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An Evaluation of Existing Methods for Quantitation of Polychlorinated Biphenyls in Environmental Samples and Suggestions for an Improved Method Based on Measurement of Individual Components*

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Some industrially produced organohalogen compounds, in particular polychlorinated biphenyls (PCB's), are distributed on a world-wide scale in soil, atmosphere, water, sediment, fish, wildlife and humans. Great concern exists about their presence in seawater, even in the open ocean. The North Atlantic is considered as the ultimate sink (ANON 1979).

PCB's have been produced for about 35 years before they were identified as environmental contaminants by JENSEN (1966). They have been produced by various industries in the form of technical formulations with overall chlorine contents roughly in the 20-80 % range depending on the manufacturing process (Table 1). Each formulation is a complex mixture of many of the more than 200 theoretically possible components, differing in the number of chlorine atoms (1-10) and also in their relative positions in the molecular structure. The average number of Cl atoms per molecule increases with overall chlorine content of the formulation (Table 1).

METHODS FOR QUANTITATION OF PCB's

In the literature, essentially two different approaches to quantitation of PCB's in environmental samples have been described intensively.

a. Quantitation in terms of technical formulation equivalents. The generally used method is based on a comparison of peaks with corresponding retention times in packed column ECD-GLC chromatograms of sample and some technical formulation. This selection is usually made on the basis of as close a fit as is possible between the chromatographic patterns. Thus, Aroclor 1242 may be selected as standard for samples with a relatively large contribution of lower chlorinated components (appearing as early eluting peaks) and Aroclor 1254 or 1260 for samples with increasing contributions of components with higher chlorine content, appearing later in the chromatograms (Fig. 1). Concentration data obtained by comparing peak heights (or areas) in chromatograms of samples and technical formulations are expressed in terms of what will be described

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Table 1

Number of chlorine atoms (n) in empirical formula of polychlorinated biphenyls $C_{12}H_{10-n}Cl_n$, the percentage chlorine (%Cl) and the number of isomers in each empirical formula and the approximate percentage composition of some commercial Aroclors. (SAFE & HUTZINGER 1973, ANON 1973).

n	% Cl	no. isomers	Approximate percentage composition of some Aroclor types 1221	1242	1254	1260
0	0	1	11	<0.1	<0.1	-
1	18	3	51	1	<0.1	-
2	31	12	32	16	0.5	-
3	41	24	4	49	1	-
4	48	42	2	25	21	-
5	54	46	0.5	8	48	12
6	58	42	-	1	23	38
7	62	24	-	<0.1	6	41
8	65	12	-	-	-	8
9	68	3	-	-	-	1
10	79	1	-	-	-	-
Average number of chlorine atoms per molecule			1.15	3.10	4.96	6.30

here as technical formulation equivalents. Many possibilities exist for the combination of peaks for comparing and quantitating. The number of peaks selected by various authors covers a wide range from the minimum value of one to up to more than ten peaks; usually the sum of an arbitrary combination of these peaks is taken for comparison.

This method for quantitation is inaccurate. This is due to a number of factors, that will be described below. Most peaks in packed column-GLC of PCB's are unresolved; they are composed of more than one individual polychlorinated biphenyl. Moreover, these have not necessarily the same number of Cl atoms (Figs. 1a, 1b). In addition, peaks in chromatograms of formulations with different overall chlorine content (e.g. Aroclor 1254 and 1260) with the same (or practically the same) retention times are not necessarily composed of the same individual components. GCMS data show that this problem exists for several peaks. (Fig. 1). Moreover, the chemical composition of the technical product is different in different batches. Another complicating factor is that in any peak, this composition may be different from the composition in the corresponding peak (i.e. with the same retention time) of environmental samples (Fig. 1). This is the result of the large range of release

mechanisms of PCB components into the environment and also of the large range of physical, chemical and biological transport, transformation and degradation processes in the environment, having different effects on the behaviour of each individual component. Finally, the response of the electron capture detector for individual components depends strongly on the number of chlorine atoms as well as (although to a lesser extent) on their relative positions in the molecular structure. The molecular response increases strongly with the number (n) of Cl atoms at low values of n (1-4) and less strongly at higher values of n. Thus, a relatively large contribution of lower chlorinated components in the sample may be underestimated as result of the relatively low intensity of their (early eluting) chromatographic peaks.

