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ANALYTICAL RESPONSE OF POLYCHLORINATED BIPHENYL HOMOLOGUES AND ISOMERS IN THIN-LAYER AND GAS CHROMATOGRAPHY

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SUMMARY

Except for pure synthetic polychlorinated biphenyl (PCB), estimation methods of PCB by thin-layer and gas chromatography with electron capture detection give comparable results. Both give a good estimate of the true mass of a biologically modified residue, but where mostly hexachlorobiphenyls and above make up the residue, the estimate will be up to 50% too high. The Coulson detector does not, in our hands, yield comparable results with modified residues; the reason for this difference is not clear at present.

INTRODUCTION

To determine the mass of polychlorinated biphenyl (PCB) present in a given matrix, standard samples of each PCB homologue or isomer must be available to calibrate the response of the detection method employed. In spite of intensive efforts, relatively few of the 70–80 compounds present in commercial mixtures have yet been synthesized because the methods available to the synthesizing chemist do not easily yield the chlorine substitution patterns produced by the commercial process of catalytic chlorination over iron[1–3]. Therefore the two methods most widely used for quantitation of PCB have depended on empirical calibration. These methods are gas chromatography with electron capture detection (GC–ECD) and thin-layer chromatography (TLC) on either silver nitrate-impregnated silica gel or alumina plates[4–7], in each case using deposition of metallic silver under ultraviolet (UV) light for chromogenesis.

For GC–ECD the most common calibration procedure is to calculate the ratio of the total height or area of the most prominent chromatogram peaks of a known mass of a commercial PCB mixture (frequently Aroclor 1254) to the peak height or area of a known mass of a pure compound such as DDE. Using the response coefficient obtained, the total mass of PCB in an unknown sample can be estimated from the measurements of the chromatogram peaks which correspond in retention time with those in the standard chromatogram. Another method is to dispense with the use of a pure standard and simply measure the total area given by a known mass of a com-

mercial mixture and relate it to the total area given by the sample after employing a clean-up procedure which removes the common interfering chlorinated hydrocarbon pesticides. A slight variation on this procedure is used in our laboratory: individual peaks given by a known mass of a commercial mixture, using Apiezon L as the stationary phase, are compared with the corresponding peak in the sample, and a value with dimensions $\mu g \cdot g^{-1}$ (notional* $\mu g/g$) is calculated. The mean of the notional $\mu g/g$ values gives an estimate of the total mass of PCB in the sample[8]; and if the values for notional $\mu g/g$ are displayed on a bar graph, the degree of modification of the original mixture brought about by passage through a biological system, or by the clean-up process, is immediately obvious.

Collins *et al.*[9] relate the mass of PCB present to the sum of

$$\frac{\text{Peak height} \times \text{retention time}}{\text{Peak height of DDE} \times \text{retention time of DDE}}$$

for all peaks in the standard and sample. They also analyze using silver-impregnated silica gel layers with reflectance densitometric measurement of the silver spot. In this laboratory densitometry is employed to give quantitation of 0.1 $\mu g \pm 10\%$ Rel.S.D. with silica gel thin layers or 0.01 $\mu g \pm 10\%$ Rel.S.D. using alumina layers[7].

Other methods of quantitation commonly employed are: pyrolysis followed by coulometry or conductance after gas chromatography (GC–Coulson)[10] and conversion to perchlorobiphenyl with antimony pentachloride followed by GC–ECD[11]. Neither method is particularly useful in toxicological studies. The former should, if pyrolysis conditions are severe enough, give a response related to the chlorine content of the residue, not to the mass of PCB present. Since the amount of chlorine present is less important in determining toxicity than is the arrangement of the chlorine substituents (which has a profound effect on the metabolism of PCB isomers[5,8]), the result of GC–Coulson will not be directly related to the toxic potential of the residue. The perchlorination method, while it simplifies clean-up problems and thus probably improves analytical precision, only gives an estimate of the total mass of biphenyl skeletal material present including unchlorinated biphenyl. Hence its usefulness for evaluating the toxicological importance of a residue is small, and of course toxicology is fundamentally the reason for doing the PCB analysis.

