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THIN-LAYER CHROMATOGRAPHY FOR QUANTITATIVE POLYCHLORINATED BIPHENYL ANALYSIS

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SUMMARY

The use of silica gel plates and hexane for identification of polychlorinated biphenyls, naphthalenes and terphenyls is discussed. Quantitation of polychlorinated biphenyls is described using a linear, thin-layer chromatographic scanner and silver nitrate/UV chromogenesis. Sensitivity is 0.5 p.p.m. in muscle tissue, egg and fat with a precision of ± 0.05 p.p.m.

INTRODUCTION

In recent years, a new group of compounds, polychlorinated biphenyls (PCB), has aroused the attention of ecologists and pesticide analysts, primarily because these compounds occur along with DDT and dieldrin throughout the ecosystem and interfere with the analysis of chlorinated pesticide residues[1,2]. PCB and the polychlorinated terphenyls are marketed in the United States under the trade name Aroclor (Monsanto); and the polychlorinated naphthalenes, which may be expected to behave similarly to the PCB in the ecosystem, are marketed by the Koppers Corporation in the United States under the trade name Halowaxes. The Aroclors are numbered 1221, 1248, 1254, etc.; the first two digits indicate the number of carbon atoms, and the last two indicate the percentage of chlorine in the compound by weight.

Analysis of all these compounds is difficult because they contain as many as sixty individual constituents[3], thanks to their production by catalytic chlorination of the relevant aromatic hydrocarbon until the required chlorine content has been obtained. As a further complication, their gas chromatographic behavior is very similar to that of the chlorinated pesticides. Thus the gas chromatogram of an extract obtained from, say, a sea bird may contain 50 to 100 zones which are separated by gas chromatography and detected by the electron capture detector; but the zones may be due to PCB, or to chlorinated pesticides, or to metabolites of these. ZITKO[4], in a recent review, has discussed this problem in detail. The use of computer-linked mass spectrometry, combined with gas chromatography (GC), has to some extent

alleviated the problem[5]. There are, however, few laboratories with sufficient resources to allow for the purchase of this type of equipment.

Thin-layer chromatography (TLC), while it is not as sensitive as GC with electron capture detection, has the advantage that the PCB migrate as two or three fairly compact spots which are well separated from all the chlorinated pesticides except DDE and aldrin. Also, the method of chromogenesis is specific for molecules containing chlorine. So far, TLC has been used only as a supplementary tool in PCB analysis: as a separation technique[6,7] before GC or as a qualitative[8] or semiquantitative method[9]. DDE and aldrin migrate similarly to the PCB in adsorption chromatographic systems on alumina, Florisil, and silica gel[2]—a problem which has been tackled by many workers. HOLDEN[10], ARMOUR AND BURKE[11], SNYDER AND REINERT[12], and others have been able to separate DDE from some PCB on columns; but other workers have reported an inability to repeat these separations (BEVENUE AND OGATA[13]). In this work, therefore, DDE has been removed chemically by oxidation to dichlorodibenzophenone. The same process will remove the other much less important interferent, aldrin, by conversion to dieldrin.

Further complications which arise in PCB analysis are the possibility of differential metabolism and elimination of various chlorinated biphenyls and their isomers during passage through the ecosystem from one species to another. It has been reported that the less chlorinated biphenyls are more rapidly metabolized than the more chlorinated biphenyls[14]. More recently it has been suggested[15] that the almost universal finding of the more chlorinated biphenyls such as Aroclor 1254 and Aroclor 1260 is due simply to elimination of less chlorinated material from all the Aroclor plasticizers reaching the environment. In addition to this apparent change in composition, there is the problem of response. It has been shown that the electron capture response is different for various isomers of the same chlorinated biphenyl[16]. Although the same problem exists in TLC analysis, it will probably be less significant than in GC analysis, thanks to the grouping of all the possible polychlorinated molecules and their isomers.

In this communication, we describe a method for the quantitative determination of PCB by TLC in the presence of chlorinated hydrocarbon pesticides including DDE. This method is particularly suited to rapid quantitative screening of biological and industrial samples containing PCB.

