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IDENTIFICATION AND DETERMINATION OF POLYCHLORINATED BIPHENYLS BY HIGH-RESOLUTION GAS CHROMATOGRAPHY

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SUMMARY

We have developed an integrated analytical procedure for polychlorinated biphenyl (PCB) multiresidues in sediment samples that enables quantitation of PCB residues at levels as low as 0.1 μg/kg in sediments. PCB residues are characterized and quantified by wall-coated open-tubular column gas chromatography with electron-capture detection. An automated data system, based on a Spectra Physics SP-4100 computing integrator, is used to select and quantitate peaks of individual isomers and homologous groups of PCBs. This procedure consistently yields results with a reproducibility within 3%.

INTRODUCTION

The most difficult task in the determination of multicomponent polychlorinated biphenyl (PCB) residues is to identify individual isomers of the PCB mixture in the presence of such contaminants as chlordanes, DDT and its analogues and toxaphene. In order to evaluate the possibility of designing a new methodology, we decided to undertake a more detailed study of PCB residues in sediment samples, which would provide data for:

(a) quantitation of individual isomers, important for toxicological and synergistic-effect studies of various congeners, in a particular PCB mixture in different matrices;

(b) quantitation of characteristic groups (homologues), based on the number of chlorine atoms in the PCB molecule, which can be used for long-term degradation and bioaccumulation studies, based on individual capacity to interact with a matrix containing PCBs;

(c) determining total loading of PCBs that would be more accurate and precise,

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since impurities would be subtracted from the chromatogram obtained.

Such a study requires a reliable analytical methodology, based on wall-coated open-tubular (WCOT) column gas chromatography (GC). Although we tried to modify previously described procedures for packed columns[1,2] and to apply them to WCOT column GC, it was impossible to improve the precision of data at μg/kg levels. These difficulties seem to be the result of many variables related to the injection techniques and the concept of quantitation using internal standardization. Therefore, we closely scrutinized each step of the GC procedure, optimized it and excluded the presence of other interfering organochlorine compounds (OCs) to obtain an acceptable methodology for determining PCBs in our samples.

The current clean-up and preconcentration procedures, found in manuals[3,4], are adequate for PCB determination in fish, sediment and biota samples. Characterization and quantitation of PCB residues obtained after clean-up can be performed by GC with electron-capture detection (ECD) and WCOT columns and, if desired, confirmation by GC–mass spectrometry (MS)[5–7]. The identification of individual congeners in technical PCB mixtures has also been studied by many authors[8–11] by means of WCOT columns; However, there is no evidence that quantitation of PCB on WCOT columns was successfully achieved.

This paper describes our methodology and provides, in our opinion, a universal method for determining PCB composition.

EXPERIMENTAL

Gas chromatography

All GC–ECD analyses were performed with a Hewlett-Packard Model 5710A gas chromatograph, equipped with split/splitless and on-column injection systems and a standard ^{63}Ni electron-capture detector. For chromatography, we used either chemically bonded (cross-linked) OV-1 or cross-linked SE-54 fused-silica WCOT columns (60 m × 0.32 mm I.D., d_f = 0.25 μm; N_{eff} = 2520 plates per m at k' = 6 for C_{13}). The carrier gas was hydrogen with a linear velocity of 40 cm/sec at maximum program temperature. An initial temperature of 75°C was held for 2 min, ballistically programmed to 140°C, programmed to 240°C at 2°C/min and finally held at that temperature for 4 min. The detector temperature was 350°C and the injector temperature was 75°C. The detector make-up gas was nitrogen at a flow-rate of 20 ml/min. To reduce the detector dead-volume, we inserted the WCOT column exit up to the detector source.

GC–MS confirmation

Mass spectrometry was performed with a Finnigan MAT 311A GC–MS instrument and SpectroSystem 200 data system in either electron-impact (EI) or chemical ionization (CI) mode with methane plasma for ionization. Samples of individual Aroclors and Aroclor mix 1:1:1 were analyzed by GC–CIMS with a WCOT column as in the GC methodology above, using the same chromatographic conditions (Fig. 1). Mass spectra were acquired with the Spectro System 200 data system, scanning from 150 to 500 a.m.u. at 2.3 sec per decade for both EI and CI.

