Journal of Chromatography, 124 (1976) 265–275

CHROM. 9120

GAS CHROMATOGRAPHIC DETERMINATION OF POLYCHLORINATED BIPHENYLS AND SOME CHLORINATED PESTICIDES IN SEWAGE SLUDGE USING A GLASS CAPILLARY COLUMN

PER E. MATTSSON and SOREN NYGREN

National Swedish Laboratory for Agricultural Chemistry, S-750 07 Uppsala (Sweden)

(First received December 11th, 1975; revised manuscript received February 16th, 1976)

SUMMARY

Gas chromatographic methods for the determination of polychlorinated biphenyls and DDT (with some of its metabolites) in sewage sludge samples and similar materials are described. The sample is extracted with a mixture of hexane, acetone and water. After separation, the hexane phase is reduced in volume and divided into two aliquots, one of which is first shaken with 7% fuming sulphuric acid to remove lipids, and then with potassium cyanide to eliminate interference by elemental sulphur. The other aliquot is evaporated to dryness and heated with ethanolic potassium hydroxide. The two aliquots are injected into a gas chromatograph fitted with a glass capillary column and an electron capture detector. Hexabromobenzene is used as an internal standard. Polychlorinated biphenyls are determined quantitatively by comparing the peaks of the sample with those of Clophen A 50 or A 60. The individual percentage composition of the chlorobiphenyls in the polychlorinated biphenyl oils is used. The capillary column is coated with silicone oil SF 96 according to a described procedure.

INTRODUCTION

The quantitation of polychlorinated biphenyls (PCBs) and DDT (including its metabolites DDE and DDD) is a problem because of the large amounts of different chlorobiphenyls in the PCB oils. In the gas chromatographic separation many of the chlorinated hydrocarbon pollutants are eluted together with the PCB components. Many papers dealing with PCB determinations do not describe the quantitation methods used, thus making comparison of the results from different investigations difficult. Jensen *et al.*[1] have reviewed some of these methods. The individual chlorobiphenyls of some common commercial PCB oils have been identified[2–5] and determined quantitatively[4]. In order to make accurate determinations of PCB in environmental samples it is necessary to determine the different chlorobiphenyls individually, because the proportions present are not identical with those in pure PCB oils. The use of capillary columns considerably improves their separation. The method used in our laboratory is described in this paper. A method for coating

glass capillary columns with the stationary phase silicone oil SF 96 is also described. The SF 96 columns are rather easy to make and have been shown to have good separation power and a long lifetime.

METHOD

Apparatus

A Varian 2700 (Palo Alto
source electron capture detectors and equipment for the use of glass capillary columns were used. Micrometering valves B22RS4 (Whitey, Oakland, Calif., U.S.A.) were used for the regulation of the gas flows through the injection splitter (Fig. 1) and of the make-up gas to the detector.

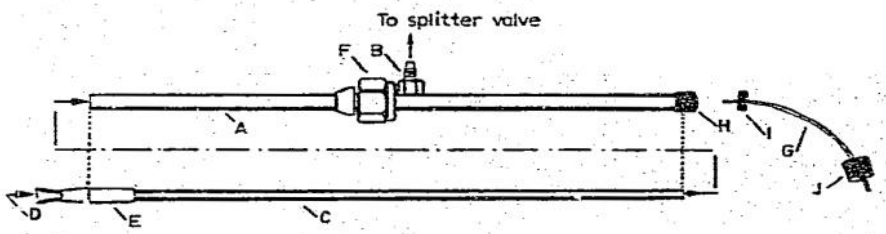

Fig. 1. Equipment for injection of samples into the capillary column. The splitter consists of a stainless-steel tube (A) with a Swagelok reducer SS-100-R-2 (B) soldered to it as outlet to the splitter valve. Inside the steel tube there is a glass tube (C), which is funnel-shaped at the injection end (D) and tightened to the steel tube by PTFE tape (E). The splitter is connected to the original injection port with a 1/4-in. Swagelok nut (F). The capillary column (G) is connected to the splitter at H with a silicone rubber septum (I) and a screw cap (J).