EXPERIMENTAL

Reagents and apparatus

Developing solvent. Nanograde hexane from Mallinckrodt Chemical Works (St. Louis, Mo. 63160, U.S.A.) was used as the developing solvent.

Spray reagent. Dissolve silver nitrate (0.5 g) in deionized water (1 ml). Add glycerol (35 ml, A.R. grade, from J. T. Baker Chemical Co., Phillipsburg, N. J., U.S.A.) and absolute ethanol (45 ml). Mix well to provide a homogeneous solution. The spray reagent should be kept in a dark bottle to avoid darkening of the solution.

Aroclors. Aroclors were kindly provided by the Monsanto Chemical Company (800 N. Linderbergh Blvd., St. Louis, Mo., U.S.A.). Stock solutions of 100 p.p.m. in hexane were prepared, and solutions of various concentrations were diluted therefrom.

Pesticide standards. *o,p'*-DDT, *o,p'*-DDE, *p,p'*-DDE, *p,p'*-TDE, heptachlor epoxide, aldrin, and dieldrin were obtained from the United States Department of Health, Education and Welfare, Public Health Service, Food and Drug Administration, Pesticide Repository, Perrine, Fla., U.S.A.

Eluents. Hexane, acetone and petroleum ether are "Nanograde" from Mallinckrodt Chemical Works (St. Louis, Mo. 63160, U.S.A.).

Instrumentation

A Farrand Vis-UV Chromatogram Analyzer (Farrand Optical Co., Mount Vernon, N.Y. 10550, U.S.A.) was used for all the absorption measurements of the TLC plates.

A Chromato-VUE irradiation chamber manufactured by Ultra-Violet Products, Inc. (San Gabriel, Calif., U.S.A.), was used for irradiating the spots after spraying with silver nitrate solution. The short-wave UV lamp (G.15 T8, 15 W from General Electric Co.) was used without the filter.

Procedure for thin-layer chromatography

Precoated silica gel plates (250 μ Schleicher and Schuell Acid "Fast"), after heating at 110° (overnight) and cooling to room temperature, were marked at 4 and 14 cm from one end of the plate, using a template.

Nine spots (20 μl per spot) were applied along the 4-cm line. The spots were allowed to dry, and the plate was placed in the developing tank. The solvent was allowed to migrate to the 14-cm line (approximately 35 min).

The plate was then air-dried, sprayed with the silver nitrate reagent, and placed in the irradiation cabinet for about 2 min. The spray and irradiation were repeated three times. After the third spray, the plate was left in the chamber until the desired darkness was obtained. Prolonged irradiation under UV light darkened the adsorbent layer of the plate.

The spots were scanned across, moving the plate from left to right, with the Farrand Vis-UV Chromatogram Analyzer in the absorption mode at a rate of 2 in. per min. The monochromator was set at approximately 550 nm. By placing an appropriate aperture in the center exciter drawer, the sleeve of the light beam on the plate was adjusted so that it covered only the area corresponding to PCB spots. The PCB could be quantified either by using the area count integrator or by cutting out the peak area from the chart and weighing it.

Analysis was carried out by placing three standard spots—100, 300 and 500 p.p.m. PCB—in hexane 4.0, 9.4 and 14.8 cm from one edge of the plate, using a template. Three unknowns were spotted 2.2, 5.8 and 7.6 cm from the edge. Duplicate unknowns were spotted 11.2, 13.0 and 16.6 cm from the same edge.

Calibration graphs were plotted using the three standards for each set of three duplicate analyses on each plate, and the concentrations were read from the graphs.

Procedure for clean-up

Extraction and clean-up procedures for fat and eggs, using dimethyl formamide (DMF) hexane partitions and Florisil columns, were as reported by RICHARDSON *et al.*[17], with some slight modifications to improve recovery. A chromic acid oxidation step[9] was included at the end of clean-up to remove interference by DDE.

With tissue samples, the chromic acid oxidation step tended to clean up effectively, so that DMF treatment and Florisil column treatment were unnecessary. Details of the procedures are, briefly, as follows:

Tissue. Frozen tissue (5 g) was cut into fine pieces, mixed with anhydrous sodium sulfate, and extracted in a Soxhlet extractor with hexane–acetone (2:1). After 4 h the solvent was evaporated from the flask just to dryness, ready for oxidation.