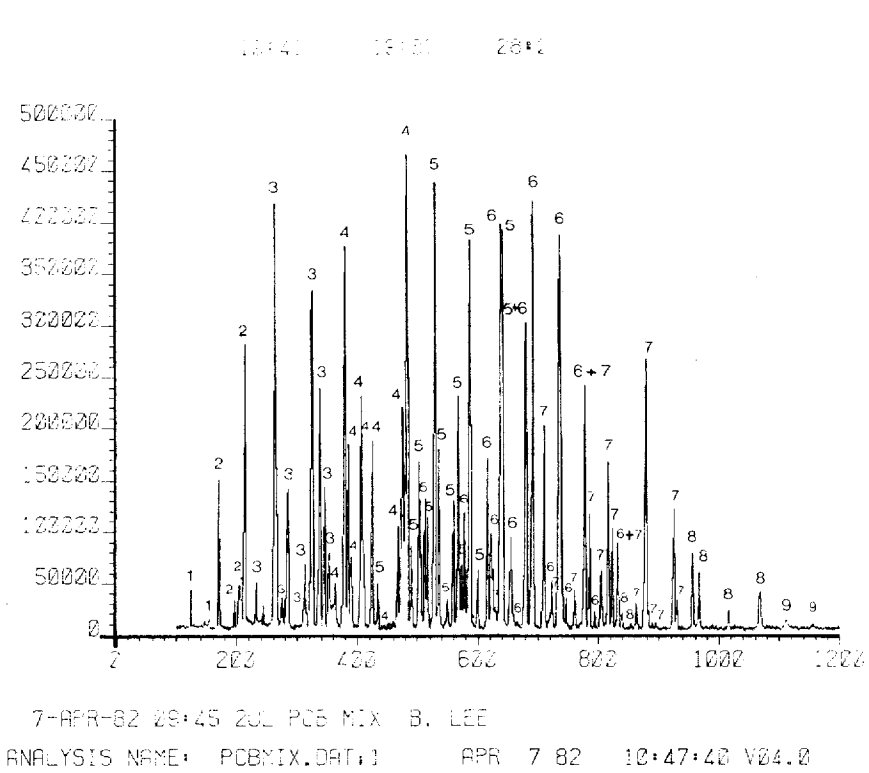

Fig. 1. GC–MS identification of the number of chlorines on the biphenyl moiety for a 1:1:1 mixture of Aroclors 1242, 1254 and 1260.

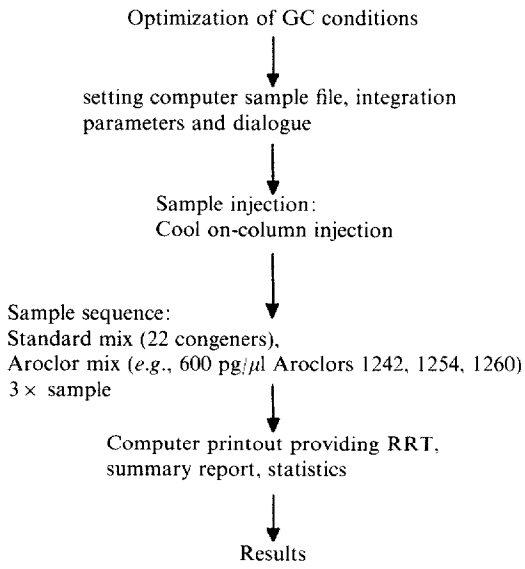

Fig. 2. Scheme of the quantification of WCOT chromatograms.

TABLE I

QUANTITATIVE RELATIVE RESPONSE FACTORS (RRFs) FOR A PCB STANDARD MIXTURE AND AVERAGED RESPONSES FOR HOMOLOGOUS GROUPS

Data in this table are for information purposes only. The operator must perform a calibration to establish RRF for his own instrument.

