Quantitative Perchlorination of Polychlorinated Biphenyls as a Method for Confirmatory Residue Measurement and Identification

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The perchlorination procedure for derivatization of PCBs described by Berg, Diosady, and Rees has been modified to achieve a micro-scale quantitative conversion (greater than 90%) of commercial PCB preparations (Aroclors) to decachlorobiphenyl. Cleaned up sample extracts containing PCB residues (1–20 μg) are allowed to react with antimony pentachloride in the presence of chloroform to form decachlorobiphenyl. This procedure converts a multicomponent mixture to a single derivative detectable by electron capture GLC, thus providing an easy method for quantitating and identifying PCB residues and at the same time increasing the sensitivity of detection. The usefulness of the perchlorination procedure is demonstrated by comparing results for environmentally contaminated samples quantitated by 2 methods: by measuring the total area of the electron capture response for the residue against the Aroclor it most closely resembles, and by measuring the single peak of the decachlorobiphenyl derivative and expressing the results in terms of the particular Aroclor.

Analytical methods normally employed for the extraction, cleanup, and detection of polychlorinated biphenyl (PCB) (1–3) residues are generally those used for organochlorine pesticides (4). Quantitative values are usually obtained by measuring the total area of a PCB residue response as shown on a gas chromatographic (GLC) tracing using an electron capture or microcoulometric detector and comparing it with the total area of a similarly obtained response of the commercial PCB preparation (Aroclor®) (5) it most closely resembles (6). This has proved to be a practical procedure for quantitation; however, it is limited because of the lack of sensitivity, the multicomponent nature of PCB residues, the alterations in residue pattern as a result of metabolic and environmental changes, and the need to rely on commercial Aroclors as reference materials.

Procedures to convert all PCB components to a single derivative, e.g., biphenyl or decachlorobiphenyl (DCB), that is indicative of the total PCB residue and that could be used for quantitative purposes have previously been reported (7, 8). In this study, the perchlorination procedure described by Berg, Diosady, and Rees (7) has been modified to achieve complete conversion on a micro-scale of all PCB components in a cleaned up sample extract to decachlorobiphenyl, thus providing a qualitative and quantitative confirmatory procedure for PCB determination. A quantitative value obtained by measuring the single GLC peak of a DCB derivative against a DCB standard of known purity reinforces the residue value achieved by the measurement of a multicomponent PCB residue. It also greatly increases the sensitivity with which PCBs can be detected and measured. Measuring the single GLC response for DCB will also minimize necessary analytical judgments involved in the physical measurement of the GLC curve of a multicomponent residue (i.e., position of baseline and method of integration) and in the discrimination between PCB and non-PCB components.

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METHOD

Reagents

(a) *Antimony pentachloride.*—Allied Chemical Reagent Code 1365.

(b) *Sodium sulfate.*—Anhydrous, granular. It may be necessary to wash with acetone and ethyl ether to remove electron-capturing substances.

(c) *HCl.*—6*N* (1 + 1 dilution of concentrated HCl in water).

(d) *Sodium bicarbonate.*—10% aqueous solution.

(e) *Solvents.*—Hexane, chloroform, and methanol 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).

Apparatus

(a) *Vacuum hydrolysis tube.*—150 × 10 mm od with No. 4 Teflon valve (Kontes Glass Co. No. K896860-0004) or Carius Pyrex combustion tube, 200 × 8 mm id, 10 mm od (Corning No. 8640).

(b) *Silicone oil bath and heating element.*—Capable of maintaining temperature at 165–175°C.

(c) *Kuderna-Danish evaporative concentrator.*—With Snyder column and collection tube, 500 ml capacity (Kontes Glass Co. No. K5470000, or equivalent).

(d) *Micro-Snyder column.*—ⅎ 19/22, column size 2–19 (Kontes Glass Co. No. K569001, or equivalent).

(e) *Gas chromatograph.*—Equipped with concentric design electron capture detector operated at a dc voltage to produce ½ full scale recorder deflection for 1 ng decachlorobiphenyl when full scale deflection is 1×10^{-9} amp, and 6′ × 4 mm id glass column of either: (*1*) 1% OV-101 on 100–120 mesh Gas-Chrom Q (W. W. Wiencke, 1972), 120 ml nitrogen/min carrier gas, column 220°C, injector 230°C, detector 200°C; (*2*) 3% Dexsil 300 on 100–120 mesh Gas-Chrom Q (W. W. Wiencke, 1972), 60 ml nitrogen/min carrier gas, column 240°C, injector 250°C, detector 200°C.

