CHROM. 6155

THE ESTIMATION OF POLYCHLOROBIPHENYLS

G. B. COLLINS, D. C. HOLMES AND F. J. JACKSON
Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1 (Great Britain)

(First received March 20th, 1972; revised manuscript received May 15th, 1972)

SUMMARY

Methods are described whereby the content of polychlorobiphenyls of sample extracts, particularly those from wildlife specimens, can be determined by gas–liquid and thin-layer chromatography, either separately or in combination.

INTRODUCTION

Since 1968, eggs and tissues of British wild birds have been regularly examined in this laboratory for their content of polychlorobiphenyls (PCB)[1], and methods for the separation of these compounds from the organochlorine pesticide residues and for their subsequent estimation have been evolved. An analytical scheme found suitable for routine purposes is reported here; although primarily designed for wildlife monitoring, it has been successfully applied to a variety of sample types. The procedure comprises three stages: partial separation of the PCB from the organochlorine pesticide residues by means of a silica gel adsorption column; oxidation of the residual *p,p'*-DDE in the PCB fraction; and determination of the amount of PCB by gas–liquid chromatography (GLC), thin-layer chromatography (TLC), or both. As a preliminary step, the sample extract should have been prepared and cleaned-up by any of the usual techniques designed for organochlorine pesticide residue determination, and should be in dry hexane or light petroleum.

PROCEDURE

Separation of PCB from the more polar pesticide residues

Apparatus. Glass chromatographic column, 300 mm long, 8 mm I.D., with a B19 ground-glass socket at the upper end and a stopcock at the lower end.

Glass funnel or reservoir to hold *ca.* 50 ml of solvent and provided with a B19 ground-glass cone at its base to fit into the upper end of the chromatographic column.

An evaporator of the Kuderna–Danish type, with interchangeable 10-ml graduated collection tubes.

Reagents. Silica gel, for chromatographic adsorption analysis, 60–100 mesh (BDH Ltd.). Heat the gel at 500° for 4 h and, when it has cooled to 150°, place it in a desiccator. When it has cooled finally to room temperature, weigh the required amount into a glass-stoppered flask and quickly add 2.5 ml of distilled water to each 100 g. Immediately stopper the flask and shake it for 1½ h on a Microid shaker. The gel is then ready for use. (When repetitive PCB surveys are being carried out, it has been found convenient to keep the muffled silica gel in an air oven at 105° until it is needed. A convenient amount can then be cooled in a desiccator and prepared as above.) Check each batch of silica gel by the method given below to determine whether or not the correct degree of activity has been obtained. The column should be able to separate both *p,p'*-DDT and *p,p'*-TDE from the PCB isomers. If necessary, adjust the amount of *n*-hexane, used as the eluting solvent, until this partition can be effected. Substitute this determined volume of *n*-hexane for that given in the method.

n-Hexane, redistilled from potassium hydroxide pellets. When concentrated 100-fold, a 5-μl portion should give no significant GLC peaks.

Diethyl ether, "Nanograde" reagent purity.

Cotton-wool, extracted with hexane and diethyl ether, dried, and stored so as to prevent its contamination.

Method. Weigh out 5.0 g of the prepared gel and rapidly transfer it, by using small amounts of hexane, to the chromatographic column, in which a plug of cotton-wool has been placed just above the stopcock. The stopcock may be moistened with solvent but must not be lubricated with grease. Allow the gel to settle in the column and remove any trapped air bubbles by stirring with a glass rod. Drain the surplus hexane from the column until its meniscus just touches the surface of the gel. Introduce the cleaned-up sample extract as a solution in 1 ml of hexane, and allow the hexane to drain until the meniscus again just touches the surface of the gel. Wash the vessel that contained the extract with two 1-ml portions of hexane, adding each washing separately to the column and allowing it to run just into the gel. Place a receiver, preferably a Kuderna-Danish evaporator, beneath the column and pass 42 ml of hexane through the column at a rate not exceeding 0.7 ml/min. Collect the eluate, stopping the elution when the meniscus reaches the top of the gel, and label this fraction 1. It should contain all the PCB. Hexachlorobenzene, aldrin, *o,p'*-DDT and *p,p'*-DDE, if present, are also eluted in this fraction. Change the receiver and pass 50 ml of a 10% solution of diethyl ether in hexane through the column. Collect the eluate and label it fraction 2. This contains the remainder of the organochlorine pesticide residues, including usually a small amount of *p,p'*-DDE, and can be used for the determination of these. Concentrate fraction 1 to *ca.* 5 ml in the Kuderna–Danish evaporator and examine it by GLC with electron capture detection, on at least two stationary phases of different polarities. (For details of suitable columns, see the later section of this paper dealing with the GLC determination of PCB.) If further concentration is necessary, fit a two-bubble, micro-Snyder column[2] to the 10-ml collection tube and remove the solvent by heating the tube cautiously in a steam-bath. If a Snyder column is not available, reduce the volume of the solution with a gentle stream of dry air or nitrogen at room temperature. Compare the chromatograms from fraction 1 with those given by solutions of commercial PCB preparations when injected on the same columns. Correspondence between peak retention times is an indication of the presence of PCB in the sample. Aroclor 1254 can conveniently be used as reference

