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Removing the Interference of DDT and Its Analogs in the Analysis for Residues of Polychlorinated Biphenyls

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A micro scale procedure is described for quantitatively separating polychlorinated biphenyls (PCBs) from DDT and its analogs. DDT and TDE are initially dehydrochlorinated with ethanolic KOH to their respective olefins, DDE and TDE-olefin (1-chloro-2,2-bis(p-chlorophenyl)ethylene). The olefins are then oxidized by CrO_3 in acetic acid to the more polar dichlorobenzophenone. PCBs are eluted from a micro Florisil column with petroleum ether. Typical recoveries in the ppm range (based on a 5 g sample) were >80% for Aroclors 1254 and 1260. Recoveries generally were poorer with Aroclors of lower chlorine content and decreased with decreasing quantity of Aroclor present. The DDT group was not quantitatively determined; recoveries averaged ≥60% for the DDT group in the ppm range (based on a 5 g sample). The procedure was successfully applied to samples of Lake Michigan chubs containing residues of PCBs and the DDT group and to extracts of human serum fortified with Aroclors and the DDT group.

The multipesticide residue methods used by the Food and Drug Administration (1, 2) recover polychlorinated biphenyls (PCBs) along with many organochlorine pesticides. PCBs are marketed in the United States under the trade name Aroclor®. Because of the similarity of gas-liquid chromatographic (GLC) retention times, the presence of chlorinated pesticides such as DDT or its analogs and the multicomponent PCBs in the same sample can hinder the identification and quantitation of PCBs and the pesticides. Various liquid-solid column chromatographic techniques have been used before GLC detection to separate PCBs from the DDT group: silicic acid and Celite (3), silica gel (4, 5), and charcoal (6). These procedures represent the ideal situation in which PCBs and the DDT group are separated without being chemically altered in the process. However, they often involve a considerable amount of time and reagents while yielding varying degrees of success.

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An earlier attempt to devise a chemical means of separating pesticides from PCBs was a nitration procedure used by Jensen and reported by Reynolds (7). A cleaned up extract containing PCBs and the DDT group was treated with concentrated HNO_3 and H_2SO_4. Reynolds reported an apparently nitrated DDT compound with a retention time of about 2 hr but concluded, similar to Armour and Burke (3), that PCBs and DDT were not separated satisfactorily. Mulhern *et al.* (8) used CrO_3 to oxidize DDE to dichlorobenzophenone (DCBP) and thus remove the interference in the thin layer chromatographic estimation of PCBs. Young and Burke (9) completely dehydrochlorinated DDT to DDE with ethanolic KOH. Aroclors were unaffected and were recovered completely through the procedure described. In order to determine DDT-type compounds in the presence of PCBs, Miles (10) dehydrochlorinated DDT and TDE with 1,5-diazobicyclo(5.4.0)undec-5-ene and oxidized the resulting olefins to DCBP. DCBP was separated from PCB on a Florisil column before determining "total DDT" by GLC of the DCBP.

Our study was directed toward KOH dehydrochlorination and CrO_3 oxidation reactions of the DDT compounds to produce the more polar DCBP, and developing a micro column chromatographic technique for separating DCBP before GLC determination of PCBs. An important objective was a micro scale procedure yielding a low reagent blank, applicable to both human tissue and food samples where PCBs must be quantitatively determined by GLC in the presence of DDT residues. The electron capture GLC response to the DDT group is substantially greater than to an equal quantity of underlying multicomponent PCBs; therefore DDT residues must be removed. Small quantities of PCB sought in human-derived samples required quantitative recovery of microgram or smaller amounts of PCBs.

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METHOD

Reagents

(a) *Solvents.*—Petroleum ether, hexane, ethyl ether, and methanol suitable for use in pesticide residue analysis; distilled-in-glass is satisfactory (available from Burdick & Jackson Laboratories, Inc., 1973 S. Harvey St., Muskegon, MI 49442).

(b) *Florisil.*—60–100 mesh PR grade (Floridin Co., Berkeley Springs, WV 25411). If received in plastic containers or paperboard boxes, transfer Florisil to glass containers with glass-stoppered or foil-covered lids and store in dark. Heat ≥5 hr but preferably overnight at 130°C before use. Store at 130°C in glass-stoppered or foil-covered bottles.