The samples were automatically injected into the column with a Varian Auto Sampler 8000 via a splitter. The glass capillary columns were 40–60 m long and 0.3 mm I.D., and were drawn on a Shimadzu glass drawing machine GDM-1 (Kyoto, Japan) and coated with the silicone oil SF 96. The column temperature was in most cases 185°, the detector temperature 210° and the injector temperature 235°. The carrier gas was nitrogen and the velocities were, through the column, 1 ml/min, and through the splitter, 40 ml/min; that of the make-up gas was 20 ml/min. The peaks were integrated on a Spectra-Physics Autolab System I (Santa Clara, Calif., U.S.A.) computing integrator.

Procedure for coating the glass capillary column

Assemble the equipment according to Fig. 2. Add 5 ml of 1% hydrofluoric acid to the vial (B) and place the column in a conventional drying oven at 70°. Force the hydrofluoric acid solution through the column with 0.1–0.2 MPa nitrogen gas gauge pressure. When the vial (B) is empty rinse it with five 10-ml portions of water. These operations take several hours. Then rinse the column with 10 ml of methanol. Increase the oven temperature to 100° and dry the column with a flow of nitrogen for 1 h. Cool the column to room temperature and disconnect at C. Pass through the column a volume of trimethylchlorosilane (TMCS) equal to one-tenth of the total col-

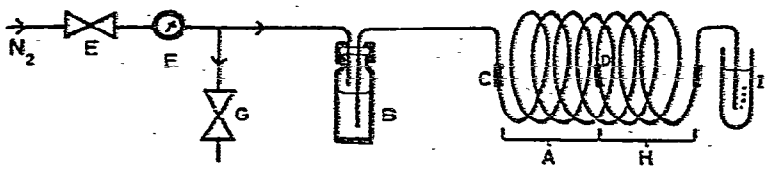

Fig. 2. Set-up for coating of glass capillary columns. The column (A) is connected at C to the vial (B) with a PTFE tube 0.8 mm I.D. and at D to a 10-m capillary column (H). The nitrogen pressure in the column is regulated with the valve (E) and read on the manometer (F) on the gas bottle. The flow through the column is regulated with the splitter valve (G). The test tube (I) serves as a collector of waste, and also as a device for checking that gas passes through the column.

umn volume. Then draw up an equal volume into the end of the column, seal this end with a microburner and place the column in the oven at 170° for 24 h.

After cooling, open the column at C, connect the PTFE tube and blow nitrogen through the column at 170° for at least 2 h. No droplets must appear when the column is cooled to room temperature. Change to a new vial (B), add 0.5 ml of a 5% solution of SF 96 in dichloromethane and let this solution move into the column, regulating the velocity of movement to 1 cm/sec with the splitter valve (F) when the pressure regulator valve (D) is at 0.05 MPa gauge pressure. Evaporate the solvent by passing nitrogen through the column overnight. Continue the conditioning by increasing the temperature by 10°/h and then maintaining at 220° for 1 day or more.

Reagents

The reagents and standards used were hexane, purum, distilled from potassium hydroxide; acetone, purum, distilled; fuming sulphuric acid with 7% sulphur trioxide; potassium cyanide solution, prepared by dissolving 0.1 g of potassium cyanide (pro analyse; Merck, Darmstadt, G.F.R.) in 5 ml of water and adding 95 ml of acetone (this solution can be used for 3 days. Store in a well ventilated hood); sodium chloride solution, prepared by dissolving 9 g of sodium chloride, pro analyse, in 1 l of water; potassium hydroxide pellets; hexabromobenzene (Aldrich-Europe, Beerse, Belgium) extraction solution (0.133 mg in 1 l of hexane); PCB standard (1.00 mg of Clophen A 50 and 0.50 mg of hexabromobenzene in 1 l of hexane); pesticide standard (0.050 mg of DDE, 0.100 mg of DDD and DDT and 0.50 mg of hexabromobenzene in 1 l of hexane); silicone oil SF 96 (Applied Science Labs., State College, Pa., U.S.A.); hydrofluoric acid (about 0.2 *M*); and trimethylchlorosilane (TMCS; Pierce, Rockford, Ill., U.S.A.).

General procedure

Homogenize carefully 1–2 l of wet sewage sludge. Take about 1000 g and evaporate the water just to dryness at 60° in a fan drying oven. Homogenize the dried sludge and shake a known amount (5–10 g) with 50 ml of acetone and 25.0 ml of the extraction solution containing hexabromobenzene in a 250-ml ground-joint flask for 20 min. Allow to stand overnight and add 200 ml of sodium chloride solution to separate the hexane phase. Evaporate a 15-ml aliquot of the hexane phase under a flow of nitrogen and dissolve the residue in 4 ml of hexane.