The oxidizing reagent—chromic oxide (9 g), dissolved in deionized water (6 ml), mixed with acetic acid (335 ml)—was added to the flask. Of the order of 25 ml of the reagent per gram of tissue was required. After 45 min at 90–100°, with constant stirring, deionized water (500 ml) was added to halt further oxidation. The quantity of oxidant required depended upon the fat content of the tissue; 5 g of liver tissue, for example, required 150 ml of oxidant. The quantity required was determined by removing 1 ml of the oxidizing mixture after 45 min, adding water (10 ml) and hexane

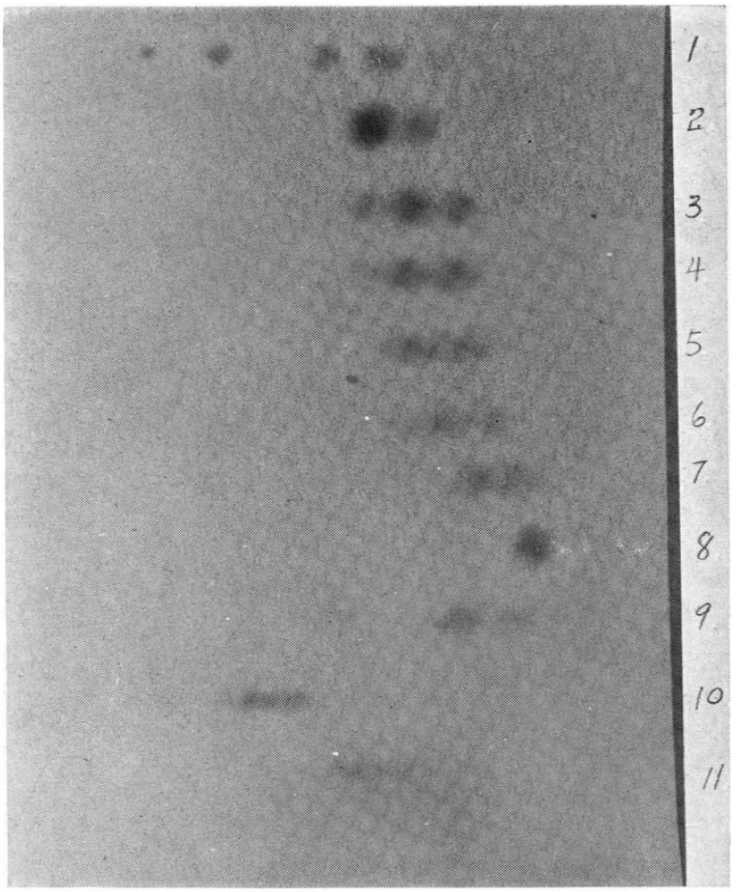


Fig. 1. Thin-layer chromatogram of Aroclor PCB, Halowaxes, and common chlorinated hydrocarbons (heptachlor epoxide, DDD, DDT, DDE, and aldrin, in ascending order of R_F value). 1 = Pesticide mixture (5 μl, 1000 p.p.m. in hexane); 2 = Aroclor 1221; 3 = Aroclor 1242; 4 = Aroclor 1248; 5 = Aroclor 1254; 6 = Aroclor 1260; 7 = Halowax 1014; 8 = Halowax 1051; 9 = Halowax 1099; 10 = Aroclor 5432; 11 = Aroclor 5460.

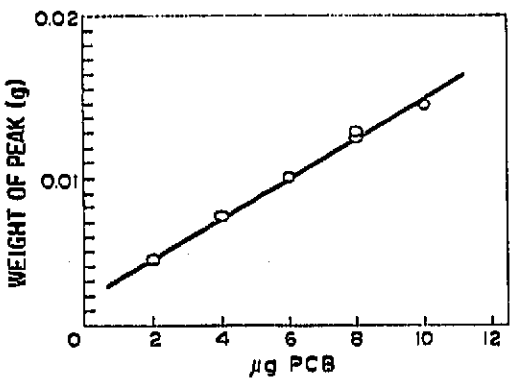
(1 ml), and shaking vigorously for about 1 min. A rapid phase separation indicated sufficient oxidation; emulsions indicated that more oxidant was required and heating should be continued for a further 20 min.