The object of the work presented here was to determine which of the three methods available to us (TLC, GC–ECD, and GC–Coulson) gives results which are most closely related to the mass of PCB present. Unfortunately, pure synthetic samples of the major components of Aroclor 1254, the mixture we have studied, are not available, and so Aroclor 1254 itself was fractionated by preparative partition and adsorption chromatography.

EXPERIMENTAL

Materials

Aroclor 1254 was kindly provided by Monsanto (St. Louis, Mo., U.S.A.). Samples of nine pure PCB homologues and isomers were purchased from Analabs

* Notional values give a notion or general indication.

(North Haven, Conn., U.S.A.). All solvents were Nanograde quality. Mineral oil was U.S.P. quality. Thin-layer plates were manufactured by Schleicher and Schuell (Keene, N.H., U.S.A.).

Fractionation of Aroclor 1254

Mineral oil (8%), was loaded on to Celite (Gas-Chrom Q, 80–100 mesh; Applied Science Labs., State College, Pa., U.S.A.) using hexane. The dried packing was tamped into a glass column (2.5 × 100 cm), and a solution of acetonitrile–methanol–acetone–water (2:2:0.9:0.1), saturated with mineral oil, was pumped upward through the column at a rate of 2 ml/min for 48 h using an AutoAnalyzer proportioning pump with Solvaflex tubing (Technicon, Tarrytown, N.Y., U.S.A.). Fractions (10 ml) were collected, and the hydrocarbon layer was monitored by TLC after the addition of 2% sodium sulfate solution (25 ml) to each tube. Fractions which appeared to be similar were bulked, and four fractions (A, B, C and D) resulted (Fig. 1).

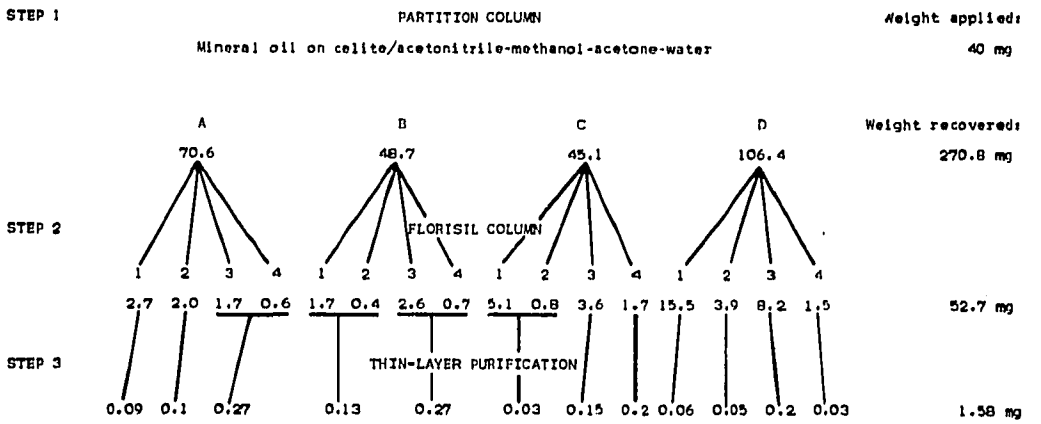

Fig. 1. Fractionation scheme for Aroclor 1254: partition fractionation followed by column adsorption and thin-layer purification.

Florisil columns were used to remove mineral oil from each fraction. The Florisil (Fisher Scientific, Fair Lawn, N.Y., U.S.A.) was activated at 450° for 4 h and packed when cool into 1 × 15-cm glass columns. For calibration, a solution of Aroclor 1254 (25 mg) plus mineral oil (25 mg) dissolved in hexane (10 ml) was applied to the top of a column previously wetted with hexane. Fractions (5 ml) were collected in weighed tubes during elution with hexane. The hexane was evaporated in a stream of filtered dry air, and the content of each tube was weighed using a Cahn electrobalance (Ventron, Paramount, Calif., U.S.A.) and assayed by TLC for PCB.