Keep an aliquot of 2 ml for subsequent treatment with potassium hydroxide.

To the other aliquot add 2 ml of 7% fuming sulphuric acid and invert the vessel containing the mixture at least 30 times. Centrifuge or freeze out the acidic phase. Transfer the hexane phase to a 50-ml volumetric flask or another narrow-necked flask and add 10 ml of potassium cyanide solution. Shake about 15 sec, wait 2 min and separate the hexane phase by adding 40 ml of water, then inject 1–5 μl into the gas chromatograph. Transfer the reserved aliquot of the hexane phase to a 10-ml centrifuge tube and add 2 ml of 95% ethanol and four pellets of potassium hydroxide and shake the tube for 30 min at 50°. Add 4 ml of water and shake the mixture for 1 min. Centrifuge, separate the hexane phase and inject it into the gas chromatograph.

Compare the two chromatograms with chromatograms of standard solutions. Quantitate the PCBs by using the percentage composition of the individual components in the PCB oils (see refs. 2–4 and Fig. 5).

RESULTS

The reproducibility of the determination is shown in Table I. The method has a detection limit for the total amounts of PCB in the dried sample of at least 0.1 mg/kg, and for DDT, DDD and DDE limits of 0.01, 0.005 and 0.005 mg/kg, respectively. The method has also been used on sediment, soil, plant material, animal tissue and, after modification of the extraction procedure, also on water.

DISCUSSION

Extraction of sample

Because of the lipophilic character of the chlorinated hydrocarbons it may be of interest to correlate their concentrations with the lipid content. Jensen *et al.*[6] used a solvent mixture that gives the same lipid yield as the Torry method[7] in the extraction of fish samples.

The Torry method is a modification of the chloroform–methanol–water extraction procedure of Bligh and Dyer[8] and was tried mainly for determination of fat in fish meal. One would expect a great deal of "bound" lipids in sewage sludge, for example calcium and magnesium salts of fatty acids, which are resistant to extraction with pure organic solvent without pre-treatment with an acidic solution. We therefore use a separate extraction procedure for determination of the fat content, *viz.*, the method officially used by the European Economic Community (EEC) for the determination of fat in feedstuffs[9]. In this method the sample is boiled with 100 ml of 3 *M* hydrochloric acid before extraction with diethyl ether. This method has been tested in our laboratory on different sample materials, such as plants, animal tissues and faeces. It has been shown that the extraction yields of lipids are higher than or equal to the yields obtained with pure solvents. If the samples contain lipids, it is possible to use this method for extraction of chlorinated hydrocarbons but if the samples do not contain lipids most of the hydrocarbons are lost in the boiling.

If the lipid content is of no interest, a simpler extraction procedure can be used. The chlorinated hydrocarbons are probably dissolved in the different lipid fractions and not bound firmly to the sample material; they are therefore more easily extracted than lipids. It is, however, important to use a solvent or solvent mixture that can penetrate cell walls. The yields of lipids and PCB with three different extraction procedures are shown in Table II.

TABLE I

RESULTS FROM FIVE PARALLEL DETERMINATIONS OF CHLORINATED HYDROCARBON POLLUTANTS IN SEWAGE SLUDGE SAMPLE TAKEN FROM A SWEDISH WASTE-WATER TREATMENT PLANT

Compound number gives identity of PCB component*. Clophen A 50 was used as reference substance. Recorded PCB components are as much as 52.5% of the total concentrations of the different chlorobiphenyls in Clophen A 50.

Compound name or No.	*Results on dried sample (mg/kg)*	
	H_2SO_4/KCN aliquot	*KOH aliquot*
6	0.044 ± 0.004	*
12	0.105 ± 0.004	0.119 ± 0.007
14	0.013 ± 0.000	0.022 ± 0.002
15	0.051 ± 0.002	*
17	0.099 ± 0.006	0.099 ± 0.005
20	0.039 ± 0.004	0.038 ± 0.002
18	0.025 ± 0.003	0.024 ± 0.003
19 + 25	0.112 ± 0.008	0.122 ± 0.005
27 + 28	0.063 ± 0.004	0.068 ± 0.004
29	0.121 ± 0.010	0.121 ± 0.008
32	0.117 ± 0.009	0.133 ± 0.007
33	0.001 ± 0.000	0.001 ± 0.000
36	0.010 ± 0.001	0.011 ± 0.001
38	0.012 ± 0.001	0.014 ± 0.003
40	0.010 ± 0.001	0.010 ± 0.002
47	0.017 ± 0.001	0.019 ± 0.001
49	0.039 ± 0.001	0.048 ± 0.002
50	0.020 ± 0.001	0.034 ± 0.003
Total	0.90	0.97**
p,p-DDE	0.23 ± 0.02	0.24 ± 0.01
o,p-DDD	<0.005	***
p,p-DDD	0.0098 ± 0.0005	***
o,p-DDT	<0.01	***
p,p-DDT	<0.01	***