	Congener	*Amount (pg/μl)*	*RRF (area per pg)*	*RRT*
1	4-Chloro	194.0	54	0.193
2	3-Chloro	239.0	76	0.199
3	2,2-Dichloro	58.4	218	0.209
4	2,4-Dichloro	24.4	1560	0.246
5	2,2,5-Trichloro	31.0	2100	0.291
6	2,4,5-Trichloro	29.0	3185	0.344
7	2,4,4-Trichloro	28.7		0.345
8	2,2,5,5-Tetrachloro	35.6	3110	0.391
9	2,5,3,4-Tetrachloro	37.7	2900	0.396
10	2,5,3,5-Tetrachloro	35.5	3335	0.432
11	2,2,4,5,5-Pentachloro	30.0	4320	0.511
12	2,2,3,4,5-Pentachloro	30.6	4235	0.542
13	2,3,4,4,5-Pentachloro	43.6	4395	0.595
14	2,2,4,4,5,5-Hexachloro	30.6	7505	0.600
15	2,2,3,4,4,5-Hexachloro	36.8	5706	0.667
16	2,2,3,3,4,4-Hexachloro	25.7	7110	0.669
17	2,2,3,4,5,5,6-Heptachloro	18.6	7780	0.718
18	2,2,3,4,4,5,6-Heptachloro	19.0	8695	0.739
19	2,2,3,4,4,5,5-Heptachloro	28.1	7165	0.770
20	2,3,3,4,5,5,6-Heptachloro	14.6	7990	0.775
21	2,2,3,3,4,4,5,5-Octachloro	13.3	11,675	0.902
22	Decachlorobiphenyl	19.8	7915	1.000
	Dichlorobiphenyls	–	540	–
	Trichlorobiphenyls	–	1670	–
	Tetrachlorobiphenyls	–	3815	–
	Pentachlorobiphenyls	–	2430	–
	Hexachlorobiphenyls	–	6730	–
	Heptachlorobiphenyls	–	3400	–

GC quantitation and data collection

The WCOT chromatograms were quantified according to Fig. 2.

The peaks appearing in the chromatogram, representing PCB congeners, were identified by a method of relative retention time (RRT) matching: we used decachlorobiphenyl with an absolute retention time in the range of 60 ± 0.5 min and assigned it a RRT of 1.000 as the marker peak (Table I), and computed RRT values for PCB congeners as the ratio of the individual peak retention time to that of decachlorobiphenyl. Retention times for decachlorobiphenyl were very reproducible (±0.1%) and permitted us to use a GC peak-matching identity range of ±0.002 RRT units for peaks in reference standards and in samples.

Peaks in Table I are representative of those in Aroclors 1016 to 1260 technical PCB mixtures. These peaks were considered for quantitation. Since not all congeners are commercially available, we used as many as 22 congeners for quantitation, knowing that they represent approximately 47% of the total concentration of PCB congeners of a 1:1:1 mixture of Aroclors 1242, 1254 and 1260. These peaks, among the

remaining 95 peaks present in a typical WCOT chromatogram, contained the most useful information for describing the similarity among PCB and PCB-like residues. RRTs and relative response factors (RRFs; area per pg) were first established for the 22 congeners in the standard calibration mixture. Afterwards, the remaining PCB peaks in the Aroclor 1:1:1 mixture of known concentration, *e.g.*, 600 pg/μl, were grouped according to the number of chlorine atoms in the PCB molecule. We will refer to this as homologue grouping. From the weight per cent of each homologue group in Aroclors 1242, 1254 and 1260, as indicated by the manufacturer, and our data given in Table II, we determined the weight of each homologue in a 600 pg/μl sample of 1:1:1 Aroclor mixture. From these homologue weights and respective integrated areas we subtracted the amounts and corresponding areas of the 22 congeners, as related to our standard calibration mixture. Using the remaining integrated areas and weights for each homologue group average, RRFs (area per pg) for the remaining homologues were determined. Approximate values are given in Table I. It is obvious that the use of as many congeners as possible in a standard mixture will reduce the systematic error inherent in the quantitation due to varying degrees of biodegradation and the varying ECD responses of PCB congeners. Once the RRT and averaged RRF are known, these data are introduced into the computing integrator memory. The dialogue is now ready for calculating grouped data, and the unknown sediment extracts can be chromatographed and quantified.

By matching RRTs and using the averaged RRF data along with precise RRFs for the 22 congeners, the amount of each PCB homologue group and the total amount of all PCBs in the sample could be calculated.

