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Composition of PCB Mixtures in Biotic and Abiotic Marine Compartments (Dutch Wadden Sea)

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INTRODUCTION

The presence of polychlorinated biphenyls in the environment was discovered by JENSEN (1966), using techniques involving GLC-separations with packed columns. Essentially the same technique has been applied since in numerous analyses of these compounds carried out on a worldwide scale.

The use of packed columns results in poor separation of the constituents of the usually complex PCB mixtures. The separations are often performed in an isothermal mode at about 200°C. Under such conditions, relatively volatile compounds elute very early, thus appearing almost unresolved from each other as well as from other volatile compounds. The importance of early eluting PCB components has been overlooked in many reports.

In combination with these packed column separations, qualitative and quantitative analyses were performed by comparison of chromatograms of sample extracts and of some technical formulation, e.g. Aroclor 1242, 1254 or 1260. The selection of the formulation to be used for comparison has been more art than science because as a rule, the chromatogram of an environmental sample differs from that of any of the formulations. The difficulties with early eluting components taken into account, it is understandable that in most cases, formulations with a high degree of chlorination have been selected, e.g. Aroclor 1254 and 1260 or the equivalents Clophen A50 and A60.

However, packed column separations are inadequate to represent the detailed composition of PCB mixtures in environmental samples. This was demonstrated by application of both capillary and packed column separation techniques to seawater extracts (DUINKER et al., 1980). In this paper we want to show that such problems exist not only for seawater extracts but in fact for a wide range of marine samples and in addition that large differences may exist between various environmental compartments in their composition of PCB mixtures. Examples will be given for water, suspended matter, homogenates of shrimp and plaice and blubber of a harbour porpoise, all from the Dutch Wadden Sea. A qualitative and quantitative evaluation will be made of temperature programmed capillary column GLC-ECD chromatograms on the basis of 102 (out of 209 theoretically possible) PCB components, available in sufficiently pure form to be used as reference compounds. In addition, use will be made of the recent capillary column GLC-ECD and GLC-MS analyses of Clophen formulations (DUINKER & HILLEBRAND, in press b).

MATERIALS AND METHODS

Samples were obtained from the Dutch Wadden Sea in the summer of 1980. Water and suspended matter (100 l) were collected with an air-lift system (TOKAR et al. 1981). Phase separation was done by pressure filtration. These forms were analyzed separately. Water was subjected to continuous liquid-liquid extraction on board ship involving 400 ml n-hexane. Stainless steel containers were used for intermediate storage and the sample was transferred through stainless steel piping and teflon tubing. Particulates were Soxhlet-extracted in the laboratory. Full details are described in DUINKER & HILLEBRAND (in press, a). All equipment was carefully cleaned before use. The entire procedure was checked for possible contamination. The liquid-liquid extraction procedure appears to be 80-100% efficient. Homogenates of shrimp and plaice (prepared in a Warren blender) and blubber of a stranded porpoise were extracted in a Soxhlet with n-hexane. The extracts were subjected to clean-up over aluminium oxide. n-Hexane used in all cases was Nanograde quality (Mallinckrodt). The extracts were eluted over silica with n-hexane, resulting in a fraction containing PCB. Several concentration steps were performed in a Kuderna-Danish concentrator equipped with a three-ball Snyder column. The entire procedure has been described in detail (EDER et al. 1981).

The extracts were analyzed by temperature programmed capillary column GLC-ECD. Conditions were as follows: 50m x 0.33 mm WCOT SE-54 fused silica column; carrier gas He 130 kPa, autosampler injection 1 µL splitless; make-up gas N_2 30 ml min^{-1}; detector 320°, injector 230°, septum and injector purge 5 and 20 ml min^{-1} He; isothermal phases at 60°C (2 min), 180°C (15 min), 210°C (5 min) and 250°C (10 min), intermediate temperature increase rates 10,4 and 4°C min^{-1} respectively; gaschromatograph Hewlett Packard model 5880A with a pulsed ^{63}Ni-electron capture detector. 102 individual PCB components were available to us as reference compounds. The assignments of peaks in the present work differs in several aspects form those given by BALLSCHMITER & ZELL (1980). Retention times and response factors were determined for all individual components. A synthetic mixture was prepared of well-separated components in known concentrations, selected as to produce similar heights for all peaks. This mixture as well as solutions of individual components were used for the qualitative and quantitative analyses of Clophens by GLC-ECD and GC-MS (DUINKER & HILLEBRAND in press, b) and of environmental samples (this work). The synthetic mixture was injected between each 9 samples and recalibration was performed automatically. For a positive identification, the fit in retention time over the entire chromatogram had to be within 0.5%. Peaks in the sample chromatograms (Fig. 1) were assigned to individual components on the basis of their retention times, taking into account the composition of the corresponding peaks in technical formulations (Clophens, Fig. 2), in the following way.