* Peaks are overlapped by impurities.
** Values for peaks 6 and 15 are taken from the H_2SO_4/KCN aliquot.
*** The compounds are degraded.

TABLE II

DETERMINATION OF YIELDS OF CRUDE FAT AND PCB WITH DIFFERENT EXTRACTION PROCEDURES IN SEWAGE SLUDGE SAMPLES FROM FOUR SWEDISH WASTE-WATER TREATMENT PLANTS

Sample No.	*Crude fat (%)*			*PCB (mg/kg)*		
	EEC	*BB*	*EE*	*EEC*	*BB*	*EE*
628	9.04	3.27	2.72	1.5	1.6	—
905	3.60	1.62	1.81	2.2	2.3	2.0
917	5.65	2.71	3.04	1.4	1.4	1.2
932	13.20	5.70	7.47	—	—	—

The classical Soxhlet extraction, or ether extraction (EE), is a rather inefficient method for extraction of lipids. Occluded lipids are not released and neither are of course the chlorinated hydrocarbons in the occlusions. Table III shows that the lipids are largely occluded or firmly bound to the sample material and that those in sludge consist mostly of free fatty acid or probably salts of fatty acids. The described extraction method, proposed by Berggren[10] (BB in Table II), gives a lower yield of lipids compared with the EEC method, but almost the same or a higher yield of PCB.

TABLE III

THE AMOUNTS OF CRUDE FAT, FREE FATTY ACIDS (FFA) AND PCB EXTRACTED FROM SEWAGE SLUDGE SAMPLES BY ETHER EXTRACTION (EE) AND BY THE EEC PROCEDURE ON THE RESIDUE OF THE SAMPLE MATERIAL FROM EE (EEC-R)

Sample No.	*Crude fat (%)*		*FFA (%)*		*PCB (mg/kg)*	
	EE	*EEC-R*	*EE*	*EEC-R*	*EE*	*EEC-R*
512	2.69	1.93	0.91	1.57	5.3	0.9
567	9.45	1.50	4.50	1.30	12.3	1.0

Clean-up

Lipids and some other impurities in the crude extracts can be destroyed by treatment with fuming sulphuric acid, either by shaking the acid[6] or by eluting on a fuming sulphuric acid–Celite column[11,12]. Dieldrin is decomposed by this treatment but DDT and its metabolites DDD and DDE are not (Table IV). Extracts of sewage sludges often contain large amounts of elemental sulphur, particularly after treatment with sulphuric acid. These interfere with early eluting compounds in the gas chromatographic step (Fig. 3A).

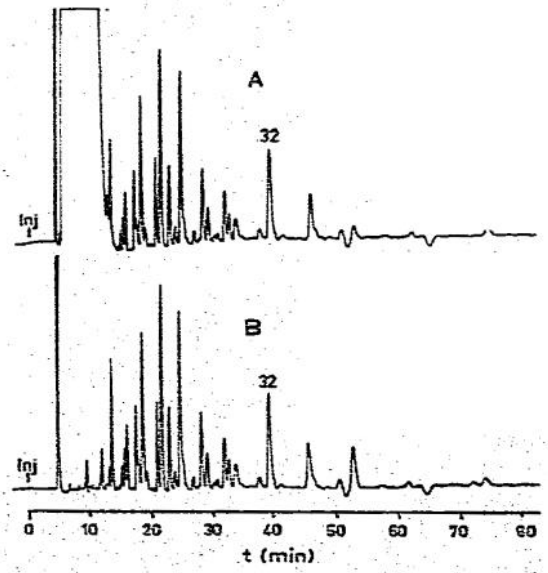

Fig. 3. Interference by elemental sulphur. Extract of sewage sludge sample treated with 7% fuming sulphuric acid (A) and also treated with cyanide (B).

