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CHLORINATED BIPHENYLS IN OPEN OCEAN WATERS: SAMPLING, EXTRACTION, CLEAN-UP AND INSTRUMENTAL DETERMINATION

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CONTENTS

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 mechanism, including a Marine Pollution Monitoring System (MARPOLMON); GIPME is since 1991 co-sponsored by IOC and UNEP. When operational, MARPOLMON 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 (1,2) is the development and proving of techniques for the collection of baseline and boundary flux data for selected contaminants, followed by intercalibration exercises.

The present report describes the results of recent 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). It builds on and replaces an earlier version (3). It takes into account significant developments in sampling, sample clean-up and instrumental analytical procedures achieved since the publication of the latter report. It will be useful primarily to scientists who have had some experience with advanced sampling and methodology. However, it will provide many scientists from developing countries with a very good idea of what they should be striving to achieve when they have access to advanced instrumentation. It is important to note that analyses for chlorobiphenyls in coastal and estuarine waters will usually not require the extensive compulsive care needed to avoid even low concentration level shipboard and laboratory contamination. This is required and set forth here for open ocean sampling and analyses.

It will serve as a guide for an open ocean baseline determination of organochlorines within the activities of the Comprehensive Plan for GIPME (1,2).

The present Report was prepared by Dr. J.C. Duinker of the Institute of Marine Science (Kiel, Germany), in response to a request by the Intergovernmental Oceanographic Commission. The Report builds on the experience initially gained at 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, on an earlier version of the present report (IOC Technical Series No. 26), continued by discussions and programmes carried out by the GIPME Group of Experts on Methods, Standards and Intercalibration (GEMSI), and completed by attempts to refine and review the present state-of-the-art for the determination of poly-chlorinated biphenyls in open-ocean waters. The Intergovernmental Oceanographic Commission wishes to acknowledge gratefully the contributions made by the author himself and his co-workers Dr. D.E. Schulz-Bull and G. Petrick and the support of the Institute for Marine Science at the University of Kiel.

The results described here have been obtained for the main part during studies in a project "Particle Flux in the North Atlantic, Organic Substances", financed by the German Research Council under DFG Du-147/1-2. The valuable comments received from Prof. Dr. J.W. Farrington, Dr. G. Topping and co-workers and other 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.

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) (4). These problems should be solved prior to any further implementation of the monitoring programmes within IOC's Global Investigation of Pollution in the Marine Environment (GIPME). Attempts to reach this goal were subsequently carried out by the Bermuda Biological Station for Research, Inc., (Bermuda) the Institute of Marine Research, Bergen, (Norway) and the Netherlands Institute for Sea Research, Texel (Netherlands). The three laboratories volunteered to work towards solving some of the problems. Preliminary results with respect to extraction, separation and analytical procedures were discussed at a GEMSI Core Group meeting at Bermuda in March 1982 (Annex X in Document IOC/GCE(MSI))-IV/3) (5). 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 (congeners) 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 GC 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 gas-chromatographic separation, as well as the standardization of the experimental conditions in order to achieve 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 a proposed 6-month activity, 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 compounds between solution and particulate matter. The distinction between solution and particulate forms is an operational definition with solution being components that pass through a 1 or 0.45 micron glass fibre filter and may contain colloid range particles. This was the subject of an earlier publication (3). Since then a considerable amount of laboratory and field work has been carried out by members of GEMSI and others. Improvements in HPLC-separation techniques, sampling procedures and instrumental analytical techniques have been achieved. The complete characterization of all chlorobiphenyls in commercial mixtures and in environmental samples has been achieved with the use of a multidimensional gas chromatographic technique (6). Using this information, a mixture of chlorobiphenyls was prepared for use as quantitative standard ("PCB-1 (IOC-KIEL)") for GC-ECD determinations of CBs using a single SE-54 column. This activity was supported by IOC. Progress in several aspects has been discussed during various GEMSI meetings.

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 HPLC-fraction as CBs (e.g., penta- and hexa-chloro-benzene); 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 microcolumn-, HPLC- and GC-separation procedures.

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 identify and eliminate sources of contamination, in particular during sampling and sampling clean-up procedures;

ii) to concentrate the compounds of interest and to separate them from interfering compounds in seawater; and

iii) to analyse the composition of the mixtures qualitatively and quantitatively in terms of well defined chemical entities.

The report will describe the progress achieved in several 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:

- The consideration of conditions to improve GC separation for accurate analyses of ultra traces of chlorobiphenyls such as those in open ocean waters;

- Improvement of procedures on board ship and in the home laboratory to identify and eliminate sources of contamination;

- The composition of commercial mixtures and of concentrated and cleaned-up PCB-extracts by GC-ECD and MDGC-ECD;

- A procedure for obtaining these extracts from water and particulates and a clean-up procedure to remove interfering compounds from the sample extracts;

- 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 open ocean waters.

III. PRESENT STATUS OF THE VARIOUS PROCEDURAL STEPS

A. Analysis of the Composition of PCB Mixtures by Gas Chromatography-Electron Capture Detection (GC-ECD & MDGC-ECD).

Whatever technique is used for sampling, sample processing, extraction and purification of extracts, the final instrumental analytical determination of organochlorines will most likely involve gas chromatographic separation either in the single column mode (GC) or in the multidimensional mode (MDGC) with electron capture detection (ECD) or mass spectrometry (MS). These techniques involving application of capillary columns will be discussed before the other aspects. More details are found in (3). The GC systems on which our experience is based are the following:

1) Carlo Erba, model 4130 equipped with an electron-capture detector and a split/splitless injection mode. A 25 m SE-54 fused silica column (Macherey and Nagel, FRG) was used (0.25 μm film, 0.25 mm i.d.), with temperature programming 40 ^{0}C (1 min), 100 ^{0}C to 250 ^{0}C at 4 ^{0}C/min.

2) Multidimensional GC was carried out with a Siemens SICHROMAT-2 gas chromatograph equipped with two independent ovens and two ^{63}Ni electron capture detectors. The PTV (programmable temperature evaporator) was modified to allow on-column injection. Conditions were: first column, 25 m fused silica SE-54 (0.25 μm, 0.32 mm i.d. and second column 30m OV-210 (0.25 μm, 0.32 mm i.d.) (ICT, FRG); gas velocity (H_2) 40 cm s^{-1}. Temperature conditions were: first oven from 140 to 250 ^{0}C at 4 ^{0}C min^{-1} and second oven at 160 ^{0}C isothermal for 20 minutes after injection and then temperature programmed to 230 ^{0}C at 4 ^{0}C min^{-1}.

3) Siemens SICHROMAT-1 with an ^{63}Ni ECD and cold on-column injector (PTV). A 50 m SE-54 fused silica column (0.25 μm, 0.32 mm i.d., ICT, FRG) was used. Temperature programming conditions were 140 ^{0}C to 170 ^{0}C at 5 ^{0}C min^{-1}, then to 240 ^{0}C at 3 ^{0}C per minute. The carrier gas: hydrogen, 40 cm s^{-1}.

1. Gas Chromatographic Separation & Detection

a. Columns

In an ideal chromatographic separation, each compound elutes as a single peak. This is difficult to achieve when dealing with environmental samples in which many compounds are present. The use of capillary columns with high

efficiency is therefore obligatory. Open tubular columns are essentially lengths of capillary tubing coated with a stationary phase. Wall-coated open tubular (WCOT) columns have a liquid phase deposited directly onto the inner surface. Good quality columns are available commercially as narrow or wide-bore (0.2-0.5 mm inner diameter), glass or fused silica, in various lengths (up to more than 100 m) and with a wide choice of coating and film thickness (0.05-0.25 μm). Support-coated open tubular (SCOT) columns are not widely used. 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 per minute. Efficiency is high: typical numbers for total effective plates are 150,000 for 50 m capillary columns. Capacity is about 100 pg per component (7-9).

It is worth noting that capillary columns have large sensitivity, signal-to-noise ratio and overall inertness. Thus, detection limits are low. The introduction and use of fused silica columns (10) has been an enormous advantage: they are flexible, inert, owing to their low metal content at the reduced surface, and they are much less fragile than glass columns.

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 determined 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) and others (10,12).

Column life and efficiency are maintained as long as the liquid phase remains as a thin, evenly distributed film. A drop in column performance occurs if the liquid phase gets 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 and in particular on-column 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 to high temperature; this is particularly the case 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 ^{0}C; they are also to some extent resistant to water and oxygen. The use of a SE-54 column remains essential as the retention properties of all CB congeners have been determined for this type of column only.

b. The Electron-capture Detector:

The electron capture detector (13) 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 may be as low as 0.02 pg s^{-1} 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 can be captured by sample molecules once their energy has been reduced to thermal level. 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 concentration of electron capturing molecules is non-linear. The useful linear range of an ECD is greatly improved if it is operated 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 electron-capturing molecules 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 so it is necessary to have an additional detector purge flow (normally nitrogen). 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 ^0C overnight assists in maintaining good detector performance. The operation of the ^{63}Ni ECD at 320 ^0C results in relatively limited contamination.

c. Operational Conditions

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 ^0C) 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 time. The temperature is commonly the maximum temperature required for the proposed analysis. To avoid destruction of the column at higher temperatures, a sufficient flow of carrier gas through the column should be maintained. During conditioning, the column should be left disconnected from the ECD in order to minimize detector contamination.

Older columns may have to be periodically subjected to higher temperatures to remove carrier gas impurities which have accumulated. This may be necessary less frequently in cases where higher temperatures are maintained for a longer period during a programmed run. Column performance can be continued over longer periods by maintaining moderate temperatures overnight (e.g., 180 ^0C). The ECD may remain connected to the column provided that it is kept at an elevated temperature (320 ^0C).

It is important to check and adjust the detector standing current at regular intervals; this can be performed automatically in modern equipment. Carrier gas must be of high purity (N_2 at least 99.999 %). Whilst 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, and if not removed result in bleed at higher temperatures which causes baseline instability and shortens the life of the column.

d. Injection of Extracts

In trace analysis, the preferred method to introduce the sample into the column involves injecting the entire sample for maximum sensitivity, without splitting off any sample to vent. This can be achieved with splitless (8,9,11) or on-column (14) injection techniques.

In the splitless mode, 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 ^0C 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 the column temperature of 10 - 30 ^0C 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 sec) 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 (47).

The splitless injection method is used in many cases because it is a convenient one. However, there are a number of risks that are not obvious. The split and splitless modes of operation require quite different volumes of the insert liner. In the splitless mode, the vapour volume of 2 μl solvent has to be accommodated quantitatively, to avoid loss of analytes. An insert of too small a volume may force part of the relatively large volume of vapour formed (about 480 μl) into the injector block or the gas tubing (14). This may result in memory peaks appearing at unexpected points of the chromatogram. Another problem arises from high boiling point contaminants remaining from insufficient sample clean-up procedures. These remain in the glass insert where polymerisation or other reactions cause the formation of undesired coatings, affecting the evaporation of sample components. Low boiling compounds of interest may reach the capillary relatively late; this affects retention properties. A continuous bleeding from the insert seriously affects the quality of the column resulting in poor stability of retention times over longer time periods. The existence of these problems can be checked and eliminated by rinsing the insert in concentrated HCl overnight, followed by intensive mechanical cleaning and heating at 350 ^{0}C.

Finally, the position of the column in the insert is critical for column performance and for the amount of sample that can be transferred to the column in e.g., 30 sec. It is important that the position of the column in the insert be determined experimentally so as to correspond with the maximum detector response in a series of repeated injections of the same solution with varying column positions.

The on-column technique allows the introduction of sample directly onto the column. The sample has to be injected into a cold injector, to allow the evaporated solvent to be taken up quantitatively by the column and the carrier gas. Injection has to take place sufficiently slowly (say in 10 sec.) to avoid an undesirable (short-term) large solvent vapour : carrier gas ratio. The needle of the syringe must reach far enough into the column to avoid the sample components being transferred back into the injector block. As the volume of the column is reduced to <0.5 mm^3 by the presence of the needle the pressure of the carrier gas has to be larger than the column pressure to enable the gas to enter the column.

We have found during several years that during slow and careful on-column injection the solvent is distributed over a distance of about 40 mm in the column before complete evaporation. The sample components are distributed in the same way. In order to use the full efficiency of the column, the sample components are transferred to a narrow band at a cold spot of the column after flash evaporation. This finding has been confirmed recently (48).

e. Gases and Gas Supplies

Gas Supply

The accurate analysis of complex mixtures of organic compounds in environmental samples by capillary GC-ECD is fraught with problems. Some of these have been discussed above in relation to parts of the equipment like injectors, detectors and columns. When analyzing chlorobiphenyls and pesticides quantitatively at high instrumental sensitivities (typically in the 2 - 10 pg range), other complicating factors have to be taken into account. One of such problems is caused by external gas supplies. Treatment of metal tubing for gas transfer by a simple rinsing procedure with solvents is insufficient because contaminants cannot be removed quantitatively. Metal tubing has to be heated (e.g., with a butane burner) under a moderate stream of N_2 gas (30 ml min^{-1}) starting at the gas supply side. A smell test at the end of the tubing is usually sufficient to check the efficiency of the treatment. Stainless steel is the preferred material: it has better mechanical stability than copper and diffusion of gases (especially of H_2) through the wall is considerably less. This is confirmed by supplier firms (e.g., Messer Griesheim, Germany).

Selection of Carrier Gases

Popular carrier gases in GC are N_2, He, Ar/CH_4 and H_2. The selection of carrier gas involves compromises between several aspects, e.g., resolution and analysis time. Although nitrogen results in higher column efficiency than either helium or hydrogen, the average linear velocity (u') of the carrier gas at this optimum is considerably lower than for He and H_2. Also, the change in efficiency with u' is smaller for the latter gases making them the preferred choice.

