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Method for Separating Polychlorinated Biphenyls from DDT and Its Analogs

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Polychlorinated biphenyls are separated from DDT and its analogs and other common chlorinated pesticides by column chromatography on silicic acid–Celite. PCB are eluted from the column with petroleum ether prior to elution of pesticides with a mixture of acetonitrile, hexane, and methylene chloride. Determination of PCB and pesticides can be made on the separate column eluates without cross interference. The method is applicable to samples prepared by multipesticide residue methodology and should be applicable to sample extracts prepared for gas chromatography by usual procedures. Recoveries of Aroclors 1251 and 1260 and of several chlorinated pesticides through the separation method ranged from 76 to 100% and 80 to 107%, respectively.

Residues of polychlorinated biphenyls (PCB) have been reported in wildlife species, notably fish and fish-eating birds, by investigators examining these specimens for residues of chlorinated pesticides (1–8). These compounds are extracted and isolated along with the pesticides, and detected by gas chromatographic (GLC) systems normally employed in analyses for chlorinated pesticides.

The multipesticide residue methods employed by the Food and Drug Administration (9) completely recover PCB. The presence of the multicomponent PCB in a sample examined for pesticide residues could interfere with or prohibit identification and measurement of some chlorinated pesticides because of similarity of GLC retention times.

The objective of this investigation was to develop a method to separate PCB from DDT and analogs so that residues of pesticides and PCB each could be determined without interference from the other.

METHOD

Reagents

(a) *Silicic acid.*—Mallinckrodt, 100 mesh powder; "specially prepared for chromatographic analysis by the method of Ramsey and Patterson," analytical reagent No. 2847. Treat as follows: Place silicic acid to depth of ca 1" in open beaker and heat ≥7 hr in 130°C oven. After heating, place beaker in desiccator and let cool to room temperature. Quickly weigh silicic acid into glass-stoppered bottle and add 3% water by pipet (97 g silicic acid + 3 ml water = 3% water). Stopper bottle tightly and seal with tape to insure that container is air-tight. Shake well until all water is adsorbed; make sure that no lumps remain. Place sealed container in desiccator and allow to equilibrate 15 hr. Return container to desiccator immediately after use. Desired activity remains for about 5 days.

(b) *Celite 545.*—Acid-washed (Johns-Manville Corp); must be dry and free of electron-capturing substances.

(c) *Solvents.*—Petroleum ether, hexane, acetonitrile, and methylene chloride, suitable for use in pesticide residue analysis; distilled-in-glass product is satisfactory (available from Burdick and Jackson Laboratories Inc., 1953 S. Harvey St., Muskegon, Mich. 49442).

(d) *Eluting mixture.*—1% acetonitrile, 19% hexane, and 80% methylene chloride (v/v/v). Pipet 10 ml acetonitrile into 1 l. volumetric flask, add 190 ml hexane, and make to volume with methylene chloride.

Apparatus

(a) *Chromatographic column.*—400 × 22 mm id with 24/40 ₸ outer joint, coarse fritted plate, and Teflon stopcock (Kontes Glass Co. K-420550, C-4, or equivalent).

(b) *Separatory funnel.*—Used for column eluant reservoir; 250 ml with Teflon stopcock, 24/40 ₸ inside joint on stem, and 24/40 ₸ outside joint at top (Kontes Glass Co. K-633030, or equivalent).

(c) *Adapter.*—Hose connector type, 24/40 ₸ inner joint at bottom, Teflon stopcock (Kontes Glass Co. K-185000, or equivalent).

(d) *Kuderna-Danish evaporative concentrator.*—With Snyder column and collection tube, 500 ml capacity (Kontes Glass Co. K-570000, or equivalent).

(e) *Air regulator.*—Or pressure-reducing device to deliver ca 1 lb pressure. Air must be dry and clean.

