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The determination of polychlorinated biphenyls in open ocean waters

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The present Report was prepared by Dr J.C. Duinker, then of the Netherlands Institute for Sea Research (Texel, The Netherlands), at present at the Institute of Marine Science (Kiel, Federal Republic of Germany), in response to a request by the Intergovernmental Oceanographic Commission. The Report builds on the experience of the Workshop on the Intercalibration of Sampling Procedures of the IOC/WMO/UNEP Pilot Project on Monitoring Background Levels of Selected Pollutants in Open-Ocean Waters, held in Bermuda, 11-26 January 1980, and attempts to review the present state-of-the-art for the determination of polychlorinated biphenyls in open-ocean waters. The Intergovernmental Oceanographic Commission wishes to acknowledge gratefully the contributions made by the author himself and the support of the Netherlands Institute for Sea Research, in collaboration with the Institute of Marine Research (Bergen).

The comments received from numerous scientists and, in particular, the members of the GIPME Group of Experts on Methods Standards and Intercalibration (GEMSI), have been taken into consideration and contributed significantly to the preparation of the final version of the report.

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Preface

A major programme of the Intergovernmental Oceanographic Commission (IOC) is the Global Investigation of Pollution in the Marine Environment (GIPME), which seeks to address the state of pollution of the world's oceans through a Marine Pollution Monitoring System (MARPOLMON), which, when operational, will comprise regional components. As a result, it is of fundamental importance that the analytical techniques and methods used produce comparable results. Therefore, the first priority in the implementation of the Comprehensive Plan for GIPME is the development and proving of techniques for the collection of baseline and boundary flux data for selected contaminants, followed by intercalibration exercises. To this end, an intercalibration exercise was conducted at the Bermuda Biological Station for Research, Inc., from 11 to 26 January 1980, where attention was given to selected trace metals and organochlorine compounds. This exercise is fully described in the IOC Technical Series No 22.

One result of the Bermuda exercise was the identification of certain questions relating to the sampling and analysis of organochlorine compounds. These questions needed to be addressed prior to initiating any monitoring activity, including training aspects, with regard to this class of marine contaminant. Consequently, the IOC supported a laboratory investigation, led by Dr. Jan C. Duinker, then of the Netherlands Institute for Sea Research, to seek answers to these questions. This report describes the results of this research, and constitutes an initial phase of a programme on the marine environmental monitoring of organochlorines which has been developed by the IOC's GIPME Group of Experts on Methods, Standards and Intercalibration (GEMSI).

Contents

I. Introduction

During the Intercalibration of Sampling Procedures of the IOC/WMO/UNEP Pilot Project on Monitoring Background Levels of Selected Pollutants in Open Ocean Waters (Bermuda, 11-26 January 1980) several problems were identified with respect to the sampling and analysis of organochlorines, in particular polychlorinated biphenyls (PCB) (1). These problems should be solved prior to any further implementation of the monitoring programmes within IOC's Global Investigations of Pollution in the Marine Environment (GIPME). Attempts to reach this goal were subsequently formulated in a detailed proposal by the Bermuda Biological Station for Research, Inc., the Institute of Marine Research, Bergen, and the Netherlands Institute for Sea Research, Texel. The proposed programme includes an intensive six-month phase of further developing the analytical method for the estimation of PCB in seawater, and the three laboratories volunteered to work intersessionally towards solving some of the problems. Preliminary results were discussed at a GEMSI Core Group meeting at Bermuda in March 1982, with respect to extraction, separation and analytical procedures (Annex X in document IOC/GCE(MSI)-IV/3) (2). At that time, much of the intersessional work had been devoted to establishing the essential steps in the qualitative and quantitative analysis for individual PCB components, including identification of peaks, determination of response factors of individual components, preparation of a calibration mixture and establishment of chromatographic conditions for reproducible and efficient separations. At this GEMSI Core Group meeting it was recommended that further work should be done on recovery efficiencies and on separation and separate analyses of dissolved and particulate forms. Further studies were considered necessary for improving the chromatographic separation, as well as the standardization of the experimental conditions that should result in better comparability of results and to finalize the outline of the analytical method for the estimation of PCB in seawater. The Core Group's recommendations were adopted at the Fourth Session of GEMSI (Curacao, 25-31 March 1982), and it was further decided that, to increase the cost effectiveness of the proposed six-month activity noted above, a detailed report would be given on the results obtained during the preliminary period with respect to identification of individual PCB components by gas chromatography and gas chromatography-mass spectrometry as well as to the distribution of individual components between solution and particulate matter. This is the subject of this publication, parts of which will also appear in the open literature.*

The methods described here are also appropriate for the estimation of several other halogenated hydrocarbons in seawater. Some of these compounds appear in the same fraction as PCB (e.g. penta- and hexa-chlorobenzene); for the estimation of more polar components, additional GC-ECD analyses are required. The extracts needed for this purpose require only relatively small additional steps in the separation procedures.

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II. Structure of the report

This report summarizes the present status of some essential steps involved in the development of the procedures for estimation of PCB in open ocean waters. The main problems are due to the complexity of the PCB mixtures and the extremely low concentration levels of all components encountered. Much effort has been devoted towards the development of methods i) to concentrate the compounds of interest and to isolate them from interfering compounds in seawater in such a way that contamination is minimized or preferably eliminated and ii) to analyse the composition of the mixtures qualitatively and quantitatively.

The report will describe the progress achieved in some of the procedural steps. The order in which these are discussed will be the reverse of that in which they are carried out in analytical procedures. This allows a more logical description of the procedures. Thus, the following steps will be discussed:

- Analysis of technical formulations and of concentrated and cleaned-up PCB-extracts by GC-ECD and GC-MS;
- A procedure for obtaining these abstracts from water and particulates by extraction into an organic solvent, and a clean-up procedure to remove interfering compounds;
- The separation of water and particulates in a seawater sample by filtration;
- Sampling and pre-filtration procedures.

In addition, the report will describe some results obtained for the North Sea and finally the work to be carried out before and/or during the six-month research period planned to take place at Bermuda will be described.

III. Present status of the various procedural steps

A. Analysis of composition of PCB mixtures by Gas Chromatography Electron Capture Detection (GC-ECD) and Gas Chromatography -Mass Spectrometry (GC-MS)

Whatever technique is used for sampling, sample processing, extraction and purification of extracts, the final analytical determination of organochlorines involves most likely GC-ECD. This technique involving application of capillary columns will be discussed before the other aspects (full details are in (3)).

1. Gas chromatographic separation and detection

a. Column and electron capture detector

The column:

In an ideal chromatographic separation, each compound is eluted as a single peak. This is difficult, if not impossible, to achieve when dealing with environmental samples. This applies especially to the low resolution obtained on packed columns that have been used almost exclusively until recently. Open tubular columns, first suggested by GOLAY (5), demonstrate a dramatic increase in resolution. These columns are essentially lengths of capillary tubing coated with a stationary phase. Wall-coated open tubular (WCOT) columns have a liquid phase deposited directly on the inner surface. Good quality columns are available commercially as narrow or wide-bore columns (0.2-0.7 mm inner diameter), glass or fused silica, in various lengths (more than 100 m) and with a wide choice of coating and film thickness (0.05-0.5 µm). Support-coated open tubular (SCOT) columns have their wall coated with a mixture of finely divided solid support and liquid phase; their internal diameter corresponds to that of wide-bore WCOT columns. Capillary columns have a high specific gas permeability and a very small amount of liquid phase. Pressure-regulated flow through the column is only a few cm^3 per minute. Efficiency is much higher than for packed columns: typical numbers for total effective plates are 5,000 and 150,000 for 2 m packed and 50 m capillary columns respectively. Capacity is considerably lower than for packed columns: about 50 ng and 10 µg per component respectively.

It is worth noting that capillary columns have increased sensitivity (as a result of an increase in peak height), signal-to-noise ratio and overall inertness (less adsorption). Thus, the detection limit may be two orders of magnitude below that of packed columns. The recent advent of fused silica columns (6) has been an enormous advantage: they are flexible, much less fragile and inert owing to their low metal content at the reduced surface.

Various column coatings have been used for the analysis of organochlorines in environmental samples. High resolution chromatograms of environmental samples are usually complex. The columns and experimental conditions preferred by each analyst have to be selected as a compromise between resolution and analysis time, usually by trial and error. Optimum conditions for one pair of peaks may be different from those for another pair. It may therefore be impossible to optimize conditions for all components of interest with the use of one column only. Depending on the problem, the coating is selected from various possibilities such as hydrocarbon apiezon-L and methyl-silicone columns (SE-30, SE-52, SE-54, CP-SIL-5, SIL-7) (7, 8).

Column efficiency and column life are maintained as long as the liquid phase remains as a thin film, evenly distributed over the wall. A drop in column performance occurs if the liquid phase becomes repelled by the surface. Thus, displacement of the liquid phase at the inlet end of the column can occur after a large number of splitless injections (removal of a few coils may bring back the original efficiency without significantly modifying retention behaviour). Deterioration of the entire column is accelerated by continued exposure of the column to high temperature and extremely so with reduced or zero flow. Column quality is also determined by the nature of injected samples (it is particularly sensitive to materials that are more strongly adsorbed than the liquid phase) and to carrier gas impurities (water and oxygen). Columns coated with methyl silicone (SE-30), 5 % phenyl (SE-52) and 1 % vinyl 5 % phenyl (SE-54) methyl silicone gums can tolerate short-term exposure at 285°C; they are also to some extent resistant to water and oxygen.

The selection of carrier gas involves a compromise between resolution and analysis time. Although nitrogen results in higher column efficiency than either helium or hydrogen, the average linear gas velocity ($\bar{u}$) of the carrier gas at this optimum is considerably lower than for He and H_2, and also the change in efficiency with u is smaller for the latter gases, which are therefore preferable.

The electron-capture detector:

The electron capture detector (9) is an essential component in the analysis of trace amounts of organochlorines for which its sensitivity is roughly 5 orders of magnitude higher than for hydrocarbons. For instance, the detection limit for lindane is as low as 0.02 pg using capillary columns.

High-energy electrons, emitted by a source within the detector (e.g. a ^{63}Ni foil, half-life 92 years), are subject to repeated collisions with carrier gas molecules, producing secondary electrons. These electrons, once their energy has been reduced to thermal level, can be captured by sample molecules. The resulting reduction in cell current is the basis of the working mechanism of an ECD as an analytical tool. However, the response function of current

versus electron capturing concentration is non-linear. The useful linear range of an ECD is greatly improved in the constant-current pulsed mode. Short voltage pulses are applied to the cell electrodes to collect the electron population in the ECD cell. The current generated by the detector cell is automatically regulated by the frequency of the polarizing pulses to maintain a certain standing current. An increase in concentration of an electron-capturing material in the cell causes a change in the polarizing pulse frequency necessary to restore the balance between the detector cell current and the standing current. The response over a voltage/frequency converter is linear with concentration over a large range. The dynamic range (covering 4 to 5 decades of concentration) depends on various parameters such as detector temperature, pulse width and standard current level. The optimum flow for an ECD (about 30 $cm^3 min^{-1}$) is much higher than the flow of carrier gas through the column; it is thus necessary to have an additional detector purge flow. Operational conditions should be optimized for all these parameters.

High-boiling-point organic compounds eluting from the column may contaminate the detector, resulting in lower sensitivity. The effects are less serious at higher detector temperature. Periodic heating to 350°C overnight assists in maintaining good detector performance. The ^{63}Ni-ECD can be used conveniently at 320°C under operational conditions, resulting in relatively limited contamination.

b. Sample injection

In trace analysis, introduction of the sample into the column preferably involves the entire sample for sufficient sensitivity, without splitting off any sample to vent. This can be achieved with a splitless injection technique (10, 11). Full efficiency of the column is realized by reconcentration of the sample components in a narrow band on the column prior to analysis, either by using a solvent effect or the effect of condensation of the solutes at the column inlet. The latter mechanism operates effectively for compounds with boiling points about 150 °C above the column temperature. Compounds with lower boiling points need a solvent effect for reconcentration. This requires a high solvent concentration at the column inlet. The solvent effect, based on stronger retention of the front than the rear of the sample plug, when encountering a liquid phase mixed with retained solvent at the inlet end of the column, is most efficient at a column temperature of 10 - 30 °C below the boiling point of the solvent. The column temperature can then be raised to the temperature required. The temperature of the injector should allow a rapid evaporation of solvent and solutes but it should be low enough to minimize septum bleed and avoid destruction of sensitive components.

The splitless mode allows a relatively large amount (0.5 - 3 mm^3) of dilute sample to be injected into a simple open glass tube liner in the injection port. The inlet flow during injection equals the (low) column flow. A relatively long period (e.g. 20 s) is required to transfer the sample into the column. The solvent that will have diffused throughout the column inlet is then vented by purging with a large volume of gas. This prevents the occurrence of a long solvent tail that might obscure early eluting components. The continuous inlet purge is interrupted only during injection. If timed properly after injection, the inlet purge can remove mainly solvent (5 - 10 %) and virtually none of the sample components.

c. Operational conditions and procedures

Like packed columns, new capillary columns have to be conditioned to remove residual traces of solvent and lower-molecular-weight fractions of the liquid phase. Carrier gas should flow at room temperature for some time to remove oxygen; the column is then subjected to moderate temperatures (80 - 100 °C) for some hours before the temperature is increased to a value that must compromise between minimum time required to achieve a stable baseline and maximum column life. The temperature limit of the liquid phase and the maximum temperature required during analysis are taken into account. To avoid destruction of the column, a sufficient flow of carrier gas through the column should be maintained at high temperatures. During conditioning, the column may be left disconnected from the ECD in order to minimize detector contamination.

Older columns may have to be subjected to higher temperatures periodically to remove slowly moving sample components or carrier gas impurities that have accumulated. This may be necessary less frequently in cases where higher temperatures during a programmed run are maintained for a longer period. Column performance can be continued over longer periods by maintaining moderate temperatures overnight (e.g. 180 °C). The ECD may remain connected to the column, provided that it is kept at an elevated temperature (320 °C).

It is important to check and adjust the detector standing current at regular intervals; it can be performed automatically in modern equipment. Carrier gas must be of high purity (N_2 at least 99.999 % but preferably 99.99999 % purity). Nitrogen is often contaminated with hydrocarbons and water. Hydrogen and helium are generally of higher purity. Impurities can saturate molecular sieve traps, gas lines and other materials. The resulting bleed at higher temperatures causes baseline instability and shortens the life of the column. All gases used in the system should be dried carefully with a molecular sieve trap. Indicating traps are useful.

Irregularities in the baseline, ghost peaks and generally poor chromatograms may result from impurities in the carrier gas. Provided that the inlet liner and septa have been excluded as possible sources, this can be checked by temperature-programmed blank runs after the column has been sitting at a low temperature for some time (overnight). Septum bleed may cause similar problems; this source can be eliminated by maintaining a septum purge during the above experiment. The septum should be changed at regular intervals according to instructions given by the manufacturer.

We have found it far from trivial to select good quality septa from those available, such as sandwiched Teflon-coated, Teflon-coated on both sides, pre-conditioned or unconditioned etc., as they are sometimes offered commercially in a variety of exotic colours. Many do not satisfy the requirements of high temperature stability, ease of penetration and long life. Septa should remain gas-tight after several (preferably many; say 30-50) needle penetrations. As the carrier gas flow through capillary columns is regulated by constant pressure at the head of the column, leakage through a hole in the

septa may not be observed readily by variations in retention properties, as is observed with packed columns. However, it will result in the loss of sample. We have found that good quality septa are being marketed. They have to be selected by trial and error.

d. The gas chromatograph

The use of high-resolution capillary columns (characterized by low flow rates, reduced sample capacity, and high degree of inertness) for the analysis of complex environmental samples places special requirements on the gas chromatograph. Availability of a GC with multi-level oven temperature programming facilities greatly assists in selecting and maintaining optimum conditions for: (a) retention times, peak shapes and separation of various peaks throughout the region of interest; and (b) reducing the time required for a chromatographic run. Reproducibility of retention times should allow identification of sample components, using the same calibration data over continued periods. These aspects can be evaluated and checked by visual inspection of chromatograms in analog form written during a chromatographic run, with retention times printed in real time. Maximum information from the chromatographic runs can be obtained when using the facilities offered by a microprocessor/keyboard-operated GC system. This enables the operator to programme and execute the entire chromatographic process, to store retention times and response factors of standards, including reference compounds, in a memory and to perform automatic post-run calculations using internal or external standard methods. Measured and expected retention times, all within a specified window, peak heights and/or peak areas are then presented in tables, including identification and quantification of components included in the calibration table. The equipment can be made completely automatic by addition of an automatic injection device. This also results in better reproducibility.

e. Optimum temperature programme conditions

Ideal separations of the components in PCB mixtures would result from a capillary column capable of separating all components completely; moreover, its ideal properties should not change with time. Such columns do not exist at present. We have found that fused silica WCOT columns coated with SE-54 can result in good separations; their life time can be very reasonable (up to several months of continuous use). The chromatographic conditions for optimum separations are slightly different for specific separations and in general a compromise is chosen to satisfy the requirements in different chromatographic regions. Examples for the separation achieved with a SE-54 column with different temperature programming conditions are presented in Figures 1 and 2. These chromatograms of mixtures of about 100 components were obtained in different laboratories (Bergen and Texel). The agreement is very good, with only one difference (no. 193). This is due to the presence of two peaks in the chromatogram of the "pure" component.