Although nitrogen and helium are not dangerous because of their inertness, the increase of their viscosity with temperature is a drawback; because the carrier gas velocity in the column decreases when pressure (as is the norm) is kept constant, rather than flow. This causes a reduction in the velocity of He as carrier gas from the optimum flow of about 2.0 ml min^{-1} at 60 ^0C to 0.5 ml min^{-1} at about 250 ^0C (pressure on the column 0.4 bar, column length 30 m, 0.32 mm diam.). Thus, the optimum ratio of carrier gas velocity: stationary phase cannot be maintained during a temperature programmed run, resulting in reduced column efficiency (increase of peak width, increased retention times, poor separations). The same applies to N_2 and Ar/CH_4. Consequently, peak broadening, longer retention times and poorer separations result. These factors become apparent and critical at concentration levels approaching detection limits. Signal peaks disappear in the baseline. Such problems do not occur when H_2 is used as carrier gas. Its viscosity is practically constant in the temperature range used during a GC run. Moreover, its high diffusion velocity allows rapid re-establishment of equilibria between vapour phase and stationary phase. Consequently, peaks are narrower and higher, and retention times shorter. Unfortunately, its use may cause reduction reactions of chlorinated compounds catalyzed by the stainless steel surface at higher temperatures. Injection in the splitless mode may also enhance this effect. The sudden evaporation of the injected volume of liquid hexane (2 μl) results in about 480 μl vapour. Ideally, this volume has to remain quantitatively in the insert. However, part of it may escape into the injector where reactions may occur, e.g., p,p'-DDT may disappear to a large extent. This problem does not occur with on-column injection, so on column injection with H_2 as carrier gas is recommended.

Carrier Gas Impurities

Irregularities in the baseline, ghost peaks and generally poor chromatograms may result from the presence of 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 kept 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 experiments. The septum should be changed at regular intervals according to instructions given by the manufacturer.

Even ultrapure gases for GC applications may still contain oxygen in the ppm range. This leads to a slow but certain deterioration of capillary columns at higher temperatures. This can be observed from shifts in retention times, in a reduction of the efficiency of the column and in baseline drift. Columns with thin layers of stationary phase (0.15 - 0.25 μm), as are used for PCB analyses, are particularly in danger.

Polyethylene and Teflon are not allowed as gas supply tubing materials because oxygen may enter the gas through diffusion or through small leaks. It is essential that a combination of an active carbon filter and oxygen scrubber gas clean filter is built into the gas supply just before the inlet of the GC. Such filters eliminate traces of oxygen and water from the gas. They also prevent any contamination that occurs when the gas supply bottles are emptied completely by accident. The gas clean filters should not be made of glass as this material may be broken easily. The laboratory room may then, within a short time, fill with hydrogen because of the high velocity of the outflowing gas. This may then result in an explosion in rooms that are not flushed properly. A change in color indicates the need for replacement of the filter/scrubber.

Leaks

Another large problem is caused by leaks in the gas system of the GC

equipment. A popular technique to trace them is by using diluted detergent solutions. These are useful, but the measures taken to eliminate the leak causes this material to be pressed from between the front ferrule and the conus into the gas supply system. It causes some sharp peaks but the bulk is transported slowly through the column at higher temperatures causing baseline drift and variations in the detector sensitivity during the day.

A precise search for leaks can be carried out as follows: 0.5 ml of a volatile chlorinated solvent, e.g., dichloromethane, is pressed slowly from a syringe onto the site situated closest to the ECD considered to be a potential leak. The detector should not react in the case where back and front ferrules are metal ones, but diffusion through graphite or vespel ferrules will cause a slowly up - followed by a slowly down-baseline drift. A clear peak is a signal for an existing leak. Whether the connection has to be renewed depends on the success of tightening with a wrench. The metal tubing may not have been positioned through both ferrules, incisions in the tubing may have been too large to be compensated for by the ferrules, etc.

During a leak search, the time required for detector response increases with increasing distance between the position of the leak and the ECD (from 10 seconds till 3 minutes). Gas supplies can be checked by covering them with a towel and saturating the atmosphere under the towel with dichloromethane. A rapid increase of the baseline indicates a peak. This system leak search can be compared with the use of Argon to detect leaks in MS systems.

It is also possible to use a hydrogen leak detector. According to our experience, it is much more difficult to locate especially small leaks with such instruments. We do not recommend leak detectors therefore.

The connections within the oven of the capillary to the detector, injector and the insert are additional and frequent problems. Two ml dichloromethane in the closed oven is useful as an overall-test. A rapid response of the detector (10-20 sec) indicates a leak at the detector side, a signal after a time that corresponds to the residence time in the column (e.g., 60 sec at 20 m column length) indicates a leak at the injector side. The signal test as discussed above is then applied (e.g., at the detector side). The ferrule for the capillary is a frequent source of problems with some equipment. The graphite or vespel ferrules must have a 0.5 mm bore for a 0.32 mm ϕ column. If a ferrule of too large a diameter is used, e.g., 0.8 - 1.0 mm too large, not enough material will be available to the gap and the capillary may well break within the connector. The use of an insert on the detector side may represent a significant and frequent source of problems. This may be the case in hardware where a transition between packed and capillary column is required or where make-up gas is added prior to the detector. Leaks may occur during a temperature programmed run because of the relatively large connnectors which are made of copper or aluminium, depending on the instrument. Radial incisions on a connector allow the undesired inflow of O_2 into the system. Polishing the contact surface assists to eliminate the problem. Additionally, heating the connector strongly with a butane burner and closing with a wrench guarantees a leakfree connection. The normal precautions when working with hydrogen gas and open flames should be considered.

Conclusions

A GC system that has been cured and tested in this way can be used without problems over long time periods (apart from electronic errors). We have used columns with even thin layers of stationary phase in such pure and leakfree systems for more than 2000 temperature programmed runs (140-250 ^{0}C) without an essential change in separation efficiency and sensitivity. The limiting factor in this ideal case is the quality of the samples injected. Samples can be prepared in such a way that the quality of the final solutions corresponds to that of standards. In such cases, capillary columns or detectors are not destroyed or contaminated, but they are of course subjected to a natural aging process. The sample preparation procedure involving the use of aluminium oxide and silica gel chromatography as well as HPLC assist in obtaining these solutions and are described in section III.C.2.c2.

f. Single Column and Multidimensional Gas Chromatography.

The use of high resolution capillary columns for the analysis of complex environmental samples is now a common procedure in many laboratories. General

aspects of GC equipment requirements are discussed in the earlier version of this document (3).

The composition of chlorobiphenyls in commercial mixtures and environmental samples is complex. There is no single GC column available that can separate all constituents of the CB mixtures as single, well-separated peaks. Until recently, it has been impossible to analyze CB mixtures unambiguously in terms of individual congeners. Mullin et al. (15) have published the necessary data to approach this goal by reporting the retention properties of all theoretically possible 209 CB congeners on one particular column, i.e., SE-54. This allows the identification of CBs that are well separated on a SE-54 column from any other CBs present in the mixture. The data of Mullin et al. (15) does not allow the the determination of which closely eluting CBs actually contribute to a peak eluting from a SE-54 column. This problem has been solved by the application of a multidimensional GC-ECD technique (6,16).

As some results of the MDGC-ECD techniques applied to commercial mixtures and seawater samples are discussed in the present document, a short description of the technique is given here.

In the MDGC-ECD mode, 2 capillary columns of differing polarities are arranged in series, such that the second column receives only small preselected fractions eluting from the first column. With appropriately selected columns, all 209 CB congeners can be baseline separated allowing their accurate identification and quantitation even at trace levels.

The equipment consists of 2 independent ovens and two ^{63}Ni electron capture detectors (the main and the monitor detector). A valveless, pneumatic control system regulates the flow of sample molecules. These are transported to the monitor detector or to the second column and the main detector, depending on pressure conditions in the live T-piece of the commercially available instrument. Details of the method have been described in the literature (6,16). Examples of compositional details of MDGC-ECD peaks in comparison to the corresponding single columns GC-ECD peaks are given in Fig.1. The application of this technique for the evaluation of commercial PCB mixtures will be discussed in section IIIB and of seawater samples in section IIIC.

2. Identification & Quantitation

a. Standards

A stock solution (about 10 cm^3) of each compound of interest is made in a concentration of 100-1000 $\mu g g^{-1}$ by gravimetry, e.g., in iso-octane. 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 approximately the same. The final volume of this mixture is adjusted with iso-octane to give concentrations that are within the dynamic range of the detector, e.g., 10-100 pg μl^{-1} for ECD. Calculation of the absolute amounts injected with 1 μl sample volumes should take into account the density of the solvent when the original stock solution has been made up on a weight/weight rather than on a weight/volume basis. All standard solutions are sealed in glass ampoules and stored at -20 ^0C. Weigths are recorded on the bottles and checked before subsampling at a later date. The quality of all concentrated stock solutions of the individual compounds should be checked by GC-FID for major other non-electrocaptive active compounds and of all diluted stock solutions by GC-ECD.

Some standard stock solutions of mixtures of CBs are available. The PCB-1 (IOC-Kiel) solution contains 16 congeners that can be analysed accurately with a SE-54 column. The Canadian National Research Council makes 4 solutions with a total of 50 congeners available.

b. Calibration

Several injections should be made of standard solutions of different 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). A more sensitive method is to plot response/mass injected against

mass injected (51). It is appropriate to adopt the practice of quantifying those peaks in sample chromatograms for which the response is not too different from that of the standard - within the linear range. The relative areas or heights of different peaks in any sample chromatogram can differ widely so it may be necessary to inject the sample extract several times in succession after the appropriate concentration or dilution.

Many compounds are completely resolved from each other using capillary columns. These compounds can be quantified accurately when standards of sufficient purity are available. A calibration mixture must be analyzed under the same instrumental conditions which are used for the sample. Differences in sensitivity of the detector for different components in the sample are accounted for by the response factors during automatic calculation. Before any quantitation is undertaken the peak has to be identified correctly. The identification is made by reference to the retention times of designated peaks in the calibration table (the reference peaks) and of course those of the peak of interest.

The external standard method uses absolute response factors, the internal standard method is calibrated in terms of response ratios. In both calibration methods, each peak is calculated independently. In external standard methods, the sample amount injected must be highly reproducible. The method is well suited to automatic mechanical methods of injection. Instrumental optimization must be maintained by frequent check of system performance and regular recalibration. The internal standard method is independent of sample size and compensates for any slight instrumental drift. When used properly, it is the most accurate method of calculation. However, the internal standard must be added to each sample in a highly reproducible way and it should include components with both low and high volatility.

There is the additional problem for seawater because any CB spike which is added to the seawater may not be in the same form which was originally present in seawater. The role of colloids is uncertain, and partitioning problems may result in differences between liquid-liquid and solid adsorbent extractions of seawater (such as have been observed by (17)). Until more information is available, we apply the internal standard method including the whole range of CBs encountered in the sample on a regular time scale (once per month) to check our procedure for recoveries. Routinely we then depend on the external standard method. We are facing the Heisenberg uncertainty principle with this issue. We will never be able to know the exact phase distribution of the analyte in the sample in a quantitative sense until we can use an effective internal standard but we cannot be certain of adding the internal standard correctly until we know the phase distribution.

c. Identification

The most widely used method of identification of a chromatographic peak is its retention time or its relative retenion time, i.e., the adjusted retention time relative to the adjusted retention time of a selected reference compound. Capillary columns have a distinct resolution advantage over packed columns with a considerably higher probability of separating interfering components. The labeling system proposed by BALLSCHMITER and ZELL (11) is extremely useful for characterizing individual components. However, there is some inconsistent numbering for some peaks (nos. 199, 200, 201). The revised list of relevant CBs (i.e., containing those that have been detected at concentration levels >0.05 % (w/w) in commercial mixtures (Clophen and Aroclors) and can therefore be expected in environmental samples, is presented in Table 1 (6).

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 distinghuish components even with identical retention properties (23). MS techniques are specific and their sensitivity (femtomole range) is comparable with that of GC-ECD techniqes (18). 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 concentraions of organochlorines, in particular seawater, will increase significantly in the near future. An additional identification technique is offered by the multidimensional GC-ECD procedure, involving consecutive elution of a compound from 2 columns with different polarities. Both retention times are involved, and the combination assists in the

identification of any compound. Only a few laboratories are presently equipped with multidimensional GC techniques. GC-ECD data obtained with a SE-54 column can be analysed in terms of CB congeners that elute as single peaks, well separated from adjacent congeners. Multidimensional chromatography can be a more powerful technique for identification than low resolution MS when the L.R. mass spectra are very similar or the mass fragments occur at the same m/z. We have not checked whether other methods e.g., GC - FTIR might work with the concentration levels that we are interested in.

d. Response factors on ECD

Response factors of individual chlorobiphenyls on ECD depend on the number and the relative positions of the chlorine atoms in the molecular framework, and also on the analytical instrument. The ECD response is affected in particular by characteristics of the detection and injection systems, such as temperature, state of contamination and geometry. Considerable differences exist between the detector responses of on-column and split/splitless injection, in particular its dependence of the amounts injected (i.e., the degree of linearity). It is not surprising, therefore, that apparently conflicting data is available in the literature (15) and also that significant differences have been found for reasonable factors between even carefully prepared standards.

The use of published data on response factors (e.g., such as the detailed list in (15) for the estimation of the response of other CBs (e.g., that are not available as reference materials) has limited value. The only reliable way to quantitate CBs in samples uses reference materials, containing the congeners of interest in known amounts, as described in the proceeding section III. a. 2.

B. Composition of Commercial PCB Mixtures by GC-ECD & MDGC-ECD

1. Introduction

PCBs had been produced for about 35 years before they were identified as environmental contaminants by JENSEN in 1966 (19). They have been produced by various industries in the form of commercial mixtures with overall chlorine contents roughly in the 20-60% range depending on the manufacturing process. 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 these formulations.

Each CB does not behave identically in the environment. To obtain accurate information on sources, transport mechanisms, sinks, accumulation, degradation and other relevant processes the analyses of polychlorinated biphenyls should be made in terms of individual components rather than of technical formulations (20). These are the dominant sources of PCBs in the environment. It is therefore essential to have detailed information on the composition of these formulations (Figures 2 and 3).

2. Recent Results

Earlier attempts to analyze the composition of commercial mixtures in terms of individual CB congeners have resulted in a wealth of information (e.g., Refs. 11, 21-23). A complete analysis has been achieved after the retention properties of all 209 congeners had been measured on a SE-54 column with the use of the individual congeners (15) and the application of multidimensional gas chromatography - ECD (on a SE-54 column + OV210 or C87), allowing the unambiguous identification and accurate determination of all 209 congeners (6). Table I lists the concentrations of all CB congeners detected in all Clophen and in most Aroclor mixtures at concentration levels >0.05 %. Although some congeners may ultimately be still detectable at even lower concentrations, their presence in environmental samples at detectable levels is very unlikely. These "absent" congeners are identified in the last column of Table 2. In this Table, the congeners have been grouped in 5 classes, reflecting the maximum concentrations in one of the mixtures (>5%, 1-5%, 0.5-1%, 0.05-0.5%, and <0.05%). Table 3 summarizes which CB congeners appear as single peaks in SE-54/ECD-chromatogramms of commercial mixtures and of environmental samples, and can therefore be analyzed accurately with this

column type. In the latter case, it is essential that other, interfering compounds, have been removed by sample clean-up.