material, but if this particular product is not available, any polychlorobiphenyl preparation with a chlorine content of 50% or more can be used instead.

Oxidation of p,p'-DDE in the PCB fraction

Apparatus. Snyder columns, two-bubble micro-columns[2], with ground-glass cones to fit the Kuderna–Danish 10-ml collection tubes.

Reagents. Acetic acid, glacial, redistilled. *n*-Hexane, redistilled from potassium hydroxide. Sodium hydroxide, a 5 *N* solution in water. Chromium trioxide, recrystallised.

Method. Adjust the volume of fraction 1 to 2 ml, concentrating, if necessary, as described above. Add 2 ml of acetic acid and, after replacing the micro-Snyder column, heat the tube cautiously in the steam-bath until all of the hexane has evaporated, as judged by the reduction in volume. Introduce 100 mg of chromium trioxide and place the tube in boiling water for 15–20 min. Cool the mixture and shake it vigorously with 2.0 ml of hexane (accurately measured) in the stoppered tube. Neutralize the acid with *ca.* 6–7 ml of 5 *N* sodium hydroxide solution. Shake the tube again and then set it aside until the two layers separate. Keep the tube well stoppered to prevent loss of hexane.

Determination of PCB by GLC

GLC conditions. These conditions are as used in organochlorine pesticide residue analysis, including one column with Apiezon L grease as the stationary phase.

In this laboratory, all columns are constructed of glass tubing, 1.8 m long and 3 mm I.D.; column packings are based on those of SIMMONS AND TATTON[3]. Operating conditions are as follows. Column 1: 1.3% Apiezon L grease + 0.15% Epikote Resin 1001 on Chromosorb G (acid-washed, DMCS treated, 60–80 mesh); column, detector and injection port temperatures 200°; carrier gas, nitrogen (oxygen-free) at a flow-rate of *ca.* 50 ml/min. Column 2: 1.3% Silicone gum GE–SE-52 + 0.15% Epikote Resin 1001, on the same support material; operating temperatures and nitrogen flow-rate as for Column 1. Column 3: 1.3% Silicone GE–XE-60 + 0.13% Epikote Resin 1001, on the same support material; operating temperature 195°; nitrogen flow-rate is adjusted to give the resolution characteristics described by SIMMONS AND TATTON[3], and is *ca.* 50 ml/min.

Electron capture detectors are used with all columns. The sensitivity of the detector and amplifier systems is such that 1 ng of *p,p'*-DDE gives a peak of half the recorder chart width after a retention time of 10 min.

Method. Inject 5 µl of the upper hexane layer of the oxidised mixture on to at least two GLC columns with different liquid phases, one of which should be Apiezon L. Compare the retention times of the peaks so obtained with those given by the PCB reference material. Agreement between the retention times of the peaks so obtained now indicates the presence of PCB compounds in the sample with a greater degree of certainty. Any *p,p'*-DDE, which was present in fraction 1, will have been converted into 4,4'-dichlorobenzophenone. Under the GLC conditions quoted, this compound has a retention time similar to that of heptachlor epoxide and so gives a peak on the chromatogram before most of the PCB isomers encountered in practice. A comparison of the traces before and after oxidation of fraction 1 will show how much *p,p'*-DDE was originally present.

Adjust the volume of the hexane solution so that the individual heights of the PCB peaks are within the linear response range of the electron capture detector. Under the prescribed GLC conditions for the Apiezon L column, the last of the PCB isomers normally found in wildlife tissues will emerge in about 120 min after injection.