2. Identification and quantification

a. Standards

A stock solution (about 50 cm^3) of each compound of interest (e.g., in n-hexane), is made in a concentration of 1000 $\mu g\ g^{-1}$ by gravimetry. Less concentrated stock solutions (50 - 100 cm^3) are produced by dilution (vol/vol) of a subsample of the concentrated stock solutions. Working standards are prepared by mixing subsamples of the less concentrated stock solutions in relative amounts that are roughly inversely proportional to the response factors of the various compounds. Peak heights (or peak areas) in the final mixture are then of the same order. The final volume of this mixture is adjusted with n-hexane to result in concentrations within the dynamic range of the detector. Calculation of the absolute amounts injected with 1 mm^3 sample volumes should take into account the density of hexane when the original stock solution has been made up on a weight/weight rather than on a weight/volume basis. All standard solutions are stored in glass bottles with Teflon-lined aluminium caps. Weights are recorded on the bottles and checked before subsampling at a later date.

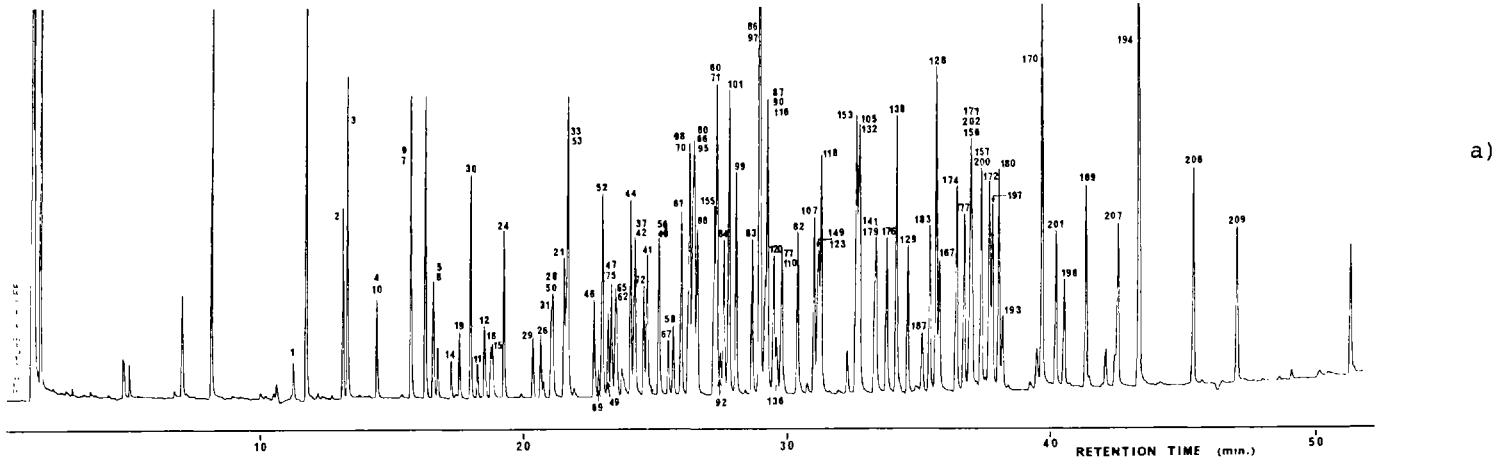
b. Calibration

Several injections should be made of standard solutions containing a range of concentrations. The results are plotted to determine the linear range of the detector response and the response factor for each component (peak area or height). We have adopted the common practice of quantifying in sample chromatograms only those peaks for which the response is not too different from that of the standard - an order of magnitude is usually accepted -in order to bring the response into the linear range. The relative areas or heights of different peaks in any sample chromatogram can differ widely. It may therefore be necessary to inject the sample extract several times in succession after appropriate concentration or dilution.

The use of capillary columns results in more or less perfect resolution of many compounds. These can be quantified accurately if standards of sufficient purity and known concentrations are available. A calibration mixture must be prepared and analysed under the same instrumental conditions as used for the sample. Differences in sensitivity of the detector for different sample components are accounted for by the response factors that are automatically calculated. Before any quantification method can be carried out on an absolute basis, a decision must be made as to whether the sample peak has been calibrated and which of the calibrated peaks it is. The identification is controlled by retention times of designated peaks in the calibration table (the reference peaks) and of course those of the non-reference peak, as well as the recognition windows for reference and non-reference peaks.

The external standard method uses absolute response factors, the internal standard method is calibrated in terms of response ratios. In both calibrated methods, each peak is calculated independently. In external standard methods, the sample amount injected must be highly reproducible. The method is well suited to mechanical methods of injection, but it is difficult with manual syringe injections. Instrumental (column) drift is not acceptable and frequent checking of system performance as well as recalibrations are essential. The internal standard method is independent of sample size and instrumental drift is compensated for. When used properly, it should be the most accurate calculation scheme for liquid samples. However, the internal standard must be added to each sample in a highly reproducible way.

Figure 1 Temperature programmed capillary column-electron capture detection chromatograms of mixture of 102 components identified by their IUPAC numbers (7).
Analysis was carried out in Bergen on a HP 5880 A gas chromatograph under the following conditions: Carrier gas H_2, 100 kPa; make up gas N_2, 30 cm^3min^{-1}; septum purge 5 cm^3min^{-1}; column 50x0.33 mm i.d. SE-54 fused silica; injector temperature 280°C; detector temperature 320°C; injection 3 mm^3 splitless.
Temperature programmed conditions were as follows: initial 100°C (hold 2 min.), 4°C min^{-1} to 170°C (hold 0 min.), 3°C min^{-1} to 280°C (hold 5 min.), (chromatogram Fig. 1a)
and initial 60°C (hold 2 min.), 25°C min^{-1} to 180°C (hold 15 min.), 4°C min^{-1} to 280°C (hold 5 min.), 4°C min^{-1} to 250°C (hold 10 min.) (Fig. 1b).



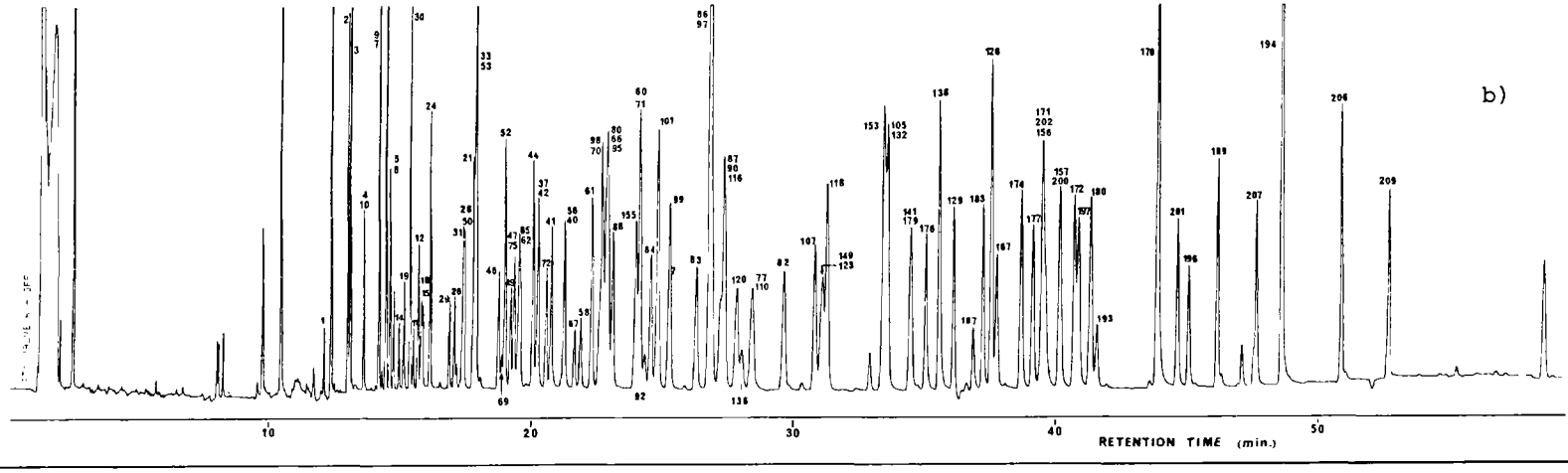
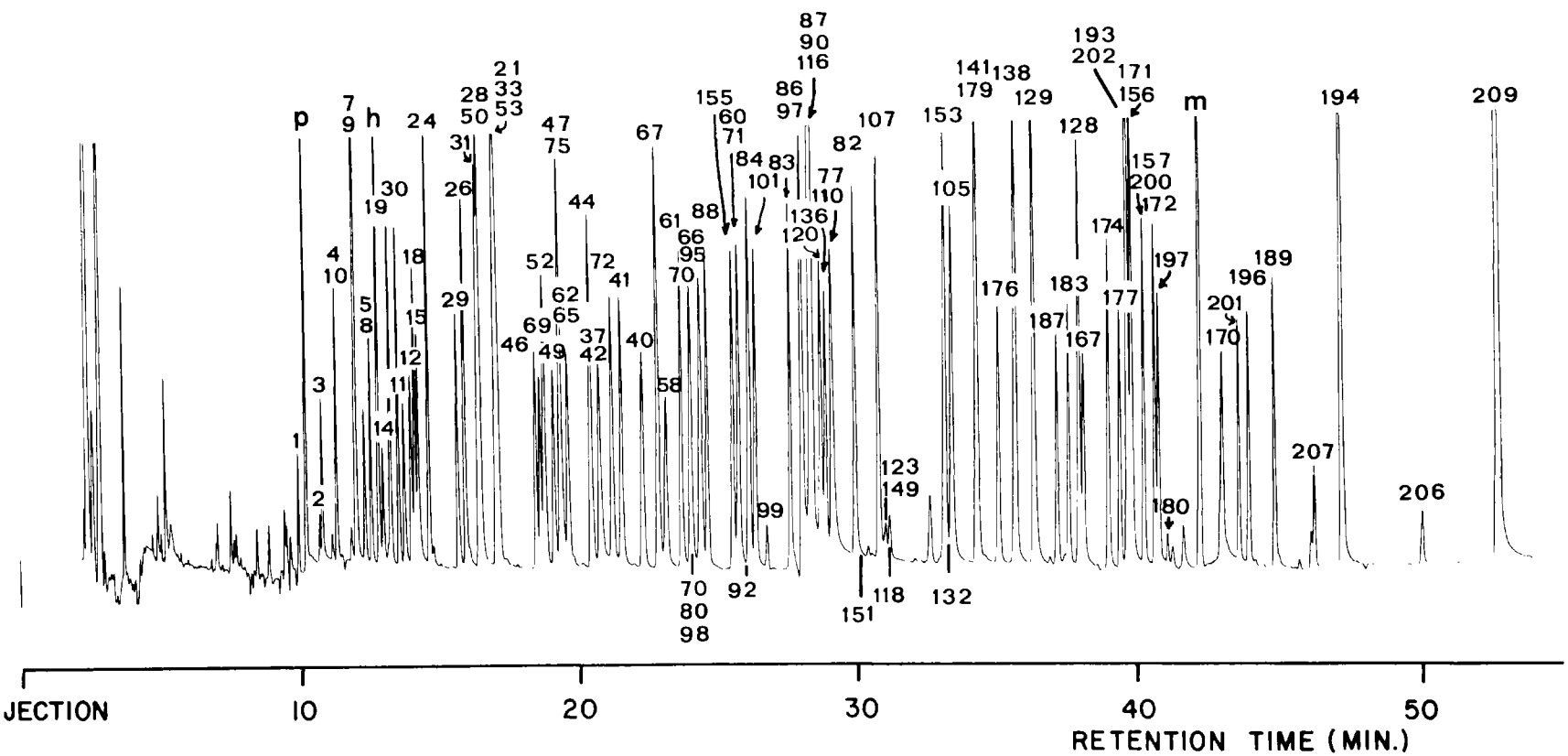


Figure 2 Temperature programmed capillary column-ECD chromatogram of the mixture of most of the 102 PCB components as in Figure I. Also included are pentachlorobenzene (p), hexachlorobenzene (h) and mirex (m). Relative amounts were selected to obtain similar peak heights in the mixture. Components that were only available as stock solutions in low concentrations have lower peak in the mixture (e.g. 123, 149, 118, 180). Peaks are identified by the IUPAC numbers of the components (co-eluting peaks given in a vertical column). Components 132, 92, 151, 141, 157, 193 were not present in the mixture at this stage because of elution close to other components. The separation of some of these components from adjacent ones is demonstrated in technical formulations (Fig. 5).
Chromatographic conditions as follows: Hewlett Packard HP 5880 A (Texel), capillary WCOT (50 m x 0.33 mm) SE-54 fused silica column, carrier gas, He (130 kPa); autosampler injection 1 mm^3 splitless; make up gas, N_2 (30 cm^3 min^{-1}); detector, 320°C; injector, 230°C; septum purge, 5 cm^3 min^{-1}; injector purge, 20 cm^3 min^{-1} He; temperature programme, isothermal phases at 60 (2 min), 180 (15 min), 210°C (5 min.) and 250°C (10 min.), with intermediate temperature increase rates of 10, 4 and 4°C min^{-1}, respectively.



c. Identification

The most widely used information about a certain peak is its retention time or its relative retention time, i.e. the adjusted retention time relative to the adjusted retention time of a selected reference compound. Capillary columns have a distinct advantage over packed columns since the probability of separating interfering components is increased considerably. The labeling system proposed by BALLSCHMITER and ZELL (7) is extremely useful for characterizing individual components (Table I).

Some techniques are available to obtain additional information about the identity of a certain peak. Mass spectrometric methods, in particular mass fragmentography, are extremely useful to identify and distinguish components even with identical retention properties (12). MS techniques are specific and their sensitivity (femtomole range) is comparable with that of GC-ECD techniques. Many marine laboratories are equipped with GC/MS systems, and most applications in the marine environmental field are related to biological samples. It is expected that the number of applications for samples with much lower concentrations of organochlorines, in particular seawater, will increase significantly in the near future.

d. Response factors on ECD

Relative response factors of individual PCB components (given as range of peak heights per pg injected) are presented in Table II.

3. Composition of technical formulations by GC-ECD and GC-MS (4)

a. Introduction

PCB were being produced for about 35 years before they were identified as environmental contaminants by JENSEN in 1966 (13). 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 III). Each formulation is a complex mixture of many of the 209 theoretically possible components, differing in the number of chlorine atoms (1 - 10) and in their relative positions in the molecular structure. The average number of chlorine atoms per molecule increases with overall chlorine content of the formulation (Table III).

The various components do not behave identically in the environment. For accurate information on sources, transport mechanisms, sinks, accumulation, degradation and other relevant processes, analyses of polychlorinated biphenyls should be made in terms of individual components rather than of technical formulations which are, however, the dominant sources of PCB in the environment. It is therefore essential to have detailed information on their composition.

SISSONS and WELTI (14) were among the first to realize the need for this approach. They separated and identified the major components in Aroclor 1254, using various analytical techniques. The retention properties of these components, as well as those of forty other components which were synthesized by them, were used to predict a complete analysis of Aroclor 1242, 1254 and 1260. JENSEN and SUNDSTRÖM (15) synthesized 90 components and identified, in Clophen A50 and A60, almost 60 components, each containing 4 or more chlorine atoms per molecule. Most reports in the literature have been concerned with formulations of either low (16 - 19) or high (20 -25) overall chlorine content, but also of both (7, 26, 27). The number of individual components available to these authors (except in ref. 14 and 15), has been up to about forty and in several cases considerably less.

Attempting to obtain information on non-available components in order to account for unidentified peaks, some authors have used the chromatographic retention indices of available components to calculate such data for missing ones. This approach has been applied to predict the composition of formulations with lower (Aroclor 1016, 1242, Clophen A30, A40) (7, 14, 17-19, 26, 28, 29) and higher (Aroclor 1248, 1254, 1260, Clophen A50 and A60) (7, 14, 25, 26) degrees of chlorination. According to some of these authors, the identity of many peaks could not however be unambiguously determined.

Some of these uncertainties have been resolved by application of other techniques than comparison of retention times; e.g., Mass Spectrometry (18, 19, 30), Nuclear Magnetic Resonance (14, 20) and Infrared Spectrometry (14, 19-21).