3. Comparison with Literature Data

Several attempts have been made in the past to unravel the complex composition of commercial PCB mixtures. SISSONS and WELTI (21) were among the first to realize the need for individual components as reference compounds. The application of several techniques enabled them to identify the major components in some commercial mixtures. Other groups have extended the list of identified peaks. (11, 22, 24-26). No single assignment has been unambiguous, however, because of 2 reasons. Firstly, in all studies, the number of individual congeners being available as reference compounds has been considerably less than the number required for unambiguous assignments (i.e., 209 congeners). Secondly, no single column has been available up till now to allow complete separation of all constituents of the commercial mixtures.

For instance, SISSONS and WELTI (21) identified 2 of the major peaks in terms of CB-95 and CB-110, respectively. It can be observed from Table 1 that this assignment has to be modified to include CB-66 (co-eluting with CB-95), and CB-77 (co-eluting with CB-110). In some cases, calculated retention times have been used to identify peaks for which no reference compounds were available to the authors (11). Major deviations occur for these calculated assignments compared with the present assignment, involving measured retention properties of all 209 congeners.

C. Determination of PCBs in Seawater

1. Introduction

The problems identified in the analysis of commercial PCB mixtures are also encountered when analyzing 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 commercial mixture. In the case of marine environmental samples, considerable differences occur between various compartments such as water and particulate matter. This makes it even more essential to analyze PCB in terms of individual compounds rather than of commercial mixtures.

The use of a single column (SE-54) allows the identification and quantitation of a large number of individual congeners, appearing as single peaks in the ECD chromatogram. It is understood that no other CBs coelute with these congeners, but other non-PCB compounds, may be present. If such compounds are ECD-active, they may interfere with the determination of the CBs. Such compounds should be eliminated by sample clean-up procedures, (see below). To each of the remaining PCB peaks, one, two or more CBs may contribute (Table 1). The composition of such composite PCB peaks differs between commercial mixtures of different overall chlorine contents as well as between environmental compartments. Their compositions can be determined with GC-MS techniques, although large volumes of seawater have to be extracted to obtain sufficient material for analysis. 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 not be determined by ECD although approximate concentrations can still be obtained on the basis of an assumed composition. The most promising technique involves the application of multidimensional GC-ECD or MDGC-MS techniques. In this case, a single peak eluting from the SE-54 column is transferred to a second column with different properties. This enables the potential separation of components which co-elute from the SE-54 column. Retention properties and MS characteristics of known and unknown compounds, respectively, will assist in identification of the compounds in such composite peaks. Results for water 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.

Table 1. Percent contribution of individual chlorobiphenyls to Clophen and Aroclor commercial mixtures. CBs which have been identified at concentration levels >0.05 % (w/w) in at least one of the mixtures are identified in the second and third columns by IUPAC number and structure, respectively. They are listed in the order of elution from a SE-54 column and grouped according to the chromatographic domain to which they belong. These are represented by numbers in the first column, as defined in Fig. 1. Those congeners at concentration levels <0.05% in all mixtures are given in the last column by their IUPAC numbers (ref. 6). These are listed according to their retention times on SE-54 (ref. 12) at the appropriate location in the table. Those congeners for which their absence (i.e. concentrations <0.05 %) could be established by retention times on SE-54 are preceded by the symbol * (this applies to all congeners following this symbol). Their IUPAC numbers appear between the data for two chromatographic domains, as defined in Fig. 1. For the other congeners in the last column MDGC-ECD was required. The present list of CB numbers is in agreement with IUPAC rules; note in particular the change in numbers for CBs Nos. 199, 200 and 201 compared to ref (6).

Weight percent contribution (%)

Domain Number	Chlorobiphenyls No.	Structure	Clophen A30	A40	A50	A60	Aroclor 1016	1242	1254	1260	Absent CBs No.
1	10	2,6	0.27	-	-	-	0.37	0.20	-	-	
	4	2,2'	3.56	-	-	-	3.89	3.01	-	-	
2	7	2,4	0.36	-	-	-	0.60	0.60	-	-	
	9	2,5	0.39	-	-	-	0.95	0.54	-	-	
3	6	2,3'	1.49	-	-	-	1.83	1.38	-	-	
4	8	2,4'	10.51	0.23	-	-	10.80	7.65	-	-	
	5	2,3	0.10	-	-	-	0.13	0.06	-	-	
											* 14
5	19	2,2',6	1.08	0.10	-	-	0.96	0.53	-	-	
											* 30,11,12,13
6	18	2,2',5	8.81	2.81	-	-	9.03	6.28	0.41	-	
	17	2,2',4	4.55	1.45			3.84	2.88	0.19		
	15	4,4'	3.05	-	-	-	2.90	1.51	-	-	
7	24	2,3,6	0.33	0.04	-	-	0.30	0.22	-	-	
	27	2,3',6	0.52	0.05	-	-	0.47	0.28	-	-	
8	16	2,2',3	3.01	0.76	-	-	2.86	2.01	-	-	
	32	2,4',6	1.41	0.41	-	-	1.34	0.88	-	-	
											* 23
9	34	2',3,5	0.10	-	-	-	0.12	0.05	-	-	54
10	29	2,4,5	0.18	-	-	-	0.19	0.10	-	-	
11	26	2,3',5	2.01	0.41	-	-	1.92	1.33	-	-	
12	25	2,3',4	1.30	0.09	-	-	1.19	0.79	-	-	
13	31	2,4',5	5.56	3.29	0.05	-	6.40	4.59	0.22	0.05	50
	28	2,4,4'	8.92	3.68	0.05	-	8.71	6.52	0.25	0.05	
14	20	2,3,3'	0.64	0.05	-	-	1.00	0.29	-	-	21
	33	2',3,4	4.30	2.76	0.11	-	6.25	4.79	0.14	-	
	53	2,2',5,6'	0.45	1.15	0.06	-	0.55	0.64	0.09	-	
15	51	2,2',4,6'	0.27	0.38	-	-	0.36	0.23	-	-	
	22	2,3,4'	5.10	1.61	-	-	4.80	3.41	-	-	
											* 36
16	45	3,3',5	1.10	1.67	-	-	1.66	1.16	-	-	
17	46	2,2',3,6'	0.52	0.74	-	-	0.70	0.49	-	-	
											* 39
18	69	2,3',4,6	0.05	-	-	-	-	0.11	-	-	
19	52	2,2',5,5'	2.80	7.26	5.53	0.75	4.46	4.04	5.18	0.56	73
20	49	2,2',4,5'	2.95	5.68	1.96	-	4.31	3.60	1.64	-	43,38
21	47	2,2',4,4'	0.80	0.19	0.18	-	1.11	0.94	0.17	0.11	
	48	2,2',4,5	0.71	0.18	0.17	-	0.98	0.82	0.14	0.09	
	75	2,4,4'6	0.07	-	-	-	0.08	0.11	-	-	
											* 65,62
22	35	3,3',4	0.11	-	-	-	0.08	0.11	-	-	
											* 104
23	44	2,2',3,5'	2.40	5.44	2.46	-	3.50	3.20	2.03	-	
24	37	3,4,4'	0.40	0.17	-	-	0.30	0.27	-	-	72,71
	59	2,3,3',6	0.39	0.64	-	-	0.29	0.34	-	-	
	42	2,2',3,4'	0.76	1.79	0.13	-	0.55	0.83	0.23	-	
25	41	2,2',3,4	1.72	3.15	0.83	-	2.24	1.86	0.64	0.14	
	64	2,3,4',6	1.53	3.35	0.71	-	1.80	1.64	0.45	-	
											* 68
26	96	2,2',3,6,6'	-	0.12	0.05	-	-	-	0.08	-	
27	40	2,2',3,3'	0.73	1.38	0.28	-	0.96	0.89	0.20	-	
											* 103,57
28	100	2,2',4,4',6	-	-	0.15	-	-	-	0.10	-	
	67	2,3',4,5	0.31	0.28	0.13	-	0.27	0.41	0.09	-	
											* 58
29	63	2,3,4',5	0.21	0.41	0.15	-	0.15	0.23	0.05	-	
30	74	2,4,4',5	1.39	3.57	1.35	-	0.89	2.17	0.78	-	94,61
31	70	2,3',4',5	2.47	6.46	3.85	0.06	1.20	3.89	3.21	0.09	76
											* 98,102
32	66	2,3',4,4'	0.82	2.86	0.50	-	1.60	1.66	0.59	-	93,80
	95	2,2',3,5',6	2.27	4.96	6.00	3.70	0.55	2.87	6.02	3.04	
33	88	2,2',3,4,6	-	0.08	-	-	-	-	-	-	
											* 121
34	91	2,2',3,4',6	0.13	0.68	0.92	-	0.15	0.17	0.83	-	55
35	60	2,3,4,4'	0.94	1.61	0.34	-	0.07	1.33	0.54	-	155
	56	2,3,3',4'	1.35	2.77	0.44	-	0.10	1.60	0.58	-	
36	92	2,2',3,5,5'	0.09	0.46	1.53	0.89	-	0.25	1.58	0.59	
37	84	2,2',3,3',6	0.19	1.24	2.08	0.40	0.14	0.72	1.95	0.25	
											* 89
38	90	2,2',3,4',5	-	0.86	0.85	0.82	-	0.32	0.93	0.56	
	101	2,2',4,5,5'	0.50	2.63	7.72	5.21	0.15	1.33	7.94	5.02	
											* 113
39	99	2,2',4,4',5	0.28	1.90	4.06	0.15	-	0.86	3.60	0.11	
											* 79

40	119	2,3',4,4',6	-	0.08	0.19	-	-	0.05	0.14	-	150
											• 112
41	83	2,2',3,3',5	-	0.26	0.53	-	-	0.12	0.45	-	78,109
											• 152
42	97	2,2',3',4,5	0.22	1.44	2.80	0.37	-	0.65	2.55	0.23	86
											* 125,145,81,117,116
43	87	2,2',3,4,5'	0.34	1.50	4.22	1.13	-	0.77	3.78	0.77	
	115	2,3,4,4',6	-	0.25	0.28	0.21	-	-	0.30	0.05	
											• 111
44	85	2,2',3,4,4'	0.17	1.16	1.85	-	-	0.53	1.66	0.05	
											• 148
45	136	2,2',3,3',6,6'	-	0.09	0.91	1.75	-	0.07	1.12	2.23	120
46	77	3,3',4,4'	0.39	0.66	-	-	-	0.45	-	-	
	110	2,3,3',4',6	0.42	2.91	6.27	2.15	-	1.53	5.85	1.90	
47	82	2,2',3,3',4	0.14	0.96	1.05	-	-	0.44	0.95	-	154
	151	2,2',3,5,5',6	-	-	1.22	2.89	-	-	1.17	3.67	
48	135	2,2',3,3',5,6'	-	0.20	1.61	3.18	-	0.08	1.62	2.56	144
											• 124,147
49	107	2,3,3',4',5	-	0.21	0.94	-	-	0.07	0.72	-	108
50	123	2',3,4,4',5	0.55	0.56	0.85	-	-	-	0.81	-	106
	149	2,2',3,4',5',6	-	0.18	4.50	8.57	-	0.63	2.21	7.83	
	118	2,3',4,4',5	0.42	2.47	10.90	1.74	-	1.62	6.39	0.57	
											• 140,139
51	134	2,2',3,3',5,6	-	0.09	0.52	0.86	-	-	0.49	0.62	143
52	114	2,3,4,4',5	-	0.17	-	-	-	-	-	-	142,133
	131	2,2',3,3',4,6	-	-	0.06	0.15	-	-	0.16	0.16	
	122	2',3,3',4,5	-	-	0.19	0.33	-	-	0.50	0.30	
											• 188,165
53	146	2,2',3,4',5,5'	-	0.08	0.80	2.07	-	-	0.83	1.49	
											• 161,184
54	132	2,2',3,3',4,6'	0.21	0.60	2.57	4.52	-	0.30	1.98	3.69	
	153	2,2',4,4',5,5'	0.55	1.15	4.17	11.43	-	0.68	4.26	10.80	
	105	2,3,3',4,4'	0.49	1.43	1.90	0.12	-	0.86	3.83	0.07	
											• 168,127
55	141	2,2',3,4,5,5'	-	0.19	0.98	3.31	-	-	1.04	2.56	
	179	2,2',3,3',5,6,6'	-	-	0.20	2.02	-	-	0.21	1.79	
56	130	2,2',3, 3',4, 5'	-	0.10	0.83	0.20	-	-	0.63	0.08	
57	176	2,2',3,3',4,6,6'	-	0.06	0.43	1.27	-	-	0.32	0.95	
	137	2,2',3,4,4',5	-	0.08	0.25	0.07	-	-	0.25	0.06	
58	160	2,3,3',4,5,6	-	-	-	0.23	-	-	-	0.05	163,164,186
	138	2,2',3,4,4',5'	0.42	0.96	3.61	8.20	0.19	0.54	3.20	6.13	
	158	2,3,3',4,4',6	-	0.16	0.98	1.35	-	-	0.77	1.55	
59	129	2,2',3,3',4,5	-	0.10	0.83	1.19	-	-	0.23	1.11	
	126	3,3',4,4',5	-	-	0.08	0.46	-	-	-	-	
	178	2,2',3,3',5,5',6	-	-	0.19	1.27	-	-	1.35	1.62	
											* 166
60	175	2,2',3,3',4,5',6	-	-	0.11	0.30	-	-	0.05	0.23	
61	187	2,2',3,4',5,5',6	-	0.09	0.30	3.55	-	-	0.32	3.97	182,159
62	183	2,2',3,4,4',5',6	-	0.05	0.21	2.56	-	-	0.17	1.76	
											• 162
63	128	2,2',3,3',4,4'	-	0,72	3.04	1.64	-	-	2.07	1.06	
64	167	2,3',4,4',5,5'	-	0.08	0.35	0.49	-	-	0.21	0.26	
65	185	2,2',3,4,5,5',6	-	-	-	0.79	-	-	-	1.34	
66	174	2,2',3,3',4,5,6'	-	0.21	0.37	3.92	-	-	0.34	3.85	181
67	177	2,2',3,3',4',5,6	-	0.12	0.21	2.36	-	-	0.21	2.21	
68	202	2,2',3,3',5,5',6,6'	-	-	-	0.14	-	-	-	0.50	
	171	2,2',3,3',4,4',6	-	0.11	0.50	1.90	-	0.05	0.50	2.16	
	156	2,3,3',4,4',5	-	0.23	1.43	1.27	-	0.09	1.62	0.88	
69	173	2,2', 3,3',4,5,6	-	-	0.09	0.14	-	-	0.09	0.36	
	157	2,3,3',4,4',5'	-	0.12	0.31	0.24	-	-	-	0.14	
	201	2,2',3,3',4,5',6,6'	-	-	0.60	1.18	-	-	0.68	0.99	
											* 204
70	172	2,2',3,3',4,5,5'	-	-	0.09	0.90	-	-	0.05	0.75	192
71	197	2,2',3,3',4,4',6,6'	-	-	-	0.09	-	-	-	0.12	
72	180	2,2',3,4,4',5,5'	-	0.26	0.53	4.60	-	0.06	0.38	7.12	
73	193	2,3,3',4',5,5',6	-	-	-	0.69	-	-	-	0.66	
74	191	2,3,3',4,4',5',6	-	-	-	0.38	-	-	-	0.25	
75	200	2,2',3,3',4,5,6,6'	-	-	-	0.28	-	-	-	0.45	
76	169	3,3',4,4',5,5'	-	-	-	-	-	-	-	0.05	
77	170	2,2',3,3',4,4',5	-	0.34	0.65	2.36	-	0.11	0.31	3.91	
	190	2,3,3',4,4',5,6	-	-	0.05	0.94	-	-	0.08	0.79	
78	198	2,2',3,3',4,5,5',6	-	-	-	0.07	-	-	-	0.09	
79	199	2,2',3,3',4, 5,5',6'	-	-	-	1.03	-	-	-	1.31	
80	203	2,2',3,4,4',5,5',6	-	-	-	0.91	-	-	-	0.99	
	196	2,2',3,3',4,4',5',6	-	-	-	0.64	-	-	-	0.69	
81	189	2,3,3',4,4',5,5'	-	-	-	0.40	-	-	-	0.11	
82	208	2,2',3,3',4,5,5',6,6'	-	-	-	0.40	-	-	-	0.17	
	195	2,2',3,3',4,4',5,6	-	-	-	0.41	-	-	-	0.68	
83	207	2,2',3,3',4,4',5,6,6'	-	-	-	0.05	-	-	-	0.05	
84	194	2,2',3,3',4,4',5,5'	-	-	-	1.47	-	-	-	1.30	
85	205	2,3,3',4,4',5,5',6	-	-	-	0.08	-	-	-	0.15	
86	206	2,2',3,3',4,4',5,5',6	-	-	-	0.09	-	-	-	0.45	
87	209	2,2',3,3',4,4',5,5',6,6'	-	-	-	-	-	-	-	0.05	
Sum (%)			100.5	102.2	108.2	109.2	100.2	98.7	97.6	103.3	