(f) *Gas chromatograph.*—Equipped with electron capture detector and 6' × 4 mm id glass column of either (*1*) 10% DC-200 on 80–100 mesh Chromosorb W HP or (*2*) 1:1 mixture of 15% QF-1 plus

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10% DC-200 on 80–100 mesh Chromosorb W HP. *Operating conditions:* nitrogen 120 ml/min, column and detector temperature 200°C, injection temperature 225°C. Concentric design electron capture detector operated at dc voltage to produce ½ full scale recorder deflection for 1 ng heptachlor epoxide when full scale deflection is 1×10^{-9} amp.

Separation Procedure

Weigh 5 g acid-washed Celite, then 20 g activated silicic acid and combine in 250 ml beaker. Immediately slurry with 80 ml petroleum ether, mixing well. Pour slurry into chromatographic column with stopcock open and complete transfer of silicic acid-Celite mixture, rinsing beaker with small portions of petroleum ether. Apply air pressure as necessary to force enough petroleum ether from column to allow space for all silicic acid-Celite. Stir material in column with glass rod to remove air bubbles. Apply air pressure to top of column to settle adsorbent and to force petroleum ether from column. Continue to apply air pressure until petroleum ether level is about 3 mm above surface of gel. (*Note:* Do not allow column to go dry or to crack at any time during the procedure. Close stopcock when air pressure is not being applied. At this point column of adsorbent should be firm and not lose its shape if tipped.)

Place 250 ml volumetric receiver under column to collect eluate. Take a suitable aliquot of cleaned up sample (6% Florisil eluate) (9) containing PCB and DDT and its analogs dissolved in not more than 5 ml petroleum ether. (*Note:* Large amounts of PCB and pesticides placed on column may result in incomplete separation. Choose aliquot to contain amounts of PCB and pesticide required for determination.) Slowly and carefully add sample to column; do not disturb top of adsorbent. Apply slight air pressure until solvent level is ca 3 mm above adsorbent. Complete transfer of sample to column, using small portions of petroleum ether, and apply slight air pressure until solvent level is ca 3 mm above absorbent. Rinse walls of column with small portion of petroleum ether and again apply slight air pressure until solvent level is ca 3 mm above adsorbent. Place reservoir containing 250 ml petroleum ether on top of column, open stopcock, and slowly apply air pressure to reservoir until an elution rate of ca 5 ml/min is established. Continue elution until eluate volume is exactly 250 ml. Stop elution. Quantitatively transfer eluate to Kuderna-Danish concentrator and reduce volume to that necessary for analysis for PCB.

Place Kuderna-Danish concentrator fitted with collection tube under column to collect second eluate and any remaining petroleum ether eluant. Apply air pressure until petroleum ether eluant level is ca 3 mm above adsorbent. Add 200 ml acetonitrile-hexane-methylene chloride (1 + 19 + 80) to reservoir, open stopcock, slowly apply air pressure, and continue elution until all eluant passes through column. Concentrate eluate to suitable definite volume for analysis for DDT and analogs.

Results and Discussion

PCB are widely used industrial chemicals. In this country a series of PCB called Aroclors® (10) are produced; each Aroclor is a mixture of chlorinated biphenyls (1200 series), chlorinated terphenyls (5400 series), or a combination of chlorinated biphenyls and terphenyls (4400 series). The last 2 digits of the identifying number indicate the weight per cent chlorine. Aroclors 1254 and 1260, biphenyls containing 54 and 60% chlorine, respectively, were chosen as representative PCB for this study.

Analytical Behavior of PCB

Aroclor 1260 was used in tests to determine the behavior of PCB in FDA multipesticide residue analytical methodology (9). The PCB were eluted from a Florisil column with 200 ml 6% ethyl ether-petroleum ether. Several common chlorinated pesticides, including DDT and analogs, are also found in this eluate. Recoveries of the PCB added to spinach and butterfat ranged from 84 to 90%. Gas chromatography of Aroclors 1221, 1232, 1254, 1260, 1262, 4465, 5442, and 5460 on columns of 10% DC-200 and 15% QF-1/10% DC-200 with electron capture detection showed the Aroclors to be multicomponent mixtures with retention times throughout and beyond the retention time range of common chlorinated pesticides. With a detector sensitivity producing ½ full scale recorder response to 1 ng heptachlor epoxide, significant responses were obtained with about 10–20 ng of the various Aroclors.