These observations that are generally valid for the analysis of any type of environmental sample, have several consequences for the reliability of results obtained with the quantitation method that we are considering here.

a. It is difficult, if not impossible, to define a mixture of technical formulations (or more common, a single formulation such as e.g. Clophen A50) with a composition of individual PCB components identical to that of any environmental sample. Their use as standards for quantitation of PCB's is not justified therefore.
b. As the relative intensities of the (unresolved) PCB peaks in sample chromatograms are usually different from those of (single) technical formulations, values calculated for PCB contents of samples in terms of technical formulation equivalents, depend on the combination of chromatographic peaks selected for quantitation. The selection is commonly made on the basis of visual inspection of the chromatograms. Anyone studying a particular sample chromatogram may select various combination of peaks without having an unbiased estimate to select the "best" set.

   It is difficult to estimate to what extent quantitative data in the literature would vary with different combination of peaks, as in many reports such important information as the chromatographic patterns have not been given. Even in various intercalibration exercises, the chromatographic conditions and the combination of peaks for quantitation have been left to the discretion of each participant, resulting in uncomparable data.
c. It should be realized that even in such rare cases where the relative intensities of the various peaks in chromatograms of technical formulation and samples are *identical or indistinguishable*, ambiguity still exists. In these cases the quantitative results depend neither on the number nor on the combination of peaks selected for comparison. However, the technical formulation will still

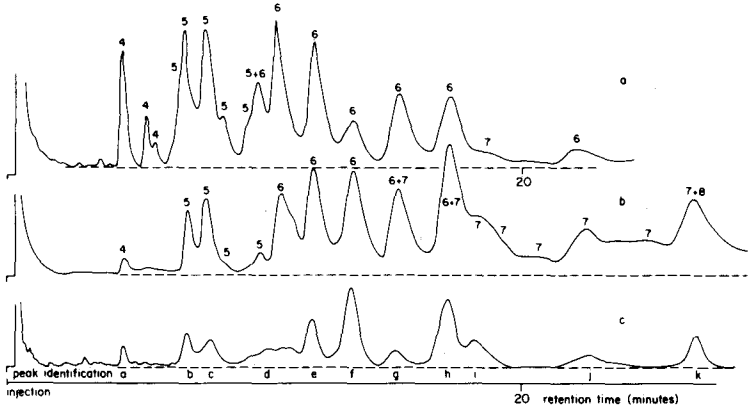

Fig. 1
ECD-chromatograms of Aroclor 1254 (a), Aroclor 1260 (b) and the first silica fraction of an extract of a kidney of a harbour porpoise from the Dutch Wadden Sea. Numbers identify the number of chlorine atoms per molecule detected by GCMS. Packed column, 1.5% SP 2250 and 1.95% SP 2401 on 100-120 mesh Supelcon (AWDMCS) 1.8 m x 0.64 cm O.D; column temperature 215°C.

