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Thin-Layer Chromatography of Polychlorinated Biphenyls

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INTRODUCTION

Polychlorinated biphenyls (PCB's) have been recognized as being industrial pollutants of the environment. Their presence in the form of residues in wildlife samples was first reported in Sweden in 1966 (1) and later in several other European countries and in North America.

PCB's have a tendency to accumulate in fatty animal tissues as for instance p,p'-DDE. They are not pesticides, but can be determined according to the usual analytical methods for detecting organochlorine insecticides. Until now only gas chromatography has been reported in the literature as a technique for separating the various components of the complicated mixtures represented by the PCB's (2,3,4,5).

Thin-layer chromatography is a useful technique in pesticide residue analysis for the qualitative confirmation of results obtained by means of gas chromatography. When an often used TLC-system like aluminum oxide/hexane is applied , however, very little information is obtained about the composition of the PCB residue. Only a diffuse and rather long-drawn spot is found with an approximate R_f-value between 0.6 and 0.8.

So far no TLC system was available that is particularly suitable for satisfactory separation of individual PCB compounds. Reversed-phase partition TLC (utilizing a non polar stationary phase and a polar mobile phase) is used for resolving closely related lipophilic materials, such as homologous series of fatty acids. We have found that this technique can be applied in separating closely related components in PCB mixtures. Details of the method, which was worked out for this purpose, are given below.

EXPERIMENTAL

1. Preparation of plates

A quantity of 25g "Kieselguhr G"(Merck no.8129) was mixed with 60 ml water and stirred until a homogeneous slurry was obtained. Any remaining air bubbles were removed by applying vacuum. The slurry was transferred to a Desaga applicator and 5 glass plates (20 x20 cm) were coated in the usual way (layer thickness 0.25mm). The plates were air-dried overnight and finally heated for one hour at 105°C. The plates were cooled and left uncovered (not in a desiccator).

A flat tray was filled with petroleum ether (b.p. 40-60°C)

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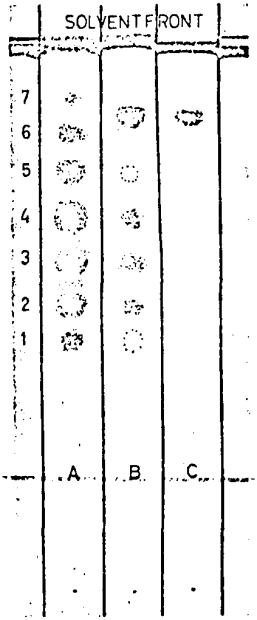

Figure 1

Thin layer chromatograms of, "A" technical PCB mixture, "B" extract of owl liver, and "C" p,p'-DDE. Kieselguhr impregnated with liquid paraffin, developed 3x with a mixture of acetonitrile,acetone,methanol, and water (40:18:40:2), and visualized with silver nitrate and U.V.

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containing 8 vol.% liquid paraffin. A coated plate was carefully soaked in this solution until the adsorbent layer was saturated all over with the solvent, which usually took 1-2 minutes. Then the plate was carefully removed from the solution. After air-drying and storage for at least 24 hours, the plate was ready for use. Plates thus prepared could be kept for a considerable time, at any rate for several weeks.

2. Spotting and development

Hexane solutions of standards and residues were spotted along the origin of a plate. The best results were obtained when a quantity of not more than 20µl was spotted.

A glass developing tank, lined with filter paper, was filled with a solvent mixture of 40 ml acetonitrile + 18 ml acetone + 40 ml methanol + 2 ml water. This mixture had previously been saturated with liquid paraffin by shaking in a separatory funnel. The tank was closed. When the solvent had reached the top of the liner, a plate was transferred to the tank. The solvent front was allowed to travel 14 cm above the origin line of the plate. After being developed the plate was air-dried, then the developing procedure was repeated two additional times. The plate was dried after each development. Three developments gave the best resolutions at moderate room temperature(± 20°C). At elevated temperatures (above + 25°C) it was found that R_f values for PCB-spots tended to be too high. In these cases better results were obtained with only two developments.

3. Visualization

After developing the plate was air-dried until the solvent had entirely evaporated. The plate was sprayed with about 10 ml of a silver nitrate spray, prepared by dissolving 1.7 g silver nitrate in 200 ml ethanol (96%). The plate was irradiated with UV-light (Philips TUV 15 watt) at a distance of about 15 cm. Black spots on a white background usually appeared within 20 min.

RESULTS AND DISCUSSION

Figure 1 shows the results obtained with the method described. A is a chromatogram of 15 micrograms of Phenochlor DP6 a commercial PCB preparation containing 60% chlorine. Other brand names for industrial preparations are Aroclor 1260 and Clophen A 60.

Residue studies in the Netherlands (2) have shown that PCB residues in fish and birds have a GLC-peak pattern very similar to that of the mixtures mentioned above. B is an example of a residue found in a wildlife sample, the liver of a long-eared owl from the northern part of The Netherlands. For the analysis the liver was ground with sand and sodium sulfate and extracted with hexane. The hexane extract was cleaned up by chromatography on two small alumina columns, as described by Holden and Marsden (6).

C is a p,p'-DDE standard which was spotted for comparison.

Fig. 1 demonstrates that also by TLC a strong resemblance is found between the spot patterns of the residue and the technical PCB mixture.