(c) *Alkali dehydrochlorination reagent.*—Dissolve 2 g KOH (ACS, anhydrous pellets) in 100 ml ethanol (USP, 95%).

(d) *Oxidation reagent.*—Dissolve 1.5 g CrO_3 (ACS, Fisher Scientific Co., Springfield, NJ 07081) in 1.0 ml water. Add 50 ml glacial acetic acid and mix. Prepare fresh daily. (*Caution:* CrO_3 is a very strong oxidant.)

(e) *Antimony pentachloride.*—Reagent grade (Matheson Coleman & Bell, 2909 Highland Ave., Norwood, OH 45212).

(f) *Carborundum chips.*—Silicon carbide grain, 20 mesh (Carborundum Co., P.O. Box 337, Niagara Falls, NY 14302).

Apparatus

(a) *Kuderna-Danish evaporative concentrator.*—K-570000. Equipped with ₸ 24/40 3-ball Snyder column (K-503000), 500 ml flask (K-570001), and ₸ 19/22 Mills concentrator tube, 10 ml size, graduated in 0.1 ml to 1.0 ml (K-570050) (Kontes Glass Co., Vineland, NJ 08360).

(b) *Tube heater.*—K-720001. Equipped with heater block (K-720003) (Kontes Glass Co.).

(c) *Disposable pipets.*—5¾" (No. 8006132, Glass Products Co., 30 Mayslanding Rd., Somers Point, NJ 08344).

(d) *Gas chromatographic systems.*—Searle-Analytic, Des Plaines, IL 60018: (*1*) Model 5360 with 6' × 4 mm id glass column packed with 10% DC-200 on 80–100 mesh Chromosorb W (HP). Operating conditions: column flow, 120 ml nitrogen/min; column and detector, 200°C; injector, 225°C; pin-cup design electron capture detector with titanium ^3H foil; detector voltage (constant dc) adjusted to cause one-half full scale recorder deflection for 1 ng heptachlor epoxide (27 ng Aroclor 1254) when full scale deflection is 1×10^{-9} amp.

(*2*) For greater sensitivity with lower quantities of PCBs: Model 4720-B with 6' × 4 mm id glass column packed with 10% DC-200 on 80–100 mesh Chromosorb W (HP). Operating conditions: column flow, 120 ml nitrogen/min; column, 200°C; detector, 253°C; injector, 225°C; parallel-plate design electron capture detector (Tracor, 6500 Tracor Lane, Austin, TX 78721) with ^{63}Ni foil; detector voltage (constant dc) adjusted to produce maximum response when full scale deflection is 1×10^{-9} amp (one-half recorder scale deflection for 0.6 ng Aroclor 1254).

Procedure

Procedure is applicable to samples extracted and cleaned up for GLC determination by usual methods for organochlorine pesticides and PCBs, such as multipesticide residue method of AOAC (*2*). Cleaned up extract should be in solvent such as hexane or 6% ethyl ether-petroleum ether eluate from Florisil. In addition to eliminating interference of DDT and its analogs in determination of PCBs, the procedure described will permit semiquantitative assay for DDT, DDE, and TDE as "total DDT," measured as DCBP derivative. If sample contains TDE as well as DDT and DDE and individual determinations are necessary, method reported by Miles (*10*) can be used to differentiate these compounds.

Dehydrochlorination

Concentrate cleaned up sample to 2 ml in Mills tube. Add 1 ml 2% ethanolic KOH dehydrochlorination reagent and 2–3 SiC chips and fit tube with micro Snyder column. With test tube clamp, hold tube over opening in steam bath in such a manner that gentle boiling ensues. After volume of solution has been reduced to ca 1 ml, insert tube completely into steam bath and heat more vigorously for 15 min or until volume is ca 0.2 ml. Remove tube from steam bath. If precipitate has formed, add few drops of 2% ethanolic KOH and heat gently in steam bath with swirling until precipitate dissolves. Let solution reach room temperature. Pipet 5 ml hexane into tube, followed by 2 ml ethanol–water (1+1). Stopper tube with glass stopper, invert, shake vigorously ca 30 sec, and let layers separate sharply. Remove by pipet 4 ml hexane (upper) layer into clean Mills tube. Repeat extraction of aqueous phase as before with additional 5 ml hexane (pipet), shaking vigorously 30 sec. Remove 5 ml hexane layer by pipet and combine with first extract.