Shaking the extract with a drop of mercury removes much of the interference[13,14]. The method is rather simple, but sometimes even repeated treatment with drops of mercury does not remove all of the sulphur. Schultmann *et al.*[15] have described a method in which sulphur is removed by refluxing for 3–4 h with a copper–aluminium alloy. In a method for the determination of elemental sulphur in hydrocarbons, Bartlett and Skoog[16] described the reaction between sulphur and cyanide in acetone solution to give thiocyanate. The reaction is rapid and quantitative and the procedure could be used on our extracts after a minor modification (Fig. 3B). BHCs are decomposed to some degree, probably to pentachlorocyclohexane[17].

An alternative procedure for the removal of sulphur has also been used in our laboratory. The procedure is as follows: take 1–2 ml of the hexane phase after sulphuric acid treatment and add 1.5 ml of 0.1 *M* aqueous barium hydroxide. Add dropwise 3 ml of acetone during 1 min. If the sample solution contains sulphur, the solution turns yellow-brown. Add a further 3-ml portion of acetone and shake the mixture. The colour changes from yellow-brown to light green. Continue to shake it until the green colour has disappeared or for 2 min. Add 10 ml of water and separate the hexane phase. If the green colour has not disappeared after 2 min of shaking with acetone, repeat the treatment. Otherwise the sample is ready for injection into the gas chromatograph. Barium hydroxide is used instead of alkali hydroxide because it does not dehydrochlorinate BHCs as easily as alkali hydroxides do.

Ethanolic potassium hydroxide[6] dehydrochlorinates DDD and DDT to DDMU and DDE, respectively, 3,4,2′,3′,6′-Pentachlorobiphenyl that is not separated from *o,p*-DDD can thus be quantitatively determined after the hydroxide treatment. Lipids and sulphur are also degraded to compounds that do not interfere with PCB in the gas chromatographic step. Lindane and its isomers are dehydrochlorinated to trichlorobenzenes[18] and are eluted together with the solvent. Cochrane and Maybury[19] have used the reaction with sodium hydroxide in methanol for the identification of BHCs. Dieldrin is not decomposed in the described potassium hydroxide treatment and can thus be detected in the chromatogram of that aliquot. Some common chlorinated hydrocarbon pollutants and the internal standard hexabromobenzene have been treated, according to the General procedure, with sulphuric acid, potassium cyanide and potassium hydroxide. The results of the recovery experiments are shown in Table IV.

When using packed columns, a pre-column of sodium and potassium hydroxides will give the same effect as the potassium hydroxide treatment described under General procedure[14,20]. We have also tested a column with a packed alkaline post-column to remove the sulphur peak from the chromatogram. In the post-column DDT and DDD are dehydrochlorinated but this does not affect their retention times.

Gas chromatography

On ordinary packed columns with, for example, silicone oil as a stationary phase and with a length of 1.6 m, 14 peaks usually appear in the chromatogram when Clophen A 50 (ref. 4) is chromatographed and detected by an electron capture detector. Jensen and Sundström[4] and Sissons and Welti[5] have used Apiezon L columns and have managed to improve the separation of the PCB compounds considerably. A 5.2-m packed column with specially purified Apiezon L has 15,000 theoretical plates[4]. Because of the poor separation with ordinary packed columns we realized that the gas chromatographic technique must be improved with capillary

TABLE IV

EFFECT OF TREATMENT OF A SOLUTION OF CHLORINATED HYDROCARBONS AND THE INTERNAL STANDARD HEXABROMOBENZENE WITH FUMING SULPHURIC ACID (I), FUMING SULPHURIC ACID PLUS POTASSIUM CYANIDE (II) AND POTASSIUM HYDROXIDE (III) EXPRESSED AS PERCENTAGES OF THE COMPOUNDS IN AN UNTREATED SOLUTION

Compound name (No.)	*Concentration (ng/ml)*	*Method of treatment*		
		I	*II*	*III*
α-BHC (1)	0.022	94	59	0
β-BHC (2)	0.072	94	70	6
Lindane (3)	0.026	92	55	11
Heptachlor (4)	0.026	94	90	104
Aldrin (5)	0.020	92	87	104
Heptachlorepoxide (6)	0.033	83	79	100
p,p-DDMU (7)	0.090	100	91	190
Dieldrin (8)	0.048	0	0	103
p,p-DDE (9)	0.022	104	100	437
o,p-DDD (10)	0.085	100	95	0
o,p-DDT (11)	0.096	98	95	0
p,p-DDT (12)	0.103	100	95	0
Hexabromobenzene (13)	0.57	(100)	(100)	(100)

columns. Occasionally, we had also noticed degradations of *p,p*-DDT in packed columns. The concentration levels of chlorinated hydrocarbons in sewage sludge allowed the use of the splitter injection technique. The splitter was made similar to that of Schulte and Acker[2]. We do not straighten out the ends of the column because we found that it was awkward to place such a column in position in the gas chromatograph.