Should the pattern of peaks given by fraction 1 after oxidation reproduce closely that given by a commercial PCB preparation, calculate the amount of PCB present in the former by direct comparison of peak heights. With many types of sample, this is unlikely to occur and, in these instances, calculate the amount of PCB by comparison of the sum of the areas of all the peaks attributable to PCB compounds with the total area under the trace given by a known amount of a commercial PCB preparation having a similar spread of peaks. A suitable standard of PCB for this calculation can be obtained by injecting a total of 20–25 ng of Aroclor 1254 on to the column. A much more convenient approach for the routine examination of wild bird and mammal samples is as follows.

Determine the peak heights and retention times of all the peaks attributable to PCB on the chromatogram from the Apiezon L column. Multiply each individual peak height by the corresponding retention time and sum all the products so obtained. Divide this sum by the product of the peak height and retention time for 1 ng of *p,p'*-DDE when injected on the same column. The result is a direct estimate of the weight, in nanograms, of the PCB injected on to the column.

Determination of PCB by TLC

Apparatus. Thin-layer plates, 20 × 10 cm. Chromatographic tank for thin-layer chromatography. Source of UV irradiation—30-W Philips UV tubes.

Reagents. Alumina G (for TLC). Silver nitrate, 0.4% solution in water. *n*-Hexane, redistilled from potassium hydroxide pellets.

Method. Slurry 25 g of Alumina G with 25 ml of silver nitrate solution and shake the slurry for 2 min before applying it to the plates as a 250-μm thick layer. There is sufficient material to cover five plates. Dry the plates at room temperature in a drawer or cupboard so that no light can reach them. Before use, place each plate in the chromatographic tank, which contains hexane, and allow the solvent to pass completely through the adsorbent material. Remove the plate from the tank and allow the solvent to evaporate.

Prepare a set of standard solutions of Aroclor 1254 at concentrations of 5, 10, 20 and 40 μg/ml.

On the assumption that the weight of sample represented in the oxidised solution is 1.0 g, adjust the volume of the upper hexane layer according to the amount of PCB determined by GLC. If the amount was between 1 and 4 p.p.m., make the volume up to 0.5 ml; for amounts between 5 and 9 p.p.m., 1.0 ml; and for 10–20 p.p.m., 2.0 ml. When larger amounts of PCB have been detected or larger weights of sample are represented, either make proportionately greater dilutions or transfer a smaller aliquot of the hexane layer to the plate.

Transfer duplicate 20-μl volumes, each as ten 2-μl applications, of the hexane solution to the centre of the baseline of a prepared plate, allowing sufficient time for the solvent to evaporate completely between each application. (The baseline should be parallel to one of the longer sides of the plate and 1.5 cm from it.) Next, transfer

10-μl volumes, as five 2-μl applications, of each standard PCB solution—a full set on each side of the sample spots. Keep all the spots 1.5 cm apart. Mark the plate on both sides at a distance of 5 cm from the baseline. Place the plate in the chromatographic tank and develop a chromatogram with hexane until the solvent front reaches the 5 cm mark. Under these conditions, the PCB isomers are not resolved from each other and keep close to the solvent front, while 4,4'-dichlorobenzophenone stays close to the origin. Remove the plate from the tank and allow the solvent to evaporate before irradiating the plate with UV light for 15 min, or until the spots have developed sufficient densities. Determine the densities of the sample and standard spots with a reflectance densitometer; if this instrument is not available, make a visual comparison, which should enable the sample spots to be evaluated to within about $\pm 30\%$. Draw a graph of the PCB concentrations against deflection readings and calculate the concentration of PCB in the sample extract from this graph. The reproducibility with this technique is 10%.

TABLE I

PCB CONTENT OF WILDLIFE SAMPLES AS DETERMINED BY GLC AND TLC

Sample	*PCB (p.p.m.)*	
	GLC	*TLC*
Guillemot egg	10	10
	5	7
Peregrine falcon egg	5	5
	2	4
	3	4
	2	3
	10	9
Razorbill egg	3	3
	17	15
Great crested grebe liver	2	2
	45	45
	5	8
	50	40
Heron liver	650	750
	10	13
Kestrel liver	2	3
	7	10
Kingfisher liver	18	20
Sparrowhawk liver	4	3
	20	21
	17	21
	5	6
	2	2

RESULTS

Table I shows the results of PCB determinations made on eggs and livers of some wild birds by both GLC (using DDE as reference standard) and TLC methods. In keeping with the possible error of the determinations, the results at the higher levels have been given to the nearest 50 p.p.m. and the lower levels to the nearest whole part per million.