Oxidation

Carefully concentrate combined hexane extracts in Mills tube to ca 0.2 ml, using Kontes tube heater at about 60°C with slight stream of nitrogen directed on hexane solution, or concentrate on steam bath after adding 2–3 SiC chips and attaching micro Snyder column to tube. Solution must

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not go to dryness. Add 1.0 ml CrO_3 oxidising reagent and 2–3 SiC chips. Attach micro Snyder column to tube and heat 15 min in steam bath (gently at first and then more vigorously). Let cool slightly and add by pipet 5 ml hexane followed by 4 ml water. Stopper tube with glass stopper, invert, and shake vigorously ca 30 sec. Let solvent layers separate completely. Remove by pipet 4 ml hexane (upper) layer into clean Mills tube. Repeat extraction of aqueous phase as before with additional 4 ml hexane (pipet), shaking vigorously 30 sec. Remove 4 ml hexane layer by pipet and combine with first extract.

Florisil Separation of PCB from Dichlorobensophenone

Carefully concentrate combined hexane solutions from oxidation to 1 ml, using Kontes tube heater with slight stream of nitrogen directed on the hexane solution, or concentrate in steam bath after adding 2–3 SiC chips and attaching micro Snyder column to tube. Solution must not go to dryness. Insert small glass wool plug into tip of disposable pipet and mount pipet as chromatographic tube. Fill disposable pipet with ca 7.5 cm (0.5 g) activated Florisil. Place ca 0.2 cm anhydrous Na_2SO_4 on top of Florisil. Wash Florisil column with 5 ml petroleum ether, discarding eluate. Place clean Mills tube under Florisil column to collect first eluate. Carefully and completely transfer concentrated hexane solution to column, using 2–3 ca 0.5 ml rinses with petroleum ether. Elute Florisil column with petroleum ether until 10 ml eluate is collected. PCBs are eluted in first eluate. Change receiver to another Mills tube to collect second eluate. Elute column with ethyl ether-petroleum ether (1+1) until 10 ml eluate is collected. DCBP is eluted in second eluate. At this point if perchlorination of PCBs to fully chlorinated decachlorobiphenyl is desired, proceed as in method of Armour (11).

Gas Chromatographic Determination

Concentrate or dilute eluates from Florisil column to suitable definite volume for GLC analysis. Determine quantities of PCBs and DCBP by reference to standards in usual manner. Adjust calculations for the fact that theoretically only 99% PCBs and DDT (DCBP) can be recovered through dehydrochlorination and oxidation steps. If dehydrochlorination step is omitted, correction for extraction recovery in oxidation step will be 96% for PCB, DDE, and TDE-olefin (1-chloro-2,2-bis(*p*-chlorophenyl)ethylene). Multiply quantity of DCBP determined by molecular weight factors (molecular wt DDT compound/molecular wt DCBP) 1.41, 1.27, 1.27, and 1.13 to convert weight of DCBP to equivalent DDT, DDE, TDE, and TDE-olefin, respectively. To approximate amount of "total DDT" from DCBP in samples containing combination of DDT-group compounds, use 1.3 as average of above molecular weight factors.

Results and Discussion

The procedure described was developed to permit quantitative estimation of PCBs in samples also containing residues of the DDT group. The stability of PCBs permits dehydrochlorination and oxidation reactions to convert the DDT-group compounds to the more polar DCBP derivative which can be separated from unreacted PCBs by column chromatography on Florisil. A micro scale procedure is less likely than a procedure requiring larger amounts of reagents to result in laboratory blank values that would inhibit the analysis for low levels (less than about 0.25 ppm) of PCB residues. The procedure should be applicable to analysis for PCBs in human and environmental media where larger scale PCB–DDT group separation procedures, such as column chromatography on silicic acid (3), can give prohibitively high laboratory blanks. At higher residue levels, the small amount of sample required permits repeat determinations by this or possibly another technique with aliquots of a cleaned up sample extract. Often the ratio of PCB to DDT-group residues is such that the latter can be measured directly by GLC, using a diluted portion of the cleaned up extract. The procedure described then provides an alternative to column chromatography to isolate the PCB residue for GLC measurement of a more concentrated solution.