Initial attempts at separating PCB from DDT and its analogs included a nitration procedure which gave poor results, consistent with those reported by Reynolds (11). A method of separating PCB from DDT and its analogs was sought which would not alter these compounds chemically but would leave them unchanged for individual analysis and which would be applicable to the 6% ethyl ether-petroleum ether Florisil eluate in FDA's multipesticide residue methodology (9). Column chromatography met these criteria and attempts at separation were made using Florisil as the adsorbent with petroleum ether eluant. Aroclor 1260 and *p,p'*-DDE were not separated and there was only a small margin

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of separation between Aroclor 1260 and p,p'-DDT. The separation was not improved by variation of parameters of the chromatography.

Silicic Acid

Column chromatography on silicic acid was then investigated. Celite was mixed with the silicic acid to achieve a faster elution rate from the adsorbent column. Initial findings indicated that PCB could be eluted from the column with petroleum ether before elution of DDT and analogs. However, there was an inconsistency in the elution pattern of Aroclor 1260 and DDT and analogs in later experiments. The only variable in these trials was the time of heating silicic acid at 130°C. Silicic acid (silica gel) is a hydrated silicon oxide ($SiO_2 . nH_2O$) whose activity will change as the amount of physically bound water varies. The 130°C temperature of heating was too low to alter the structure of surface active sites but would have caused elimination or partial removal of physically bound water (12–14).

The effect of water content of silicic acid on elution and separation of Aroclor 1260 and DDT analogs is shown in Fig. 1. Silicic acid was heated at 130°C for several days before equilibrating with 0–10% (w/w) water. A mixture of Aroclor 1260, *o,p'*- and *p,p'*-DDT, *p,p'*-TDE, and *p,p'*-DDE were eluted from columns of silicic acid of various moisture content, using petroleum ether as the eluant. The maximum margin of separation of PCB from pesticides, with good reproducibility of elution pattern, was obtained with silicic acid containing 3% water. A more polar eluant was required for complete elution of *p,p'*-DDT and *p,p'*-TDE when water content was 5% or less. Aroclor 1260 was completely eluted with 200 ml petroleum ether; 250 ml eluant was required for complete recovery of Aroclor 1254. When more than 5% water was added, activity was decreased to the extent that retentiveness was lost.

In early experiments it appeared that the separation of PCB and pesticides was affected by

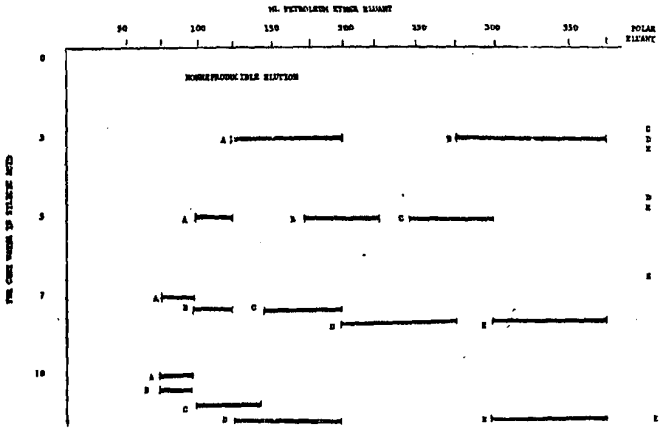

FIG. 1.—Effect of silicic acid water content on elution and separation of Aroclor 1260 and DDT and analogs.
25 ml fractions of petroleum ether eluate analyzed by GLC; respective compound found present in volumes crossed by horizontal line. Various polar eluants used to elute pesticides C, D, and E. A, Aroclor 1260; B, *p,p'*-DDE; C, *o,p'*-DDT; D, *p,p'*-DDT; E, *p,p'*-TDE.

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physical variables attendent to the preparation and elution of the silicic acid-Celite column. It was possible to observe some undesirable effects and their causative factors by eluting D&C Green No. 4 dye from columns of silicic acid-Celite. Uneven adsorption and widening of the elution band occurred whenever the column of adsorbent was allowed to go dry or to crack. Similar effects on the elution band were noted when water was not first added to the silicic acid. It was assumed that elution of PCB and pesticides was also adversely affected by these conditions.