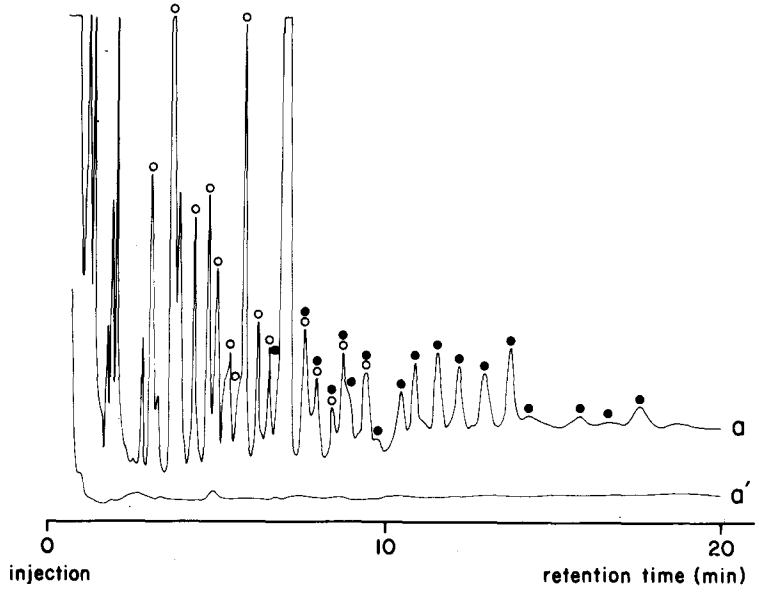

Fig. 2
ECD-chromatogram of first silica fraction of seawater extract from the Southern Bight (a). Same column as in Fig. 1, temperature programmed from 160°C to 215°C with a 4°C. min^{-1} increase. Peaks corresponding to Clophen A30 are indicated by O and those in Aroclor 1254 by ● Chromatogram of blank obtained by solvent extraction of water that had already been extracted twice is given by a'.

not represent the composition of the sample adequately.

d. Additional problems arise when components with low and/or high degree of chlorination have a higher contribution to the composition of the sample than is suggested by the chromatographic pattern of the technical formulation that reflects the most prominent peaks in the sample chromatogram. Fig. 2 demonstrates the recently reported observation on the significant contribution of lower chlorinated compounds in seawater (North Sea). The "concentration" in the sample will then depend critically on the selection of the technical formulation to be used as standard. In the case of Fig. 2, the concentration of PCB's in the seawater sample in terms of Clophen A30 equivalent (4.1 $ng.L^{-1}$) was considerably above the value in terms of Clophen A50 (0.7 $ng.L^{-1}$). As demonstrated before, both numbers would be different when other, equally reasonable combinations of peaks had been selected for quantitation.

e. In the most sophisticated approach for quantitation in terms of technical formulation equivalents, linear combination of technical formulations with different overall chlorine content have been used to improve the similarity between the chromatograms of sample and mixture used for quantitation (EDER 1976). However, the results are not necessarily more accurate because of the same reasons as under a).

b. Quantitation after perchlorination

A different approach to PCB quantitation reported in the literature involves perchlorination of PCB components in sample extracts, resulting in only one peak (of decachlorobiphenyl) in ECD-GLC (BERG et al. 1972, ARMOUR 1973). Thus, quantitation from the resulting very simple chromatogram is a straightforward procedure. However, the simplification of this part of the interpretation does not solve the problems; in fact, the uncertainties introduced are even larger. The technique causes loss of all information on the relative contribution of PCB components with different chlorine content. As components with a low degree of chlorination have low specific response on the electron capture detector, the relative contribution of these components to the decachlorobiphenyl peak and consequently to the quantitative results may be considerably larger than in the quantitation technique based on technical formulations. Further complications arise because PCB components with different degree of chlorination are perchlorinated with different efficiencies (60-100 %, with higher efficiencies for components with a higher number of chlorine atoms). Serious limitations in the applicability of the technique, especially at low concentrations such as in water, are caused by contamination of the chlorination agent ($SbCl_5$) with decachlorobyphenyl, resulting in high blanks (TROTTER & YOUNG 1975). Moreover, bromine presents

as contaminant in $SbCl_5$ causes the formation of bromononachlorobiphenyl, interfering with an accurate estimation of decachlorobiphenyl. Finally, a contribution to the amount of decachlorobiphenyl after perchlorination may result from compounds not belonging to the class of PCB components. For instance, biphenyl is perchlorinated by this procedure. This will interfere seriously with an accurate PCB analysis.