We acquired chromatographic data with a Spectra Physics SP-4100 computing integrator with the programs supplied by the vendor for peak detection, integration and quantitation.

RESULTS AND DISCUSSION

Some similarities in peak pattern fingerprints in both sediment samples and the technical mixture of Aroclors 1242, 1254 and 1260 are apparent. No significant environmental degradation has taken place, although there is evident loss in the relative intensity of several peaks (Fig. 3).

TABLE II

COMPOSITION OF AROCLORS AND A (1:1:1) MIXTURE OF AROCLOR 1242, 1254 AND 1260 (IN wt.-%)

Homologous group	*1242*	*1254*	*1260*	*Mixture*
Monochlorobiphenyl	1.0	0.05	–	0.3
Dichlorobiphenyl	16.0	0.1	–	5.4
Trichlorobiphenyl	43.0	0.5	0.1	14.5
Tetrachlorobiphenyl	27.0	10.0	2.0	13.0
Pentachlorobiphenyl	9.0	70.0	9.0	29.3
Hexachlorobiphenyl	4.0	14.0	37.5	18.5
Heptachlorobiphenyl	–	5.35	41.0	15.4
Octachlorobiphenyl	–	–	8.5	2.8
Nonachlorobiphenyl	–	–	1.0	0.7

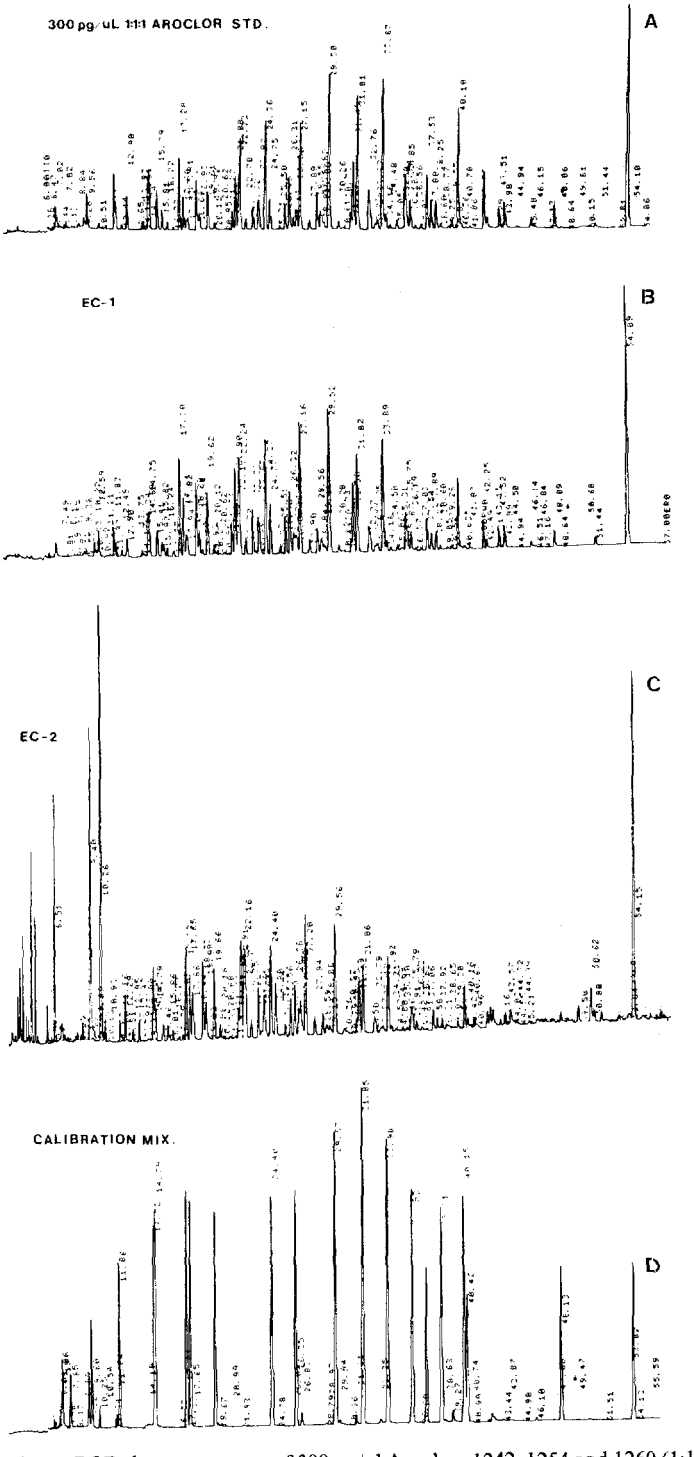

Fig. 3. PCB chromatograms of 300 pg/μl Aroclors 1242, 1254 and 1260 (1:1:1) (A), EC-1 sediment samples (B), EC-2 sediment samples (C) and a calibration mixture containing 22 congeners (D). The last peak on all chromatograms is decachlorobiphenyl.

TABLE III

RELATIVE RETENTION TIMES OF ORGANOCHLORINE COMPOUNDS

Compound	*RRT*
α-Benzene hexachloride	0.238
Hexachlorobenzene	0.254
Lindane	0.268
Heptachlor	0.365
Aldrin	0.409
Heptachlor epoxide	0.456
γ-Chlordane	0.486
α-Endosulfan	0.504
α-Chlordane	0.511
Dieldrin	0.540
DDE	0.551
Endrin	0.563
β-Endosulfan	0.567
pp'-DDD	0.601
op'-DDT	0.612
pp'-DDT	0.661
Methoxychlor	0.749
Mirex	0.790
Decachlorobiphenyl	1.000; t_R = 59.53 min

High-resolution GC analyses of the samples revealed differences in the individual components. Since the RRT are reproducible to ±0.002 units, these alterations would not cause difficulties in attempts to determine which components of the mixture are environmentally persistent[9,11]. In addition, interferences from the DDT group, organochlorine compounds and Mirex can easily be identified and subtracted from the chromatogram (Table III).

Various WCOT columns are suitable for separation of PCB residues. We tested Apiezon L[10], OV-17, OV-1 and SE-54. OV-1 and SE-54 columns, especially, yielded useful data from environmental samples. These columns also resolved DDT analogues, Mirex and its degradation products and OCs under the conditions found best for resolution of PCB components.