Recoveries of Aroclors 1242, 1254, and 1260, each in the presence of DDT, DDE, or TDE, were determined after fortifying hexane, hexane extracts of human serum, and cleaned up extracts of Lake Michigan chubs and proceeding with the above separation procedure. Aroclor recoveries were determined by comparing the sum of the heights of 5 major peaks for the sample and reference Aroclor. Table 1 lists the recoveries of Aroclors from hexane with no sample extract present. Table 2 lists the recoveries of Aroclors from the hexane extracts of human serum. Three 15 ml hexane extracts of 5 g serum were combined and concentrated before fortification. The total extract was carried through the procedure. Aroclor recovery did not depend on

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Table 1. Recoveries of Aroclors in hexane solution after separation from a DDT-group compound[a]

Aroclor	Added, μg	Perchlorinated	Rec., %	No. of trials	Range, %
1242	6	yes	49	3	42–55
1242	9	no	79	3	77–82
1242	56	yes	59	3	56–65
1242	56	no	82	4	78–86
1254[b]	0.08	no	60	3	56–63
1254	4	yes	71	3	65–74
1254	4	no	83	5	77–90
1254	6	no	84	3	83–84
1254	17	no	84	4	77–109
1254	40	yes	80	3	78–82
1254	40	no	86	3	85–87
1260[b]	0.07	no	53	3	52–66
1260	4	no	85	4	82–90

[a] Hexane solution of Aroclor and DDT compound; 2–33 μg *p,p'*-DDT, *p,p'*-DDE, or *p,p'*-TDE.
[b] Corrected for reagent blank, 9.6 ng.

Table 2. Recoveries of Aroclors in hexane extract of human serum after separation from a DDT-group compound[a]

Aroclor	Added, μg	Perchlorinated	Rec., %	No. of trials	Range, %
1242[b]	0.17	no	46	2	41–46
1242[b]	0.56	yes	35	2	30–39
1242	5.6	yes	39	3	35–42
1242	5.6	no	80	3	77–82
1254[b]	0.08	no	75	2	74–75
1254[b]	0.40	yes	73	3	71–75
1254[b]	0.40	no	82	3	81–83
1254	4.0	no	87	4	82–91
1260[b]	0.07	no	71	3	69–74

[a] Added to hexane extract of 5 g human serum; 2–33 μg *p,p'*-DDT, *p,p'*-DDE, or *p,p'*-TDE.
[b] Corrected for serum blank, 19 ng.