For all peaks in the sample chromatograms, PCB components with the appropriate retention times (as determined by GC-ECD) and the appropriate number of chlorine atoms (as determined by GC-MS), are identified, in Table 1 in order of elution, by their IUPAC numbers as given by BALLSCHMITER & ZELL (1980). The corresponding peak in Figs 1 and 2 is labelled by the IUPAC number of the component involved. In cases of two or more co-eluting components, only one is used for labelling the peak. For some peaks of the Clophens, no individual component was available as reference compound. These peaks are labelled "u" (unidentified PCB component) in the Clophens and in the sample chromatograms. Finally, chromatograms of the samples also contain some peaks which do not appear in the Clophens and thus do probably not represent PCB components. These are not labelled at all in Fig. 1. Response factors for quantitative estimates were based on peak heights of individual components in a synthetic mixture containing 102 components. Although a quantitative analysis is generally not possible for peaks in which two or more components co-elute, quantitative estimates of the contribution of PCB components in such peaks can still be reasonably accurate because the response factors of components with chlorine numbers which do not differ by more than one, are similar.

RESULTS

Qualitative aspects

Chromatograms of the PCB fractions of water, suspension shrimp, plaice and porpoise are represented in Fig. 1. The constancy of retention times allows corresponding peaks to be correlated accurately by vertical lines in the Fig. as well as with Fig. 2. Early eluting PCB components are important in water. They become relatively less important in suspension and even less in biological samples, where components appearing later in the chromatograms, become progressively more important in the series shrimp, plaice and porpoise. However, the trend within the entire series of samples differs between the various peaks. In all samples, unidentified (u)peaks happen to be relatively weak and their number (2-7) is in the order of 10% of the number of identified peaks in each sample. Some peaks, although corresponding to at least one individual PCB component available as reference compound, have not been found or only in trace amounts in Clophens (e.g. peaks corresponding to components 1,3,14,12 and 30). Although these have been identified in Fig. 1 for comparison, they may not represent PCB components. Based on the comparison with the 102 available components, 49 out of the total number of 60 peaks are composed of only one component; 43 of which are known, the remaining six yet being unidentified.11 peaks correspond to Clophen peaks consisting of more than one component.

Quantitative aspects

Quantitative results are given in Table 1. Accurate data were obtained for the 43 single-component peaks for which the component was available as reference compound. Peaks consisting of more than one component were evaluated on the basis of average response factors of the components likely to be involved on the basis of the compositions of the Clophen formulations. The components considered in the calculations are underlined in Table 1. For each sample, total PCB concentration (last row in Table 1) was taken as the sum of the individual concentrations. Peaks labelled u, 1,2,14,12 and 30 were not taken into consideration in the calculations. The individual concentrations in each peak are given as percentage of total PCB concentration. It should be observed that the distinction of closely eluting components is given in much more detail for the Clophen formulations due to availability of GC-MS data in this case.

DISCUSSION

The chromatograms of Fig. 1 are characteristic for a large series of samples that we have analyzed for each of the compartments considered in this paper. Many peaks are present in all compartments. Early eluting peaks contribute significantly only in water and suspension while peaks eluting later in the chromatograms become progressively important in the series shrimp-plaice-porpoise.

Capillary column separation is still incomplete and the analysis of PCB mixtures in terms of individual components is not yet ideal therefore. Some aspects might be solved by GC-MS, e.g. the relative contributions of components with different chlorine numbers to a particular peak, but such problems associated with co-eluting components with identical chlorine numbers remain unsolved. However, these problems do not limit the validity of the qualitative and semi-quantitative arguments presented here and moreover, the number of peaks for which such problems exist, is relatively small.

We shall investigate the compositional similarities and differences between the environmental compartments as well as between each compartment and the various Clophen formulations. The chromatographic region where dominant peaks occur in water, correspond roughly with that of Clophen A30. However, the strongest peaks in water (labelled 1,14 and 12) were not found in Clophen A30 and the strongest peaks of A30 (5,18,15,u,31,28,u) are relatively weak in water. Here, most of the significant peaks occur with similar height until component 138. Several components with chlorine numbers as high as 5 and 6 are present in water at a higher fraction of total PCB concentration than in A30.