Fractions A, B, C, and D were purified by an identical Florisil column procedure. Several distinct fractions resulted as indicated by their TLC chromatograms (Fig. 1). The total mass recovered indicated that some mineral oil was still present, particularly in the D fractions, and so the last traces of oil were removed from all fractions by TLC on silica gel G 1500 layers, eluting with hexane, scraping zones

from the plates, and eluting with hexane into weighed tubes. Four fractions large enough to be weighed resulted (Fig. 1).

Because of the disappointing yield (1.58 mg from 40 mg) from this experiment, another fractionation was carried out solely by adsorption chromatography on silica gel thin-layer plates. Aroclor 1254 dissolved in hexane (1 g/l) was applied to ten G 1500 plates as streaks. After development in hexane for 20 cm, the six separated zones were located by chromogenesis of markers, scraped from the plate, and extracted from the adsorbent with hexane.

Weighing

The ten resultant fractions (4 partition, 6 adsorption) were weighed accurately with the Cahn balance by taring a small aluminum foil cup (5-mm diameter, 2.5 mm deep), adding the material in hexane, evaporating in a stream of filtered dry air, and re-weighing, taking care not to jar the balance pan downward. The precision achieved was $\pm$ 0.02 mg. The masses of the fractions obtained by this process and by preparative TLC are given in Table I.

TABLE I

MASS OF AROCLOR 1254 FRACTIONS

Fraction (partition)	*Mass (mg)*	*Fraction (adsorption, TLC)*	*Mass (mg)*
A	0.46	1	0.28
B	0.40	2	0.22
C	0.38	3	0.45
D	0.34	4	0.10
Total	1.58	5	0.23
		6	0.18
		Total	1.46

Gas chromatography–electron capture detection

A Hewlett-Packard 7600A system with electron capture detector was used. Glass columns (1.5 m $\times$ 0.2 mm I.D.) were packed with 2% Apiezon L on Gas-Chrom Q (80–100mesh). As carrier gas argon–methane (95:5) was employed. The column temperature was 205°. A gas chromatogram of Aroclor 1254 on Apiezon L is shown in Fig. 2.

The integrator read-out was processed in one of two ways: (1) the data were printed and converted into bar charts using a programmable calculator (Wang, Tewksbury, Mass.,) U.S.A. or (2) the data were recorded on paper tape using the Hewlett-Packard data acquisition package and the minicomputer incorporated into the 7600A system, the tape being used with a Fortran program to calculate the notional μg/g values and their mean.

Gas chromatography–Coulson detection

A Fisher-Victoreen Series 4400 gas chromatograph (Fisher Scientific, Pittsburgh, Pa., U.S.A.) was used with a Coulson detector (Tracor, Houston, Texas, U.S.A.) for the conductivity detection. The glass column (2 m $\times$ 1 mm I.D.) was