Generally, the efficiency of packed columns in GC is insufficient to allow their use for accurate analysis of the complex PCB mixtures in technical formulations and environmental samples (31). However, they have been used succesfully in the analysis of Aroclor 1254 fractions that were preseparated on alumina columns (14), and of Clophen A50 and A60 fractions containing 4, 3 and less than 3, respectively, of o,o'-chlorines in the molecular framework that were preseparated on charcoal columns (15). Another approach to the use of packed columns involves the use of columns with varying selectivities. In this way, complete analysis of all components in Aroclors 1242 and 1016 has been claimed using up to 12 columns (18, 25, 29). The strongly increased GC separation offered by capillary columns has been used to advantage in the analysis of technical formulations (7, 14, 17, 19, 23, 24, 26-28); in some cases the eluate was also analysed by MS (14, 17, 19, 23).

We have studied the composition of PCB formulations, both qualitatively and quantitatively, by analysing the eluate of the same capillary column by electron capture detection (ECD) and by directly coupled computerized mass spectrometry (MS).

Results will be presented for a characteristic series of formulations with chlorine contents in the 30 - 60 % range. We have observed only minor differences in chromatograms of Clophen and Aroclor formulation with the same chlorine contents; e.g., Clophen A50 and Aroclor 1254. Data will be reported here in detail for the Clophen series; i.e., A30, A40, A50 and A60. The number of individual components available to us as reference compounds (102) was larger than in earlier reports. This allows a detailed comparison with the existing literature data on predicted compositions of such mixtures that have been based on retention index calculations that were, in turn, derived from a relatively limited number of available components.

Table I

Systematic numbering of PCB compounds. The number is used as a synonym for the corresponding PCB compound in tables and figures (7)

No.	Structure	No.	Structure	No.	Structure	No.	Structure
	Monochlorobiphenyls		**Tetrachlorobiphenyls**		**Pentachlorobiphenyls**		**Hexachlorobiphenyls**
1	2	52	2,2',5,5'	105	2,3,3',4,4'	161	2,3,3',4,5',6
2	3	53	2,2',5,6'	106	2,3,3',4,5	162	2,3,3',4',5,5'
3	4	54	2,2',6,6'	107	2,3,3',4',5	163	2,3,3',4',5,6
	Dichlorobiphenyls	55	2,3,3',4	108	2,3,3',4,5'	164	2,3,3',4',5',6
		56	2,3,3',4'	109	2,3,3',4,6	165	2,3,3',5,5',6
4	2,2'	57	2,3,3',5	110	2,3,3',4',6	166	2,3,4,4',5,6
5	2,3	58	2,3,3',5'	111	2,3,3',5,5'	167	2,3',4,4',5,5'
6	2,3'	59	2,3,3',6	112	2,3,3',5,6	168	2,3',4,4',5',6
7	2,4	60	2,3,4,4'	113	2,3,3',5',6	169	3,3',4,4',5,5'
8	2,4'	61	2,3,4,5	114	2,3,4,4',5		**Heptachlorobiphenyls**
9	2,5	62	2,3,4,6	115	2,3,4,4',6		
10	2,6	63	2,3,4',5	116	2,3,4,5,6	170	2,2',3,3',4,4',5
11	3,3'	64	2,3,4',6	117	2,3,4',5,6	171	2,2',3,3',4,4',6
12	3,4	65	2,3,5,6	118	2,3',4,4',5	172	2,2',3,3',4,5,5'
13	3,4'	66	2,3',4,4'	119	2,3',4,4',6	173	2,2',3,3',4,5,6
14	3,5	67	2,3',4,5	120	2,3',4,5,5'	174	2,2',3,3',4,5,6'
15	4,4'	68	2,3',4,5'	121	2,3',4,5',6	175	2,2',3,3',4,5',6
	Trichlorobiphenyls	69	2,3',4,6	122	2',3,3',4,5	176	2,2',3,3',4,6,6'
		70	2,3',4',5	123	2',3,4,4',5	177	2,2',3,3',4',5,6
16	2,2',3	71	2,3',4',6	124	2',3,4,5,5'	178	2,2',3,3',5,5',6
17	2,2',4	72	2,3',5,5'	125	2',3,4,5,6'	179	2,2',3,3',5,6,6'
18	2,2',5	73	2,3',5',6	126	3,3',4,4',5	180	2,2',3,4,4',5,5'
19	2,2',6	74	2,4,4',5	127	3,3',4,5,5'	181	2,2',3,4,4',5,6
20	2,3,3'	75	2,4,4',6		**Hexachlorobiphenyls**	182	2,2',3,4,4',5,6'
21	2,3,4	76	2',3,4,5			183	2,2',3,4,4',5',6
22	2,3,4'	77	3,3',4,4'	128	2,2',3,3',4,4'	184	2,2',3,4,4',6,6'
23	2,3,5	78	3,3',4,5	129	2,2',3,3',4,5	185	2,2',3,4,5,5',6
24	2,3,6	79	3,3',4,5'	130	2,2',3,3',4,5'	186	2,2',3,4,5,6,6'
25	2,3',4	80	3,3',5,5'	131	2,2',3,3',4,6	187	2,2',3,4',5,5',6
26	2,3',5	81	3,4,4',5	132	2,2',3,3',4,6'	188	2,2',3,4',5,6,6'
27	2,3',6		**Pentachlorobiphenyls**	133	2,2',3,3',5,5'	189	2,3,3',4,4',5,5'
28	2,4,4'			134	2,2',3,3',5,6	190	2,3,3',4,4',5,6
29	2,4,5	82	2,2',3,3',4	135	2,2',3,3',5,6'	191	2,3,3',4,4',5',6
30	2,4,6	83	2,2',3,3',5	136	2,2',3,3',6,6'	192	2,3,3',4,5,5',6
31	2,4',5	84	2,2',3,3',6	137	2,2',3,4,4',5	193	2,3,3',4',5,5',6
32	2,4',6	85	2,2',3,4,4'	138	2,2',3,4,4',5'		**Octachlorobiphenyls**
33	2',3,4	86	2,2',3,4,5	139	2,2',3,4,4',6		
34	2',3,5	87	2,2',3,4,5'	140	2,2',3,4,4',6'	194	2,2',3,3',4,4',5,5'
35	3,3',4	88	2,2',3,4,6	141	2,2',3,4,5,5'	195	2,2',3,3',4,4',5,6
36	3,3',5	89	2,2',3,4,6'	142	2,2',3,4,5,6	196	2,2',3,3',4,4',5',6
37	3,4,4'	90	2,2',3,4',5	143	2,2',3,4,5,6'	197	2,2',3,3',4,4',6,6'
38	3,4,5	91	2,2',3,4',6	144	2,2',3,4,5',6	198	2,2',3,3',4,5,5',6
39	3,4',5	92	2,2',3,5,5'	145	2,2',3,4,6,6'	199	2,2',3,3',4,5,6,6'
	Tetrachlorobiphenyls	93	2,2',3,5,6	146	2,2',3,4',5,5'	200	2,2',3,3',4,5',6,6'
		94	2,2',3,5,6'	147	2,2',3,4',5,6	201	2,2',3,3',4',5,5',6
40	2,2',3,3'	95	2,2',3,5',6	148	2,2',3,4',5,6'	202	2,2',3,3',5,5',6,6'
41	2,2',3,4	96	2,2',3,6,6'	149	2,2',3,4',5',6	203	2,2',3,4,4',5,5',6
42	2,2',3,4'	97	2,2',3',4,5	150	2,2',3,4',6,6'	204	2,2',3,4,4',5,6,6'
43	2,2',3,5	98	2,2',3',4,6	151	2,2',3,5,5',6	205	2,3,3',4,4',5,5',6
44	2,2',3,5'	99	2,2',4,4',5	152	2,2',3,5,6,6'		**Nonachlorobiphenyls**
45	2,2',3,6	100	2,2',4,4',6	153	2,2',4,4',5,5'		
46	2,2',3,6'	101	2,2',4,5,5'	154	2,2',4,4',5,6'	206	2,2',3,3',4,4',5,5',6
47	2,2',4,4'	102	2,2',4,5,6'	155	2,2',4,4',6,6'	207	2,2',3,3',4,4',5,6,6'
48	2,2',4,5	103	2,2',4,5',6	156	2,3,3',4,4',5	208	2,2',3,3',4,5,5',6,6'
49	2,2',4,5'	104	2,2',4,6,6'	157	2,3,3',4,4',5'		**Decachlorobiphenyl**
50	2,2',4,6			158	2,3,3',4,4',6		
51	2,2',4,6'			159	2,3,3',4,5,5'	209	2,2',3,3',4,4',5,5',6,6'
				160	2,3,3',4,5,6		

Table II Relative ECD-response factors of individual PCB components, given as range of peak heights per pg component injected, for m available components with chlorine number n_{Cl}. * the range is 8.0 - 15.3 when the data for component 97 is deleted.

n_{Cl}	m	relative factors
1	3	0.07 - 2.2
2	8	1.4 - 7.7
3	9	4.7 - 14.3
4	22	4.4 - 11.1
5	18	8.0 - 31.2*
6	11	7.1 - 16.5
7	10	4.9 - 10.6
8	6	6.1 - 10.9
9	2	2.0 - 18.1
10	1	41

Table III 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.

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

b. Experimental part

Clophens, labelled 16571 (A30), 16557 (A40), 16572 (A50), 16573 (A60) and chloro-biphenyl standards were partly gifts from Bayer (Leverkusen, FRG), and Analabs Inc. (Northhaven, USA).

A Hewlett Packard gas chromatograph model 5880A, equipped with a pulsed ^{63}Ni-electron capture detector was used for GC-ECD analysis in the splitless mode. Solvent was n-hexane (Nanograde, Mallinckrodt Inc., St. Louis, USA). All individual components were injected individually to determine response factors and retention times. Synthetic mixtures of various compositions were made to determine temperature-programmed conditions for optimum separation. The conditions for the capillary WCOT (50 m x 0,33 m) SE-54 fused silica column, compromising between the various chromatographic regions, were as follows: carrier gas He 130 kPa; autosampler injection 1 mm^3 splitless; make-up gas N_2 30 cm^3 min^{-1}, detector 320°C, injector 230°C, septum purge 5 cm^3 min^{-1}, injector purge 20 cm^3 min^{-1} He; temperature programme: isothermal phases at 60°C (2 min.), 180°C (15 min.), 210°C (5 min.) and 250°C (10 min.), with intermediate temperature increase rates of 10, 4 and 4°C min^{-1}, respectively. The separation and co-elution of the available components under these conditions is represented in Figure 2. Retention times were very reproducible (within 0.000 - 0.005 minutes over prolonged periods for retention times up to 1 hour). This allowed accurate correlations.

A Finnigan 1020 automated GC/MS System with a Data General Nova 3 computer was used to obtain the mass spectral data. Column as above (156,000 theoretical plates, C_{14}). Injector 230°C, inlet 60°C (1 min.), 6°C min^{-1} up to 240°C. Separator oven at 240°C and analyzer at 90°C; electron energy 70 eV; scan range 150 - 550 amu; 1 sec scan time.

c. Results

Mass spectrometric analysis:

Reconstructed single ion mass chromatograms in combination with the reconstructed total ion current chromatogram (RIC) are shown for Clophen A60 in Figure 3. The selected ions characterize strong peaks in each of the clusters M^+, $(M-35)^+$ and $(M-70)^+$. Usually for PCB components, the strongest peaks belong to even masses in M^+, followed by even ones in $(M-70)^+$; much weaker peaks characterize the $(M-35)^+$ cluster (odd masses). The chlorine number of the component(s) in each peak of the RIC were evaluated by comparing the relative contributions of strong peaks characterizing the various clusters. For example, the characteristic ratio of peaks with m/e = 360, 326 and 290 for hexachloro-components can be observed for several well-separated peaks in Figure 3 (These define the peak numbers given completely in Figure 4; these should not be confused with scan numbers). Any significant contribution from a pentachloro-component results in a larger contribution of m/e = 326. The distinction between such components is illustrated in Figure 3 for the practically co-eluting pairs of components in peak numbers 65, 66 (hexa, penta), 73, 74 (hexa, hepta) and 88, 89 (hepta, hexa). Distinction is still possible in cases where the peak maxima coincide, provided that the chlorine numbers differ by one. Two peaks originating from components with equal chlorine numbers could only be distinguished if the peak maxima were separated by at least 2 - 3 scan numbers in cases of similar contribution, or by at least 5 scan numbers in cases of very dissimilar contributions, where one peak appeared as a weak shoulder on another one.

Single ion mass chromatograms have also been analysed for the other formulations (using m/e = 220, 221, 222, 254, 255, 256 etc.). The total number of positions throughout the series of Clophens where a peak was detected in total ion current and/or in single ion mass chromatograms of at least one of the formulations, was 106. These define the peak numbers in Figure 4. Chlorine numbers of the components in each peak were obtained from reconstructed single ion mass chromatograms and full mass spectra and, in more complex situations with more than one component contributing to a peak, from a study of the evolution of mass spectral details around each peak. This information could also be used as the proof that the peaks of interest were actually derived from PCB components. For closely eluting (or co-eluting) components with different chlorine numbers, the relative contributions of such components were evaluated (semi-quantitatively) from single ion mass chromatograms. Chlorine numbers and relative contributions of components in each peak are given in Table IV.

In the following, some specific findings will be considered. The chlorine numbers of the major constituents are 2 and 4 but mainly 3 in A30, 3 and 5 but mainly 4 in A40, 4 and 6 but mainly 5 in A50, and 5 and 7 but mainly 6 (and also significant contributions of 8 and 9) in A60. Several peaks (e.g., no. 7 and 13, Figure 4) appeared only as minor contributions in one or more formulations. Early eluting peaks are strong in A30 and very weak in A60 whereas late peaks are strong in A60 and very weak in A30. The contributions of all peaks show systematic trends within the series A30-A60 but with considerably different rates of change. Some peaks have maximum contributions in A30 (e.g., peak no. 4), others in A40 (e.g., 27), etc. The structure of partly resolved and composite peaks (i.e., with proven contributions of two or more co-eluting components) are therefore also expected to differ appreciably between the formulations. The single ion mass chromatographic data are essential in this respect. For instance, whereas peak 9 has similar contributions from chlorine numbers 2 and 3 in A30, the contribution of 3 dominates in A40; peak 45 changes its composition from 90 % tetra- 10 % pentachloro in A30 to practically 100 % penta in A60. The practically co-eluting peaks 65 (hexa) and 66 (penta) increase and decrease strongly in the Clophen series and the contributions of peaks 70-72 (hexa-, hexa- and pentachloro PCB) increase, remain constant and decrease in the series. Similar observations were also made for weaker peaks. For example, peaks no 75, 76 and 78 showed the presence of hexa in A50 and hepta in A60.

Finally, it was determined which of the available individual PCB components could be assigned to each Clophen peak, taking into account chromatographic retention and chlorine number constraints. Individual components and the synthetic mixture were subjected to GC-MS under the same conditions as the Clophens. Although temperature-programme conditions during GC-MS were different from those in GC-ECD analysis (Figure 2), the order of elution was identical and the separation of adjacent components differed slightly, for a few pairs of peaks only. Thus, the analysis of the synthetic mixture by GC-MS was fairly straightforward. For each peak, the number of chlorine atoms actually found agreed with the assumed content of the sample vials. It is still possible that some components have not been labelled correctly. However, no errors were found in the chlorine numbers. The components listed in the third column of Table IV, satisfy retention and chlorine number constraints.

Figure 3 Relative abundances of mass peaks in reconstructed single ion and total ion current mass chromatograms of Clophen A60. Peak numbers are defined in Figure 4.