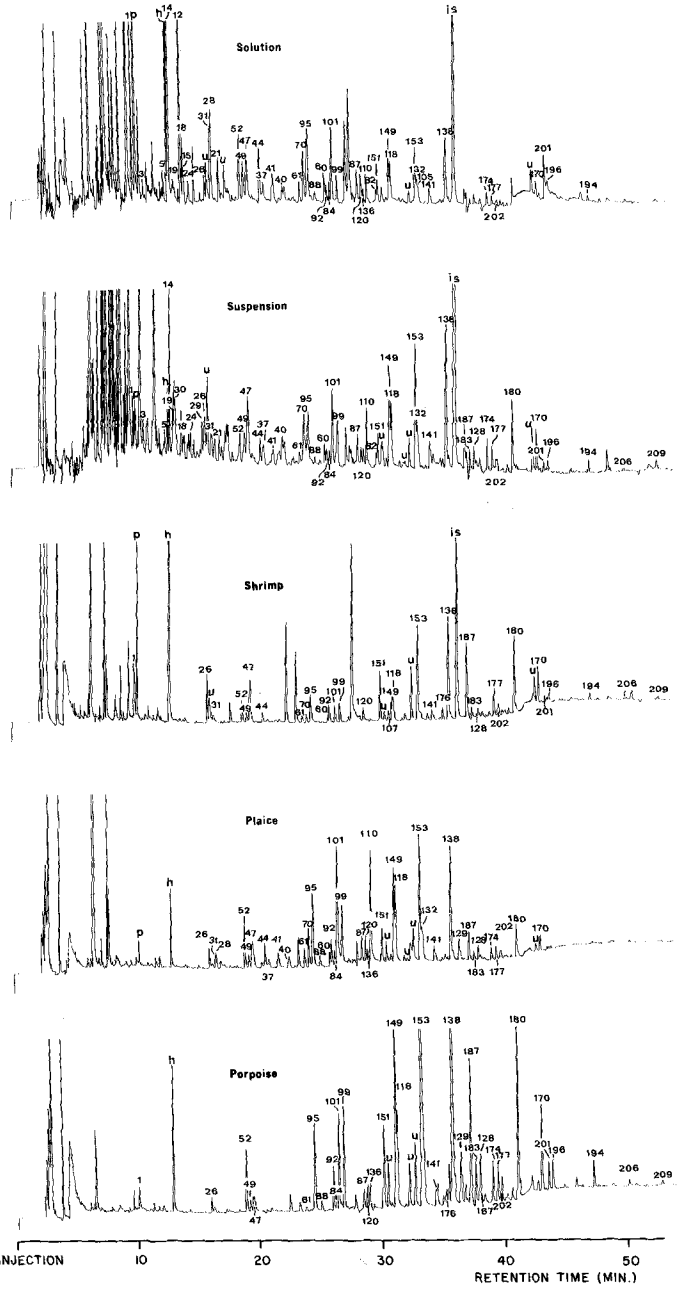

Fig. 1.
Temperature programmed capillary ECD chromatograms of water, suspended matter, homogenates of shrimp and plaice and blubber of a stranded harbour porpoise (Dutch Wadden Sea). is=internal standard; p,h=penta- and hexachlorobenzene; PCB component identification by IUPAC numbers and by "u" if yet unknown.