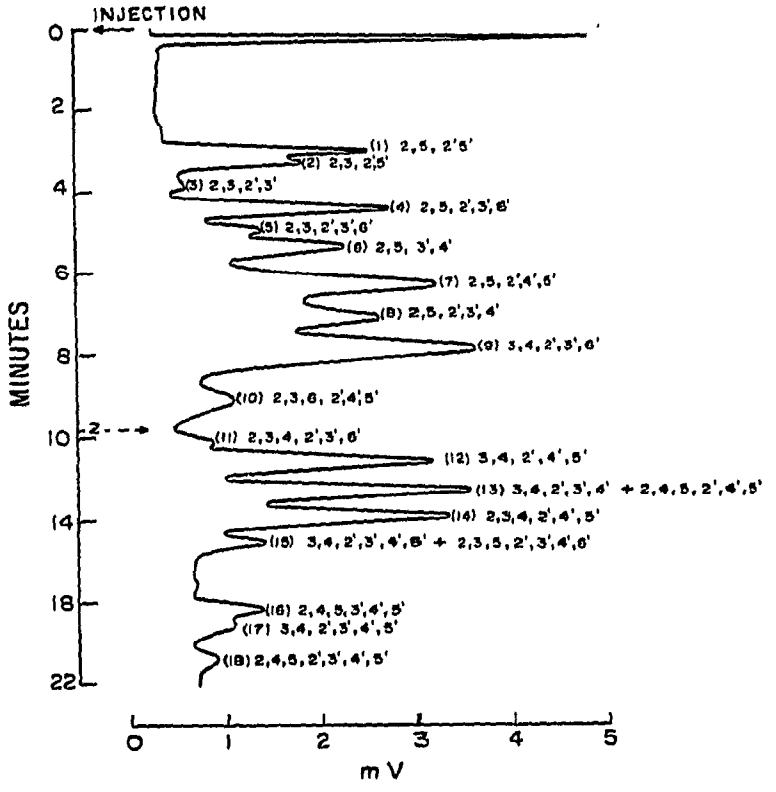

Fig. 2. Gas chromatogram of Aroclor 1254 on Apiezon L. At point Z the chart speed was changed from 0.5 to 0.25 in./min.

packed with 2% Apiezon L on Gas-Chrom Q (80–100 mesh). Detector conditions were as follows. Mode: reducing, no catalyst. Temperatures: column 200°, inlet 210°, transfer 220°, furnace 900°. Gases: carrier, nitrogen at 40 p.s.i., 60 ml/min; reactant, hydrogen at 20 p.s.i., 40 ml/min. Conductivity bridge: 30 V; attenuation, 2. Recorder: 1 mV at 30 in./h.

RESULTS

The relative responses of the three systems for the chromatographic and pure synthetic fractions are shown in Table II and summarized in Table III. The relative performance of the methods was also tested with real samples. When wildlife samples were analyzed, DDE was removed by oxidation with a mixture of chromium trioxide and acetic acid[7]. Chlordane and dieldrin are not removed by this clean-up procedure, and residues of the former are common in grain-eating birds. The nine peaks of commercial chlordane could, however, be separated from the eighteen peaks of Aroclor 1254 mixture by using a temperature program: 4 min at 160°, then 10°/min to 200° and hold for 30 min. Only a slight shift in baseline was observed, and the integrator is designed to compensate for such changes. Residues were confirmed by TLC. Fig. 4

TABLE II

RESPONSE RATIO SAMPLE: AROCLOR 1254

TCBP = tetrachlorobiphenyl; PCBP = pentachlorobiphenyl.

Sample fraction	*TLC*	*GC–ECD*	*GC–Coulson*
Partition			
A	0.28	0.44	0.37
B	0.61	0.82	0.60
C	1.07	0.63	0.44
D	1.62	1.48	0.45
Adsorption			
1	0.60	0.38	0.42
2	0.70	0.32	0.49
3	0.90	0.57	0.75
4	1.10	0.80	0.81
5	0.75	0.61	0.60
6	0.81	0.56	0.39
Pure synthetic PCB			
2,3-2′,3′-TCBP	0.64	0.60	1.01
3,2-2′,5′-TCBP	0.66	0.50	1.01
3,4-2′,4′-TCBP	1.10	0.50	1.09
2,3-5′,6′-TCBP	0.60	0.95	1.04
2,5-2′,5′-TCBP	0.58	0.40	0.86
3,4-3′,4′-TCBP	0.67	0.50	0.69
2,5-3′,4′-TCBP	0.84	0.80	1.38
2,3,4,5,6-PCBP	1.01	0.90	0.81
2,3,4-2′,5′-PCBP	0.80	0.95	1.52

TABLE III

MEAN RESPONSE RATIO SAMPLE: AROCLOR 1254

Fractions	*TLC*	*GC–ECD*	*GC–Coulson*
Partition fractions	0.90	0.84	0.47
Adsorption fractions	0.81	0.54	0.58
Synthetic PCB	0.65	0.80	1.00

shows bar charts of typical samples (wildlife and experimental toxicology) handled by this laboratory.

The ratio of response of each modified fraction to the response of a known mass of Aroclor 1254 was determined by TLC. The spot response to a known mass of each fraction was compared with the spot response to an identical mass of Aroclor 1254 on the same plate, taking the mean of the ratios of five pairs of spots to determine the ratio for each fraction.

For the Coulson detector, the total area under the response curve for each modified fraction was compared with the total area given by an identical mass of Aroclor 1254. The detector has too high a dead volume to allow for resolution of the individual peaks of the chromatogram.