Table 2. Representation of concentrations of all 209 individual chlorobiphenyl congeners as determined in Clophen (A30,A40,A50,A60) and Aroclor (1016),1242,1254,1260) commercial mixtures. Each congener occurs with different concentrations in the commercial mixtures. Its maximum concentration is used as a criterion to assign the congener to one of five groups, that are characterized by the concentration range: >5%, 1-5%, 0.5-1%,0.05-0.5% and < 0.05%. This facilitates a comparison of chemical structure characteristics and concentration level in any of the commercial mixtures.

Conc. >5%		1-5%		0.5-1%		0.05-0.5%		<0.05%	
Nr.	Structure	Nr.	Structure	Nr.	Structure	Nr.	Structure	Nr.	Structure
1	2	4	2,2'	7	2,4	5	2,3	11	3,3'
2	3	6	2,3'	9	2,5	10	2,6	12	3,4
3	4	15	4,4'	20	2,3,3'	24	2,3,6	13	3,4'
8	2,4'	16	2,2',3	27	2,3',6	29	2,4,5	14	3,5
18	2,2',5	17	2,2',4	46	2,2',3,6'	34	2',3,5	21	2,3,4
22	2,3,4'	19	2,2',6	48	2,2',4,5	35	3,3',4	23	2,3,5
28	2,4,4'	25	2,3',4	59	2,3,3',6	37	3,4,4'	30	2,4,6
31	2,4',5	26	2,3',5	77	3,3',4,4'	51	2,2',4,6'	36	3,3',5
33	2',3,4	32	2,4',6	83	2,2',3,3',5	63	2,3,4',5	38	3,4,5
44	2,2',3,5'	40	2,2',3,3'	90	2,2',3,4',5	67	2,3',4,5	39	3,4',5
49	2,2',4,5'	41	2,2',3,4	91	2,2',3,4',6	69	2,3',4,6	43	2,2',3,5
52	2,2',5,5'	42	2,2',3,4'	107	2,3,3',4',5	75	2,4,4',6	50	2,2',4,6
70	2,3',4',5	45	2,2',3,6	123	2',3,4,4',5	88	2,2',3,4,6	54	2,2',6,6'
95	2,2',3,5',6	47	2,2',4,4'	130	2,2',3,3',4,5'	96	2,2',3,6,6'	55	2,3,3',4
101	2,2',4,5,5'	53	2,2',5,6'	134	2,2',3,3',5,6	100	2,2',4,4',6	57	2,3,3',5
110	2,3,3',4',6	56	2,3,3',4'	172	2,2',3,3',4,5,5'	114	2,3,4,4',5	58	2,3,3',5'
118	2,3',4,4',5	60	2,3,4,4'	190	2,3,3',4,4',5,6	115	2,3,4,4',6	61	2,3,4,5
138	2,2',3,4,4',5'	64	2,3,4',6	193	2,3,3',4',5,5',6	119	2,3',4,4',6	62	2,3,4,6
149	2,2',3,4',5',6	66	2,3',4,4'	195	2,2',3,3',4,4',5,6	122	2',3,3',4,5	65	2,3,5,6
153	2,2',4,4',5,5'	74	2,4,4',5	196	2,2',3,3',4,4',5',6	126	3,3',4,4',5	68	2,3',4,5'
180	2,2',3,4,4',5,5'	82	2,2',3,3',4	202	2,2',3,3',5,5',6,6'	131	2,2',3,3',4,6'	71	2,3',4',6
		84	2,2',3,3',6	203	2,2',3,4,4',5,5',6	137	2,2',3,4,4',5	72	2,3',5,5'
		85	2,2',3,4,4'			157	2,3,3',4,4',5'	73	2,3',5',6
		87	2,2',3,4,5'			160	2,3,3',4,5,6	76	2',3,4,5
		92	2,2',3,5,5'			167	2,3',4,4',5,5'	78	3,3',4,5
		97	2,2',3',4,5			169	3,3',4,4',5,5'	79	3,3',4,5'
		99	2,2',4,4',5			173	2,2',3,3',4,5,6	80	3,3',5,5'
		105	2,3,3',4,4'			175	2,2',3,3',4,5',6	81	3,4,4',5
		128	2,2',3,3',4,4'			189	2,3,3',4,4',5,5'	86	2,2',3,4,5
		129	2,2',3,3',4,5			191	2,3,3',4,4',5',6	89	2,2',3,4,6'
		132	2,2',3,3',4,6'			197	2,2',3,3',4,4',6,6'	93	2,2',3,5,6
		135	2,2',3,3',5,6'			198	2,2',3,3',4,5,5',6	94	2,2',3,5,6'
		136	2,2',3,3',6,6'			200	2,2',3,3',4,5,6,6'	98	2,2',3',4,6
		141	2,2',3,4,5,5'			205	2,3,3',4,4',5,5',6	102	2,2',4,5,6'
		146	2,2',3,4',5,5'			206	2,2',3,3',4,4',5,5',6	103	2,2',4,5',6
		151	2,2',3,5,5',6			207	2,2',3,3',4,4',5,6,6'	104	2,2',4,6,6'
		156	2,3,3',4,4',5			208	2,2',3,3'4,5,5',6,6'	106	2,3,3',4,5
		158	2,3,3',4,4',6			209	2,2',3,3',4,4',5,5',6,6'	108	2,3,3',4,5'
		170	2,2',3,3',4,4',5					109	2,3,3',4,6
		171	2,2',3,3',4,4',6					111	2,3,3',5,5'
		174	2,2',3,3',4,5,6'					112	2,3,3',5,6
		176	2,2',3,3',4,6,6'					113	2,3,3',5',6
		177	2,2',3,3',4',5,6					116	2,3,4,5,6
		178	2,2',3,3',5,5',6					117	2,3,4',5,6
		179	2,2',3,3',5,6,6'					120	2,3',4,5,5'
		183	2,2',3,4,4',5',6					121	2,3',4,5',6
		185	2,2',3,4,5,5',6					124	2',3,4,5,5'
		187	2,2',3,4',5,5',6					125	2',3,4,5,6'
		194	2,2',3,3',4,4',5,5'					127	3,3',4,5,5'
		199	2,2',3,3',4',5,5',6					133	2,2',3,3',5,5'
		201	2,2',3,3',4,5',6,6'					139	2,2',3,4,4',6
								140	2,2',3,4,4',6'
								142	2,2',3,4,5,6
								143	2,2',3,4,5,6'
								144	2,2',3,4,5',6
								145	2,2',3,4,6,6'
								147	2,2',3,4',5,6
								148	2,2',3,4',5,6'
								150	2,2',3,4',6,6'
								152	2,2',3,5,6,6'
								154	2,2',4,4',5,6'
								155	2,2',4,4',6,6'
								159	2,3,3',4,5,5'
								161	2,3,3',4,5',6
								162	2,3,3',4',5,5'
								163	2,3,3',4',5,6
								164	2,3,3',4',5',6
								165	2,3,3',5,5',6
								166	2,3,4,4',5,6
								168	2,3',4,4',5',6
								181	2,2',3,4,4',5,6
								182	2,2',3,4,4',5,6'
								184	2,2',3,4,4',6,6'
								186	2,2',3,4,5,6,6'
								188	2,2',3,4',5,6,6'
								192	2,3,3',4,5,5',6
								204	2,2',3,4,4',5,6,6'

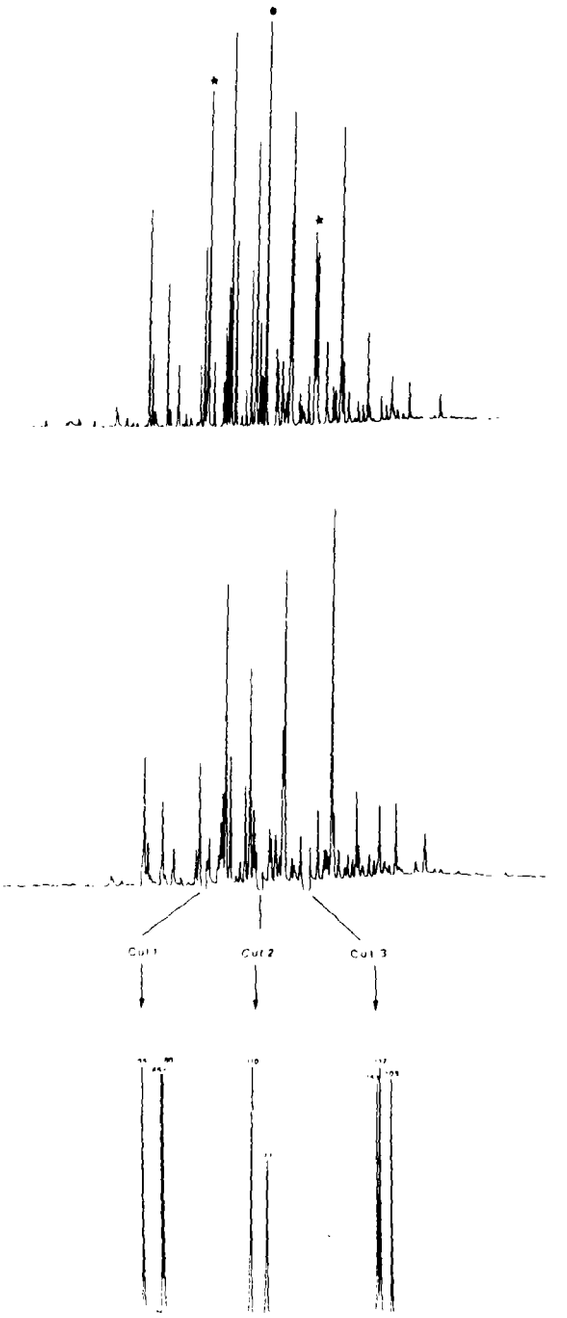


Fig. 1

a: Chromatogram of Aroclor 1254 recorded by the monitoring ECD in the MDGC mode (the ECD-chromatogram of Aroclor 1254 recorded by the monitoring detector without heart cutting is shown in Fig. 2b.) First column: SE-54; second column: OV-210. Three consecutive cuts were made, involving the chromatographic domains 32, 46 and 54, indicated as cut 1, cut 2 and cut 3.

b: Chromatograms recorded by the main ECD in the MDGC mode, reflecting the compositions of the domains, cut from Aroclor 1254 (a). Congener identification as in Table 1.

c: Chromatogram recorded by the main detector in the MDGC mode, reflecting the separation of all possible congeners in the three cuts 1,2 and 3, with the aid of a synthetic mixture. Cut conditions as in a and b.