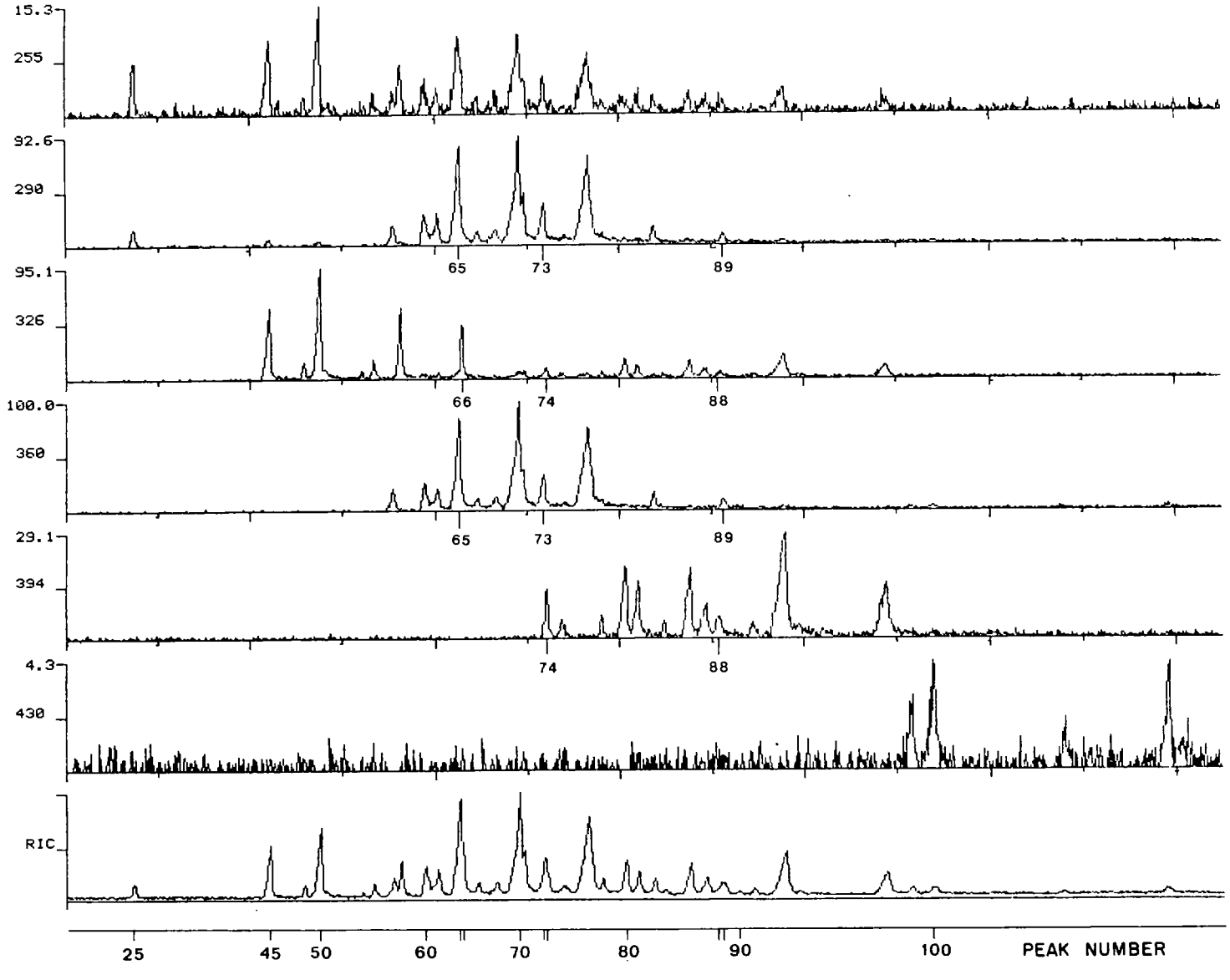


Figure 4 Relative abundance of mass peaks in reconstructed total ion current mass chromatograms of Clophen A30, A40, A50 and A60.
Peak numbers identify the positions at which a peak was detected in total ion and/or single ion mass chromatograms of at least one of the formulations.

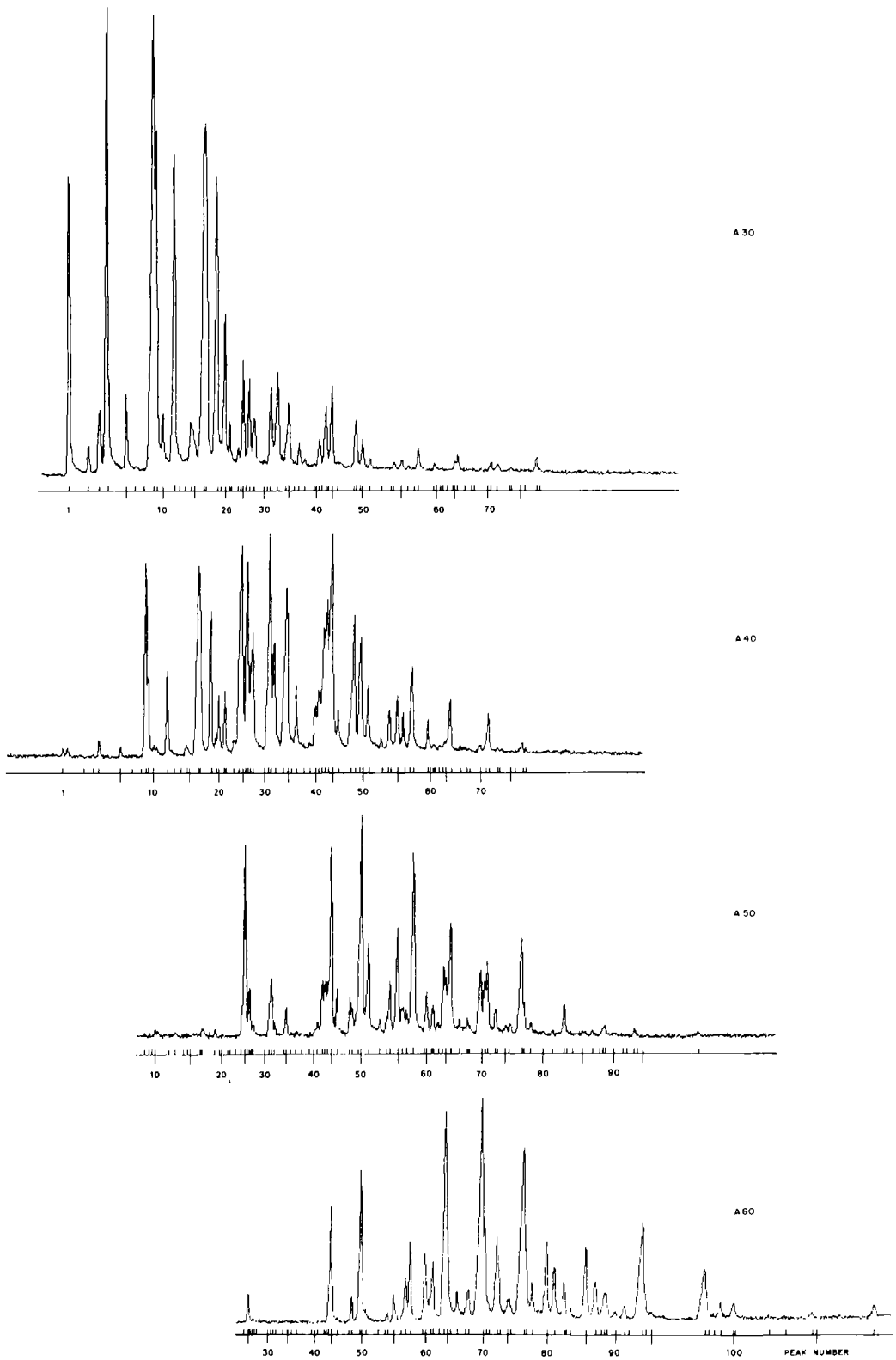


Table IV Composition of peaks in ECD chromatograms of Clophen A30 - A60 as represented in Figure 5. PCB components with retention times corresponding to each Clophen peak within ± 0.005 min are listed in order of elution. Well separated components are given in consecutive lines.
At least partly separated components are given in consecutive lines, connected by the symbol {. Exactly co-eluting components are given in the same line. In some cases, such components could be distinguished by MS: for clarity, these are also given in consecutive lines, connected by the symbol [.

Column 1: IUPAC numbers of PCB components (u if yet unidentified).
a: component no 56 resulted in two strong peaks, co-eluting with 40 and 60.

Column 2-5: for each formulation, peak heights relative to the highest peak: ++++ ≈ 100 %; +++ ≈ 75 %; ++ ≈ 50 %; + ≈ 25 %; (+) ≈ 10 %; tr = trace, sh = shoulder.
Information derived from MS is included as percentage contributions for each set of closely eluting or co-eluting components.

Column 6: chlorine number(s) of component(s) detected by MS

Column 7: the corresponding peak number(s) as defined in the mass chromatograms (Fig. 4)

Column 8: IUPAC numbers of co-eluting components with the inappropriate chlorine number (in brackets).

Components (IUPAC numbers)	Relative peak heights in ECD chromatograms and if applicable, relative contributions of co-eluting components, determined by MS				Chlorine number(s) of component(s) detected by MS	Corresponding peak numbers in mass-chromatograms (Fig. 4)	Co-eluting components with inappropriate chlorine numbers
	A30	A40	A50	A60			
4,10	+	-	-	-	2	1	
7, 9	+	-	-	-	2	2	
u	+	-	-	-	2	3	2(1)
5, 8	++++	(+)	(+)	-	2	4	
19	+	(+)	-	-	3	5	
18	++++	++	(+)	-	3	.8	
[15 / [u	++[-50 / -50	+[-20 / -80	(+)	-	[2 / [3	9	

Table IV continued

Components (IUPAC numbers)	Relative peak heights in ECD chromatograms and if applicable, relative contributions of co-eluting components, determined by MS				Chlorine number(s) of component(s) detected by MS	Corresponding peak numbers in mass-chromatograms (Fig. 4)	Co-eluting components with inappropriate chlorine numbers
	A30	A40	A50	A60			
24	+	tr	-	-	3	10	
u	++++	++	tr	-	3	11	
u	tr	-	-	-	3	12	
29	(+)	-	-	-	3	13	
26	+	(+)	-	-	3	14	
u	+	tr	-	-	3	15	
31	++++	++++	(+)	-	3	16	
28	++++	++++	(+)	-	3	17	50(4)
21,33	90	70	50		3		
[53	++++ [10	++ [30	(+) [50	-	[4	18	
u	tr	tr	-	-	4	19	
u	++++	+	-	-	3	20	
u	+	+	tr	-	4	21	
46	(+)	(+)	-	-	4	23	
52	++	++++	++++	+	4	25	
49	++	++++	+	(+)	4	27	
47	30	30			4	28	
{75	+ {70	++ {70	+	+	{4	29	
u	tr	-	-	-	5	30	
44	++	+++	++	(+)	4	32	
37	90	50			3		
{42	{10	{50	-	-	{4	33	
u	50	40	20		4	34	
{41	++ {50	++++ {60	+ {80	-	{4	35	
u	-	tr	tr	-	4	36	
40 a)	+	+	(+)	-	4	37	
67	(+)	(+)	-	-	4	38	
u	tr	(+)	tr	tr	4	39	
61	+	+++	+	-	4	41	

Table IV continued

Components (IUPAC numbers)	Relative peak heights in ECD chromatograms and if applicable, relative contributions of co-eluting components, determined by MS				Chlorine number(s) of component(s) detected by MS	Corresponding peak numbers in mass-chromatograms (Fig. 4)	Co-eluting components with inappropriate chlorine numbers
	A30	A40	A50	A60			
70	-	30	30		4	42	
{80	++ 100	++++ {30	+++ {30	(+)	{4	43	
98	-	30	30		5	44	
[66	90	90	40	+	[4	45	
[95	++ [10	++++ [10	++++ [60	+	[5		
{88	(+)	+	+	(+)	{5	46	
{u	tr	-	-	-	{5		
60 a)	++	++++	+	-	[4	48	
92	+(sh)	+(sh)	++	(+)	[5		
84	(+)	+	++	(+)	5	49	
101	+	++	++++	++	5	50	
99	(+)	+	+++	(+)	5	51	
83	-	(+)	+	-	5	52	
86,97	tr	(+)	++	-	5	54	
87,90,116	+	++	++++	+	5	55	
120	(+)	+	++	(+)	5	56	
136	(+)	(+)	+	+	6	57	
77	20	20	10	-	4	58	
[110	+ [80	+++ [80	++++ [90	++ [100	[5		
82	+	+	+	(+)	5	59	
151	-	-	(+)	+	6	60	
u	tr	tr	(+)	+	6	61	
u	tr	tr	(+)	-	6	62	
u	tr	tr	(+)	tr	6	63	107(5)
149	(+)	(+)	+++	++++	6	65	
118	(+)	++	++++	+	5	66	
u	tr	tr	(+)	(+)	6	67	
u	tr	tr	tr	tr	6	68	
u	tr	tr	tr	tr	6	69	
153	(+) 20	(+) 10	+++ 30	++++ 60	6	70	
{132	(+) {20	(+) {30	++ {30	++ {30	{6	71	
105	(+) 60	+ 60	+++ 30	(+) 10	5	72	
141	tr	tr	+	++	6	73	
179	tr	tr	-	+	7	74	

Table IV continued

Components (IUPAC numbers)	Relative peak heights in ECD chromatograms and if applicable, relative contributions of co-eluting components, determined by MS				Chlorine number(s) of component(s) detected by MS	Corresponding peak numbers in mass-chromatograms (Fig. 4)	Co-eluting components with inappropriate chlorine numbers
	A30	A40	A50	A60			
u	-	-	(+)	-	6	75	
u	-	-	-	tr	7		
u	-	-	(+)	-	6	76	
176	-	-	-	(+)	7		
138	+	(+)	++++	++++	6	77	
u	tr	tr	(+)	+	6	78	
129	-	-	(+)	(+) 50	6	79	
u	-	-	tr	(+) 50	7		
187	-	-	tr	++	7	80	
183	-	-	tr	+	7	81	
128	-	-	+	+	6	82	
167	-	-	tr	tr	6	83	
u	-	-	-	tr	7	84	
174	-	-	tr	+	7	85	
177	-	-	tr	(+)	7	86	
u			+	-	6		
193	-	-	-	+	7	87,88	
202			-	tr	8		
156		-	+	+	6	89	171(7)
200	-	-	-	tr	8	90	157(7)
u	-	-	-	tr	8		
172	-	-	(+)	(+)	7	91	
197	-	-	-	tr	8	92	
180	-	-	+	++++	7	93	
u	-	-	-	tr	7	94	
u	tr	tr	tr	tr	8	95	
170	-	-	+	+++	7	96	
u	-	-	-	tr	7	98	
201	-	-	-	+	8	99	
196	-	-	-	+	8	100	
189	-	-	-	(+)	7	102	
u	-	-	-	+	8	103	
194	-	-	-	(+)	8	104	
u	-	-	-	tr	8	105	
206	-	-	-	tr	9	106	
209	-	-	-	+	10		

GC-ECD analysis:

ECD chromatograms of the Clophen series are represented in Figure 5. All peaks observed in at least one of the Clophens have been listed in order of elution in Table IV. Individual PCB components with retention times compatible with the chromatograms of the formulations within ± 0,01 min are identified for each peak by their IUPAC number(s) or by u, if no component with the appropriate properties was available. One of these numbers or the symbol u is given at the apex of the corresponding peak in Figure 5. Components with inappropriate chlorine numbers, as determined by GC-MS, are given in the last column of Table IV. It is interesting to note that ECD chromatograms of the Aroclor formulations that are available to us, were practically identical to those of the Clophens with corresponding chlorine contents.

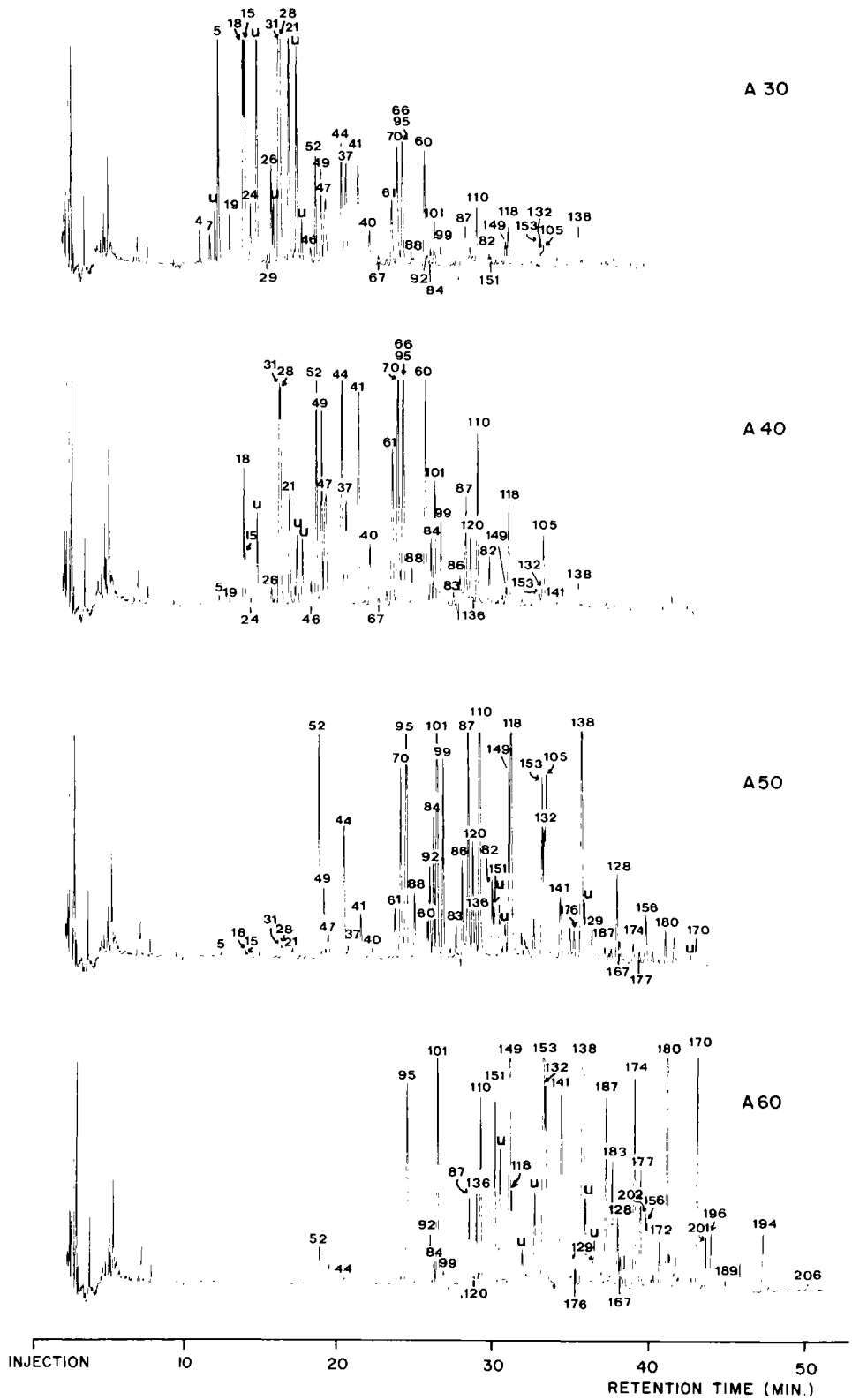
Despite the very different temperature programme conditions the elution order of the PCB components in the ECD chromatograms was identical to that in the total ion current mass chromatograms presented in Figure 4. Equally important is the fact that the relative peak heights were very similar, except for the mono-chloro components which are characterized by a considerable lower response on ECD.