DISCUSSION

It is clear from Table II that for pure synthetic PCB the Coulson detector gives the best estimate of the mass of PCB present, using Aroclor 1254 as the standard. With the chromatographic fractions, however, GC–Coulson yields variable results. TLC is least accurate with pure samples, perhaps because pure compounds give compact circular zones which would be expected to cause a TLC scanner response different from that caused by the diffuse elongated zone given by Aroclor 1254 and the chromatographic fractions.

ECD appears to give a good estimate of the mass present for three of the partition fractions but not for the adsorption fractions. This may be due to the different characteristics of the two fractionating systems. The partition system has been shown to separate according to degree of chlorination[5] (Fig. 2), whereas adsorption systems differentiate more according to steric arrangement around the biphenyl skeleton. Table II (synthetic PCB) illustrates how dependent is the electron affinity on substitution pattern.

Fraction D most resembles Aroclor 1254 after modification by living organisms (Figs. 3 and 4). It consists mostly of hexa- and heptachlorinated biphenyls, and the high response of both TLC and GC–ECD to this fraction (Table II) indicates that our analysis of such samples, while consistent by either method, will be approximately 50% too high.

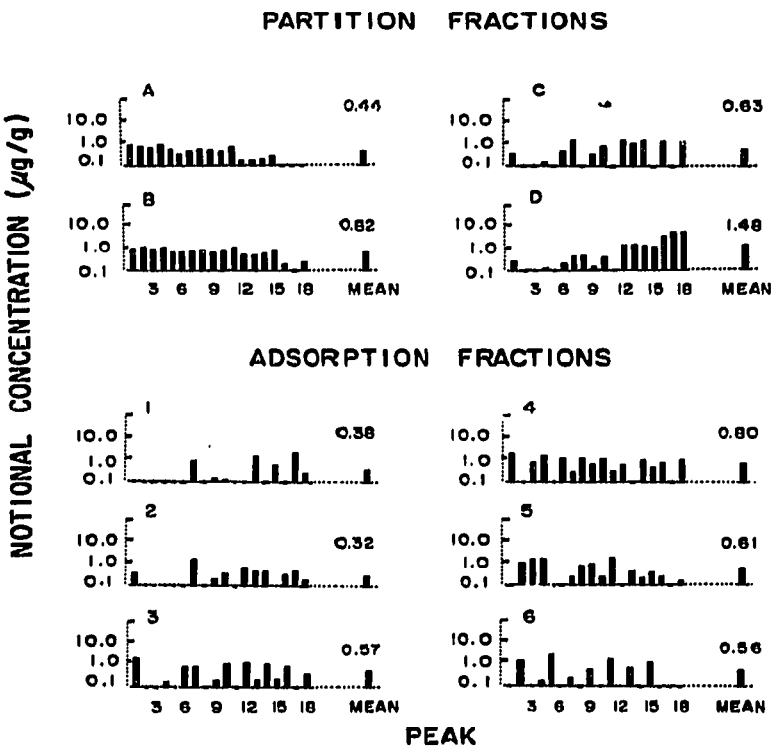

Fig. 3. Bar charts of the composition of fractions determined by GC–ECD.