the DDT-type compound separated. The results show that the procedure is applicable to the quantitative determination of the more chlorinated PCBs (Aroclors 1242, 1254, and 1260). Recoveries for at least 4 μg Aroclor 1254 or 1260, without perchlorination, were greater than 80%; with 0.07 μg Aroclor 1254 or 1260, recoveries were at least 60%. Less than 100% recovery is probably attributable to losses due to volatility and to reaction during oxidation of the less chlorinated components of the Aroclors studied. Diminution of the less chlorinated components of Aroclor 1242 was evident in the GLC curve following CrO_3 treatment. Volatility losses must be guarded against at all times. It is imperative that no solution containing compounds to be determined be allowed to reach dryness. In Fig. 1A, the gas chromatogram of a 6% Florisil eluate (1) from a Lake Michigan chub sample contains 2 large peaks due to 1.3 ppm *p,p'*-DDE and 2.2 ppm *p,p'*-DDT. These amounts of DDE and DDT permitted their determination without separation from the PCB residue which was also present. However, it was not possible to estimate the quantity of PCBs without removing the DDT residues. After applying the above method to another portion of the 6% Florisil eluate which represented 8.8 g chubs or 1.5 g fat, the chromatogram in Fig. 1B is free of peaks for DDT and analogs; PCBs present were determined as 2.3 ppm by comparison with Aroclor 1254. To verify applicability of the procedure to extracts of fish which often contain residues of both PCBs and DDT, PCB recovery was determined. Portions of the 6% Florisil eluate representing 8.8 g Lake Michigan chubs were fortified with 3.5 ppm Aroclor 1254. After removing DDE and DDT by the above procedure and subtracting for 2.3 ppm PCB residue found in the chubs, recoveries averaged 83% with a range of 80–85% for 3 replicate fortifications. Recovery of "total DDT" was determined by applying the procedure to the 6% Florisil eluate, representing 8.8 g chubs fortified with 2.7 ppm *p,p'*-DDE and 5.0 ppm *p,p'*-DDT. Recoveries averaged 52% with a range of 47–55% for 3 replicate determinations after subtraction for 1.3 ppm *p,p'*-DDE and 2.2 ppm *p,p'*-DDT found in the chubs. Table 3 gives average recoveries from hexane solution for *p,p'*-DDT, *p,p'*-DDE, and *p,p'*-TDE after derivatization and separation from PCBs, with no sample extract present. The recovery did not depend on the amount of pesticide in the range studied. Recoveries are not quantitative; results averaged 60%. Recoveries under the same conditions for *o,p'*-DDT, *o,p'*-DDE, and *o,p'*-TDE were all erratically low (5–43%) with 4–12 μg pesticide in hexane.

When the procedure was carried out with reagents only, the reagent blank gave a gas chromatogram resembling that of Aroclor 1254 and averaged 1.9 ppb PCBs by comparison with Aroclor 1254, based on a 5 g sample with a range of 1.7–2.0 ppb PCBs for 4 replicate determinations. A hexane extract of human serum gave a gas chromatogram which also resembled that of Aroclor 1254 and averaged 1.9 ppb

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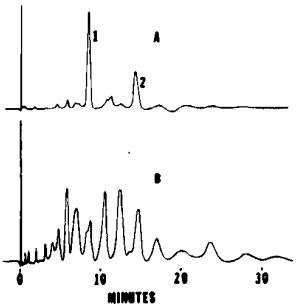

FIG. 1—Electron capture GLC curves from 6% Florisil eluate of Lake Michigan chubs, before and after separation of PCBs from p,p'-DDE and p,p'-DDT, using the described procedure:

A, Before separation, 0.4 mg equivalent fish injected, containing 1.3 ppm p,p'-DDE (Peak 1), 2.2 ppm p,p'-DDT (Peak 2), and PCB residue; B, after separation, petroleum ether eluate from micro Florisil column, 7 mg equivalent fish injected, containing 2.3 ppm PCBs calculated as Aroclor 1254.

PCBs above the reagent blank by comparison with Aroclor 1254, based on a 5 g sample with a range of 1.8–2.1 ppb PCBs for 3 replicate determinations. Contamination by PCBs or electron-capturing substances, as evidenced by the reagent blank, can come from reagents, glassware, laboratory equipment, etc. Care must constantly be exercised to keep contamination at a minimum. In analysing for low amounts of PCBs, it may be necessary to wash the Florisil, Na_2SO_4, and glass wool with petroleum ether to remove contamination. The Florisil used in this study was received in amber glass bottles with Teflon-lined caps. Glassware was cleaned thoroughly with a saturated solution of $K_2Cr_2O_7$ in concentrated H_2SO_4 and carefully rinsed with distilled water, followed by acetone and petroleum ether of distilled-in-glass quality.

Table 3. Recoveries of p,p'-DDT, p,p'-DDE, and p,p'-TDE after separation from an Aroclor PCB[a]

Pesticide	Added, μg	Rec., %	No. of trials	Range, %
p,p'-DDT	2–32	80	10	41–82
p,p'-DDE	30	63	3	58–67
p,p'-TDE	3–33	63	5	49–86

[a] Hexane solution of DDT-group compound and Aroclor; 0.07–56 μg Aroclor 1242, 1254, or 1260.

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