	302 (7)	324 (6) 302 (7)	302 (7)	302 (7)	302 (7)	302 (7)	426 (8)
1	Greenfinch (*Chloris chloris*) total body extracts 1968	+	+	+	+	+	+	+	+	+	+	+
2	Mussel (*Mytilus edulis*) 1966	+	+	+	+	+	+	+	+	+	+	+
2	Lugworm (*Arenicola marina*) extract 1966	+	+	+	+	+	+	+	+	+	+	+
3	Sandwich tern (*Sterna sandvicensis*) tissue and egg extracts 1965, 1966	DDE 318 (4) 316 (5)		316 (5) 314 (5)	316 (5)		316 (5)	302 (7)	302 (7)	302 (7)	302 (7)	426 (8)
3	Eider (*Somateria mollissima*) tissue and egg extracts 1966, 1967	DDE 318 (4) 316 (5)		316 (5) 314 (5)	316 (5)		316 (5)	302 (7)	302 (7)	302 (7)	302 (7)	426 (8)
	Phenochlor DP 6	316 (5)	316 (5)	316 (5)	316 (5)	302 (7)	324 (6)	302 (7)	302 (7)	302 (7)	302 (7)	426 (8)
	Aroclor 1260	316 (5)	316 (5)	316 (5)	316 (5)	302 (7)	324 (6)	302 (7)	302 (7)	302 (7)	302 (7)	426 (8)
	Clophen A 60	316 (5)	316 (5)	316 (5)	316 (5)	302 (7)	324 (6)	302 (7)	302 (7)	302 (7)	302 (7)	426 (8)

With the exception of DDE all compounds identified are conformable to PCBs. + All the given relative time a peak is present in the electron capture gas trace.

See notes under Table 1.



Fig. 2. Location of sampling areas: Texel White (1), coastal area along the west coast (2) and the Wadden Sea (3).

developed hydropericardia with a dose of 2,000 p.p.m. Fitch *et al.*[10] also discovered hydropericardia in White Leghorn cockerels dosed with 400 p.p.m. in their diet. No hydropericardia were observed in birds which died in the Wadden Sea area. Details of this study will be published separately.

The peculiar physical properties and chemical stability of PCBs make it likely that the application will increase and that their release into the environment will continue. Although fatal concentrations are probably not developed yet along the Dutch coast, we suggest that monitoring of the environment should be carried out regularly in order to keep an eye on the further trends in the level of contamination. Apart from being a potential hazard in the natural environment, these PCBs also represent an important source of interference in the detection of insecticides such as DDE, *o,p'*-DDT, *p,p'*-DDT and DDD.

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1 *New Sci.*, **22**, 612 (1964).
2 *Monsanto Company Tech. Bull. No. PL-305* (1970).
3 *Encyclopedia of Chemical Technology* (Edit. by Eller Kirk, R., and Othmer, D. F.), revised rev. ed., **5**, 339 (1964).
4 Holmes, D. C., Simmons, J. H., and Tatton, J. O'G., *Nature*, **216**, 227 (1967).
5 Ecklen, A. V., and Monakov, K., *Nature*, **214**, 1274 (1967).
6 Koeman, J. H., Ockhamp, A. A. M., Veen, J., Brouwer, F., Roels, J., Zwart, P., v.d. Broek, E., and van Genderen, H., *Meded. Rijksfaculteit Landbouwwetenschappen Gent*, **32**, 511 (1967).
7 ten Noever de Brauw, M. C., and Brunace, C., *R. Dutch. Chem.*, **219**, 321 (1967).
8 de Faubert Maunder, M. J., Egan, H., Goulty, E. W., Hammond, E. W., Roberts, J., and Thomson, J., *Analyst*, **88**, 166 (1963).
9 Vos, J. G., Broekhuizen, Hetty A., and Notebelt, p. H., *Meded. Rijksfaculteit Landbouwwetenschappen Gent*, **35** (in the press).
10 Koeman, J. H., and van Genderen, H., *J. Appl. Ecol.*, **3** (suppl.), 93 (1966).
11 Koeman, J. H., Veen, J., Brouwer, F., de Goeij, J., Hoekman de Leeuw, L., and Ysebaert, J. L., *Bijdr. Wetenschap. Mededelingen*, **47**, 1 (1968).
12 Zeeman, E. H., *Proceedings of the Conference on Oil Pollution of the Sea, Rome, October 7–9, 1968*, Paper No. 21.
13 Fitch, Ch. T., Gish, L. G., and Childs, V. A., *Poultry Science*, **44**, 1079 (1965).

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