Experiments with time of heating showed that silicic acid should be heated a minimum of 7 hr at 130°C before re-establishing the water content to 3%. Silicic acid remained satisfactory for use after heating for periods up to 30 days at 130°C. However, low recoveries of pesticides were obtained from adsorbent heated for 40–80 days.

J. T. Baker, Merck, and Mallinckrodt, "analytical grade," 100 mesh, silicic acids, with similar statements of impurities, were found to perform satisfactorily after treatment as described under *Method*. Mallinckrodt silicic acid (a) was the adsorbent of choice, since special testing by the manufacturer insures a specific performance and should lessen lot-to-lot variation. Six lots of this material manufactured over a 2-year period have been evaluated; all were satisfactory and reproducible in their performance.

Performance of Separation

An adsorbent column of 20 g silicic acid (containing 3% added water) plus 5 g Celite was determined to be the best for separating PCB from DDT and analogs. The separation was accomplished by eluting PCB with 250 ml petroleum ether followed by elution of DDT and analogs with 200 ml eluant of acetonitrile-hexane-methylene chloride (1 + 19 + 80). DDT and analogs are eluted by the first 100–125 ml of the second eluant. In addition to *o,p'*- and *p,p'*-DDT, *p,p'*-DDE, and *p,p'*-TDE, complete recovery of lindane, heptachlor, heptachlor epoxide, dieldrin, and endrin was obtained by the second elution. Aldrin was eluted with PCB. With the exception of dieldrin and endrin, all these pesticides and PCB are found in the 6% ethyl ether-petroleum ether Florisil eluate. The inability to separate aldrin from PCB is not a problem, as aldrin is rarely present as a residue in environmental samples and various techniques are available for determining aldrin. Recoveries of Aroclors 1254 and 1260 and pesticides are given in Table 1. Aroclors 1221, 1232, 1262, 5442, and 5460 were also completely separated from pesticides by the petroleum ether elution. The multicomponent pesticides, chlordane and toxaphene, were eluted by the polar eluant and did not interfere with PCB.

Table 1. Recoveries and separation of Aroclors and chlorinated pesticides added to silicic acid column

Chemical	Added, µg	Av. Found, %	No. Trials	Range, %
Eluate 1[a]				
Aroclor 1254	40	95–100[b]	10	95–100[b]
Aroclor 1260	20–40	95–100[b]	18	95–100[b]
Aldrin	1	103	4	100–103
Eluate 2[c]				
Lindane	0.5	96	4	94–100
Heptachlor	1	93	4	88–98
Heptachlor epoxide	1	100	4	96–104
Dieldrin	1	104	2	102–105
Endrin	1	94	4	93–96
p,p'-DDE	5	99	16	88–105
o,p'-DDT	10	98	14	91–101
p,p'-DDT	10	98	18	90–105
p,p'-TDE	5	97	18	87–107

[a] 250 ml petroleum ether.
[b] Recoveries estimated; no Aroclor present in second eluate.
[c] 200 ml acetonitrile-hexane-methylene chloride (1 + 19 + 80).

There is only a small margin of separation between PCB and *p,p'*-DDE. The elution of PCB with petroleum ether should be stopped just as the eluate volume reaches 250 ml. A trace of polar solvent in the sample placed on the column will result in elution of some *p,p'*-DDE with PCB. Samples should be in petroleum ether solution and placed on the column in a volume not greater than 5 ml. Separation of PCB and pesticides may not be complete if excessive amounts of these compounds are present in the sample placed on the silicic acid column. Estimation of the amount of PCB present can be made from gas chromatography before separation. The sample aliquot should be chosen to contain only the amounts of pesticide and PCB required for determination. For example, with the GLC sensitivity described earlier, we would choose an aliquot containing about 20 µg PCB. If the eluate from the silicic

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Table 2. Recoveries and separation of Aroclors and chlorinated pesticides added to trout extract and put through the silicic acid column