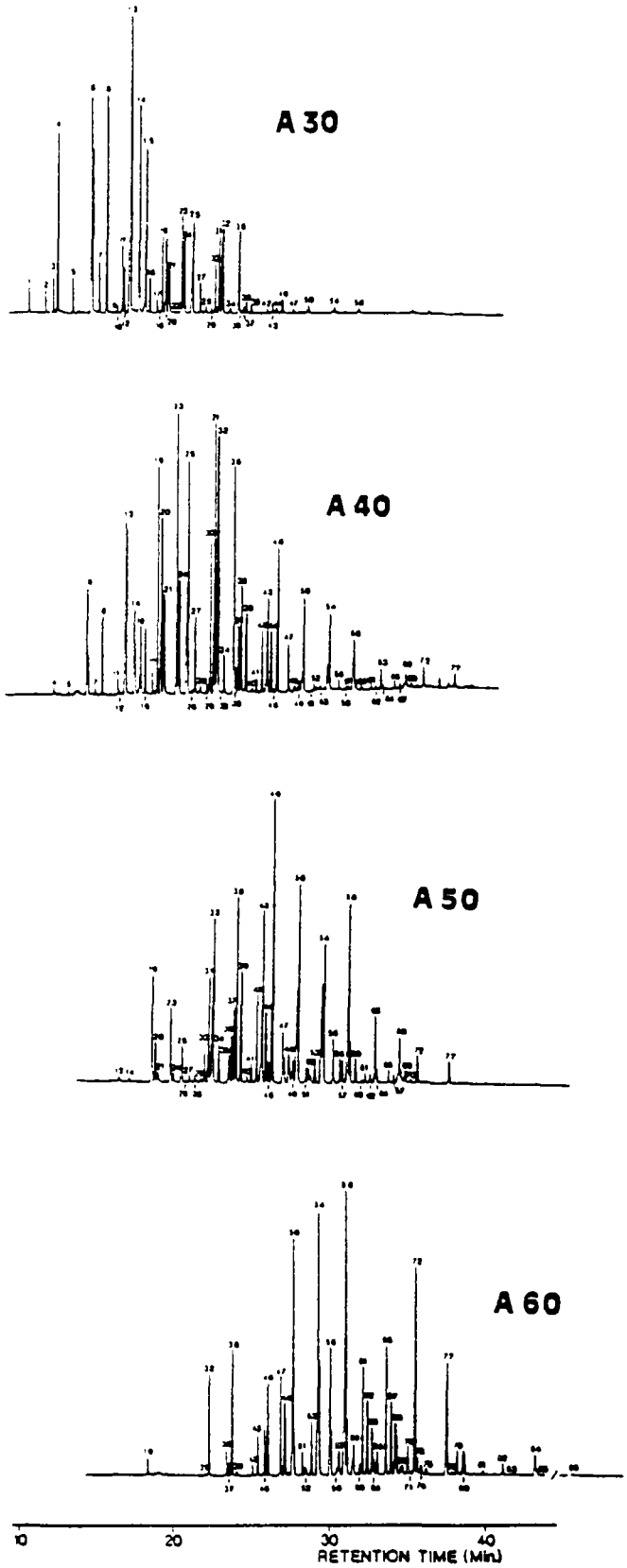


Fig. 2 a

ECD chromatograms of Chlophens A 30, A 40, A 50 and A 60 on a single SE-54 column. For chromatographic conditions see Ref. 6. Numbers identify the chromatographic domains, defined to include a series of peaks that are not base-line separated. Corresponding domains in each of the Clophens and Aroclors (Fig. 2 b) have the same numbers.

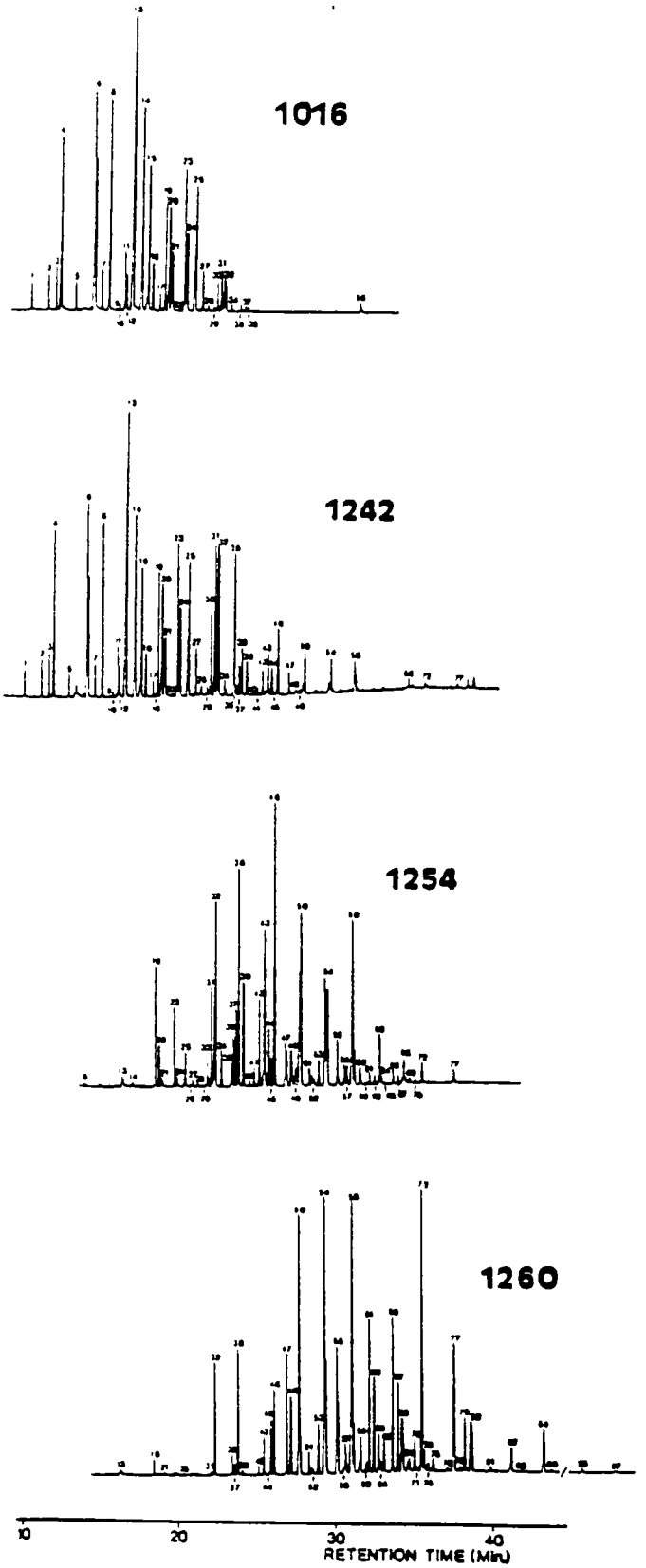


Fig. 2 b

ECD-chromatograms of Aroclors 1016, 1242, 1254 and 1260 on a single SE-54 column. For chromatographic conditions see Ref. 6. Numbers identify the chromatographic domains, defined to include a series of peaks that are not base-line separated. Corresponding domains in each of the Aroclors and Chlophens (Fig. 2 a) have the same numbers.

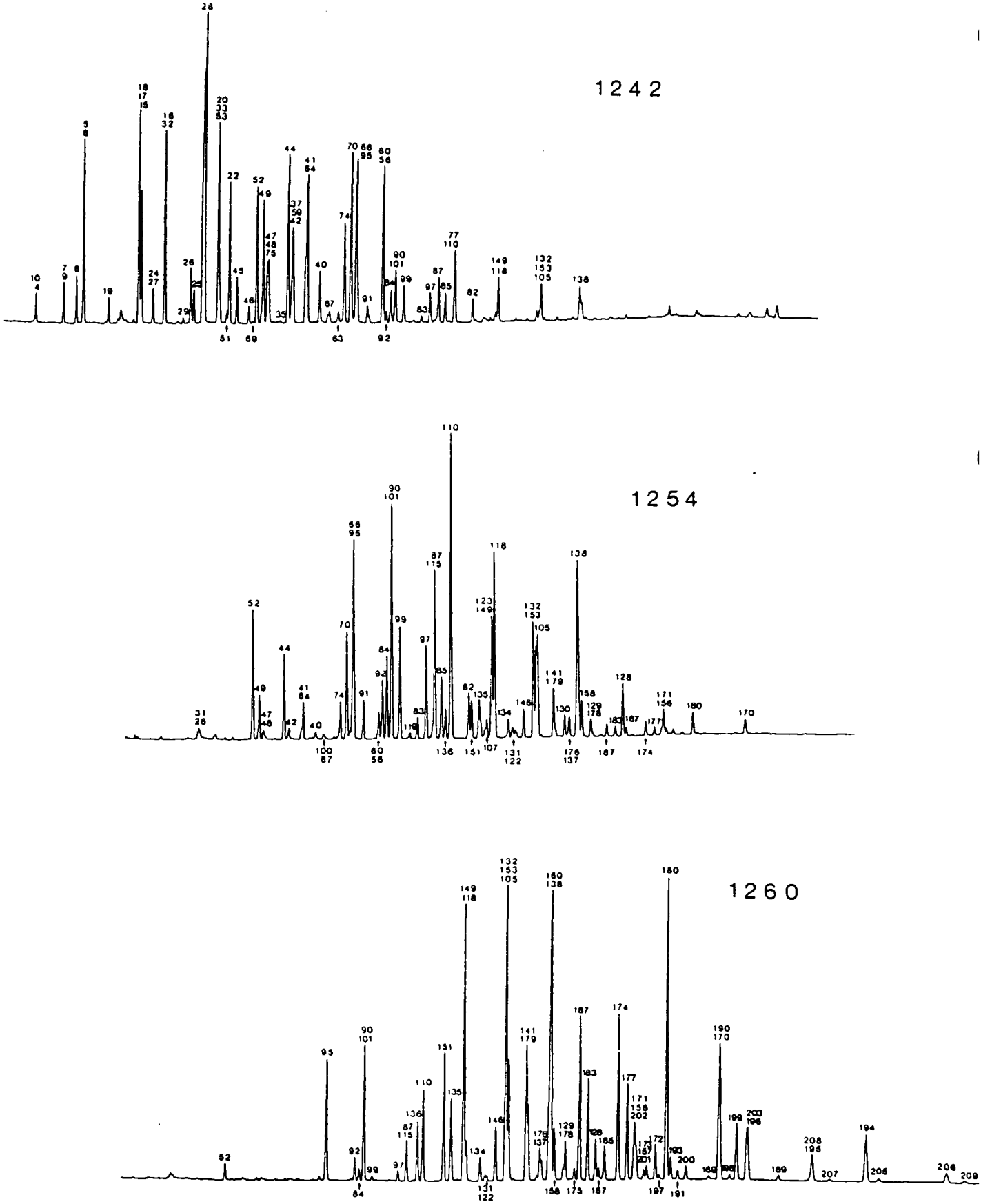


Fig. 3

ECD-chromatograms of Aroclors 1242, 1254 and 1260 (as in Fig. 2b). Peaks are labeled in terms of those CBs that contribute to the peak. Corresponding peaks in different mixtures may have different labels. For instance, CB-77 contributes to Aroclor 1242, but not to Aroclor 1254.

2. Extraction, Separation and Clean-up Procedures

a. Methodology for Extraction from Water

The extremely low concentrations at which organochlorines usually occur in seawater solution require their concentration over many orders of magnitude prior to GC-ECD analysis. Basically, 2 methods are available: sorption onto a solid adsorbent and solvent extraction.

a1. 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. A continuous extraction system for the extraction of essentially unlimited volumes of water with a relatively small volume of solvent (300-400 ml) and some application has been described (3). The method is attractive, but it has a serious drawback. The main problems are the small sample volumes that can be processed in a reasonable time (about 5 dm^3 h^{-1}), and the incomplete separation of solvent and water (solubility). Up to 30% of n-hexane may be lost in even multistep separators. This loss has to be compensated for during an extraction of 300 - 500 dm^3 water. The advantages of a possibly complete extraction is lost in this way. Additional problems are caused by the high concentrations of colloids, particularly in coastal waters. These form stable emulsions with the organic solvent phase, resulting in additional losses. A final problem is the need for continuous attention to be paid to the extraction process at sea over many hours. We have devoted considerable efforts to develop a technique using adsorbents for extraction of dissolved apolar compounds from seawater since the formulation of the former document (IOC Technical Series No. 26 (3)). We shall concentrate our discussions in this document to the possibilities and limitations of the use of XAD-2 columns.

a2. Sorption onto Adsorbents

Various sorbents have been used to adsorb organochlorine compounds from natural waters. These include activated carbon (27), urethane foam plugs (28, 29), polyurethane foam coated with adsorbents (30), a porous polymer Tenax (31), a mixture of activated carbon powder, MgO powder and refined diatomaceous earth (32), Carbowax 4000 and n-undecane on Chromosorb DMCS (33), and Amberlite XAD resins (34-37). The method using XAD resin has been described in detail by DAWSON (38). It was also used during the multilaboratory 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 (4). Problems were encountered in the cleaning procedure of this material. These have been solved in the meantime. Extremely low blank values can now be obtained for XAD-2 columns that have been treated with great care. Their use allows reliable determinations of CBs present in seawater at levels as low as 10-100 femtogram per dm^3.

The advantages to use XAD-2, as we see them today, are the larger volumes that can be processed per unit time (28 - 30 dm^3 h^{-1}, i.e., 5 bedvolumes/ min), and the feasibility to use several extractors in series. The extraction efficiency of a single column is 70 - 90%, if total capacity of the column is not exceeded. This is no problem in open sea waters. Extraction is carried out in air tight equipment under a N_2 blanket. The entire procedure needs much less attention than the liquid-liquid extraction procedure that was described before (3). In addition, no solvents have to be added during extraction. The various steps required to obtain reliable seawater extracts for the analysis of OCs, in particular chlorinated biphenyls with the aid of XAD-2 columns will be described in detail below. Elution of the sorbed material requires only about 100 ml acetonitrile.

b. Laboratory procedures

b1. Cleaning XAD-2

Even p.a. quality of commercially available XAD-2 (technical grade, e.g., of Supelco) has to be cleaned thoroughly in order to remove the often considerable amounts of contaminants. The following procedure appears to be effective: about 2 dm^3 water are added to not more than 500 g resin in a 3 dm^3 narrow-necked glass bottle and shaken vigorously for 2-3 minutes. The smaller undesired particles are allowed to separate: this may take 1-10 hours (depending on quality of the resin between 1 and 5% mass of the total amount separates out). In case no wetting takes place, thus preventing the larger particles from settling, acetone is added until settling takes place. Water is then introduced through a tube, at below the level of the small floating particles, in order to remove them from the flask. This procedure should be repeated until no more fine particles float to the top. This may require many repetitions of the procedure (10 or more). Removal of the small particles is necessary to prevent resistance to water flow building up to such an extent that the required flow rate (5-6 bedvolumes/min) cannot be achieved, or achieved only with great difficulty. Another advantage is the cleaning effect. In this way, technical XAD can be used reducing the cost to only 10% of p.a. material. The resin treated as above is then slurried into an extraction column (39). An acetonitrile-water mixture containing 10-20 % water is used as the extraction solvent. The amount of sea water (20-60 ml) remaining in a column previously used for sea water extraction is usually sufficient. If too small an amount of water is present, the resin may "dry out" and undergo a volume reduction of about 10%. This decreases the internal pore size of the resin and limits the ability of solvent and contaminant molecules to adsorb.