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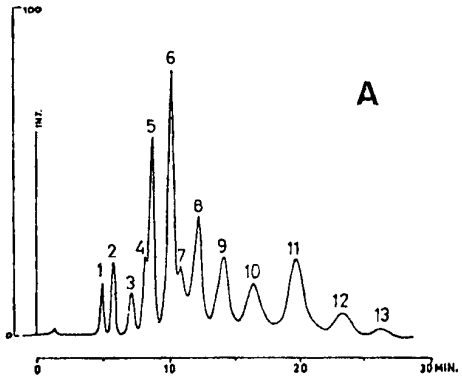

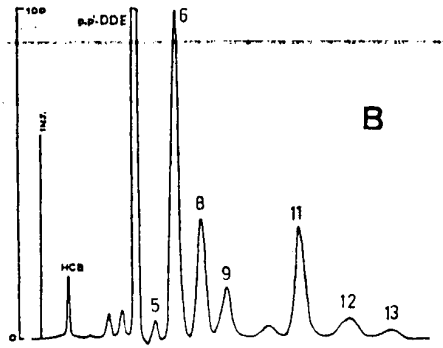

FIG. 2

Gas chromatograms of "A" Phenochlor DP6 (0.6 µg) and "B" hexane extract of owl liver. Instrumental conditions are given in Table 1.

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Gas chromatograms of Phenochlor DP6 and the liver extract are shown in Figs. 2A and B. A dohrmann microcoulometer, equipped with a silver cell, was used as a detection system. Further instrumental conditions and relative retentions of the peaks in Fig. 2A are summarized in Table 1.

The PCB-residue (estimated by microcoulometry) in the liver sample was 350 ppm. Besides this residue, hexachlorobenzene (6.5 ppm), heptachlorepoxide (3.6ppm) and p,p'-DDE (290 ppm) were also detected. The sample was selected for demonstration purposes; the detected residues are high and cannot be regarded as general background residues in Dutch birds.

TABLE 1

Relative retentions (dieldrin = 1.00) of PCB-peaks 1-13 in Fig. 2 A.

Peak No.	Rel. retention[a]
1	0.71
2	0.84
3	1.03
4	1.18
5	1.23
6	1.47
7	1.59
8	1.73
9	2.01
10	2.33
11	2.80
12	3.33
13	3.76

Instrument: Microtek MT 220. Column: glass, 6' x1/4").D., packed with 3% OV-1 on Gas Chrom Q, 80-100 Mesh. Operating temperatures: column 200°C; injector 220°C; transfer line (Teflon coated aluminum) 220°C Detection system: Dohrmann C 250 A microcoulometer, with oxidative pyrolysis furnace and T 300 S titration cell. Sensitivity: 200 ohms. Gain: low (200). Recorder sensitivity: 1 mv. Chart speed: 1 cm/min.

Peak numbers 5, 6, 8, 9, 11, 12, and 13 in Fig. 2 B have the same retention times as the corresponding peaks in Fig. 2 A.

Previous investigations, carried out at our institute with a gas chromatograph - mass spectrometer combination (2), have revealed that in the chromatogram of the technical mixture containing 60% chlorine, peaks 1 and 2 are produced by pentachlorobiphenyls, peaks 3, 4, 5, 6, and 8 by hexachlorobiphenyls, peaks 7, 9, 10, 11, and 12 by heptachlorobiphenyls, and finally "peak" 13 by octachlorobiphenyl(s).

In order to ascertain the relation between spots A 1 to # 7 on the plate (Fig. 1) and peaks 1-13 in Fig. 2 A, several aliquots of Phenochlor DP6 solution in hexane were spotted along the origin

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of a TLC plate. After developing, as described before, small vertical areas on the plate were treated with silver nitrate and irradiated to locate the various rows of spots. Horizontal zones corresponding to spots 1-7 were scratched out. The absorbent fractions obtained were eluted and analysed by gas chromatography under conditions as stated in Table 1. The results are reported in Table 2.

This table shows that spots with lower R_f-values generally correspond to peaks with higher retention times, or in other words to PCB's with a higher degree of chlorine substitution (as follows from the already mentioned mass spectrometric observation). Spot A 1 corresponds to peak 13, but also to a peak eluting very late with relative retention 5.6 (dieldrin = 1.00). This peak is normally not visible in the gas chromatogram, unless more concentrated solutions are injected. The identity of this peak has not yet been investigated by means of mass spectrometry.

TABLE 2
Relationship between spots obtained by thin-layer chromatography (Fig. 1 A) and peaks obtained by gas chromatography (Fig. 2 A) of Phenochlor DP6

spot no.	corresponding peak numbers
1	13
2	11
3	6, 9, 12
4	7, 8, 10
5	2, 4, 5
6	3, 6
7	1, 3

When observing the peaks and spots found with the liver extract it can be seen that there is a relationship similar to that found with the technical PCB mixture (Table 2).

Of the chlorinated pesticides that are frequently detected in wildlife samples only the fungicide hexachlorobenzene(HCB) interferes with one of the PCB spots (no.3). In the liver sample interference is negligible, since the HCB residue is very low compared with the PCB residue. p,p'-DDT, p,p'-DDE, p,p'-TDE, dieldrin, heptachlorepoxide, and lindane all migrate faster than PCB spot no. 7 (Fig. 1); most of these compounds are found quite close to the solvent front.

The lowest detectable amounts obtained with the reported TLC-system are of the order of 100 ng of single compounds like DDE and HCB, and a few micrograms for PCB mixtures such as Phenochlor DP6.

In summary it can be stated that the reversed phase partition TLC system described was found satisfactory for the separation of the components of a PCB mixture and can be used in combination with gas chromatography for the study of the composition of PCB residues.

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