DISCUSSION

Several other procedures for the separation of PCB from organochlorine pesticide residues have been described. Of these, adsorption chromatography on columns of Florisil[4] or of "dry" silica gel[5] both yield PCB fractions that also contain *p,p'*-DDE. ARMOUR AND BURKE[6] have published details of a method that is said to be capable of effecting a complete separation of PCB from *p,p'*-DDE, but in our hands this has not been entirely successful. Recently, SNYDER AND REINERT[7] have also used a silica gel column to separate PCB from *p,p'*-DDE, but warn that poor results may be obtained if the amounts applied are too large. In the development of the procedure described here, the difficulty in achieving a complete separation between PCB and *p,p'*-DDE, particularly when they are present in amounts such as may be encountered in wildlife samples, has been accepted and preference given to the elimination by oxidation of the interference caused by *p,p'*-DDE.

The method for the oxidation of *p,p'*-DDE to 4,4'-dichlorobenzophenone is based on one used earlier by HOLMES (see the paper by MULHERN *et al.*[8]), but includes modifications introduced recently by SISSONS[9] to give virtually quantitative conversion. The PCB isomers are unaffected by the oxidation.

Because the patterns of PCB peaks given by sample extracts on examination by GLC rarely correspond with that of any commercial PCB preparation, and because most of the peaks on the chromatograms represent mixtures of PCB isomers, the problem of determining the amount of PCB present is very real. It has been found, however, that when various commercial PCB preparations are injected onto the Apiezon L column, the total peak area per nanogram corresponds closely with the area given by 1 ng of *p,p'*-DDE, particularly when the preparations contain larger proportions of the more highly chlorinated biphenyls. Thus, although the individual PCB isomers have different electron capturing powers[10,11], the mean electron capturing power of the more highly chlorinated biphenyls as grouped by the specified GLC conditions is similar to that of *p,p'*-DDE. The use of the product of peak height and retention time as a measure of the peak area saves much time and has been shown to yield essentially similar conclusions (see Table II). As sample extracts, particularly those from wildlife specimens, usually give peaks that correspond with the more highly chlorinated biphenyls, it is argued that the mean electron capturing power of these will also be similar to that of *p,p'*-DDE.

The TLC method is based on that of ABBOTT *et al.*[12], modified so as to eliminate any resolution of the PCB isomers. It has the advantage over the GLC method of indicating the halogenated nature of the isolated compounds. Under the conditions

TABLE II

COMPARISON OF WEIGHT OF AROCLOR INJECTED ON TO APIEZON L COLUMN WITH WEIGHT CALCULATED FROM PEAK HEIGHT × RETENTION TIME DATA, WITH *p,p'*-DDE AS REFERENCE STANDARD

Aroclor preparation	*Amount injected (ng)*	*Amount calculated (ng)*
1254	20	17
1260	20	19
1262	20	20

described, equal weights of commercial PCB preparations containing 48% or more of chlorine give single spots of approximately equal intensities. Close agreement has been found between results obtained by this method and by GLC (see Table I). A similar procedure has been proposed recently by MULHERN *et al.*[8], but, as judged from the standard concentrations advocated, their method is less sensitive than that described in this paper.

Behaviour on the adsorption column, resistance to oxidation, agreement of retention times on the various columns and behaviour on TLC, give mutually supporting evidence that the compounds being dealt with are polychlorobiphenyls. For more conclusive proof of identity, techniques such as NMR[13] or mass spectrometry[14] or carbon-skeleton chromatography[15] can be used, provided that sufficient material is available.

When used in conjunction with the sample extraction procedures that are normally used in this laboratory[16,17], the procedure described has given recoveries of PCB from spiked samples ranging from 85 to 100%.

ACKNOWLEDGEMENTS

We would like to thank all our colleagues in the Pesticide Residues Survey Section, particularly Mr. J. R. COWLES, for their help in developing and proving these methods. We are also indebted to the Nature Conservancy for the provision of suitable material for analysis; to Monsanto Chemicals Limited for providing samples from their range of Aroclor products; and to the Government Chemist for permission to publish this paper.

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