It is important to pay attention to discrimination occurring in the split/splitless injection port, which can cause significant errors in the quantitation of mixtures with a wide boiling range, such as PCB, at picogram levels. On-column injection devices are the only ones that yield satisfactory results[9].

In general, analysts prefer methodologies certified by collaborative studies and

TABLE IV

PCB QUANTITATION (CONCENTRATION IN pg/μl) BY SPLITLESS AND ON-COLUMN INJECTION

Mean of five samples and relative standard deviation.

Method	*EC-1*	*EC-2*
Splitless injection	356.0 (8%)	375.4 (9%)
On-column injection	272.4 (2%)	252.6 (1.8%)

which show the advantages of WCOT columns over packed columns. However, there are no data with which to compare results for PCB residue analyses on both types of columns. Data from our studies provide a preliminary comparison of the precision of different injection techniques, as shown in Table IV.

The results obtained indicate good precision, but it was impossible to obtain accuracy data, since the sample extracts received were not spiked with known amounts of PCB mixture and, as these were real samples, the actual concentration was unknown.

The disadvantage of using ECD is evident when lower chlorinated PCB congeners are present: fewer early peaks can be used for quantitation, and a larger error must be expected for monochlorobiphenyl homologues, since their relative responses are very small (Table I).

CONCLUSIONS

Characterization and determination of environmental samples for PCB residues is complicated by several generic problems. The contaminants must be separated from the matrix, and this is a most time-consuming and difficult task. Further, other interfering materials, such as OCs, must be removed or separated from PCB residues to enable accurate and precise qualitative and quantitative analyses.

Our multiresidue PCB Methodology is capable of accomplishing this task for a variety of contaminants. High-resolution GC resolves PCB from other interfering compounds and aids identification and quantitation. Resolution is sufficient to determine the relative concentrations of homologous groups. In general, this methodology is applicable to the examination of PCB residues in the environment. The discrepancy between the on-column and splitless injection results is not fully understood, and more work on this question is in progress.

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