The column is installed in the apparatus as shown in Figure 4 and this is attached to a round bottomed flask containing the solvent. The resin is extracted under reflux overnight, with a continuous N_2 flow of 10 ml per minute flushing the system (compare with section III c.2.b5 on distillation and storage of organic solvents). It is important that solvent enters from below, thus forcing any remaining air out of the resin. Air pockets in the resin form channels for the solvent which leads to inefficient extraction.

b2. XAD-2 Test

On completion of the extraction, the resin is left in contact with the solvent in the apparatus for a further 2 hours. This solvent, contained in the extraction column (80 ml) is transferred to a round-bottom flask and the acetonitrile removed on a rotary evaporator. The remaining aqueous solution is extracted with 5ml n-hexane, dried with anhydrous Na_2SO_4 and concentrated to 50 μl. Analysis of 2 μl by GC/ECD should not show any peak greater than that of 1 pg lindane.

b3. Cleaning Aluminium Oxide, Silica Gel and Sodium Sulphate.

Sodium sulphate can be cleaned conveniently in an oven at 350 ^{0}C in 10 hours. It is stored in ground stoppered bottles.

Silica gel has to be treated chemically. It is first refluxed with dichloromthane in a Soxhlet for about 24 hours, then with n-hexane for the same period. The solvent is then removed from the solid in a rotary evaporator (Fig. 5). The glass flask should be rotated at minimum speed to avoid mechanical breaking of particles into smaller units. The silicagel is removed, as soon as it starts "raining" down as fine particles, and dried in a drying oven (e.g., Büchi TO-51, Flawil, Switzerland) at 10^{-2} mbar. Initial temperature should be 40 ^{0}C. The final temperature of 120 ^{0}C is achieved in steps of 30^0, 30^0 and 20 ^{0}C increases, whereby each of the intermediate temperatures and the final temperature (40, 70, 100, 120 ^{0}C) is maintained for one hour. With this slow temperature increase a polymerisation of acetonitrile is avoided. The silicagel is allowed to cool under vacuum and sealed in glass ampoules in the necessary amounts, immediately afterwards to avoid contamination.

As active silicagel attracts water and contaminants from the atmosphere, care must be taken to avoid deactivation prior to and during sealing. This

problem can be avoided by controlled partial deactivation of the silica gel (48). This involves adding 3-5% by weight of water to the fully active silica gel. In this form, it is less liable to undergo changes in activity during storage and ensures good reproducibility. If this method is used the elution pattern described in Section C2 will not apply.

b4. Cleaning Glass Fibre Filters

The collection of sea water particles on XAD resin during the extraction should be avoided as such particles will cause clogging of the resin greatly reducing the flowrate under hydrostatic pressure. Particles with a high organic content can also burst when in contact with the organic adsorbent. The compounds thus released will be extracted and erroneously included in the dissolved phase.

For these reasons filtration is essential. Filtration is effected with a clean glass fibre filter. Removal of any contamination present is necessary as the filters may be in contact with sea water for up to 72 hours. Cleaning with solvent is impracticable as large amounts of solvent are required and the filters are brittle when wet. An efficient method is heating at 350-370 ^{0}C. The filters should be well separated from each other in an oven to allow efficient evaporation of contaminants. They should not be treated with solvents afterwards. The cleaned filters can be kept in clean Petri dishes.

b5. Distillation and Storage of Organic Solvents

Commercially available solvents like acetone, acetonitrile, dichloromethane, hexane and pentane are invariably contaminated with ECD-active substances: their concentrations varying with the quality ordered, the batch number and supplier. This should be checked by injection of 2 μl of a 100 ml batch of solvent, after concentration to 50 μl in a rotary evaporator. No peak in the GC-ECD chromatogram (90-250 ^{0}C) should be larger than that of 1 pg lindane. Otherwise, the solvent must be distilled. The following procedure has been found to be very effective, as well as cost-effective, as it allows the use of technical grade solvents as the basic material (reducing the cost by an order of magnitude). 130-150 cm columns are required, the filling material must be glass to allow a cleaning procedure with oxidizing acid. The entire equipment is cleaned by 2 consecutive distillation procedures with 500 ml water in each case. It is essential that a current of nitrogen gas (15 ml/min) flows from the distillation flask during distillation of the organic solvents: the cooler serves as exhaust. Room air has no contact with the solvent in this way. Problems are associated with other methods of excluding room air (e.g., active carbon or molecular sieves), the most important one being discontinuity (see next section). The condensate is distilled into a 1 dm^{3} flask at a 1:20 ratio. This large volume allows the direct transfer into the appropriate solvent containers. These should consist of glass of sufficient size to provide solvent for not more than 2 analyses. A bottle with sufficient solvent for 10-15 analyses has to be opened and closed many times. Even when kept closed in between, contamination of the bottle content from the surrounding atmosphere takes place. The following example will illustrate this finding. Dichloromethane was distilled in a nitrogen atmosphere as indicated above. 100 ml were concentrated to 100 μl and 2 μl were injected on-column. The ECD-GC corresponded to a total amount of CBs < 40 pg in 100 ml. The solvent was left in a glass-stoppered flask in the laboratory for 4 hours. The same procedure resulted then in a total amount of 10 ng total CBs in 100 ml solvent. All bottles that are closed with screw-on devices show "leakage" between bottle content and atmosphere because of "breathing" under variable temperature conditions resulting in astonishingly rapid contamination of solvents. This can be minimized by storage at constant temperature (-20 ^{0}C). This problem of contamination from the atmosphere is one of the more serious threats of solvent integrity. We have found this problem invariably in the laboratory, in outside atmosphere and on research vessels.

We have found that the only way in which ultrapure organic solvents can be stored for unlimited time and during transport is achieved by sealing in glass ampoules. The solvent is transferred into the ampoules directly from the receiver of the distillate. They must be cooled by liquid air to allow solidification of their content. The ampoules are flushed with nitrogen gas, removing oxygen. The ampoules can then be sealed while still in the Dewar bottle, thus avoiding volume increases in the volume of vapour, which would not allow proper sealing. The ampoules can be stored safely in appropriate

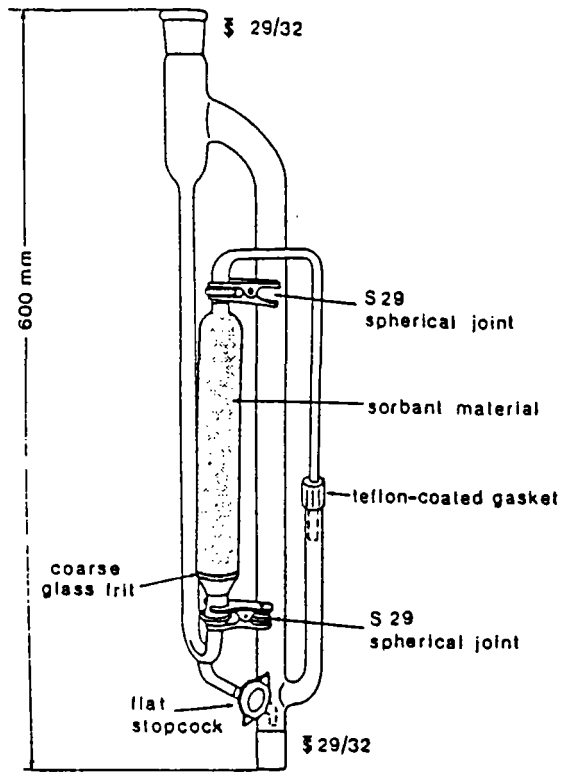


Fig. 4

Equipment used for extracting seawater with a XAD-2 column according to Ehrhardt (39).

Figure 5

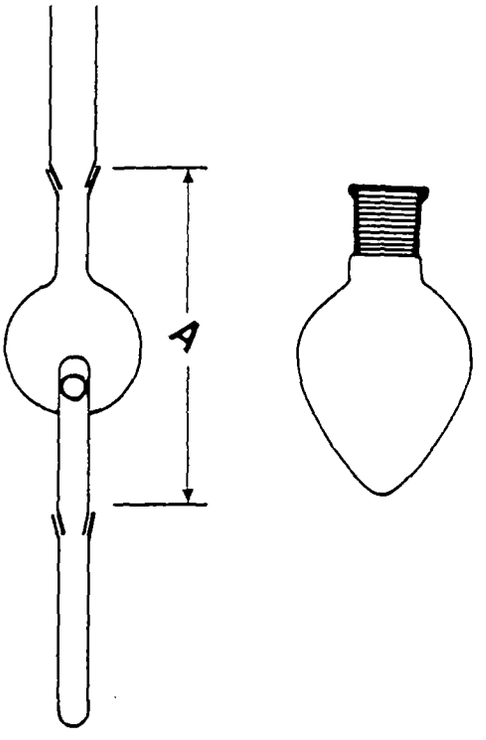


Figure 6

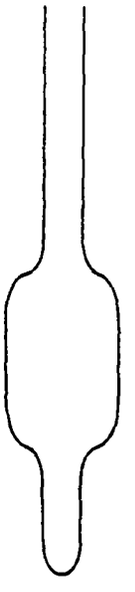


Fig. 5

Left: Distillation flask section of a rotary evaporator for removing organic solvents. Part A is included when concentrating sample extracts. Its use avoids the loss of solutes because of boiling point retardation effects.
Right: small vials for concentrating down to small volumes.

Fig. 6

Glass ampoule to concentrate down to volumes of a few microliters.

wooden boxes for unlimited time periods. Care must be taken when the ampoules are opened at room temperature, because of pressure buildup. No danger arises when they are opened under a protecting towel.

b6. Volume Reduction of Organic Solvents and Solutions

A rotary evaporator is useful to reduce the volume of an extract in e.g., hexane. We have found it extremely useful to include an extra part into commercially available rotary evaporators, to control and eliminate the possible loss of solutes during fast or explosion-like distillation of the solvent (part A in Fig. 5). The initial temperature of the solvent should be lower than room temperature, to avoid rapid initial boiling particularly when using pentane and dichloromethane as solvents. This high speed of volatilization can result in loss of low boiling fractions of solutes. The danger diminishes during progress of the procedure because of the temperature decrease of the solvent as result of the required heat of evaporation. When the precautions mentioned are taken into account, volume reduction of a C9-C24 mixture can be carried out with this procedure from 100 ml to 0.5 ml without discrimination. This procedure takes 30 minutes at 1.0 mbar. A blanket of N_2 gas is required to avoid contamination of the solvent by atmospheric components. The use of active carbon or a molecular sieve for cleaning ambient laboratory air before its introduction into the rotary evaporator is inappropriate in many cases, as the contact time between the air and the adsorption agent is often too short for efficient contaminant removal. Moreover, it is difficult to observe the condition of the sorption column, which changes with time, at a rate depending on the quality of the laboratory air. A rotary evaporator treated with N_2-gas can easily remain clean for periods of 1-2 years as was shown by running blanks and this procedure is preferred.

The final reduction of volume from 0.5 ml to 20 μl can be accomplished in narrow glass ampoules, with a narrow glass tube (Fig. 6). N_2 is passed through a piece of fused silica capillary (70 mm), connected to a polyethylene tube (0.2 mm i.d.). The capillary can be moved in the tubing easily after slight heating of the latter with a conventional torch or even a cigarette lighter. The removal of 500 μl at 0 ^{0}C may take typically 15 minutes. Too fast removal may result in discrimination. Cooling is applied during solvent removal. The ampoule can thus be sealed immediately after the desired final column has been obtained. As soon as the tip of the ampoule has taken the form of a sphere, the sealing procedure has been completed successfully and the content of the ampoule can be stored safely. The ampoules are made from normal Pasteur pipettes.

c. Sample Treatment

c1. Sample Eluates from XAD-2 Columns

For elution of the sample constituents, the XAD-2 column is extracted with acetonitrile-water (10:2) for 6 hours. This ratio is achieved by extracting with 100 cm^3 pure acetonitrile, while the corresponding amount of water is present in the XAD-2 column already. Boiling stones must be present in the flask. A nitrogen gas flow of 10 ml min^{-1} through the cooler should be maintained during this period. The flask is then cooled at -20 ^{0}C for one hour and the solvent is removed in a rotary evaporator at 1.0 mbar. A progressively increasing layer of ice on the outside of the flask reduces the evaporation of the solvent (boiling point 82 ^{0}C). It can be removed by immersion in a water bath at 10 ^{0}C after 15 minutes. It is important to allow for this period as this solvent tends to 'bumping' or rapid initial boiling rather strongly. This is decreased by the presence of the boiling stones.

Although this is a manual for CBs, scientists analysing CBs may also be interest in polar compounds. The following note may be useful therefore. It is important that acetonitrile is removed quantitatively. This becomes apparent when water drops appear at the wall of vessel P in Fig. 5 (acetonitrile, remaining in the water would seriously limit the extraction of more polar seawater-dissolved constituents during the extraction of the remaining waterphase with n-hexane. This effect for polar compounds would be reduced significantly by extraction in an acid or basic regime, and it could be insignificant for apolar compounds).

The remaining waterphase is extracted with 3 consecutive portions (10 ml each) of n-hexane. The extract is dried with as little Na_2SO_4 as possible. Small portions are added, until the added Na_2SO_4 no longer sticks to the Na_2SO_4 already present. The solution is then removed with a Pasteur pipette, and transferred to a small flask as in Fig. 5b. (Contact of the tip of the pipette and the mouth of the flask must be avoided because of possible contamination). It is cooled at -20 ^{0}C. The extract is concentrated to 0.4 ml in this flask with the rotary evaporator in about 20 minutes without additional heating. The final concentrated extract is removed with a 500 μl syringe. It can be subjected to sample clean-up, or alternatively, stored in an ampoule that is sealed immediately.

c2. Clean-up of Extracts involving HPLC

Extracts of most types of environmental samples in an organic solvent require clean-up before they can be analyzed reliably by GC-ECD. If interfering compounds, usually occurring at much higher concentrations than the OCs of interest are not removed, several problems are bound to arise in the GC-ECD procedures. These have been described in detail in preceding sections.

Normal treatment of the sample extracts involves clean-up over an Al_2O_3 microcolumn, followed by class separation over a silica gel microcolumn (40). We have found that, following Al_2O_3 clean-up (4 x 0.5 cm i.d.), a simple HPLC method can be used very efficiently for the elimination of interfering compounds in extracts of various sample types, including sea water. At the same time, different fractions of organic compounds of interest are obtained (aliphatics, PAHs, OC-pesticides, Toxaphene, PCBs). These can be analyzed accurately by GC methods, without contamination or deterioration of injector, columns and detector (41).