The oxidized product was mixed with water (1 l) and hexane (50 ml) and shaken for 2 min. The organic phase was separated, and partition was repeated three more times with 50-ml portions of hexane. The hexane solution, filtered through anhydrous sodium sulfate, was evaporated just to dryness with filtered air, and the residue was transferred to a 3-ml swab tube by rinsing with hexane. The solution in the tube was again evaporated almost to dryness. Then 0.1 ml of hexane was added, using a Hamilton syringe; the tube was rinsed carefully during the addition.

Eggs and fat. These materials were treated by the method of RICHARDSON *et al.*[17], with the oxidation step after clean-up. Oxidation before clean-up, as described for less fatty tissues, is not practicable with these materials due to the large quantity of oxidant required and the resultant large volumes of liquid.

RESULTS

Thin-layer chromatograms of the Aroclor PCB and the common chlorinated insecticides are shown in Fig. 1 along with other chlorinated hydrocarbon materials which are used similarly to PCB. (R_F values of PCB zones are shown in Table I.) Figs. 2 and 3 show typical calibration curves, the former using peak weight measure-



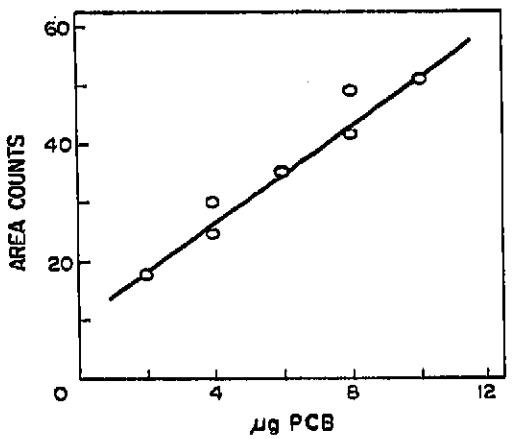


Fig. 2. Typical calibration curve by cutting and weighing.

Fig. 3. Typical calibration curve using integrator.

ment, the latter using the area counter of the scanning instrument. Table II shows the precision of the analysis at four different levels, using pure hexane solutions for calibration of each plate. Table III shows the same at two levels; but instead of using pure hexane standards, portions of a cleaned-up extract of PCB-free egg were spiked with PCB. In this way, if any material not shown up by the silver nitrate reagent were present at the same R_F value as the PCB zone, or if the presence of fatty material during the spotting operation had changed the light absorption characteristics

TABLE I

R_F VALUES OF POLYCHLORINATED BIPHENYLS AND ORGANOCHLORINATED PESTICIDES

Material	*Hexane as developing solvent*	*5% benzene in hexane as developing solvent*
Aroclor 1221	0.42–0.48, 0.50–0.55	0.57–0.63, 0.66–0.69
Aroclor 1242	0.50–0.55, 0.56–0.62	0.66–0.73, 0.74–0.79
Aroclor 1248	0.52–0.55, 0.56–0.62	0.67–0.72, 0.73–0.80
Aroclor 1254	0.50–0.56, 0.57–0.64	0.68–0.73, 0.74–0.81
Aroclor 1260	0.56–0.63, 0.64–0.72	0.73–0.80, 0.82–0.87
Aldrin	0.59–0.60	0.78–0.82
p,p'-DDE	0.51–0.57	0.70–0.75
o,p'-DDE	0.42–0.48	0.61–0.67
p,p'-DDT	0.32–0.38	0.37–0.43
p,p'-DDD	0.17–0.21	0.11–0.15
Heptachlor epoxide	0.06–0.08	0.03–0.07
Dieldrin	0.02–0.04	0.03–0.07

of the final zone, comparison with the results obtained with pure hexane standards (Table II) would have shown the error.

Fig. 4 shows typical spiked tissue samples before and after chromic acid oxidation. Figs. 5 and 6 show the scanner response to various Aroclors in the method. Table II gives the precision of analysis at various quantities of material spotted. The most difficult clean-up problem was considered to be eggs; recovery from six spiked samples is shown in Table IV.