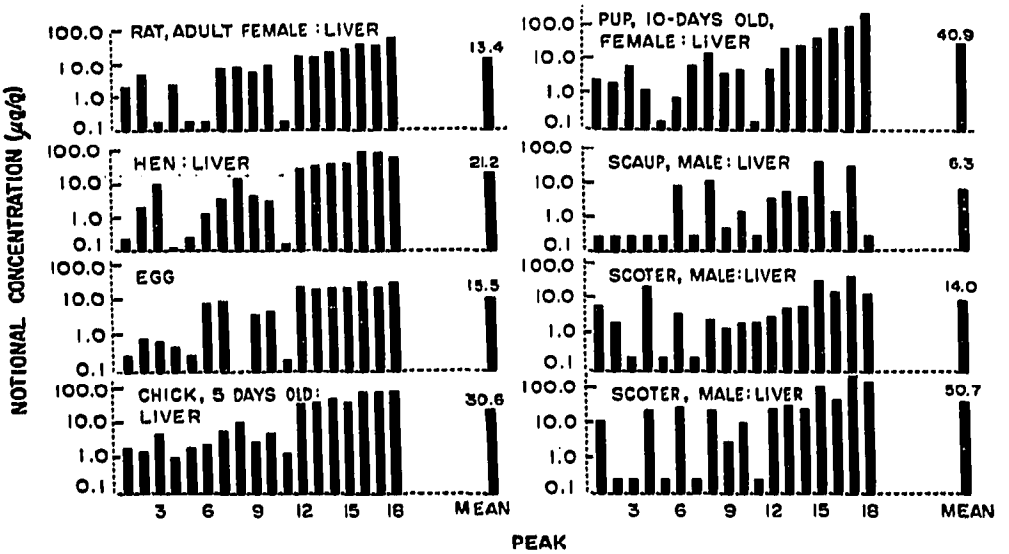

Fig. 4. Typical bar charts of biologically modified PCB determined by GC–ECD.

Correlation tests[12] using the Wang Statistical Package on the Wang Programmable Calculator (Table IV) show that the TLC and GC–ECD methods generally agree but the Coulson detector does not respond to PCB residues in the same way at all. Although the intercept figures (a_0) are small, except for the rat tissues the slopes of the regressions (a_1) are significantly different from unity, which would be obtained from two populations yielding exactly similar results. In all cases except for the rat tissues, TLC gives a lower estimate of PCB concentration than GC–ECD. If the synthetic samples are eliminated from the comparison, the value of a_1 rises to 0.88 (r = 0.80) for the ten chromatographic fractions.

TABLE IV

STATISTICAL COMPARISON OF TLC, GC–ECD AND GC–COULSON RESULTS

Symbols: ν, degrees of freedom: $N-2$, where N is the number of pairs of results: r, coefficient of correlation between the two populations; a_1, regression slope: first-named population *vs.* second; a_0, regression intercept on axis of first-named population; P, probability that the observed correlation does not occur by chance.

Population	*Assays compared*	*Statistical values*				
		ν	r	a_1	a_0	P
Fractions plus synthetic samples	TLC *vs.* GC–ECD	17	0.65	0.67	0.35	>0.99
	TLC *vs.* GC–Coulson	17	0.33	—*	—	<0.90
	GC–ECD *vs.* GC–Coulson	17	0.15	—	—	<0.90
Wildlife samples	TLC *vs.* GC–ECD	14	0.77	0.37	2.3	>0.99
Rat tissues from a PCB toxicology experiment	TLC *vs.* GC–ECD	13	0.94	1.0	0.5	>0.99
	TLC *vs.* GC–Coulson	13	0.24	—	—	<0.90
Hen eggs from a PCB toxicology experiment	TLC *vs.* GC–ECD	18	0.93	0.79	10	>0.99

* Blanks indicate no correlation.

REFERENCES

1 O. Hutzinger, S. Safe and V. Zitko, *Bull. Environ. Contam. Toxicol.*, 6 (1971) 209.
2 A. C. Tas and R. H. Devos, *Environ. Sci. Technol.*, 5 (1971) 1216.
3 A. C. Tas and R. J. D. Kleipool, *Bull. Environ. Contam. Toxicol.*, 8 (1972) 32.
4 L. Fishbein, *J. Chromatogr.*, 68 (1972) 345.
5 P. B. Hammond, *Environ. Res.*, 5 (1972) 249.
6 J. Reinke, J. F. Unthe and H. O'Brodovich, *Environ. Letters*, 4 (1973) 201.
7 B. Bush and F.-C. Lo, *J. Chromatogr.*, 77 (1973) 377.
8 B. Bush, F. Tumasonis and F. Baker, *Arch. Environ. Contam. Toxicol.*, 2 (1974) 195.
9 G. B. Collins, D. C. Holmes and F. J. Jackson, *J. Chromatogr.*, 71 (1972) 443.
10 R. W. Risebrough, P. Reiche and H. S. Olcott, *Bull. Environ. Contam. Toxicol.*, 4 (1969) 192.
11 O. W. Berg, P. L. Diosady and G. A. V. Rees, *Bull. Environ. Contam. Toxicol.*, 7 (1972) 338.
12 C. G. Paradine and B. H. Rivett, *Statistical Methods for the Technologist*, English Univ. Press, London, 1964, p. 196.