SUGGESTIONS FOR IMPROVEMENT

The main reason for the ambiguity of PCB data in terms of technical formulation equivalents, using data obtained by application of packed column-chromatography is the insufficient resolution of eluting peaks. Literature on PCB's in seawater and other environmental samples so far has been based almost exclusively on gas-liquid chromatography in the isothermal mode. The standard for quantitation of PCB in seawater has been Aroclor 1254 in practically all cases. A significant contribution of lower chlorinated biphenyls to the composition of seawater (resembling Clophen A30) was described recently on the basis of packed column ECD-GLC in the temperature programmed mode (DUINKER & HILLEBRAND 1979). The significant contribution of PCB components with low degree of chlorination is supported by recent analyses of subsurface seawater obtained at Bergen (Norway) and in the Mediterrenean off Monaco (PALMORK & WILHELMSEN 1979) and in the central part of the southern Bight (unpublished results) by application of temperature programmed capillary ECD-GLC). This technique offers the ultimate separation obtainable at present, allowing identification and quantitation of individual PCB components. These ideas formed the basis for the design of the "Outline of the method to be used for the determination of chlorinated hydrocarbons in seawater" (IOC/WMO/UNEP Pilot project on monitoring background levels of selected pollutants in open-ocean waters Bermuda January 1980).
Fig. 3 shows a chromatogram of a XAD-2 extract of surface water sample (10m depth) of Panulirus station (i.e. 12 miles southeast of Bermuda, $32^{o}10'$ N $64^{o}31'$ W), including the identification of six individual components on the basis of retention times. Their concentrations in the original seawater are given in Table 2; these have been calculated by comparison with peaks of standard solutions of individual components and by taking into account (relatively small) XAD-2 blanks. It was assumed that these peaks in the sample chromatogram are composed of the quantitated peaks only. The amount of sample extract was insufficient to support this assumption by GCMS data. However, such data obtained in the selected ion monitoring mode of Aroclor 1254 confirmed that the components eluting at the particular retention times have in fact the appropriate number of Cl atoms according to the identification given. The question as to whether two tetrachlorobiphenyls (and similarly two penta- or

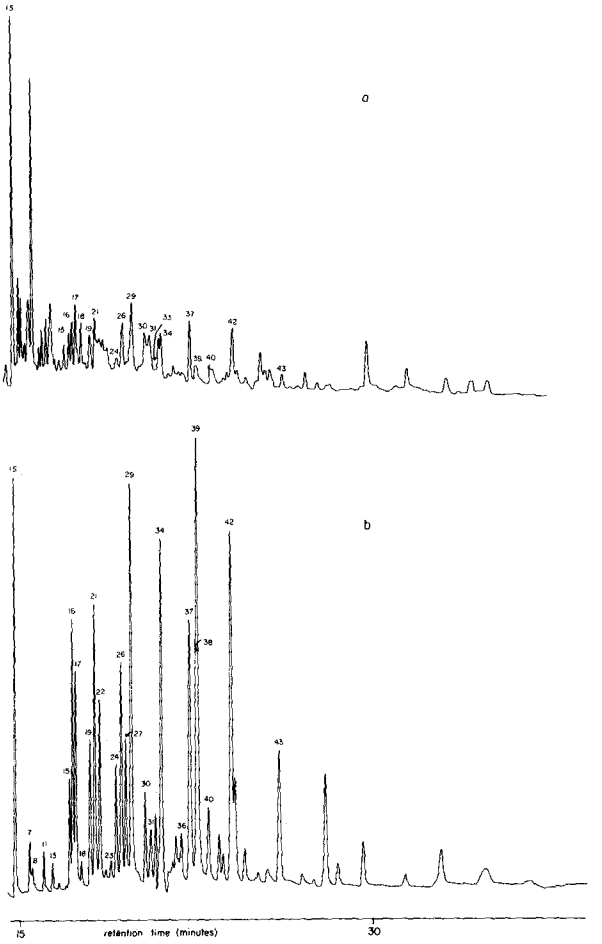

Fig. 3
Temperature programmed capillary column ECD-gaschromatogram of (50 m SE-54) a: seawater extract (see text) and b: Aroclor 1254. I.S. = internal standard. 100-230°C, 8°C. min^{-1}.