TABLE II

DETERMINATION OF AROCLOR 1254

μg applied	*μg measured*			*Mean*	*Coefficient of variation (%)*
10	9.7	10.5	10.4		
	10.7	9.8	8.6		
	10.4	10.7		10.1	7.1
8	8.0	8.7	8.8		
	7.5	9.3	7.7	8.3	8.6
5	5.2	4.8	5.3		
	3.9	4.8	3.9		
	4.6			4.6	13.4
4	4.9	3.4	4.4		
	4.9	4.4	3.4		
	5.1	5.4		4.5	12.5

The composition of each of the Aroclor spots on the thin-layer chromatograms has been examined, using GC with electron capture detection. The carrier gas was helium; the stationary phase was silicone oil, SE-30 (10% on Gas-Chrom Q 80–100 mesh); and the temperature was 170°. Zones were removed from the plate by scraping, and the Aroclors were eluted from the scraped material, using diethyl ether. Figs. 7, 8, and 9 show the chromatograms obtained with our column for each zone.

TABLE III

EGG UNKNOWNS DETERMINED BY EGG STANDARDS

μg applied	*μg measured*			*Mean*	*Coefficient of variation (%)*
7	8.0	6.2	7.7		
	6.7	7.5	7.5		
	6.8	6.1		7.1	9.0
4	4.6	3.4	4.0		
	4.5	4.7	3.7		
	3.8	4.6		4.2	11.9

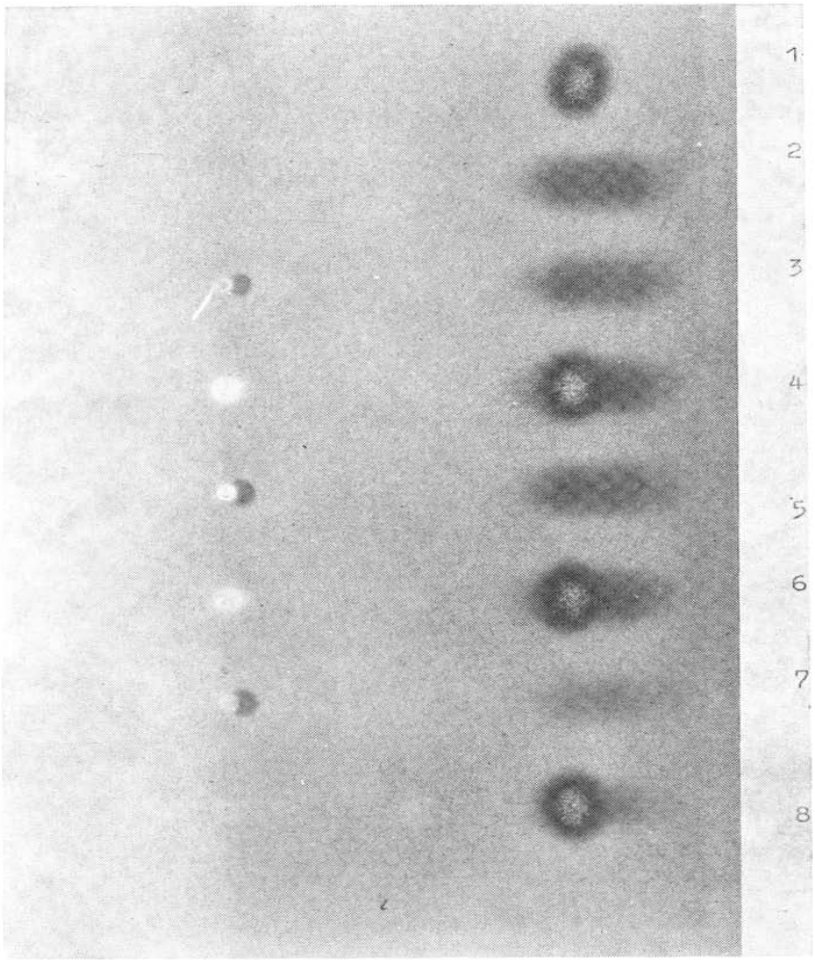


Fig. 4. Typical spiked tissue samples before and after chromic acid oxidation. 1 = 8 μg *p,p'*-DDE; 2 = 8 μg Aroclor 1254; 3 = 8 μg Aroclor 1254 and 8 μg *p,p'*-DDE in 5 g oxidized pork tissue; 4 = 8 μg Aroclor 1254 and 8 μg *p,p'*-DDE in 5 g unoxidized pork tissue; 5 = same as 3; 6 = same as 4; 7 = 4 μg Aroclor 1254 and 8 μg *p,p'*-DDE in 5 g oxidized egg yolk; 8 = 4 μg Aroclor 1254 and 8 μg *p,p'*-DDE in 5 g unoxidized egg yolk.