Chemical	Added, ppm	% Recovery: Eluate 1[a] Trial 1	Eluate 1[a] Trial 2	Eluate 1[a] Trial 3	Eluate 2[b] Trial 1	Eluate 2[b] Trial 2	Eluate 2[b] Trial 3
Level 1							
Aroclor 1254	2.0	100	—[c]	95	0	—	0
Aroclor 1260	2.0	—	76	—	—	0	—
p,p'-DDE	0.15[d]	0	0	0	100	107	96
p,p'-TDE	0.15	0	0	0	96	93	90
p,p'-DDT	0.30	0	0	0	100	90	96
Level 2							
Aroclor 1254	20.0	—	76	—	—	0	—
Aroclor 1260	20.0	100	—	—	0	—	—
p,p'-DDE	2.5[d]	0	0	—	80	102	—
p,p'-TDE	2.5	0	0	—	90	101	—
p,p'-DDT	5.0	0	0	—	88	100	—

[a] 200 ml petroleum ether.
[b] 700 ml acetonitrile-hexane-methylene chloride (1 + 19 + 80).
[c] No fortification.
[d] Trout contained 0.19 ppm *p,p'*-DDE. Recovery determined on amount added plus residue.

acid column was concentrated to 5 ml this would be a suitable concentration for GLC determination.

Application of Method

Separation of PCB from DDT and analogs and recovery determinations were made on mixtures of Aroclor 1254 or 1260 and *p,p'*-DDT, *p,p'*-TDE, and *p,p'*-DDE added to cleaned up extracts of brown trout. The trout was analyzed by multipesticide residue methods (9) and found to contain only *p,p'*-DDE at 0.19 ppm. Five portions of the 6% ethyl ether-petroleum ether Florisil column eluate, each equivalent to 20 g fish, were fortified with a PCB-pesticide mixture. Fortification was at 2 levels: (*1*) 40 μg (2.0 ppm) Aroclor 1254, 6 μg (0.30 ppm) *p,p'*-DDT, 3 μg (0.15 ppm) *p,p'*-TDE, and 3 μg (0.15 ppm) *p,p'*-DDE; (*2*) 400 μg (20.0 ppm) Aroclor 1254, 100 μg (5.0 ppm) *p,p'*-DDT, 50 μg (2.5 ppm) *p,p'*-TDE, and 50 μg (2.5 ppm) *p,p'*-DDE. In a second trial at each level Aroclor 1260 was substituted for Aroclor 1254. Three trials were made at the lower fortification level. The fortified Florisil column eluates were placed on the silicic acid column, separated into 2 fractions, and analyzed by electron capture GLC as described under *Method*. Results are given in Table 2. Gas chromatograms of the mixture before and after separation are shown in Fig. 2.

Lake Michigan coho salmon with residues of PCB and pesticides resulting from environmental exposure was extracted and cleaned up by multipesticide residue methodology (9) prior to separation and measurement of PCB and pesticides. A 1 ml aliquot of the concentrated 6% ethyl ether-petroleum ether Florisil column eluate, equivalent to 5.4 g fish, was placed on the silicic acid column, separated into 2 fractions, and analyzed by electron capture GLC as described. The gas chromatograms in Fig. 3 show a good separation of PCB from DDT and analogs. Determination by electron capture GLC showed the salmon to contain 14.6 ppm PCB, 3.2 ppm *p,p'*-DDT, 1.3 ppm *o,p'*-DDT, 10.4 ppm *p,p'*-DDE, and 0.4 ppm *o,p'*-DDE. PCB residue was measured in terms of Aroclor 1254, using total area of the gas chromatographic response. GLC retention times, a positive microcoulometric halogen response for all GLC peaks, and behavior on the silicic acid column showed the residue in the first eluate to be PCB. Retention times and a positive microcoulometric halogen response indicated the identity of DDT and analogs. Thin layer chromatography and dehydrohalogenation with alkali confirmed the identity of the DDT isomers. Mass spectrometry identified *p,p'*-DDE.