Figure 5 shows that several components eluting at close intervals can be distinguished at least qualitatively, such as the pairs 18-15; 31-28: 60-92-84; 82-151; 149-123-118; 153-132-105. Strong variations can be observed in the ratios between 149 and 118 and between 153, 132 and 105 within the Clophen series. Whereas 118 is the dominant peak in A30-A50, 149 dominates in A60. Components 153 and 132 increase in the series A30-A60, 105 dominates in A40 and is practically absent in A60. On the basis of missing (or very weak) peaks at the expected retention times, the absence (or presence in only trace amounts) of several components can be determined (Table V). These findings are supported by the GC-MS data, which showed the absence of three more components (Table IV).

Table V Components (IUPAC numbers) in the Clophen mixtures, detected at only trace concentrations or below the detection limit of mass spectrometry (MS) and electron capture detection (ECD) technique in this work.
-: absent and tr: in trace amounts.
* no distinction from co-eluting component by ECD.

Component	MS	ECD
1	-	-
2	-	-
3	-	
7	tr	tr
9	tr	tr
11	-	-
12	-	-
14	-	-
29	tr	tr
30	-	-
46	tr	tr
50	-	*
58	-	-
62	-	-
65	-	-
67	-	tr
69	-	-
71	tr	-
72	tr	-
107	-	-
123	-	*
155	-	-
157	-	*
171	-	*
197	tr	tr
207	tr	tr

Figure 5 Temperature programmed capillary ECD chromatograms of Clophen A30, A40, A50, and A60. Peaks are identified in terms of individual PCB components by the IUPAC number of the component or one of the components (if applicable, with the largest contribution) with appropriate retention time. u = unknown. Full details are presented in Table IV. Experimental conditions as for Figure 2.



Combination of MS and ECD data:

The good agreement between both the elution patterns and relative peak heights of all four Clophens in the ECD - and total ion current mass - chromatograms allowed a relatively straightforward correlation between the results of the two detection techniques (Table IV). Several ECD peaks include possible contributions from co-eluting components with equal chlorine number. The relative contributions of at least partly separated components with equal and different chlorine numbers, and of co-eluting components with different chlorine numbers, were evaluated from single ion mass chromatograms. These were taken into account when considering the composition of peaks that are labelled 15, 21, 47, 37, 70, 95 and 110 in the ECD chromatograms. This has assisted in obtaining a quantitative estimate of the contributions of as many individual components as was possible in each of the Clophen formulations (Table VI), based on ECD response and compositional data obtained from GC-MS analyses.

d. Comparison with literature data

Qualitative analyses:

SISSONS and WELTI (14), using NMR, MS and IR and solid-liquid chromatographic preseparation techniques, identified the major components of Aroclor 1254: 52, 44, 95, 84, 70, 101, 99, 87, 110, 149, 132, 118, 105, 153, 138, and using solely NMR, components with smaller contributions: 128, 156 and 170. Their major components also appear in the presently reported assignment of the strongest peaks in Clophen A50. The present assignments also include 66 (co-eluting with 95), 80 and 98 (with 70), 86 (with 97), 116 (with 87) and 77 (with 110).

JENSEN and SUNDSTRÖM (15) using 90 components synthesized by them as well as some literature data, were able to calculate the percentage contributions of 46 components in Clophen A50 and A60. There is reasonably good agreement between their assignment and ours with respect to components that were available as reference compounds to them and to us.

WEBB and McCALL (19) separated 27 PCB components in Aroclor 1221, 1242 and 1254; the GC retention times and IR spectra were compared with those of known, prepared compounds. The identification of components eluting from their SCOT SE-30 column agrees with our findings for the components which were available to them. These authors also reported 20 components to be absent from Aroclors 1221-1254, including 37, 42, 26, 90, 92, 120. However, these components appear in our assignments. TAS and DE VOS (20) and TAS and KLEIPOOL (21) synthesized seven components (153, 138, 180, 170, 136, 149 and 174) and proved their presence in Phenoclor DP6 by application of NMR, IR and GC techniques. SCHULTE and ACKER (23) analysed Aroclor 1254 on a 60m SE-30 capillary column with ECD. Their elution pattern was identical to that reported here for the 20 components which they identified by GC and GC-MS analysis. The reported elution order is different, however, for 128, 187, 202 and 200.

Recently, some authors have made extensive use of retention indices of available components to obtain estimates of retention data of unavailable components. Thus, complete quantitative characterization of the Aroclor series 1016-1260 has been claimed (18, 25, 29). With the use of retention indices of all PCB components computed for 13 GC phases, six packed columns with liquid phases of varying selectivities were needed for 1221 and twelve for 1242 and 1016, based upon 38 available components. Four capillary columns with different selectivities were needed for Aroclor 1248, 1254 and 1260, using 45 available components (25). It is difficult to compare their data because no detailed information on chromatograms and available components was presented. BALLSCHMITER et al. presented the most recent and detailed results for the analysis of technical formulations (7, 26), as a basis for analysis for various types of environmental samples (32, 33). They have presented chromatograms obtained with ECD on SE-30 columns, resulting in the same elution pattern as on the present SE-54 column. The chromatograms differ little from those presented here, except for a few details in separation. Using calculated retention indices, and experimental data for 45 available components, detailed assignments of the Clophen series were given (7, 26). The agreement between their assignment and ours is good, but not perfect, for these 45 components. Significant differences exist for other components. Because it is the most recent and detailed assignment presently available in the literature, and it has been applied to various types of environmental samples (32, 33), it may be useful to specify where differences exist on the basis of experimentally determined retention times and chlorine number constraints. This then applies to components 14, 29, 33, 36, 42, 51, 54, 62, 79, 86, 90, 128, 130, 132, 137, 160, 163, 165, 167, 173, 174, 177, 196, 200, 201, 202 and 208. For these components, bottles may have been wrongly labelled or calculated retention times may not be sufficiently reliable. Future work in different laboratories will assist in distinguishing between these possibilities.

Quantitative analyses:

Table VI summarizes the results of quantitative estimates of available components in Clophen formulations A30-A60 evaluated in the present work and those in A50 and A60 analysed by JENSEN and SUNDSTRÖM (15). Generally the agreement is good, differences being within a factor of 2, with the exception of component 105 in A50. The sum of all contributions that we were able to determine, is practically 100 %, particularly if the contribution of the few strong and unidentified peaks in A30 and A40 are taken into account (their aggregate contribution was estimated to be 10 % on the basis of estimated response factors, Table II). A comparison with other detailed quantitative data is more complicated, despite the agreement for many components, because available reference components have not been specifically identified (25). Recently, the synthesis of the octa- and nonachlorobiphenyls and their quantification in Aroclors was reported (34). Agreement for the components available to us is reasonable; quantitative data for six additional components contributing about 6 % (34) cannot be confirmed by us because of lack of reference compounds.

e. Conclusions

As on other capillary columns several PCB components remain unresolved on the present SE-54 fused silica column. The application of both electron capture and mass spectrometric techniques for the

Table VI Percentage contents (%) of individual components (identified by their IUPAC numbers) in Clophen A30 - A60 formulations, taken from the literature (JENSEN & SUNDSTRÖM, right) and evaluated in the present work on the basis of single component-ECD response factors (left). Values <0.1 not given.
a: average response factors used for co-eluting components with equal chlorine numbers;
b: response factors evaluated with the use of GC-MS data as given in Table I. {: not fully separated

IUPAC number	comment	Present work				Literature	
		Clophen					
		A30	A40	A50	A60	A50	A60
4+10	a	1.5					
7+ 9	a	0.6					
5+ 8	a	6.1	0.2				
19		1.2					
18		9.9	3.8				
15	b	9.0	0.8				
24		0.5					
29		0.2					
26		2.1	0.4				
31	b	6.8	2.4	0.1			
28	b	9.9	4.0				
21+33	a	4.6	1.3				
53		0.5	0.5				
46		0.5	0.8				
52		3.1	7.3	6.8	1.1	5.0	
49		1.2	4.1	1.5		1.4	
47	b	0.4	0.7	0.1			
75	b	0.9	1.7	0.2			
44		3.0	6.6	3.3		1.9	
37	b	3.2	1.2				
42	b	0.4	1.2	0.3			
41		1.6	3.5	0.7		1.29	
40		0.7	1.2	0.2			
67		0.2	0.2				
61		0.9	2.2	0.8			
70	b		1.9	1.2		3.9	
80	b	2.5	1.8	1.2			
98	b		1.8	1.1			
95	b	0.3	0.6	2.3	3.9	4.4	2.9
66	b	2.3	5.7	1.6			
88		0.2	0.4	0.4	0.1		
60		1.5	3.1	1.6	0.8		
92				1.3	0.5		
84		0.4	1.3	2.7	0.4	2.5	0.3
101		0.7	2.3	6.1	4.1	7.0	5.6
99		0.3	1.1	2.5	0.2	1.8	

Table VI continued

IUPAC number	comment	Present work				Clophen	Literature	
		A30	A40	A50	A60		A50	A60
83		0.2	0.3	0.7				
86+97	a	0.2	0.6	1.3	0.2			
87+90+116	a	0.4	1.1	3.5	0.9			
120		0.3	1.0	1.9				
136		0.1	0.1	0.7	1.5		0.5	1.0
77	b	0.3	0.7	1.1				
110	b	1.0	2.8	9.7	3.6		7.6	2.9
82		0.2	0.9	1.4	0.1		1.0	
151		0.1	0.1	0.6	4.7		1.3	3.3
149		0.5	0.4	4.1	9.6		2.0	6.5
118		2.5	6.7	10.5	1.0		5.0	1.6
153	b	0.5	0.3	3.2	8.6		4.2	12.9
132	b	0.2	0.5	3.1	4.6		1.8	3.2
105	b	0.5	1.5	0.7	0.2		3.6	
141	b			0.7	1.8			
179	b				0.7			
176				0.7	1.3		0.1	0.37
138		0.8	0.5	6.0	11.3		5.1	11.3
129				0.6	1.1			
187		0.1		0.3	3.8			
183				0.3	3.1		0.39	3.3
128				1.4	1.2		1.3	2.0
167				tr	tr		0.47	1.0
174				0.3	4.9		0.33	3.7
177				0.3	3.4		0.27	2.1
202	b				0.8		tr	0.07
193	b				0.8			
156				0.8	0.8		0.81	1.5
200				0.2	0.8		tr	0.09
172				0.1	1.1		0.23	0.9
197					tr		tr	tr
180		0.3	0.2	0.2	8.9		0.98	7.6
170		tr	tr	0.6	5.2		0.72	4.1
201					1.4		0.1	0.74
196					1.4		0.1	0.44
189					0.4			
194					1.3		0.35	0.67
206					0.1			
209					0.2			
Sum		85 %	82 %	91 %	100 %			

detection of components present in Clophen A30, A40, A50 and A60 and the availability of 102 individual components as pure reference compounds has revealed many previously unobserved details of the qualitative and quantitative composition of chromatographic peaks in these formulations. It was found that the contribution and composition of any given chromatographic peak may vary considerably within the Clophen series.

Assignments were made for most major peaks and many of the smaller peaks. For some peaks, ambiguities in the assignments still remain, in particular with respect to co-eluting components with equal chlorine numbers. The separation of some pairs can be improved by selection of other operational conditions such as pre-separation by solid-liquid chromatography (15, 27) or by using a number of columns with different selectivities, either in parallel or in series (18, 25, 29). Until all individual components are available, we cannot be sure that for each peak or component, no other co-eluting component(s) exist. The GC-MS results show that this is not likely to be a major problem for most peaks, at least not for those components having different chlorine numbers.

4. Composition of PCB mixtures in environmental samples analysed by GC-ECD and GC-MS

The problems identified in the analysis of technical formulations of PCB mixtures are also encountered when analysing environmental samples, but in the latter case, additional problems exist.

The composition of the PCB mixture in a sample will generally be different from that of any technical formulation. In the case of marine environmental samples, we have also found considerable differences between various compartments such as water and particulate matter. This makes it even more essential to analyse PCB in terms of individual compounds rather than of technical formulations. This is, however, not yet possible for all peaks and/or components. We know that for several peaks, co-eluting compounds exist. Although it has not been found in all such cases, for several peaks of the Clophens the presence of two or more co-eluting components has been established. Morever, the composition of such a composite peak was found (on the basis of GC-MS) to differ between the various Clophens with different overall chlorine contents. The composition of the corresponding peak in an environmental sample extract can only be determined with GC-MS techniques. Although their application is possible in principle, for seawater, large volumes have to be extracted to obtain sufficient material for analysis. However, the success of this approach is limited because other organic compounds, which are present in the extract at usually considerably higher concentrations than the PCB components, obscure the mass spectral signals of trace components. The situation is much more favourable when using the electron capture detecting system because of its high specific sensitivity for chlorine-containing compounds. The compositions of such composite peaks can then not be determined. However, approximate concentrations can still be obtained on the basis of an assumed composition.

Results for water and suspended particle analyses will be discussed after having considered the various steps to which seawater has to be subjected prior to the final analysis of its extract by GC-ECD.

B. Extraction and Separation procedures

1. Extraction from water

The extremely low concentrations at which organochlorines usually occur in seawater require their concentration over many orders of magnitude prior to GC-ECD analysis. Basically, two methods are available: sorption onto a solid adsorbent and solvent extraction.

a. Sorption onto adsorbents

Various solids have been used to adsorb organochlorine compounds from natural waters. These include activated carbon (35), urethane foam plugs (36, 37), polyurethane foam coated with adsorbents (38), a porous polymer Tenax (39), a mixture of activated carbon powder, MgO powder and refined diatomaceous earth (40), Carbowax 4000 and n-undecane on Chromosorb DMCS (41), and Amberlite XAD resins (42-45). The method using XAD resin has been described in detail by DAWSON (46). It was also used during the multi-laboratory IOC/WMO/UNEP workshop on intercalibration of sampling methods in Bermuda (January 1980), resulting in the identification and analysis of several individual PCB components in Sargasso Sea water (1).

b. Solvent extraction

Solvent extraction can be carried out in a batch or in a continuous extraction mode. Batch procedures may be convenient and adequate in cases where sufficient material for analysis - i.e. well above the detection limit - can be extracted from small water volumes. They become inconvenient when processing large volume samples. Moreover, the quality of the solvent becomes critical as large amounts of solvents are required; contaminants in the solvent limit the applicability of batch procedures for water with low concentrations of organochlorines.