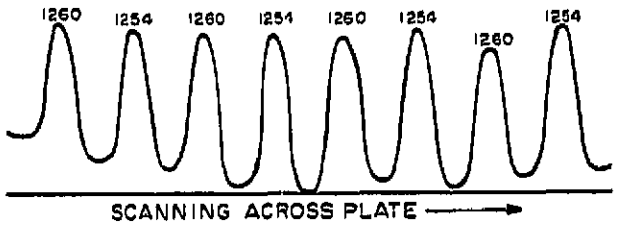


Fig. 5. Scanner response to Aroclor 1260 and Aroclor 1254, 8 μg per spot (Plate 62, Table V).

DISCUSSION

Qualitative analysis

It is clear from Fig. 1 that the method provides an "at a glance" system for screening samples for PCB and other chlorinated hydrocarbon materials. The method has been designed primarily to analyze for PCB; hence, DDE and aldrin have

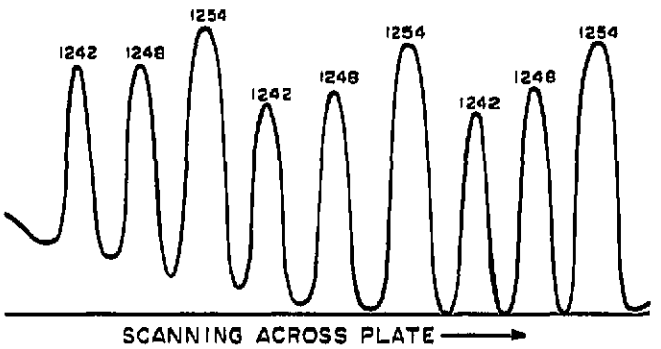


Fig. 6. Scanner response to Aroclors 1242, 1248, and 1254 (Plate 57, Table V).

been eliminated during clean-up in the interests of good quantitation. However, the method may be used for qualitative work, and in many instances the presence of DDE, the chief DDT-related material found in wildlife, may be distinguished (Fig. 4). The presence of aldrin would be difficult to establish, but it is rarely found in wildlife samples because of its metabolic oxidation to dieldrin.

TABLE IV

PERCENTAGE OF RECOVERY OF PCB IN EGGS FOR THE ENTIRE PROCESS (EXTRACTION, DMF PARTITION, FLORISIL COLUMN TREATMENT)

Sample number	*% recovery*
1	96.0
2	94.8
3	97.9
4	98.8
5	100.4
6	93.3

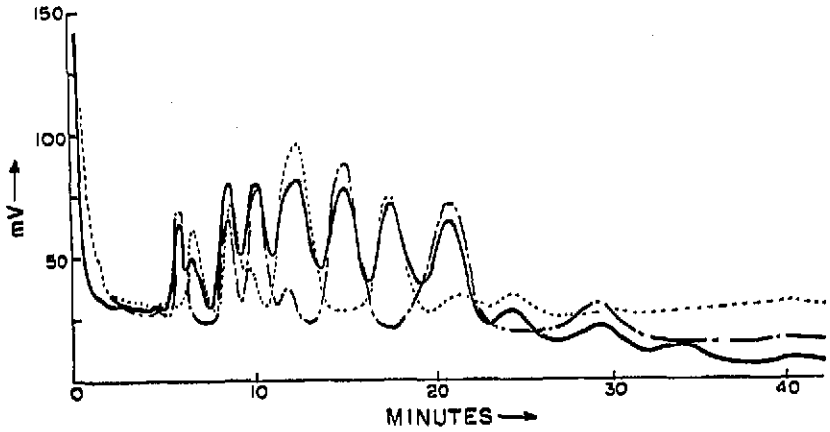


Fig. 7. Gas chromatograms of thin-layer zones (Aroclor 1254). ———, Aroclor 1254; — — —, 1254 zone I; — · —, 1254 zone II.