The following procedure is recommended:

Between 20 to 200 μl of solution are eluted on a stainless-steel column (200 x 4 mm i.d.) packed with Nucleosil 100-5 (Machery-Nagel, Düren, Germany), with n-pentane, 20 % dichloromethane in n-pentane and finally dichloromethane. Experiments showed that for an n-heptane flowrate of 1 ml/min, the column efficiency for anthracene was 57,000 (benzene 55,000) theoretical plates per meter. HPLC is performed with a Pump-Constametric III with a Rheodyne injector at a flowrate 0.5 ml/min. The eluate is collected in 0.5 ml fractions. Each fraction is analyzed by GC-ECD and GC-FID (flame ionization detection). It is determined which fractions contain the following classes of compounds:

fraction 1: n-hydrocarbons and alkenes
" 2: PCBs
" 3: PAHs and Toxaphene
" 4: Pesticides and Toxaphene
" 5: Acids, etc., (polar compounds).

When elution takes place with n-pentane (0-11.0 ml), 20 % dichloromethane in n-pentane (11.0 - 15.0 ml), and 100 % dichloromethane (from 15.0 ml onwards), the fractions 1 - 5 correspond roughly to the following elution volumes: 1:(0.5 - 2,0 ml), 2:(2.0 - 4.5 ml), 3:(4,5 - 11.0 ml), 4:(11.0 -15.0 ml) and 5:(15.0 - 25.0 ml).

Full details can be found in the literature (41).

c3. Efficiency of XAD Columns

In an attempt to check the efficiency of XAD-2 columns, 3 seawater samples were extracted, each with 2 XAD-2 cartridges in series. The work was carried out during R.V. POSEIDON cruise in the North Atlantic (47 ^{0}N, 20 ^{0}W) in June 1987. Samples were taken with a 400 dm^3 stainless steel sampler (Hydrobios Kiel) from 250 m, 750 m and 4000 m depths. Seawater was filtered using GF/C filters (Fig. 7). The filtrate was passed through 2 XAD-2 resin columns in series (flow rate was 500 ml, 5 resin bedvolumes per minute). The resin was eluted with 150 ml of boiling acetonitrile. The eluate was treated as described in c1 and cleaned-up with HPLC as in c2. The resulting ECD-chromatograms of the first and second XAD-column extraction of the 250 m-depth sample are given in Fig. 8. The results are presented quantitatively for 13 individual CBs in Table 4. The apparent extraction efficiencies

decrease with decreasing concentrations (17 - 1.7 pg dm^{-3} for ΣCBs). This is mainly due to the fact that the signals of the second extract are similar to those of the procedural blank at the lowest concentration levels detected in the deepest sample. It appears however, that the efficiency of the first column is about 90%. This is in agreement with the results of a recent study (17).

d. Precautions on Board Ship

Conditions and activities on board represent the largest potential sources of contamination for the samples. Significant sources of contamination are in particular the engine room, the storage rooms for fuel and oils, and the painting and waste incineration activities. Exhaust particles accumulate on deck during longer station periods, from where they are then distributed over the entire ship.

It is essential therefore to test the sampling and sampling processing equipment carefully. This may be considered as the main task on board. The research team typically spends the first few days on board cleaning the entire system, and a good deal of the available time during the cruise is spent repeating the checking and correcting procedures, realizing that few good data is worth more than many bad data.

The laboratories have to be cleaned before the equipment is brought in and assembled, as in most cases the history of the laboratory space is unknown. The extraction units should be protected against the ships' atmosphere by continuous flushing with N_2 (see section on XAD-2 extraction). The use of a clean bench is essential to eliminate dust particles during sampling processing. All glassware actually needed is stored in the clean bench and all chromatography involving Al_2O_3, SiO_2 and HPLC techniques is only carried out here. Care has to be taken that introduction of materials and/or personnel that have been in contact with contaminant-rich air (e.g., from the engine room) is minimized or prevented.

Organic solvents solutions are concentrated in a rotary evaporator with N_2-flushing. All activities must be checked by capillary column GC-ECD analyses. This allows the identification and elimination of specific contamination sources, e.g., part of the bench, atmosphere in the bench, glass containers, etc.

The samples are extracted and analyzed by GC-ECD on board under the same conditions. The signals of individual CBs should be at least 5 times higher than the blank values. A final check on data validity is supplied by the comparison of the compositions of the CB mixtures in samples and in the blank determinations: the presence of significant contamination is unlikely if the peak patterns are different.

3. 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 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, therefore, operationally defined.

In open ocean waters with low content of suspended matter, concentrations of PCB in particulate forms are considered to be small (about 10 %) with respect to those in solution (42-45). However, in natural waters with high suspended matter concentrations, they may be comparable with, or even higher than, the concentrations of PCB in solution (46). In such cases, it is essential to perform phase separation, preferably on board ship immediately after sampling (3, 46). This extra step is an additional possible source of contamination.

Figure 7 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 filtrated is derived from the 400 dm^3 sampler or from the pumping system. It is sucked through the glass

Table 3.

List of CB congeners that elute as single peaks from a SE-54 column. They are identified by their IUPAC numbers and chlorine numbers (n_{Cl}).

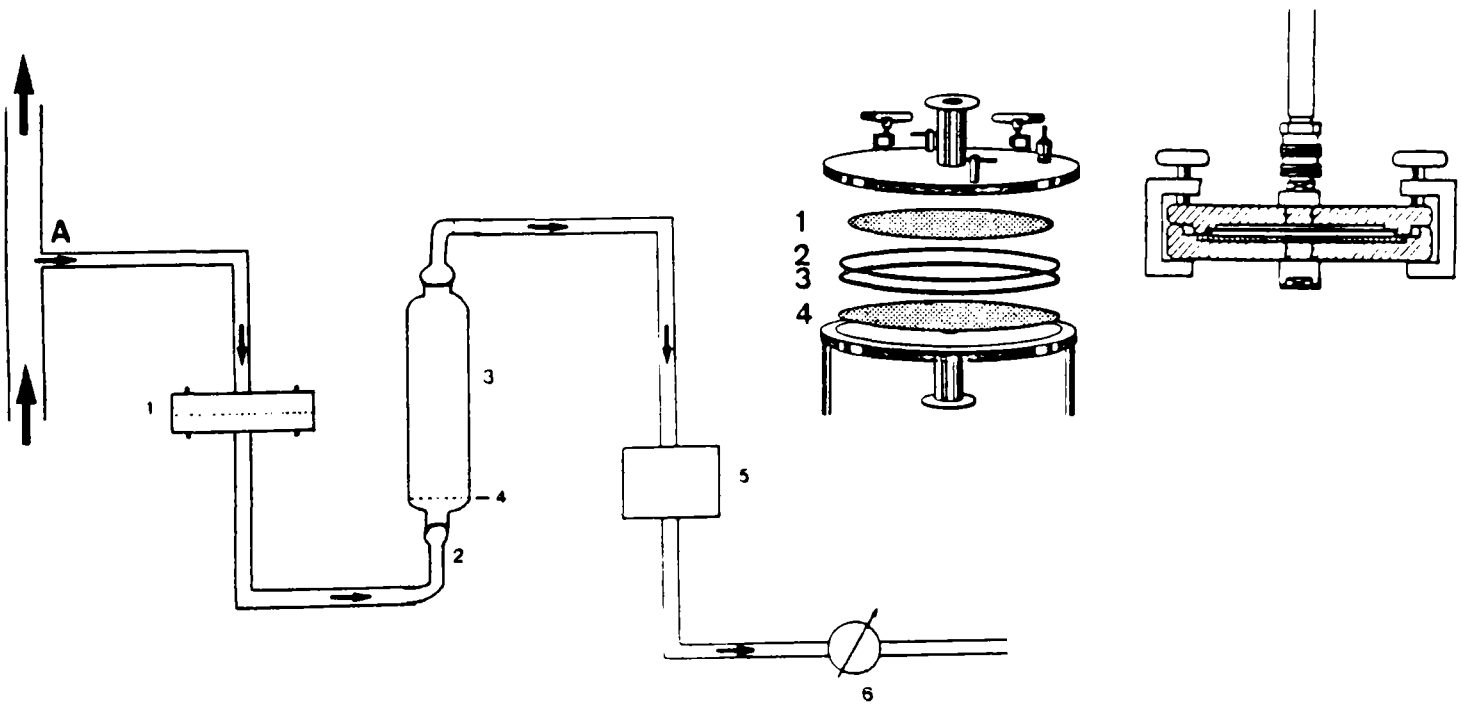
n_{Cl}	IUPAC number
1	1, 2, 3
2	6
3	19, 25, 26, 29, 34, 35
4	40, 44, 45, 46, 49, 52, 63, 69, 70, 74
5	83, 84, 85, 88, 91, 92, 96, 97, 99, 107, 119
6	128, 130, 134, 135, 136, 146, 167, 169
7	172, 174, 175, 177, 180, 183, 185, 187, 189, 191, 193
8	194, 197, 198, 199, 200, 205
9	206, 207
10	209

Table 4

Results of the efficiency of XAD-2 columns to extract 13 selected CBs from seawater. Samples were obtained at 250, 750 and 4000 m depth (details given in text). They were filtered and extracted with two consecutive XAD-2 columns. The concentrations of individual congeners were summed (sum CB). This sum of concentrations determinated in the 1.st and 2.nd XAD-2 extractions are given in the last row as % of the sum of concentrations in the first and second extractions.

Depth	250 m		750 m		4000 m	
column	first	second	first	second	first	second
CB No.	Concentrations in pg dm^{-3}					
18	1.60	0.10	0.30	<0.05	0.19	<0.05
31	0.70	0.09	0.20	<0.05	0.17	<0.05
28	0.60	0.08	0.10	<0.05	0.13	<0.05
52	0.90	0.06	0.20	0.08	0.17	0.08
49	0.50	<0.05	0.05	<0.05	0.09	<0.05
44	0.50	<0.05	0.10	<0.05	0.07	<0.05
101	1.70	0.10	0.50	0.05	0.16	0.08
149	1.60	0.11	0.50	<0.05	0.18	0.05
118	0.60	<0.05	0.20	<0.05	0.08	<0.05
153	2.70	0.11	0.80	0.05	0.15	0.05
138	4.50	0.16	1.20	0.07	0.26	0.10
180	1.70	0.19	0.50	0.05	0.13	<0.05
170	0.80	0.08	0.30	<0.05	0.07	<0.05
Sum CB	18.40	1.08	4.95	0.30	1.85	0.36
extracted	95%	5%	94%	6%	84%	16%

Figure 7



Left: Arrangement of filtration-extraction unit. Connection to the pumping system or the large volume sampler at A. 1 = filtration unit, 3 = XAD-2 column, 4 = grid, 5 = pump, 6 = flow meter.

Right: details of filtration unit 1 - 4 = stainless steel plate, 2 = Teflon O-ring, 3 = filter

fibre filter (Fig. 7b), followed by an XAD-2 column according to the arrangement in Fig. 7a, at a rate of 30 dm^3 h^{-1}.

4. Sampling

The equipment used to sample seawater and suspended particles for the analysis of trace organic contaminants is presently the weakest chain in the entire analytical procedure because its role as possible source of contamination cannot be determined unambiguously.

We have used i) a stainless steel gas-lift system for surface water from a vessel kept at a fixed position, ii) a stainless steel/polyethylene pumping system for surface samples from under the hull of the ship while in transit (47) and iii) stainless steel 400 dm samplers for deep water.

The gas-lift system has been described before (3). The pump in the system (ii) must have a capacity >0.5 m^3 h^{-1} to keep any contamination as result of subsampling to a minimum. The head of the pump may be ceramic. A magnet-coupled pump made of metal, PTFE or Teflon causes less contamination problems. We have used a teflon membrane pump, driven by compressed air. A special metal reinforced teflon tube is positioned in the hydrographic well extending 1.5 m under the hull. This guarantees continuous sampling under practically all conditions at sea from a depth between 5 and 13 meters.

Samples from larger depths require the use of a sampler. We have been using a 400 dm^3 stainless steel sampler (Hydrobios, Kiel, Germany). It is lowered through the water-atmosphere interface while opened. This is a serious potential source of contamination, because of a usually greasy cable, water dropping from the wheel rod or arm, or the seawater surface film. We are presently working on an improved version allowing the sampler to pass through the water-atmosphere interface while closed, and to be opened at 10 m depth. Also, we are working on an in-situ extraction system, using XAB-2 resin at depths up to 6 km.

During lowering, the sampler content is exchanged every 30 meters. Its journey to large depths allows intensive rinsing with ambient seawater: the sampler is rinsed effectively with 40 m^3 water when lowered to 3,000 m depth. This system is less suitable for surface samples, when taken directly, but the sampler can be lowered to a large depth first for rinsing purposes, then raised to above the sampling depth, then lowered again to the sampling depth where it is finally closed.

The sampler, once on deck, is connected with a nitrogen gas supply to avoid contamination from the atmosphere especially during temperature changes. During emptying the sampler, nitrogen is used to aerate the sampler. In no case should ambient air be used.

Other similar devices have been described in the literature such as a glass sampler (49) and the stainless steel and anodized aluminum Bodman bottle (50); both are closed when passing through the surface.