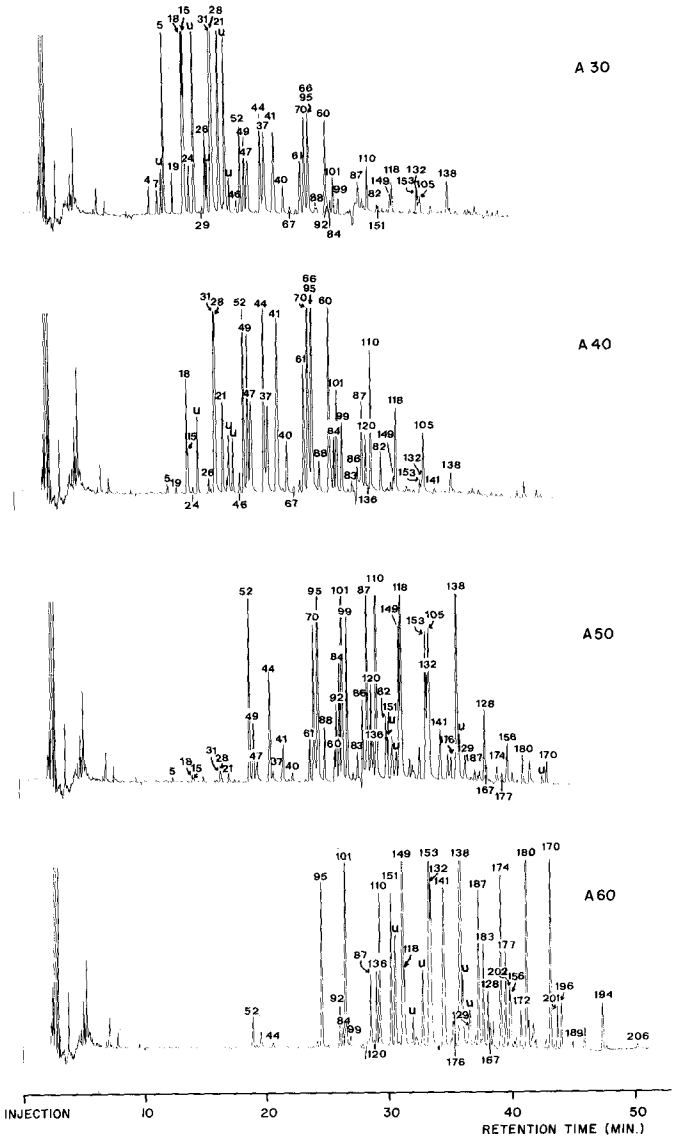

Fig. 2.
Temperature programmed capillary ECD chromatograms of Clophen A30, A40, A50 and A60. Component identification by IUPAC numbers and by "u" if yet unknown.

Table 1

Concentration of individual PCB components in samples from the Dutch Wadden Sea: water, suspended matter (seston), homogenates of shrimp and plaice and blubber of a harbour porpoise. The sum of the individual concentrations is given in the last row. Individual concentrations are expressed as percentage of the sum for the samples and for the Clophen series (Duinker & Hillebrand, in press b). For the calculations involving co-eluting components, response factors were calculated as average values of the underlined components. Contributions below 0.1% are omitted.

IUPAC numbers (chlorine numbers) of PCB components. Co-eluting components in the same row.		Fraction in % of total PCB content in					IUPAC NUMBER	Fraction in % of total PCB content in Clophen			
		Water	Seston	Shrimp	Plaice	Porpoise		A30	A40	A50	A60
5 (2)	8 (2)	2.0	1.6				5+8	6.1	0.2		
19 (3)		1.9	2.6				19	1.2			
18 (3)		6.0	2.1	0.5			18	9.9	3.8		
15 (2)	u (3)	4.7	2.1	0.8			15	9.0	0.8		
24 (3)		0.7	2.1				24	0.5			
29 (3)		0.3	2.1				29	0.2			
26 (3)		1.4	2.8	7.8	1.1	0.6	26	2.1	0.4		
u (3)											
31 (3)		1.1	1.4	1.0	0.4		31	6.8	2.4	0.1	
28 (3)	50[a] (4)	1.8	1.4	1.2	0.3		28	9.9	4.0		
21 (3)	33(3),53(4)	1.0	1.4				21,33	4.6	1.3		
							53	0.5	0.5		
u (4)											
52 (4)		6.0	2.1	8.5	4.2	4.1	52	3.1	7.3	6.8	1.1
49 (4)		2.7	2.8	4.4	0.9	0.7	49	1.2	4.1	1.5	
47 (4)	75 (4)	1.2	4.9	0.4	1.0	0.2	47	0.4	0.7	0.1	
							75	0.9	1.7	0.2	
44 (4)		3.9	2.1	2.8	0.5		44	3.0	6.6	3.3	
37 (3)	42 (4)						37	3.2	1.2		
		2.4	2.1	1.0	0.2		42	0.4	1.2	0.3	
41 (4)		1.8	1.4	2.2	0.3		41	1.6	3.5	0.7	
40 (4)		1.1	1.4	1.4	0.2		40	0.7	1.2	0.2	
61 (4)		0.9	0.7	1.2	0.8	0.1	61	0.9	2.2	0.8	
70 (4)	80(4),98(5)	4.8	4.9	6.0	3.4		70		1.9	1.2	
							80	2.5	1.8	1.2	
							98		1.8	1.1	
95 (5)	66 (4)	5.5	3.5	6.9	4.0	2.9	95	0.3	0.6	2.3	3.9
							66	2.3	5.7	1.6	
88		0.4		0.4		0.1	88	0.2	0.4	0.4	0.1
60 (4)		1.1	1.4	0.5	0.6		60	1.5	3.1	1.6	0.8
92 (5)		0.6		0.7	0.7	1.2	92			1.3	0.5
84 (5)		0.8	0.7	0.1	1.0	0.1	84	0.4	1.3	2.7	0.4
101 (5)		6.1	4.2	6.0	7.0	3.3	101	0.7	2.3	6.1	4.1
99 (5)		1.6	2.1	6.0	3.3	3.5	99	0.3	1.1	2.5	0.2
87 (5)	90,116(5)	0.5	0.7	0.6	0.4	0.3	87,90,116	0.4	1.1	3.5	0.9
120 (5)		0.3	0.7	3.0	0.5	0.3	120	0.3	1.0	1.9	
136 (6)		0.8			0.2	0.3	136	0.1	0.1	0.7	1.5
110 (5)	77 (4)	9.4	8.5		16.8		110	1.0	2.8	9.7	3.6
							77	0.3	0.7	1.1	
82 (5)		0.3	0.3		0.2	0.2	82	0.2	0.9	1.4	0.1
151 (6)		1.0	1.4	6.0	1.9	2.2	151	0.1	0.1	0.6	4.7
u (6)											
149 (6)	123 (5)[a]	3.2	2.1	1.0	15.8	7.0	149	0.5	0.4	4.1	9.6
118 (5)		1.6	1.4	3.0	14.0	2.2	118	2.5	6.7	10.5	1.0
u (6)											
u (6)											
153 (6)		3.6	4.2	6.4	6.6	22.5	153	0.5	0.3	3.2	8.6
132 (6)		2.3	2.8		1.4	2.6	132	0.2	0.5	3.1	4.6
105 (5)		2.6	2.1		0.9	0	105	0.5	1.5	0.7	0.2
141 (6)		0.8	1.4	1.0	0.9	0.2	141			0.7	1.8
179 (7)		0.8	0.5	0.2	0.9	0.3	179				0.7
176 (7)				0.3		0.4	176			0.7	1.3
138 (6)		3.7	4.9	6.4	6.1	16.7	138	0.8	0.5	6.0	11.3
187 (7)			1.4	3.0	2.3	4.0	187	0.1		0.3	3.8
183 (7)			0.7	0.2	0.5	1.8	183			0.3	3.1
128 (6)		0.6	1.4	0.2	0.1	1.6	128			1.4	1.2
174 (7)		0.7	1.4	0.2	0.1	1.8	174			0.3	4.9
177 (7)		0.7	1.4	1.0	0.2	1.8	177			0.3	3.4
202 (8)	193 (7)	0.1	0.2	0.2	0.2	2.0	202				0.8
							193				0.8
180 (7)		1.6	3.5	2.0	1.9	7.5	180	0.3	0.2	0.2	8.9
u (8)											
170 (7)		1.1	2.8	1.4	1.1	3.5	170			0.6	5.2
201 (8)		2.4	0.7	0.2		1.5	201				1.4
196 (8)		1.6	0.6	0.2	0.1	1.8	196				1.4
194 (8)		0.2	0.6	0.1		1.8	194				1.3
206 (9)			0.1			0.4	206				0.1
209 (10)			0.5	0.1		0.3	209				0.2
Sum PCB content		620 pgL^{-1}	140 $ng\ g^{-1}$	500 $ng\ g^{-1}$	2150 $ng\ g^{-1}$	22700 $ng\ g^{-1}$	(Lipid basis).				