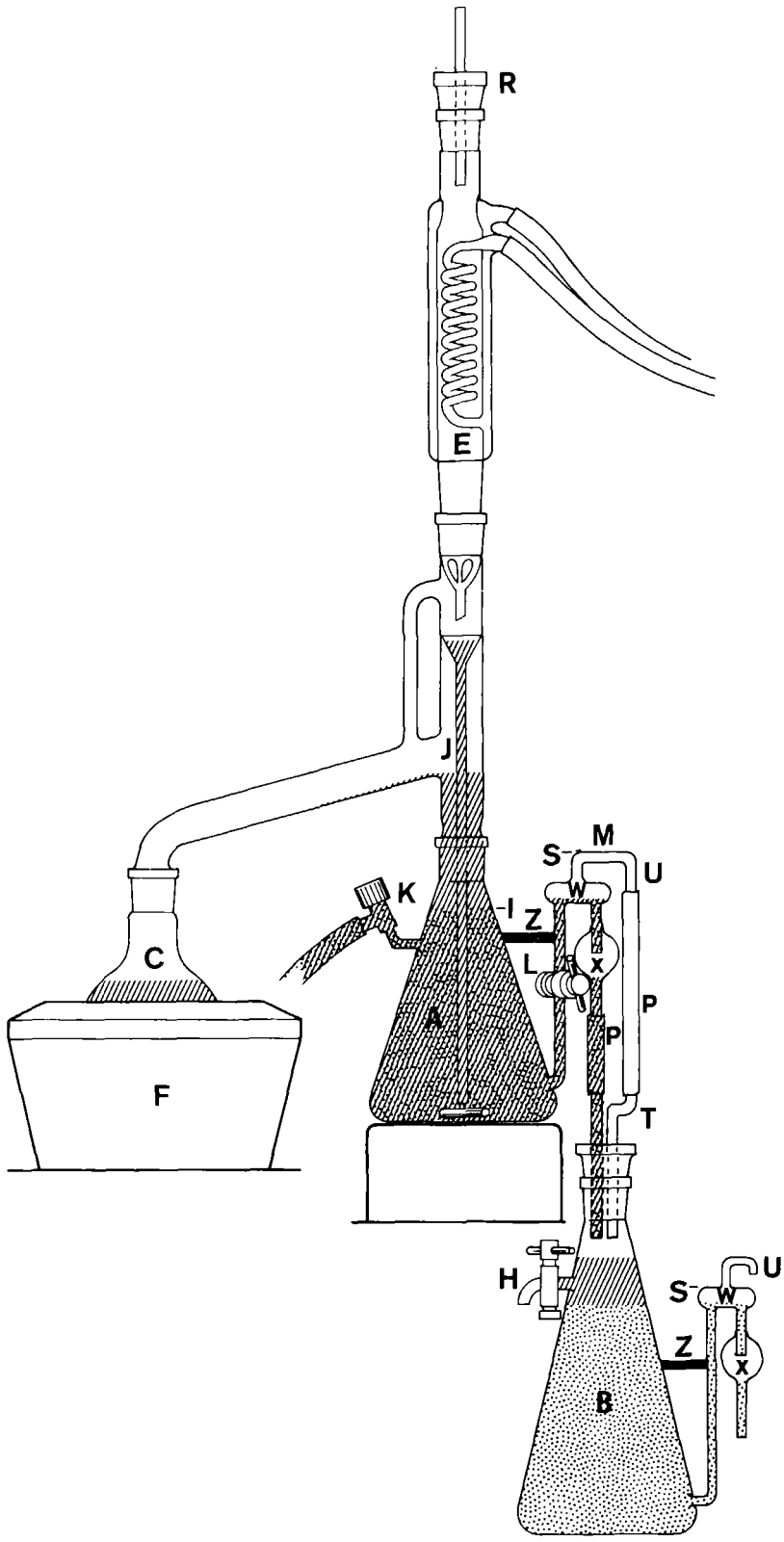
The continuous extraction system described below allows the extraction of essentially unlimited volumes of water with a relatively small volume of solvent (300-400 ml). Some basic aspects are derived from the design of KAHN and WAYMAN (47). In this chapter, we shall pay attention to extraction efficiencies. Some results will be discussed in Section IV.

Extraction system:

The extraction system consists of several units (Fig. 6) that can be used both individually as well as in series. Each unit consists of an extraction (A) and a separation (B) chamber. The solvent is evaporated at its boiling temperature in the round-bottom flask (C) by an electric heater (F), condensed at E and transported through a glass tube J, extending to just above a Teflon-coated magnetic stirrer on the bottom of A, where it is dispersed into fine droplets in water to be extracted. It flows back into C, together with extracted components, thus concentrating in C.

Water is fed into the extractor through a Teflon needle valve (K) from a stainless steel drum under nitrogen pressure or, if units are used in series, from another extracting unit by gravity. All connections consist of Teflon tubing. The setting of K controls the water flow rate. The water outlet of

Figure 6 Continuous extraction unit for water samples. Description is given in the text.



each chamber is an inverse U glass tube with valve L and reservoirs (X, W, with W open to vent U). These prevent syphoning of the content of the chambers each time the liquid level has reached S. The U tube is connected to the chamber by a solid glass bar Z. The setting of valve L is adjusted to produce a stable water-solvent interface at level I that should be high enough to leave sufficient solvent in C and sufficiently low to leave time, in case of flow irregularities, to make adjustments to prevent water flowing into C. The connection between the water outflow tube of A and chamber B is made with Teflon tubing (P).

Solvent, separating out on top of the water phase in B can be removed through Teflon tap H and fed back into the extractor through R at regular intervals. Solvent vapour pressure building up in the space above liquid B can interfere with proper water flow from A to B. This problem is eliminated by vent T. Loss of solvent by evaporation through T is prevented by connecting T and U by a Teflon tube that should be easily removable from U to vent for a few seconds when required. Another important feature of the design is the counter current system whereby the flow of water opposes that of the solvent.

The initial procedures for an extraction are as follows: Fill A to appropriate level, close K and L, initiate the solvent cycle, start stirring, wait for equilibrium; disconnect T and U, open K and adjust L. Measure flow rate from water outflow and adjust K and L according to flow rate desired. Connect T and U when B is filled and starts overflowing.

The units need attention during extraction. The production of a version that does not need attention is presently in progress. Thorough cleaning between samples is essential; it is done by taking the entire apparatus apart, washing all parts with hot water and soap, drying with acetone, and finally washing with n-hexane. Each unit is attached to a separate rigid metal frame. Consecutive extractions in series are made with two frames separated vertically over about 1 m; the outlet flow of B of the first unit is used as the inlet for the second extracting unit (gravity flow).

Extraction efficiency: The extractors, in conjunction with the sampling and filtration units to be discussed below, have been tested during several cruises at sea. In the various stages of the development of the extractors toward their final form, it was attempted to maximize the extraction efficiency and to minimize the loss of solvent with the outflowing water. The residence time of water in the extraction chamber was optimized by determining of the effect of flow rate by simultaneous, independent extractions of different samples from the same water mass. It appears that most compounds of interest could be extracted with a high optimum extraction efficiency (80-100 %), at flow rates < 5 $dm^3\ h^{-1}$. This compares favourably with the equipment designed by AHNOFF and JOSEFSSON, where larger losses were observed (48). Contamination from the ship's atmosphere and the equipment used was kept at a negligible level if extreme precautions were taken. For example, all openings of tubes, drums and sampling equipment remained covered with clean aluminium-foil until use. Loss of compounds of interest to the wall of the stainless steel drum was negligible. These conclusions were established during several cruises. They will be illustrated below for seawater, sampled at 54°12'N 3°50'E in September 1982. A 100 litre sample was filtered. The filtrate was extracted in three extractors in succession (3 x 400 ml hexane). Each extract was processed and analysed separately. The GC-ECD chromatograms of the first silica fractions are given in Fig. 7 A-C.

The potential loss of dissolved components to the wall of the drum was tested as follows. A 100 dm^3 filtered sample was left in a drum, for 24 hours; after it had been emptied, the wall was washed with three portions of 135 ml n-hexane in succession. These were combined, and analysed like the 400 ml water extracts. The chromatogram is given in Fig. 7D. Corresponding peaks can be correlated accurately by vertical lines. For clarity, these are also labelled by identical peak numbes in each chromatogram.

In Table VII, the height of each peak in the first silica fraction of the second and third water extracts and in the drum washings are related to its height in the first water extract, and given as percentage thereof. The compounds contributing to each peak and their concentrations in the seawater sample are included in the Table. Identification of compounds was done on the basis of fit in retention times of $\pm$ 0.01 min between sample and reference compounds. The concentrations of most of the compounds identified in the water sample are extremely low: 1-350 fmol dm^{-3}.

The chromatograms of the second and third extractions are very similar; they appear to represent the blank chromatogram of 400 ml n-hexane, concentrated to 1 cm^3 in a Kuderna-Danish (KD) evaporator. Moreover, the heights of most peaks in the 2nd and 3rd extractions are low with respect to those in the first extraction (0-20 %). For these peaks, one extraction is therefore sufficiently effective and also accurate for their determination even at the fmol dm^{-3} level. A few peaks (e.g. numbers 2, 7, 8, 47, 49, 50) cannot be determined accurately in the present data. For these peaks, larger water samples and/or the use of solvents with still lower levels of interfering components are required.

Interfering compounds were also present in the second fraction, e.g., peak numbers 2, 4, 14, 15 and six peaks beyond peak 30. These also originate from the solvent. The extraction efficiency is 100 % for practically all peaks (95 % for peak no 25, γ-HCH). Therefore, no table was constructed.

The extractors need more attention during operation than the automatic extractors of AHNOFF and JOSEFSSON (48). However, the efficiency is better and no solvent is lost. The chromatograms show that adsorption to the drum wall was not observed for any component.

The extraction procedure appears to be a suitable one for waters with even very low concentrations. Background levels in the solvent even get progressively less important when analysing natural waters with larger concentrations of organochlorines than in the example given here.

c. Comparison of continuous solvent and XAD-2 resin extraction

In this chapter we shall describe work on isolation of PCB and PAH from sea water using the AHNOFF and JOSEFSSON continuous extractor compared to direct and XAD-2 extractions. The work was carried out using seawater spiked with a solution of

Figure 7 ECD-chromatograms of the first silica fractions of the first (A), second (B) and third (C) extracts of a 100 dm^3 seawater sample from the North Sea (see text). Chromatogram (D) represents the extract involving the material adsorbed to the container wall during a 24 hours exposure to a sample from the same water mass as (A), see text.
Corresponding peaks are labelled by the same peak number (1 - 62) in the four chromatograms.

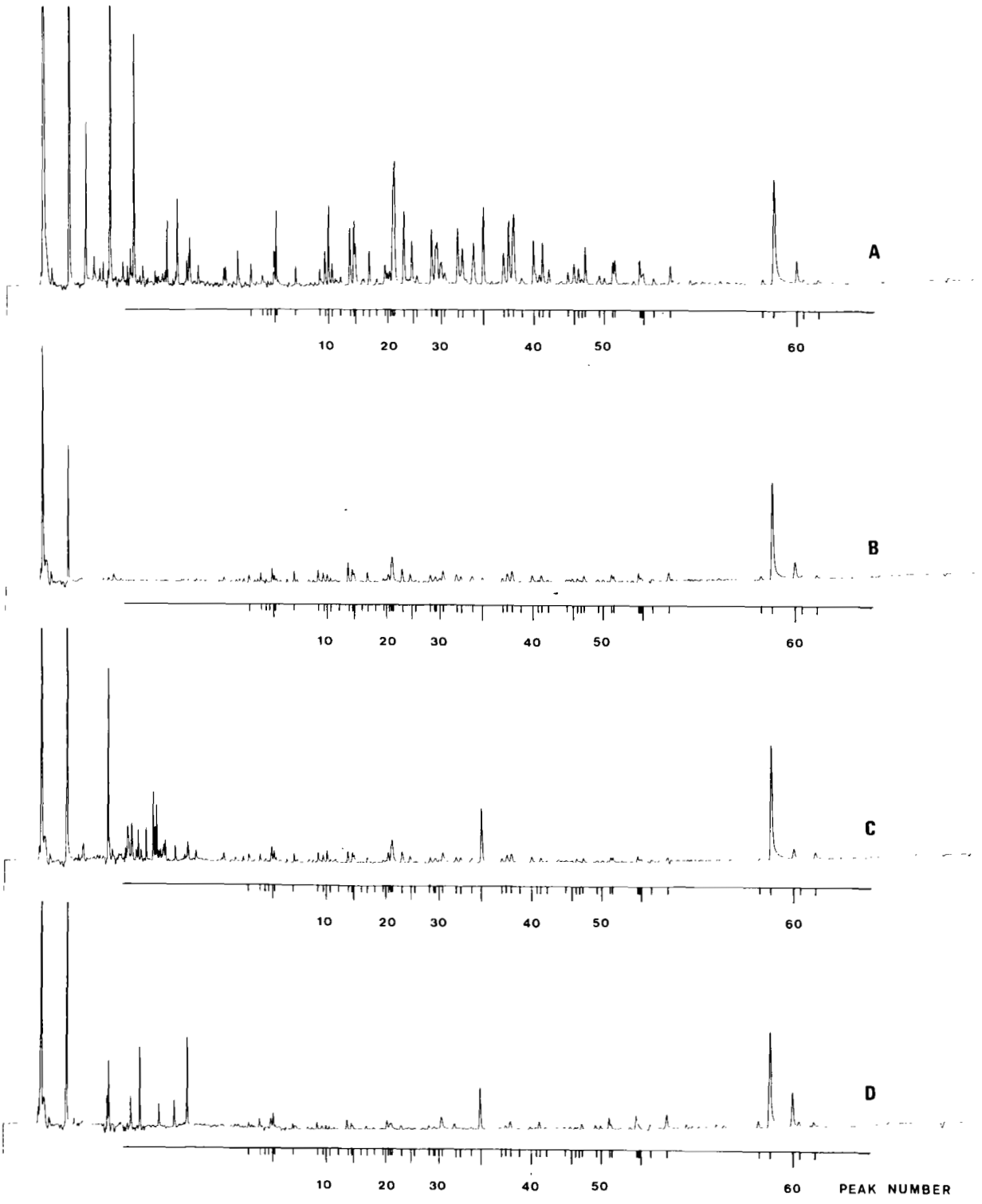


Table VII Qualitative and quantitative data on the peaks in the first silica fractions of consecutive extractions of a 100 litre filtered water sample from the North Sea.
First column: Peak numbers as defined in Figure 7;
Second column: Peak heights in arbitrary relative units;
Third and fourth columns: Peak heights in 2nd and 3rd extracts, expressed as % of peak heights in first extraction;
Fifth column: Peak heights in extract of drum wall, expressed as % of peak heights in first extraction;
Sixth column: assignment of available PCB components in terms of IUPAC numbers (5) (co-eluting components are given in the same line; p,h = penta- and hexa-chlorobenzene);
Seventh column: Concentration of these compounds in water sample in fmol dm^{-3}; for co-eluting components, concentrations were evaluated from the response factor of one component only (i.e. the first one given in each row).

Peak number	Peak height in 1st extract (arbitrary relative units)	Peak height in 2nd extract (in % of 1st extract)	Peak height in 3rd extract (in % of 1st extract)	Peak height in extract of drum wall (in % 1st extract)	Assignment	Concentration (fmol dm^{-3})
1	41	29	32	22		
2	22	68	59	64		
3	11	0	0	0		
4	5	0	0	0	1	4
5	56	41	46	30		
6	125	10	14	20	p	1
7	32	59	47	28		
8	26	73	65	42		
9	57	25	19	9	5,8	5
10	134	9	14	5	h	1
11	35	0	0	0	14	35
12	13	0	0	0		
13	96	34	25	17		
14	108	20	15	8	18	9
15	68	22	16	13	15	13
16	10	0	0	0		
17	56	0	0	0		
18	10	0	0	0		
19	32	0	0	0	26	2
20	18	0	0	0		
21	21	57	76	62		
22	165	15	14	7	31	5
23	206	20	18	4	28,50	7
24	122	18	15	8		
25	71	14	14	3		
26	14	10	0	0		

Table VII continued

Peak number	Peak height in 1st extract (arbitrary relative units)	Peak height in 2nd extract (in % of 1st extract)	Peak height in 3rd extract (in % of 1st extract)	Peak height in extract of drum wall (in % 1st extract)	Assign-ment	Concen-tration (fmol dm^{-3})
27	91	12	12	6	52	7
28	64	10	10	7		
29	70	12	10	6	49	4
30	36	100	100	100	47,75	
31	17	100	82	100	65,62	1
32	94	13	10	9	44	6
33	60	8	8	0	37,42	6
34	70	13	9	4	41	3
35	130	GHOST		PEAK		
36	52	12	10	8	61	2
37	106	13	11	9	70,90,98	7
38	117	15	12	9	95,66	4
39	10	0	0	0		
40	74	15	14	4	60,71	2
41	16	16	10	16	84	1
42	70	14	15	14	101	3
43	24	0	0	0	99	1
44	20	10	10	8	86,97	0.5
45	34	5	5	5	87,90,116	0.5
46	26	25	20	20	120	1
47	20	20	20	20	136	0.5
48	63	17	17	17	110,77	7
49	15	20	20	20	82	0.5
50	10	10	10	12	149	1
51	36	33	20	50	118	7
52	40	20	15	15		
53	38	37	24	61	153	1.9
54	17	40	20	20	132	1
55	19	35	20	20	105	1
56	12	20	20	20	141,179	0.5
57	32	44	0	72	138	1
58	9	80	80	100	180	1
59-62	GHOST PEAKS					

0.5 cm^3 PCB-1254, (ICES = 5IIB (2.2 µg PCB-1254) and 0.2 cm^3 PAH-mixture 6.54 µg Naphthalene
6.52 µg 2.6-dimethylnaphthalene
5.34 µg 2.3.6-trimethylnaphthalene
5.02 µg fluorene
6.38 µg phenanthrene
6.50 µg fluoranthene
in 10 cm^3 methanol. 1 cm^3, containing 220 ng PCB was added to 10 dm^3 seawater.