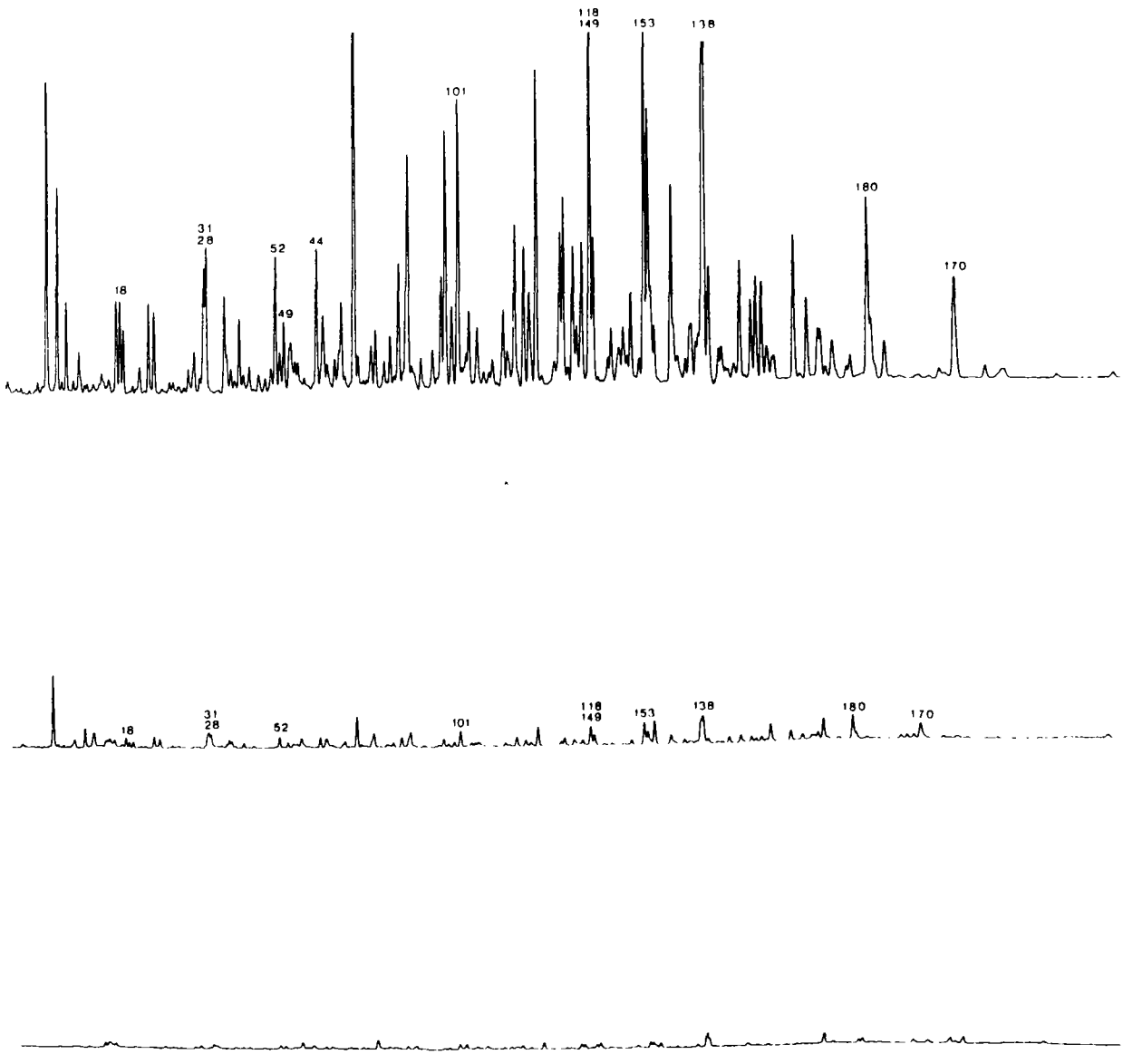
D. Results on the Distribution of CBs in Seawater

In this chapter we shall describe some results on the distribution and composition of chlorinated biphenyls in open ocean water (44). Samples were taken in the northeast part of the North Atlantic at position 47 ^0N, 20 ^0W in June 1986. Water were obtained from 250 m, 750 m, and 3,500 m depths with the 400 dm^3 sampler. Seawater was filtered and CBs adsorbed on XAD-2 resin directly from the sampler. CBs were separated from interfering compounds and analyzed by GC-ECD. To avoid contamination, the sampler was cleaned by flushing in deep water for several hours before being closed at the selected depth. Deep water samples were taken before those at shallower depth.

The ECD-chromatogram of sample 1 (250 m) is shown in Figure 9, the results for 23 CBs at the 3 depths are represented in Table 5. Early eluting congeners (with n_{Cl} =2,3 and 4) with relatively large contributions to ΣCBs were present in all samples (Figure 10). This contrasts to other sample types (particles, organisms).

Qualitatively, the CB mixture in the water reflects the average world production of commercial PCB mixtures of different overall degree of

Figure 8



Top: ECD-chromatogram of extract of open ocean water sample (400 dm^3)

Middle: Chromatogram of extract of second XAD-2 column, as described in the section on efficiency of XAD-2 columns

Bottom: Procedural blank. XAD-2 extracted with 150 ml acetonitrile; further treatment as with samples: 150 ml concentrated to 100 μl, clean-up by HPLC, second fraction concentrated to 20 μl and 1 μl injected on SE-54.

Table 5

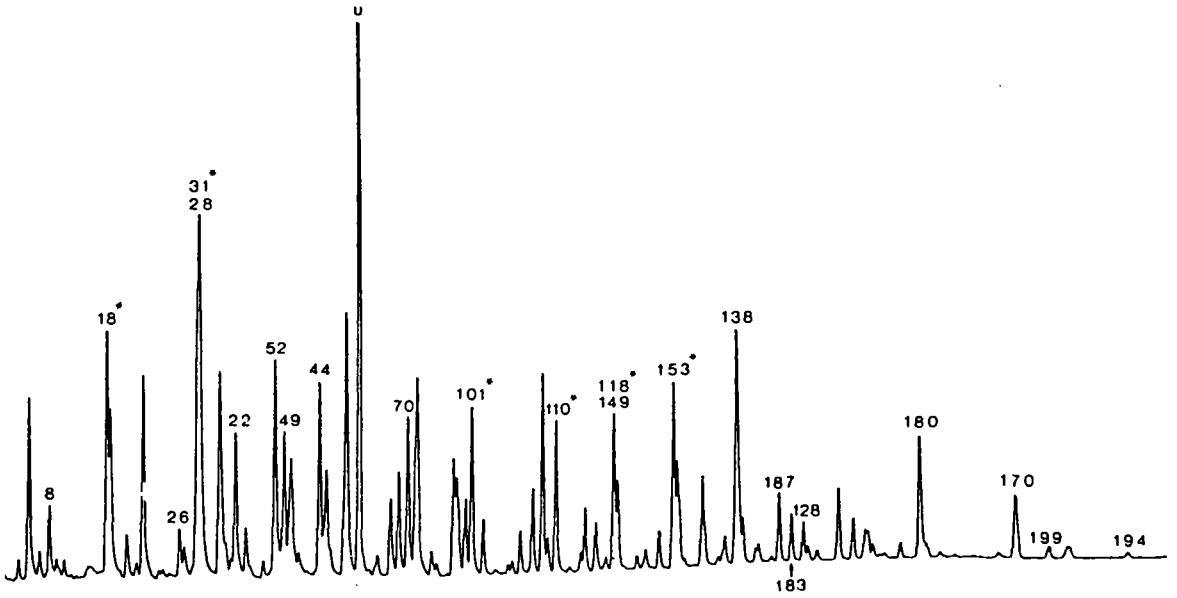
Concentrations of 23 Chlorobiphenyl congeners (pg/dm^3) at different depths in North Atlantic water (details given in text).

	Concentrations in pg dm^{-3}		
Depth	250 m	750 m	3500 m
CB No.			
8	0.70	0.12	0.06
18	1.60	0.30	0.20
26	<0.05	<0.05	<0.05
31	0.70	0.20	0.10
28	0.60	0.10	0.08
22	0.75	0.16	0.08
52	0.90	0.20	0.09
49	0.50	0.05	0.05
44	0.70	0.10	0.05
70	0.61	0.24	<0.05
101	1.70	0.50	0.07
110	1.13	0.24	<0.05
149	1.60	0.50	0.17
118	0.60	0.20	0.08
153	2.70	0.80	0.13
138	4.50	1.20	0.24
187	0.90	0.30	<0.05
183	0.50	0.15	<0.05
128	0.60	0.20	<0.05
180	1.70	0.50	0.10
170	0.80	0.30	0.06
199	0.20	0.05	<0.05
194	0.20	0.06	<0.05
Σ CB	24.19	6.47	1.56

chlorination because the highest peaks in each of them (6) are also found in the samples. The 23 congeners reported here contribute more than 60 % of total CBs, estimated on the basis of ECD responses. Unresolved peaks (* in the chromatogram) were quantitated after MDGC separation.

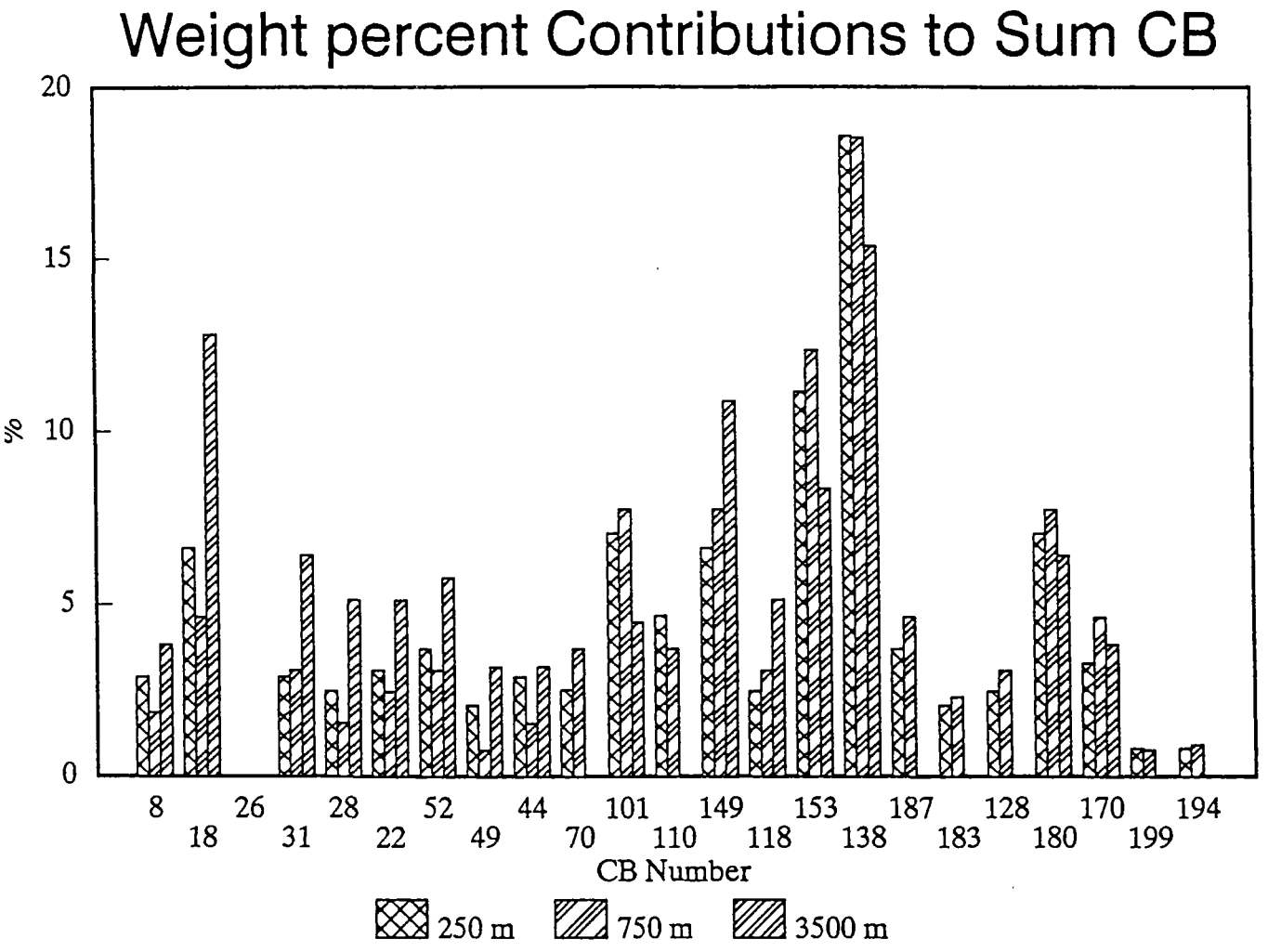
Deep water concentrations were lower than at smaller depths (Figure 11). The contributions of early eluting congeners to the mixture of CBs increased with depth. This may be due to preferential release from sinking particles, as result of the larger solubility of the early eluting fraction. Considerable more work is needed to check this hypothesis (44), contrasting with other findings (45).

Figure 9



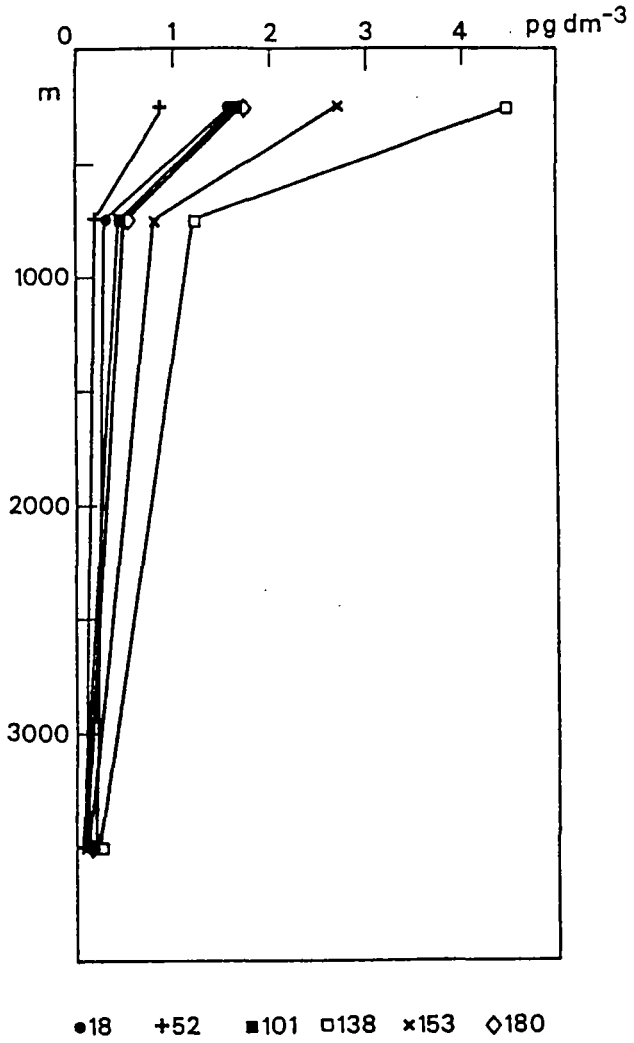
ECD-chromatogram of a XAD-2 extract of a water sample at 250 m depth (Table 5). CB congeners are identified by numbers (Table 1). Those marked with * have been determined by MDGC-ECD. u = unknown, non-CB.

Figure 10



Weight % contributions of the concentrations (pg/dm^3) of 23 individual CB congeners (identified by numbers) to their mixtures in samples from three depths in the Atlantic (47 ^{0}N, 20 ^{0}W; data in Table 5).

Figure 11



Concentrations of CBs Nos. 18, 52, 101, 138, 153, 180 as function of depth (250, 750 and 3500 m) at 47 °N, 20 °W in the Atlantic (data Table 5).

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