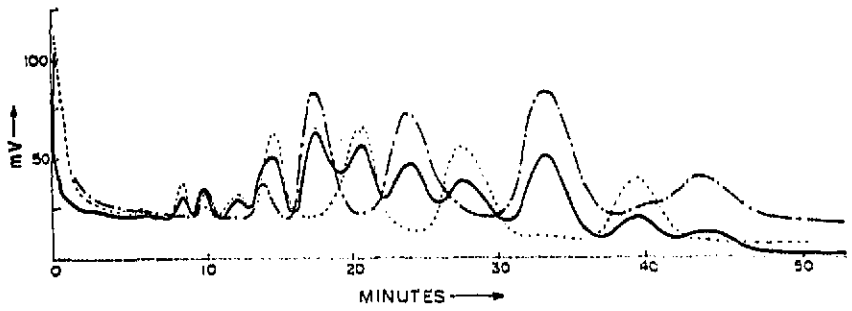


Fig. 8. Gas chromatograms of thin-layer zones (Aroclor 1260). ———, Aroclor 1260; — — —, 1260 zone I; — · —, 1260 zone II.

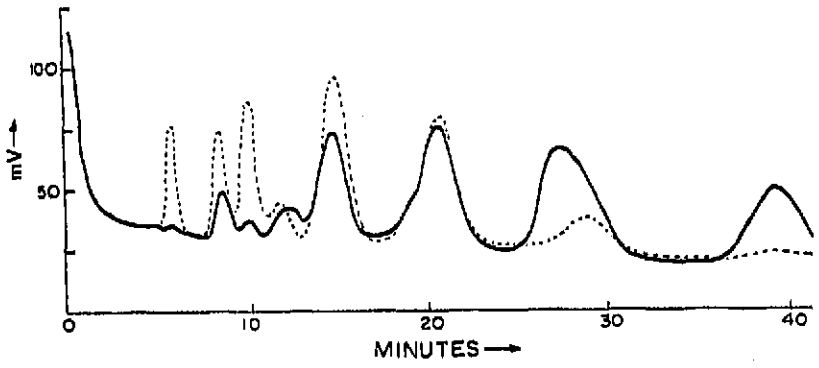


Fig. 9. Comparison of composition of material from zone with R_F 0.56–0.64 from Aroclors 1254 and 1260. ———, Aroclor 1260 zone I; — — —, Aroclor 1254 zone II.

The main advantage of the thin-layer technique at the qualitative level is the simple pictorial presentation which is achieved. Furthermore, spots may easily be removed from thin-layer chromatograms[18], and mass spectrometry or GC may be used to corroborate the TLC identification. The sensitivity of the method is between 0.5 and 1.0 μg of Aroclor 1254 or 1260 per chromatogram spot. In our procedure the residue from 1 g of material after clean-up is applied per spot, so that the minimum concentration detectable by the method is 0.5 to 1.0 p.p.m.

Comparison of the R_F values of the various Aroclors yields the expected trend; that is, the introduction of more chlorine atoms into the aromatic ring decreases the adsorption affinity of the compound[19], with the highly chlorinated PCB migrating farther than the less chlorinated PCB. However, when there are several substituted chlorine atoms in the same molecule, their influence on the adsorption affinity is only approximately additive. We therefore do not expect to find the molecules grouped simply by chlorine content, as the steric factor may be relatively strong in this group of compounds.

Mass spectrometry has been used to find the composition of the main peaks shown by GC. Of course separations differ from column to column, but the majority of work has been done with silicone oil stationary phase[5,20]. Unfortunately the major study, carried out by SISSONS AND WELTI[3], was done with a hydrocarbon stationary phase. From the work of BIROS *et al.*[20] it appears that peaks 1 and 2 in the chromatogram of Aroclor 1254 are due to tetrachlorobiphenyls; peaks 3–5, to pentachlorobiphenyls; and peaks 6–9, to hexachlorobiphenyls. Ignoring the minor peaks, this assignment appears roughly correct when compared with that obtained at high resolution on hydrocarbon stationary phase by SISSONS AND WELTI.