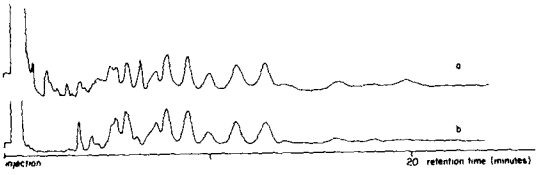

Fig. 4
Isothermal packed column chromatograms of same open ocean sample as in Fig. 3 (a) and Aroclor 1254 (b); column and conditions as in Fig. 1.

hexachlorobiphenyls) might have exactly the same retention times, i.e. within the time necessary for one or two mass-spectrometric scans, remains unanswered for the moment. However, this is not essential for the approach itself, as future work is expected to result in still better separations. Moreover, the limiting factor in problems of this type is sensitivity and this should be much less important in samples with higher content of chlorinated biphenyls than we encountered in the extremely low level-open ocean sample.

Packed column chromatography resulted in a peak pattern distribution of the PCB fraction that resembles that of Aroclor 1254 very closely (Fig. 4), However the inter-peak relations in the capillary column chromatograms of the open ocean sample are different from those in Aroclor 1254 (Fig. 3). The information in Table 2 based on this chromatogram could not be derived from packed column chromatograms. Future data on the composition of ocean water in terms of individual components such as in Table 2 may assist in explaining temporal and spatial trends, even if data of only a few components are made available. This information is essentially lacking for all environmental compartments on the basis of the present data. Chromatograms of extracts of samples of various types from the marine environment (sediments, organisms, water) investigated in our laboratories recently, demonstrate considerable differences from those of the formulations and from each other.

The compositions of sample and standard used for quantitation in terms of individual chemical compounds should be identical, in order to obtain accurate analytical data. Although the use of packed column chromatography has proved to be valuable for obtaining qualitative and semi-quantitative data in the past, the method results in ambiguous analytical PCB data that supply insufficient information on important aspects related to studies of sources (atmosphere, land drainage), transport pathways (biotic and abiotic compartments), biogeochemical transformations (metabolism, photo-oxidation), toxicity, effects and ultimate fate of well defined environmental contaminants. (BALLSCHMITER & ZELL, 1980).

Table 2. Concentration of individual chlorinated biphenyls in an open-ocean water sample (Fig. 3).

Peak no.	Compound		$ng.L^{-1}$
16	2,5,3',4',	-tetrachlorobiphenyl	0.038
24	2,4,5,2',3',	-pentachlorobiphenyl	0.010
26	2,3,4,2',5',	-pentachlorobiphenyl	0.030
37	2,4,5,2',4',5',	-hexachlorobiphenyl	0.052
42	2,3,4,2',4',5',	-hexachlorobiphenyl	0.042
43	2,3,4,2',3',4',	-hexachlorobiphenyl	0.009

It was shown above that the application of combined temperature programmed capillary gaschromatography and gaschromatographic/mass spectrometric techniques to environmental samples will assist in the identification and quantitation of individual PCB components. This is to be preferred over the use of mixtures of unknown composition resulting in ambiguous data.

It is important that high quality standards of individual polychlorinated biphenyls will become widely available as in several cases, temperature programmed ECD-chromatograms of solutions of presently commercially available, so-called individual PCB components, show extra peaks. In some cases, peaks with very large response occur, causing unknown uncertainties in identification of components as well as in quantitative results.

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