We also found it to be very easy to remove condensed high-boiling substances in the first part of the column simply by cutting off a short piece. The main part of the high-boiling impurities of injected sample extracts condenses in the glass tube of the splitter. These impurities, together with the impurities in the first part of the column, cause poor separations and lower sensitivity[21]. The effect of changing the glass tubes of the splitter is shown in Fig. 4. Grob and Jaeggi[21] have given a detailed description and discussed the techniques of sample injection into a capillary column. The columns we have manufactured have a HETP between 0.5 and 1 mm measured on *p,p*-DDT, with an average carrier gas flow-rate[22] of just below 1 ml/min and a column temperature of 185°. Nearly 70 peaks were detectable when a PCB oil (Clophen A 50) was chromatographed.

When making our first columns we washed the walls of the glass capillaries with 1.25 *M* aqueous sodium hydroxide to remove metal impurities from the glass surfaces and to prepare suitable surfaces for further treatment with silylating reagents. With these columns we obtained leading peaks of DDT and DDD. We think that these compounds are partially dehydrochlorinated during the chromatographic run because of residues of sodium hydroxide on the column walls in spite of careful rinsing with water. Some columns were treated with 0.02 *M* hydrofluoric acid to counter the alkaline nature of the glass walls. We later found that the alkali and acid treatments could be replaced simply by washing with 0.2 *M* hydrofluoric acid. The

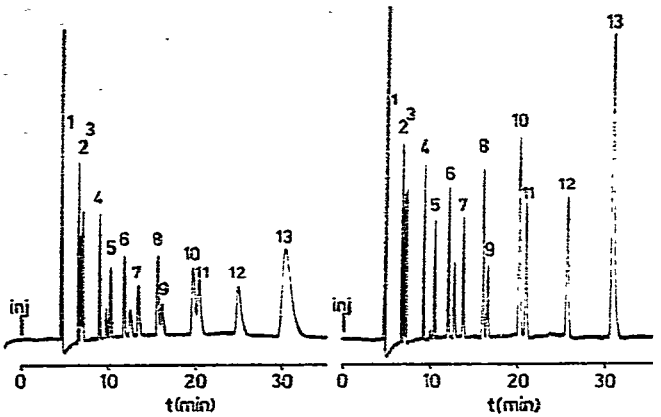

Fig. 4. Illustration of the effect of changing injector glass tube. Left: standard solution run after about one hundred injections of sewage sludge extracts. Right: the same solution run after changing the injector glass tubes. For identities of the peaks in the standard solution see Table IV.

glass walls were then silylated with TMCS and we assume that it can polymerize on the glass walls as in the reaction with Gas-Chrom Q described by Al-Taiar *et al.*[23].

At the detector end, the column is joined together with a straight capillary tube with a modified Swagelok union (SS-100-6) and silicone rubber septa. Inside the union a small glass-mantle covers the ends of the column and the capillary tube so as to prevent the sample from making contact with the metal surface. One of the union nuts is soldered to a stainless-steel tube, which serves as a support for the capillary tube and also as an entrance for the make-up gas to the detector. This gas must be regulated to give optimum sensitivity and resolution of the peaks.

Most packed columns give 14–35 PCB peaks and capillary columns give twice as many peaks in the same chromatographic time, about 45 min. However, there are still some PCB peaks that are not resolved from each other or from those of some other compounds[14,24]. As we largely use silicone oil SF 96 as a stationary phase, the works of Schulte and Acker[2] and Jensen and Sundström[4] are of great value for the identification of the individual components in the PCB oils Clophen A 50 and A 60. We did not carry out any mass-spectrometric examinations of our own in order to confirm the identities of the peaks in our chromatograms, which might lead to errors in the identification. Therefore the identities of the PCB peaks and the percentage composition used for quantitation of PCB are shown in Fig. 5.

Jensen *et al.*[1] have reviewed some different quantitation methods. We prefer that in which the peaks of the individual components of the samples are compared with the corresponding peaks in PCB oils of known composition.