Continuous extraction:

10 dm^3 of spiked sea water was extracted with 150 cm^3 cyclohexane in an AHNHOFF-JOSEFSSON extractor at a rate of 4 dm^3 h^{-1}, speed 4 on the Cole-Parmer pump (Catalog no. 7546-00 30, 600 RPM, Cole-Parmer Inst. Co., Chicago, IL. 60648).

Direct extraction:

10 dm^3 of spiked seawater (in a glass flask) was extracted directly in the flask using 150 cm^3 pentane and a Turax (Ultra-Turax Type TP 18/2N, Janke & Kunkel KG, IKA WERK, Staufen i. Breisgau) at full speed 20,000 RPM for 1-2 min. The pentane-phase was pipetted off after 1 hour.

As external standard for the recovery experiments for PCB, IUPAC no. 53 was used. 50 mm^3(= 40 ng) was added. Theoretical amount PCB = 220 ng, 8 peaks were used for the calculation of the recovery percentage. Table VIII shows the results of the recovery of PAH; Table IX shows the results of the direct and continuous extraction of PCB; Table X shows the factors used to calculate total PCB.

Table VIII Recovery of polycyclic aromatic hydrocarbons

Direct extraction				% Recovery					
cm^3 Solvent	Mixing time	Settling-time	recovery of solvent in %	N	DMN	TMN	FL	P	F
150	1 min	60 min	85	12	21	37	47	66	82
150	2 min	60 min	89	10	24	40	50	62	71
2x75	2x1 min	2x30 min	84	16	34	49	55	69	77

Continuous extraction								
cm^3 Solvent	Flow rate dm^3h^{-1}	recovery of solvent in %	N	DMN	TMN	FL	P	F
128	4	87	15	18	24	42	49	49
150	4	96	14	27	32	47	59	63
150	4	90	31	27	33	45	60	66

N = naphthalene
DMN = 2.6-dimethylnaphthalene
TMN = 2.3.6-trimethylnaphthalene
FL = fluorene
P = phenanthrene
F = fluoranthene

Recovery of PCB and PAH from seawater using XAD-2:

10 dm^3 seawater was spiked with 1 cm^3 "Spike" (220 ng PCB). The spiked seawater was pumped through the XAD-2 column at the speed of 200 cm^3/min (speed 6). The sample was eluted with 200 cm^3 of boiling acetonitril going directly into 600 $cm^3$of seawater. This solution was then extracted with 2 x 50 cm^3 pentane, the pentane evaporated to approximately 1 cm^3 and sub-mitted to a clean-up on a short silica column (pasteur-pipette) and eluted with 1 cm^3 hexane to which was added 40 ng IUPAC no. 53 (see factors Table X).

Figure 8 shows the numbered peaks that have been used for the calculation of the recovery percentage.

2. Extraction from particulate matter

The filter and its content are treated in a Vortex with 50 cm^3 n-hexane and then Soxhlet-extracted with n-hexane for 8 hours. The cleaning and separation procedures resulting in fractions of different polarities are identical to those described for water extracts.

3. Separation of PCB from interfering compounds

Clean-up of extracts is necessary to remove co-extracted organics that will interfere in the GC-ECD analysis, either by masking components of interest or by detrimental effects on the column or detector. Such interfering compounds can be removed by numerous solid-liquid chromatographic methods; for

Table IX Recovery of PCB by direct and continuous extraction of spiked seawater. Peak numbers as defined in Figure 8

	Direct extraction % Recovery			Continuous extraction % Recovery		
Peak no.	1	2	3	1	2	3
1	79	78	79	29	42	40
2	81	67	82	24	41	47
3	92	88	91	31	49	57
4	94	93	77	33	57	61
5	102	101	109	35	60	63
6	111	98	104	35	53	55
7	98	98	105	33	54	58
8	107	98	104	33	52	56
average	96	90	94	32	51	55
± %	12 %	13 %	14 %	11 %	13 %	14 %

Table X Factors for the calculation of total PCB with the external standard as reference. Peak numbers as in Figure 8

	Replicates			Mean value
Peak no.	1	2	3	x
1	9.22	9.17	8.82	9.07
2	7.28	7.25	7.00	7.18
3	11.16	10.62	10.22	10.62
4	6.26	6.09	5.86	6.07
5	8.37	8.44	7.70	8.17
6	16.05	15.44	14.62	15.37
7	11.60	11.41	10.60	11.20
8	8.88	8.51	8.37	8.59

Figure 8 Chromatogram of Aroclor 1254 and numbering of peaks used for the calibration of the recovery percentage.

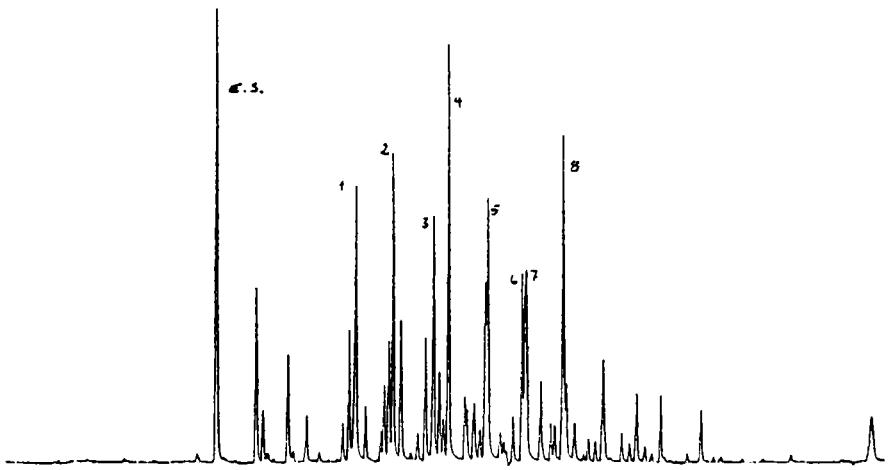


Table XI Recovery of PCB and PAH from the XAD-2 experiment. Peaks as in Figure 8

	Theoretical values 220 ng PCB			
Peak no.	Found	% recovery	Found	% recovery
1	117.8 ng	54 %	115.2 ng	53 %
2	108.2	49	106.1	48
3	109.0	50	109.5	50
4	115.6	53	110.8	50
5	118.8	54	123.6	56
6	111.8	51	120.3	55
7	108.6	49	107.1	49
8	105.6	48	110.6	50
average	111.9	51 %	112.9	51 %
±	4 %		6 %	

Sample	Naphth	dimet. naphth	trimet-naphth	fluoren	phenan-threne	fluoranthen
(1) 26.10.81	-	46 %	56	69	74	76
(2) 27.10.81	-	46 %	46	47	52	42

Naphthalene disappears in a forest of peaks.

instance, FLORISIL (49) and alumina microcolumns (50). We have investigated the use of High Performance Liquid Chromatography (HPLC) for the separation of the extracts into broad groups.

Isocratic HPLC on silicagel micropack with back flushing has proved to be effective as a separation technique for petroleum hydrocarbons. Preliminary work indicates that to the PCB/toxaphene split there is no easy method for separation of these compounds using an isocratic mode. Therefore, binary and ternary solvent systems with solvent programming are being investigated as possible separation techniques.

We have, like others, adopted essentially the method involving alumina microcolumns. The cleaned extracts are then separated on silica microcolumns to obtain two fractions, the first containing low-polarity compounds such as PCB, chlorobenzenes and p,p'-DDE and the second containing more polar compounds such as DDD and DDT components and dieldrin. This separation, originally designed to separate components that interfere in the analysis on packed columns is not superfluous when working with capillary column GC-ECD.

We have slightly modified the basic HOLDEN and MARSDEN method (50) so that analysis time is reduced and, more important, the quite serious contamination arising from the microcolumns is practically eliminated by treatment with dichloromethane (see below).

Apparatus and chemicals: Glass columns, 30 cm long, the lower section 22 cm long, with a bore of 0.6 cm, are joined at the upper end to a section of 3 cm bore tubing, 8 cm long. The lower end of the narrow bore end is drawn to a tip and plugged with glass wool;

10 cm^3 stoppered glass tubes graduated to 0.1 cm^3
20 cm^3 stoppered glass tubes graduated to 0.1 cm^3
25 cm^3 measuring cylinder;
1 cm^3 sample vials;
2 cm^3 Pasteur pipettes;
10 % diethylether in n-hexane (v/v) dried with Na_2SO_4 in a glass stoppered flask;
n-hexane;
dichloromethane dried with Na_2SO_4.

Solvents should be of very high purity. Evaporation of a volume, representing at least the volume used in the analytical procedures, should not contain electron-capturing compounds that interfere in the GC-ECD determination. Glass wool is extracted overnight in a Soxhlet extractor with 1:1 acetone-hexane, dried at 300°C and stored in a beaker covered with aluminium-foil. Glassware is cleaned in hot water with soap, rinsed with distilled water, baked in an oven at 300°C overnight and rinsed with n-hexane before use. It might be necessary to treat the glass wool with HCl solution before extraction, to remove adherent dust and inorganics. To alumina powder (Woelm B-activity Super 1) 10 % water is added; it is shaken for 30 minutes, equilibrated overnight and kept in a glass-stoppered air-tight flask in a low-volume desiccator.

Silicagel, Merck 7754 (70 - 230 mesh, ASTM) is washed with hot, distilled water (200 g with 4 dm^3), washed, when still hot, with 400 cm^3 diethylether on a Büchner funnel, dried on a water bath, activated in an oven at 120°C overnight and cooled in a desiccator; after addition of 7 % organic-free water, it is shaken for 30 minutes, equilibrated overnight and stored in a desiccator.

Method for separation of impurities: After addition of some clean boiling chips, concentrate

the sample extract in n-hexane to 1 cm^3 in a Kuderna-Danish (KD) apparatus equipped with a three-ball Snyder column on a water bath. Remove the upper parts, collect the n-hexane that is still refluxing and evaporate any excess volume of solvent with a moderate stream of nitrogen down to 1 cm^3.

For the preparation of an alumina microcolumn, weigh 2.00 g alumina, fill column and elute with 10cm^3 dichloromethane followed by 10 cm^3 n-hexane. Alumina should be exposed to air for as little time as possible to maintain activity. Transfer the 1 cm^3 extract to the top of the column with a Pasteur pipette, wash the tube with another 1 cm^3 n-hexane, transfer it to the column and elute with 13.5 cm^3 n-hexane to remove 100 % of the components of interest. β-HCH is not eluted completely unless 40 - 50 mg lipid is present on the column (51). Concentrate the eluate in KD equipped with a micro Snyder column on a water bath to 1 cm^3 as before. Transfer it to the top of a 2.00-g silica column, prepared in the same way as the alumina column and elute the first fraction into a graduated 10 cm^3 tube with the experimentally determined volume of n-hexane that is just sufficient to elute p,p'-DDE (about 6 cm^3). This (first) fraction will include chlorobenzenes, aldrin, p,p'-DDE and PCB quantitatively. If other components are of interest as well, a second fraction is eluted with 13.5 cm^3 10 % diethylether in n-hexane, containing hexachloro-cyclohexane isomers (α, β and γ), heptachlorepoxide, dieldrin, endrin and DDD and DDT components. Both fractions are concentrated to 1 cm^3 in the graduated tubes equipped with a micro Snyder column. Store these extracts in sample vials with aluminium caps and Teflon-faced liners using a hand-crimper. The use of Teflon liners is essential for avoiding sample contamination (52). Standard or reference compounds may be added at this stage; e.g., when post-run automatic identifications and/or quantifications, requiring a reference peak, are to be performed.

Injections can be made directly from these vials. It may then be necessary to replace cap and liner for storage of the remaining sample over longer periods. Removable conical inserts are useful when storing reduced volumes ≤ 200 mm^3.

Blanks are run by subjecting 1 cm^3 n-hexane (instead of sample extract) to the entire procedure. Our experience is that perfect blanks can be obtained from microcolumns that have been treated with dichloromethane (53). This is an important improvement because blanks, if present, may be highly irreproducible, and thus difficult to deal with in quantifications.

The extracts thus obtained can usually be subjected to GC-ECD analysis without further treatment. Further cleaning may be done by treatment (which may be destructive to some components of interest) or by repeated clean-up over alumina (46).

C. Filtration of seawater

Like other chemicals, organochlorines in seawater occur in a continuous series of dissolved, colloidal and discrete particulate forms. A distinction is usually made on the basis of a separation technique such as filtration or centrifugation. The separation depends on size and density of the particles; it may also depend on the composition of the suspension: particles smaller than the nominal pore size of the filter may be retained on the filter when clogged. This can occur in suspension of high concentrations of inorganic particles and/or phytoplankton. The distinction is operationally defined therefore.

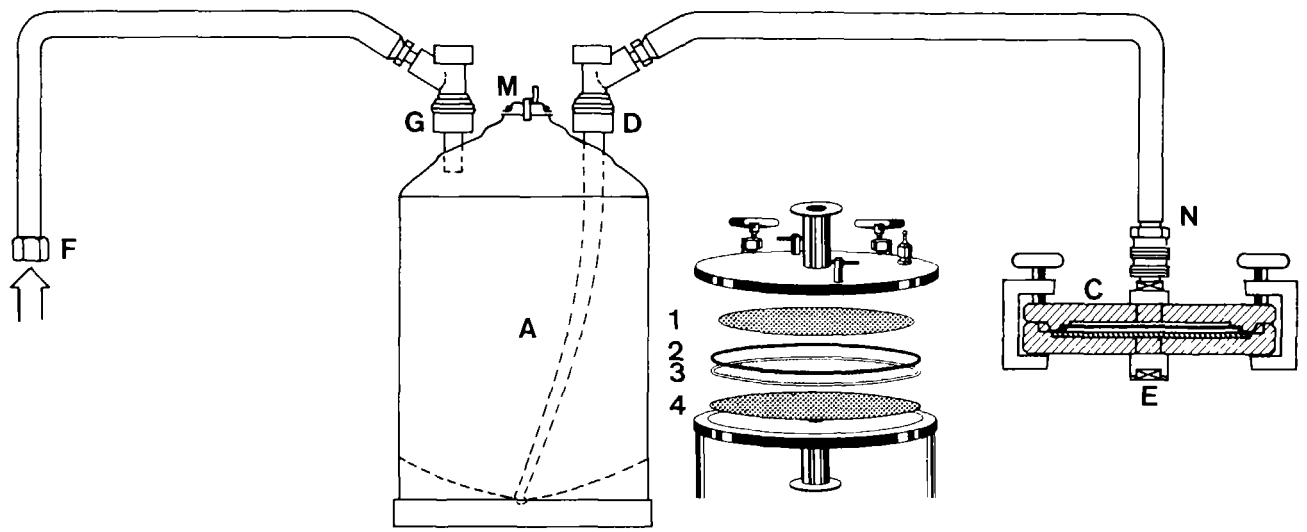
In open ocean water with low contents of suspended matter, concentrations of PCB in particulate forms are considered to be small (about 10 %) with respect to those in solution (54, 55). However, in natural waters with high seston concentrations, they may be comparable with, or even larger than the concentrations of PCB in solution. In such cases, it is essential to perform phase separation, preferably on board ship immediately after sampling (56). However, this extra step is a potential source of contamination.

Figure 9 is a diagram of a system that allows sample handling under well-controlled clean conditions. It involves a closed system practically excluded from the ship's atmosphere. The sample to be subjected to phase separation is stored in a 100 - 200 dm^3 stainless steel container (A). Its content is discharged into a filtration unit (C) through opening (D), connected with the lowest part of the container by means of a stainless steel pipe. The filtrate is received through E in a second stainless steel drum from which it is fed into the extractor. Each sample transfer is achieved under controlled pressure at G with nitrogen gas from a tank. Pressure regulator, drums, and filtration units are connected with standard length Teflon tubing, protected with stainless steel wiring on the outside. Connections are made with stainless steel conical male and female connectors (fixed with stainless steel nuts) on both ends of the tubings. Each drum has an oval central port (about 12 x 6 cm) for cleaning and inspection purposes; it can be closed with an oval lid (M) fixed on the outside while sitting inside the container. The drums have two threaded tube connectors: from one a stainless steel pipe extends to a few millimeters above the lowest part of the curved bottom. This allows the drums to be emptied almost completely, including particles that may have settled on the bottom.