The gas chromatograms of the PCB fractions obtained using TLC allow several conclusions to be drawn as to the composition of material in the thin-layer zones. It is clear from Figs. 6 and 7 that the adsorption behavior of tetra-, penta-, and hexachlorobiphenyls is determined more by steric effects than by total chlorine content. It is interesting to note (Fig. 9) that although the first zone from Aroclor 1260 and the corresponding zone from Aroclor 1254 show GC peaks of the same retention time, the proportion of the materials present differs in the two products.

Quantitative analysis

The precision of the method shown in Tables II and III is satisfactory for this type of residue analysis and is almost certainly superior to the precision obtained by GC, primarily because of two deficiencies in GC analysis at the present time—the uncertainty of peak identity, and the changes in the proportion of each peak from sample to sample[10,11,14].

The problem of peak identity may also arise in the present method. Table V shows the response of Aroclor 1254 and 1260 on four different plates with a total of 26 measurements. Because the level of response varies from plate to plate, they are best compared by examining the ratio of response as given in the table. It is clear that, within the experimental error ($\pm$ 10%) of the method, the response given by Aroclor containing 60% chlorine is not distinguishable from that of Aroclor containing 54% chlorine. Materials with less chlorine, however, show an appreciable decrease in response.

Another problem that may cause systematic errors in both GC and TLC anal-

TABLE V

RELATIVE SENSITIVITIES OF POLYCHLORINATED BIPHENYLS BY TLC

Aroclor	*μg per spot*	*Plate number*	*Average of peak weight*	*Ratio of Aroclor 1260 : Aroclor 1254*	*Number of determinations*
1254	20	55	0.0354		4
1260	20	55	0.0326	0.92	3
1254	5	56	0.0086		4
1260	5	56	0.0105	1.22	3
1254	8	63	0.0118		3
1260	8	63	0.0136	1.15	3
1254	8	62	0.0253		3
1260	8	62	0.0250	0.99	3
1254	20	57	0.0576		3
1248	20	57	0.0354	0.67	3
1242	20	57	0.0305	0.53	3

ysis is the existence of materials which have similar retention characteristics to the materials being assayed but which arise from the tissue and do not respond to the detection system. In theory different molecules should migrate independently, but interference could occur either with the efficiency of the detection method or (in the TLC technique) during the spot application step. The study shown in Table III was designed to test this possibility. Comparison with the results in Table II shows clearly that the use of standards containing egg does not change the results obtained and that hexane standards are acceptable for the analysis. Recovery yielded by the process of clean-up is as high as the standard normally accepted for organochlorine pesticide residue analysis.

The method is particularly easy to use for screening non-fatty tissues for PCB, and it can be used for screening industrial materials for PCB simply by grinding the material and extracting into hexane. The question of relative response for the different industrial PCB preparations (Table V), while relatively unimportant with wildlife samples due to the elimination of low-chlorinated members[14], is more important with industrial products. Should a lower chlorinated PCB be present, it will be immediately obvious due to its smaller R_F value, and quantitation may then be achieved by using the requisite standard commercial products.

With regard to the clean-up method, the oxidation procedure with non-fatty tissue is very rapid and convenient. (Application of the method in wildlife will be considered in a separate paper.) The method is also useful for in-house toxicological work for monitoring changes in level of PCB in various tissues of populations maintained on daily intakes of PCB and other chlorinated hydrocarbons. It should be particularly attractive to biological workers, who are accustomed to obtaining quantitative results from layer methods such as electrophoresis. Also, scanning apparatus are widely distributed in biochemical laboratories, whereas GC, with electron capture detection, is very much less widely available.

CONCLUSION

Besides being simpler, cheaper, and more rapid, the quantification and identification of PCB by TLC is more reliable than GC. With the present technique, we are able to detect 0.5 p.p.m. of PCB in 5 g of tissue, egg, or fat. We are also able to identify other polychlorinated residues, and the method is being extended to quantitation of these. An increase in sensitivity will become possible only by the development of a better chromogenic system than the universally used silver nitrate system. The present sensitivity is, however, adequate for enforcement of the present USDA food standards.

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