In our laboratory we identify and determine as many biphenyls in the sample as the chromatographic separation allows. They are calculated from known data of concentrations and responses of the biphenyls in Clophen A 50 or A 60 (ref. 4). The unidentified components in the sample are approximated to have the same percentage composition as the corresponding components in the Clophen standards. The results of PCB analyses are usually given as the total amount of chlorinated biphenyls.

Occasionally, two compounds do not separate at a certain temperature, but

Fig. 5. Chromatogram of Clophen A 60 run on a 60-m SF 96 glass capillary column at 185°. The identities are taken from Schulte and Acker[3,5] and Jensen and Sundström[4] and the numbering and percentage composition from Jensen and Sundström[4].

separate above and below this temperature, with reversed peak order. We observed this effect with dieldrin and *p,p*-DDE; at 200° there was no separation. We also noticed that the order of elution of some PCB peaks is influenced by the temperature. This effect must be taken into consideration when the chromatographic conditions are chosen. The problems can be avoided by changing the experimental conditions, *viz.*, the temperature, the stationary phase and even the sample size[25]. The thermodynamic principles, which are the basis of the reversal of peak order at different column temperatures, have been discussed by Littlewood[26].

ACKNOWLEDGEMENT

The authors are indebted to G. Ekström and W. J. Kirsten for stimulating discussions and suggestions, and to S. Andersson, T. Bergh, R. Ekelund and U. Josefsson for skilful technical assistance.

REFERENCES

1 S. Jensen, L. Renberg and R. Vaz, in S. Lundström (Editor), *PCB Conference II*, Stockholm, National Swedish Environment Protection Board Publication 1973: 4E, p. 7.
2 E. Schulte and L. Acker, *Z. Anal. Chem.*, 268 (1974) 260.
3 E. Schulte and L. Acker, *Naturwissenschaften*, 61 (1974) 79.
4 S. Jensen and G. Sundström, *Ambio*, 3 (1974) 70.
5 D. Sissons and D. Welti, *J. Chromatogr.*, 60 (1971) 15.
6 S. Jensen, A. G. Johnels, M. Olsson and G. Otterlind, *Ambio Special Rep.*, 1 (1972) 71.
7 S. W. F. Hanson and J. Olley, *Biochem. J.*, 89 (1963) 101P.
8 E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.*, 37 (1959) 911.
9 *Amtsblatt Europäischen Gemeinschaften*, L 279 (1971) 17.
10 B. Berggren, personal communication.
11 *Methods of Analysis of the Association of Official Analytical Chemists*, Association of Official Analytical Chemists, Washington, D.C., 10th ed., 1965, p. 393.
12 K. Erne, *Acta Pharmacol. Toxicol.*, 14 (1958) 158.
13 D. F. Goerlitz and L. M. Law, *Bull. Environ. Contam. Toxicol.*, 6 (1971) 9.
14 P. E. Mattsson, Å. Norström and C. Rappe, *J. Chromatogr.*, 111 (1975) 209.
15 R. L. Schultmann, D. W. Woodham and C. W. Collier, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 1117.
16 J. K. Bartlett and D. A. Skoog, *Anal. Chem.*, 26 (1954) 1008.
17 G. Westöö, K. Norén and M. Andersson, *Vår föda*, 10 (1971) 341.
18 B. Zimmerli, H. Sulser and B. Marek, *Mitt. Geb. Lebensmittelunters. Hyg.*, 62 (1971) 60.
19 W. P. Cochrane and R. B. Maybury, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 1324.
20 G. A. Miller and C. E. Wells, *J. Ass. Offic. Anal. Chem.*, 52 (1969) 548.
21 K. Grob and H. J. Jaeggi, *Chromatographia*, 5 (1972) 382.
22 S. Nygren and P. E. Mattsson, *J. Chromatogr.*, 123 (1976) 101.
23 A. H. Al-Taiar, J. R. Lindsay Smith and D. J. Waddington, *Anal. Chem.*, 46 (1974) 1135.
24 D. C. Holmes and M. Wallen, *J. Chromatogr.*, 71 (1972) 562.
25 T. H. Schultz, T. R. Mon and R. R. Forrey, *J. Food Sci.*, 35 (1970) 165.
26 A. B. Littlewood, *Gas Chromatography*, Academic Press, New York, London, 1962, p. 72.