The filtration unit (Schleicher and Schüll, F.R.G., Fig. 9) consists of two solid Teflon or stainless steel plates (various diameters are available, 14 - 29 cm), separated by three Teflon-covered perforated stainless steel plates, a filter and a Teflon O-ring and kept together by means of clamps on the edge of the plates. The upper plate (on the inlet side) takes care of an even distribution of material on the filter; in other two are filter support plates. Large volumes of seawater (~ 100 dm^3) containing several mg seston per dm^3 can be filtered in a reasonable time (30 - 60 min) under low pressure (100 - 150 kPa, 1 - 1.5 atm). If seston concentrations are to be determined, the filters should be pre-weighed in the laboratory under standard conditions, washed with organic-free distilled water after filtration to remove sea salt, dried and weighed under standard conditions in the laboratory.

Prior to the cruise, the whole system must be thoroughly cleaned in the laboratory with soap and hot water, water, acetone, and hexane. The containers can be cleaned with pressurized steam, followed by acetone and hexane. All parts that will come into contact with samples are to be protected against continuous direct contact with the atmosphere by covering with clean aluminium foil. Glass fibre filters must be rinsed intensively until blanks are sufficiently low. It may be necessary to extract the filters in a reflux system of appropriate size.

Figure 9 Arrangement for pressure filtration of large water samples (100 - 200 dm^3). The stainless steel container A is filled with samples from air-lift through FG with lid M open: water is filtered (under pressure through FG) by C through DN. C consists of two stainless steel plates, separated by Teflon covered stainless steel plates, a filter and a Teflon O-ring (1 - 4).



During the cruise the filtration unit is rinsed between successive samples with n-hexane and hexane-extracted distilled water.

D. Sampling procedures

Published values for concentrations of organochlorine compounds range over several orders of magnitude (reviews are given in 44, 57). These variations may be real, but they may also, at least partly, reflect the effects of inadequate sampling and analytical procedures. In fact, there are numerous sources of contamination. Sampling is probably the least easily controlled phase in the entire procedure, for a number of reasons.

Generally, reported levels appear to be lower for large volume samples (up to 300 dm^3) than for small volumes (down to 1 dm^3). Considering the concentration levels in seawater and the uncertainties (or errors) in the entire procedure, it is hard to rely on data based on analyses of samples of only 1 or 2 dm^3.

An appropriate sampling procedure for analysis of organochlorines in the water column should eliminate:

a) the effects of the surface microlayer, for which considerably higher concentrations of several organochlorines have been reported (58, 59);
b) adsorption of compounds of interest onto the sampler wall;
c) contamination from components of the sampling device, chemicals used, the ship's atmosphere and ship's hull (60).

Glass tubes packed with resin (XAD-2) in a submersible "fish" at 1 - 2 m depth from a boom mounted towards the bow of the vessel have been used for *in situ* extraction of organochlorines from seawater (44).

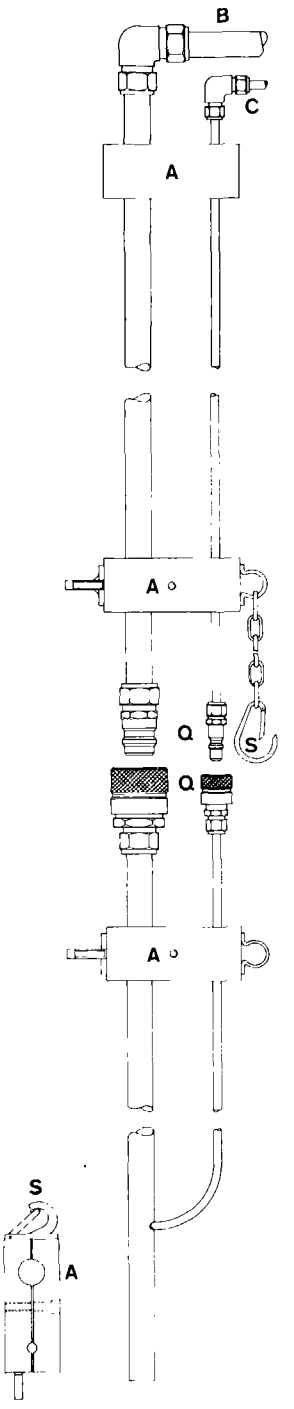
Anodized samplers, passing through the surface in closed position (1, 43) can be used to obtain clean ($\simeq$ 200 dm^3) samples at any depth, according to the original design by BODMAN *et al.* (61).

A sampling system for the upper 50 m: The use of solvent extraction for isolation and concentration of organochlorines from large volumes of seawater requires collection in containers such as those described before. A procedure that allows appropriate measures to be taken against contamination during sampling is illustrated in Figure 10. A system of two stainless steel tubes, connected by metal clamps (A) and attached to the hydrographic wire, extends from a few meters above the sea surface to well below the ship's hull. The wider tube (15-mm inner diameter) is open at the lower end; the narrow tube (3-mm inner diameter) is welled into the wide tube about 15 cm above its lower end. The system consists of a number of sections, each 2.5 m long. The lower section is attached to the hydrographic wire in a fixed position above its lower end which carries a heavy weight. The other sections are added consecutively to the system, which is lowered before addition of the next section. The sections fit together through Swagelock quick connectors (Q) attached to both ends of the tubes, and they are connected loosely to the wire by means of snaphooks (S). The upper section allows lengths of tubing to be connected for water

transport from the wide tube to the container and for application of pressure to the narrow tube from a nitrogen tank at C. Connecting Teflon tubings are identical to those desribed in Section III C. An intermittent flow of water is obtained on deck with only a moderate pressure (100 - 200 kPa, 1 - 2 atm). This is essentially the gas lift system described by TOKAR et al. (62). Water can also be obtained from depth by evacuating the container with a vacuum pump on deck through FG (Figure 9) with M (Figure 9) closed and N (Figure 9) connected to B (Figure 10).

Before each cruise, the entire system is rinsed thoroughly with water and organic solvents. When not in use, all open ends should be covered with rinsed aluminium foil to avoid contamination from the atmosphere. At sea, the entire system is flushed with new sample. If sufficient time is allowed for equilibration, the effects of sorption processes that might take place should be insignificant.

Figure 10 Gas-lift sampling system (see text, section III C.)



IV. Results on the distribution of PCB in seawater

In this chapter we shall describe some results on the distribution of PCB in water and suspension of North Sea samples. These were taken in September 1981 at positions 51°58'5"N, 3°51'E (A) and 55°21'N 0°58'E (B) under stormy conditions at position B. These samples represent a nearshore (A) and offshore (B) position in a larger series of samples taken during that cruise from the R.V. AURELIA (Texel). 100 dm^3 water and seston were obtained from 6 - 10 m depth with the gas-lift system (Fig. 10), discharged into clean stainless steel drums (Fig. 9), filtered through 15 cm ϕ, 0.45 µm GF/C glass fibre filters (Fig. 9) and discharged into another clean drum. The filtrate was extracted with n-hexane in a continuous extraction system (Fig. 6) and the filter content in a Soxhlet extractor with n-hexane for 8 hours. Suspended matter concentrations were 13 mg dm^{-3} (A) and 0.8 mg dm^{-3} (B). PCB were separated from interfering compounds (III.B.3), separated and analysed by GC-ECD as in III.A.

Chromatograms are represented in Figure 11. The concentrations of as many individual components as could be estimated are given in Table XII. Water is the dominant carrier for components with a lower degree of chlorination; this suspension becomes more important for components with greater degrees of chlorination. For offshore samples, 100 litres are hardly sufficient for accurate analyses.

Recently, we have investigated the vertical transport of individual PCB components by sediment traps at 3,200 m in the Sargasso Sea. The results are now being finalized and will be part of a future intersessional report.

Figure 11 Chromatogram of extracts of water (w) and suspended particles (s) of the Southern Bight (North Sea); 100 dm^3 sample; positions A (51°58'NB 3°51'EL), B (55°20'NB 0°58'EL); September 1981.

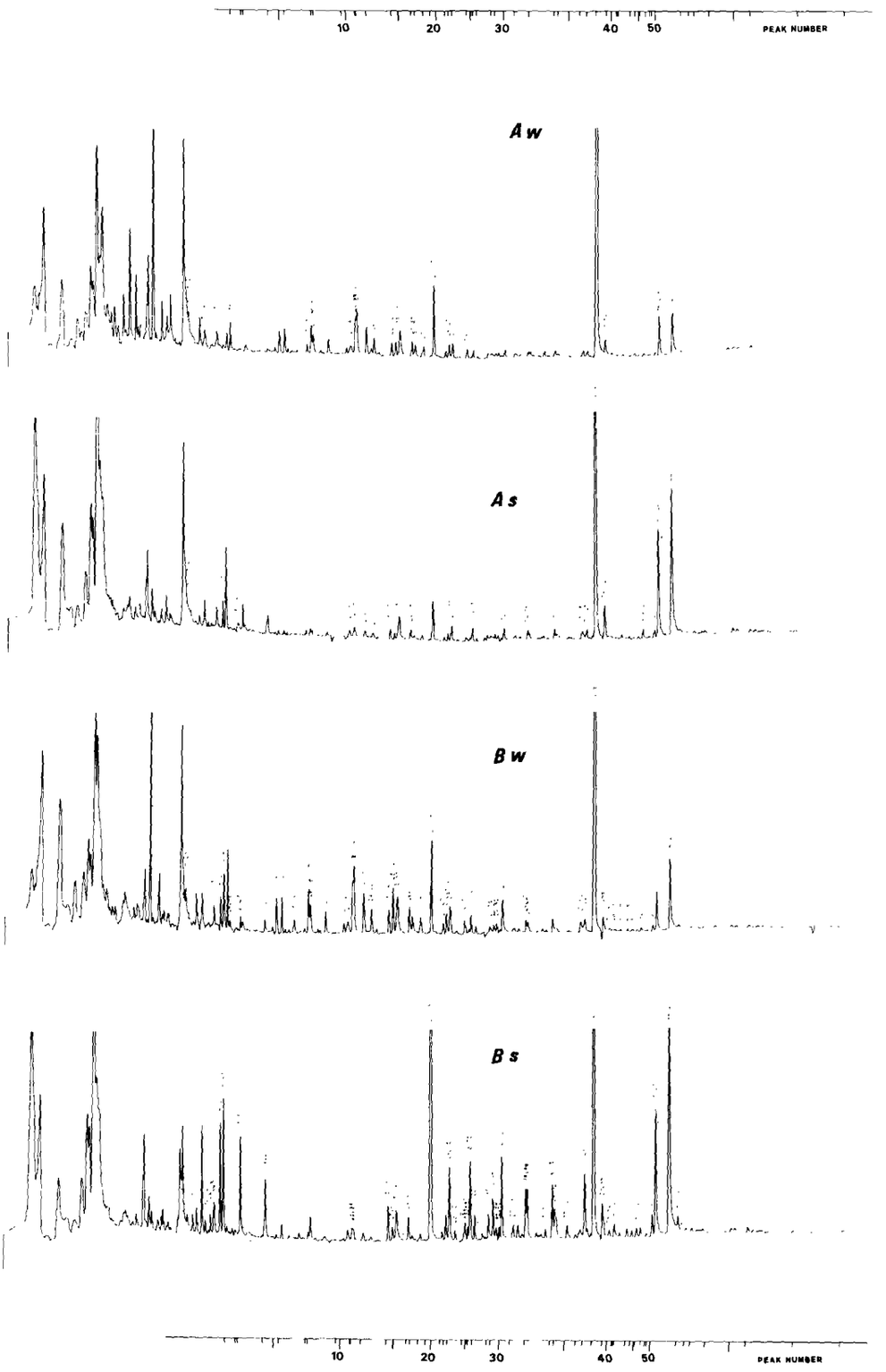


Table XII Individual components in water and suspension in samples A and B (chromatograms in Figure 11). Concentrations of individual components were evaluated from single component response factors. For peaks with possible contribution from two or more coeluting components the response factor of one component (the first one of the set specified in the first column) was taken for quantification. Concentrations were added, resulting in total PCB concentrations. Concentrations of all peaks have been expressed as percentage of total concentration.
a): IUPAC numbers of individual components (ref 7)
b): Concentrations as percentage of total summed concentrations of individual contributions (bottom)
c): Peak numbers as defined in Figure 11

Components[a]	Concentration[b]				Peak number[c]
	Sample A		Sample B		
	water	suspension	water	suspension	
7,9	0.5	1.7	-	3.4	4
5,8	7.9	-	9.6	-	5
18	10.4	2.8	13.2	-	7
15	16.8	8.3	23.9	-	8
26	1.8	-	-	-	10
31	4.4	0.7	6.1	3.1	11
28,50	7.1	0.9	7.1	-	12
21,33,53	3.8	-	5.7	2.8	13
52	4.8	4.8	5.7	8.6	14
47,75	2.6	1.4	3.6	6.6	15
44	3.7	2.4	4.6	6.5	16
37,42	4.0	-	6.4	-	17
41	1.5	-	1.8	-	18
70,80,96	3.7	3.1	5.0	-	22
95,66	2.6	4.5	2.6	6.0	23
60,56,71	11.7	0.4	1.6	-	24
84	-	1.1	-	-	25
101	2.0	5.2	-	5.6	26
99	-	1.8	-	-	27
86,97	0.5	0.9	-	-	28
87,90,116	0.3	0.7	-	-	29
110,77	11.2	18.3	-	16.8	30
82	-	1.1	-	-	31
149,118	-	10.8	-	-	32
153	1.6	4.1	-	5.1	33
132,105	-	3.0	-	-	34
176	1.8	-	-	6.1	36
138	1.7	4.9	-	4.7	37
187	2.2	2.6	-	16.3	39
183	0.2	0.7	3.6	-	40
128	0.3	1.2	-	-	42
174	0.3	0.8	-	-	43
177	0.3	-	-	-	44
200,157	0.3	0.6	-	-	46
180	0.7	2.3	-	-	50
209	-	1.4	-	-	56
Sum.	100 %	100 %	100 %	100 %	
Total concentration	547 pg dm^{-3}	180 ng g^{-1}	280 pg dm^{-3}	146 ng g^{-1}	
suspended matter concentration		13 mg dm^{-3}		0,8 mg dm^{-3}	

V. Future work

At this point, we shall summarize the present state of art with respect to our attempts to analyse PCB in seawater. This will be the basis for the description of future work required for analyses in open ocean waters.

Chromatographic separation, identification and quantification of individual components

At present it is not yet possible to separate all possible individual PCB components on one column. It has been claimed that this should be possible by using more columns, but this cannot be proved unambigously before all components have been prepared in sufficiently pure form to allow their retention properties to be determined.

However, it seems unreasonable to assume, on the basis of GC-MS analyses of technical formulations, that the number of peaks for which co-eluting components have to be considered is significantly larger than the number we have found up till now. Distinction of co-eluting components in a water sample is presently a problem owing to the low concentrations with respect to the amounts required for GC-MS analysis and to the presence of other organic compounds in much higher concentrations, thus interfering in the GC-MS analyses. Improvements are expected to result from the use of more than one column with different stationary phases. However, the extra work involved when using more than one column for analysing actual samples may be a limiting factor. Rather than aiming at a complete analysis of all detectable PCB components, a selection of peaks to be analysed might be preferable at least for the time being. Further work is needed to improve the possibilities for analysis of PCB in water samples by GC-MS techniques. This may involve removal of interfering compounds by destruction techniques (e.g., H_2SO_4 treatment) or chromatographic separation methods.

Extraction

Several extraction techniques are presently available, for which reasonable extraction efficiencies have been reported. However, the solvent-solvent extraction and the adsorption techniques have to be investigated further. Also, work is required to compare the various techniques with respect to efficiency and signal-to-noise ratio.

Separation of dissolved and particulate forms

At least one technique (filtration) has been reported for separation of dissolved and particulate suspended forms in combination with PCB determinations in seawater. Further work is required on separation techniques, involving filters of various pore sizes and preferably centrifugation techniques. This may result in an appreciation of the role of colloidal material particularly in waters with low SPM concentrations.

Sampling

Available methods for sampling include large volume samplers (Bodman type), the gas-lift system, foam sampling and in-situ extraction by XAD resin, including the automatic sampler designed by EHRHARDT, being now commercially available (it can be operated up to 200 m depth and it allows in-situ prefiltration). These techniques have to be investigated in more detail and they must also be intercompared, preferably in water with very low SPM concentrations. It may also be rewarding to investigate the role of the surface microlayer.

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