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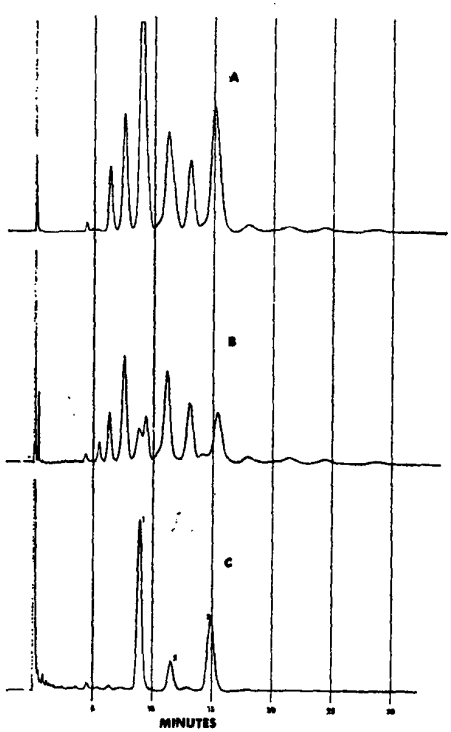

FIG. 2—GLC curves of brown trout extract fortified with 2.0 ppm Aroclor 1254, 0.3 ppm *o,p'*-DDT, 0.13 ppm *p,p'*-TDE, and 0.13 ppm *p,p'*-DDE (plus 0.19 ppm residue) before and after separation on silicic acid column: A, before separation; B, petroleum ether eluate from silicic acid column containing PCB; C, polar eluate from silicic acid column containing (1) *p,p'*-DDE, (2) *p,p'*-TDE, and (3) *p,p'*-DDT. 10 mg sample injected for each curve. GLC conditions given under *Method*.

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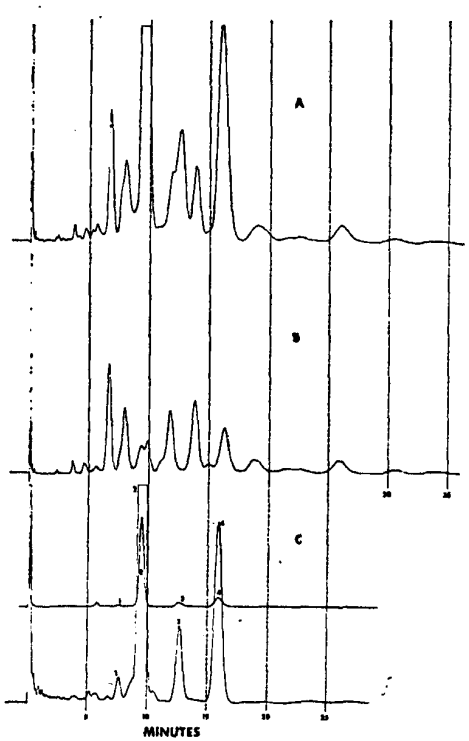

FIG. 3—GLC curves of Lake Michigan coho salmon extract containing residues of PCB and DDT and analogs before and after separation on silicic acid column: A, before separation; B, petroleum ether eluate from silicic acid column containing 14.6 ppm PCB (as Aroclor 1254); C, polar eluate from silicic acid column containing (1) 0.40 ppm *o,p'*-DDE, (2) 10.4 ppm *p,p'*-DDE, (3) 1.3 ppm *p,p'*-DDT, (4) 3.2 ppm *p,p'*-DUT. 2.7 mg sample injected for curves A, B, and C; 0.16 mg sample injected for curve C, used for *p,p'*-DDE quantitation. GLC conditions given under *Method*.

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Conclusion

The method developed for separating PCB from important chlorinated pesticides is routinely applicable to samples prepared for GLC pesticide residue analysis. Its use by laboratories analyzing for pesticide residues should eliminate any doubt of the validity of residue data, particularly those data for DDT and analogs. The separation of PCB from chlorinated pesticides will also permit an accurate assessment of the PCB contribution to environmental pollution. The coho salmon example presented here is additional evidence of the presence of PCB in some parts of the aquatic environment in this country.

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