The region of significant peaks in suspension reflects that of Clophen A40 until component 95 and Clophen A50 beyond 95. However, several penta- and hexachloro components have larger contributions in suspension than in Clophen A50. The number of chlorine atoms of the significant components ranges 3-7. In both regions differences can be observed in the relative heights of adjacent peaks. e.g. 52-49-47-44; 149-118, etc.
In shrimp, some of the early eluting components are present. The important components have chlorine numbers in the range 4-7. The strongest peaks correspond with the strongest ones in A60. However, several differences from A60 are observed for e.g. 149-118 and 153-132-105. Comparable conclusions can be drawn for the similarity and differences between plaice and A50 and porpoise and A60. Of the entire series of samples, porpoise blubber shows the most striking similarity to a particular formulation. The main components have chlorine numbers in the range 5-8. Early eluting components are missing, the first components is 52 and the percentage contributions to total PCB are similar to Clophen A60 for practically each component. However, they are not identical. Thus, the composition of the PCB mixtures in any of these marine compartments cannot be described accurately in terms of any single Clophen, Aroclor (or Phenoclor etc.) formulation. Also, the individual components differ widely in their distribution over water, suspension and various organisms. These different distribution patterns result from intercomponent differences in physicochemical properties which affect solubilities, adsorption, evaporation, etc. Individual components also have interspecies differences in uptake, transformation and excretion mechanisms. Laboratory experiments will assist in further understanding the relative importance of mechanisms involved in the distribution patterns of each compound. These observations support the idea that, in order to understand their sources, transport mechanisms, sinks and effects, analyses of PCB should be carried out in terms of individual components rather than in technical formulations (DUINKER et al., 1980).

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