Perchlorination Procedure

Extract and clean up sample to be perchlorinated by procedures (such as the FDA multiresidue pesticide procedure (9)) leaving the residues in solvents that can be selectively evaporated from chloroform (6% ethyl ether-petroleum ether from Florisil or petroleum ether fraction from silicic acid). Transfer 1–2 ml cleaned up extract containing 1–20 μg PCB residue to vacuum hydrolysis tube (a) (Carius tube may be substituted). Add several drops chloroform and 1–2 20-mesh carborundum chips, and reduce volume to 0.1 ml by carefully heating tube on steam bath. Let tube cool to room temperature, add 2 ml chloroform and 1–2 carborundum chips, and reduce volume to 0.1 ml on steam bath. Repeat addition of chloroform, concentrate to 0.1 ml, and cool to room temperature. Working in well ventilated hood, carefully add 0.2 ml antimony pentachloride to tube. Immediately seal tube tightly to ensure closed system reaction. (*Note:* It may be necessary to cool Teflon valves in ice before fitting into vacuum hydrolysis tube. Narrowed bottom of valve should fit flush against constriction in glass when sealed. Carius tubes should be sealed in an oxygen flame.) Immerse tube 2–3″ in oil bath heated to 165–175°C and let reaction proceed overnight (ca 15 hr). Remove tube from bath and cool to room temperature. In hood, and while pointing the venting side arm of vacuum hydrolysis tube away from face, open by slowly unscrewing valve and pulling it out of tube. (Carius tube should be scored with a file and snapped open in hood at arm's length away from face; use extreme precaution since there is a rapid release of pressure and sometimes splintered glass.) Slowly add 1 ml 6*N* HCl to reaction mixture, tap tube lightly to mix, and quantitatively transfer mixture to 30 ml separatory funnel, rinsing with several small portions of HCl: use a total of 5 ml 6*N* HCl. (*Note:* It may be necessary to rock tube back and forth or tap gently in order to drain.) Rinse reaction tube with several small portions of hexane, using a total of 15 ml hexane, and quantitatively transfer rinsings to the 30 ml separatory funnel containing the HCl. Shake funnel vigorously 1 min; let layers separate. Drain lower HCl layer into second 30 ml separatory funnel containing 15 ml hexane and shake 1 min. Drain HCl layer into third separatory funnel containing another 15 ml hexane and repeat extraction. Discard HCl layer and combine the 3 hexane extracts in 125 ml separatory funnel. Wash hexane extract successively with two 20 ml portions of water, one 20 ml volume of 10% $NaHCO_3$ solution, and finally 2 additional 20 ml portions of water. Discard all washes. Dry washed hexane extract by passing through 2″ column of anhydrous sodium sulfate and rinse column with 100 ml hexane, collecting hexane extract and rinse in Kuderna-Danish concentrator. Add few drops methanol and concentrate on steam bath to ca 3 ml. Separate volumetric receiver from concentrator, attach micro-Snyder column to receiver, and concentrate extract to final volume <0.5 ml to remove traces of chloroform. Dilute to suitable definite volume for GLC determination. Perform GLC assay and calculations as described in *Discussion*.

Discussion

The micro-scale perchlorination procedure of Berg, Diosady, and Rees was evaluated to determine its utility as a routine laboratory procedure for confirmatory PCB quantitation. Parameters for quantitative extraction of DCB from the reaction mixture were not clearly defined by these authors and needed to be established prior to testing the procedure for reproducibility. Even after we developed a method of extraction that would recover greater than 95% of DCB standard from the reaction mixture, perchlorination using the reaction conditions of Berg *et al.* resulted in 90–100% conversion of Aroclor 1254 to DCB but only 30–70% conversion of Aroclor 1242. Conditions contributing to inconsistency in behavior between Aroclor 1242 and Aroclor 1254 needed to be determined and modifications needed to be made to achieve conversion of all PCB components in the various Aroclors to DCB.

Perchlorination

The perchlorination procedure was designed for use following extraction, cleanup, ancillary (separation), and detection procedures used in multipesticide residue methodology. In the Berg *et al.* procedure, cleaned up extracts containing PCBs were evaporated to dryness before reaction with

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antimony pentachloride. The observed nonreproducible conversion of Aroclor 1242 to DCB with the original procedure is attributed to volatilization during evaporation to dryness of the lower molecular weight PCB components present in Aroclors with less than 54% chlorine substitution. To reduce the loss, evaporation is discontinued while a small amount of solvent (0.1 ml) still remains. However, this limits the types of solvents which can be used since they may interfere during the perchlorination step. It was found that solvents with more than one carbon atom reacted exothermically with antimony pentachloride to form a solid black product. Chloroform as the solvent, however, minimized volatility, reacted favorably at the given conditions, and permitted selective evaporation of all solvents used in cleanup and separation procedures.

Perchlorination is accomplished in a sealed reaction tube at elevated temperature and pressure. Increased pressure produced by the presence of chloroform vapors increased the hazards of handling the Carius tubes recommended for use by Berg *et al.* In place of the Carius tube that required sealing in an oxygen flame and careful opening, a reaction tube with the same volume specifications, tight Teflon sealing valve, and venting side arm is recommended for use (*see* Fig. 1).

Parameters for extraction of DCB from the reaction mixture were developed to completely recover (greater than 95%) DCB standard. Traces of chloroform present in the extract must be removed before electron capture GLC determination. This can be done by adding methanol which forms an azeotrope with chloroform and evaporating to a small volume before diluting. The resulting extract is sufficiently clean for electron capture GLC determination of DCB.

In experiments designed to determine the feasibility of a shorter reaction time, iron was tested as a catalyst. One mg samples of Aroclor 1242 were reacted and extracted as described for overnight conditions except that 2 mg iron powder was added after the addition of antimony pentachloride, the tube was sealed, and the sample was reacted for 4 and 6 hr. Results are discussed under *Recoveries*.

By-products that would interfere with quantitative derivatization were not evident in our perchlorination experiments; however, D. L. Stalling (1972) has found that a brominated by-product is formed when the antimony pentachloride is contaminated with bromine. Care should be taken to avoid use of contaminated antimony pentachloride.

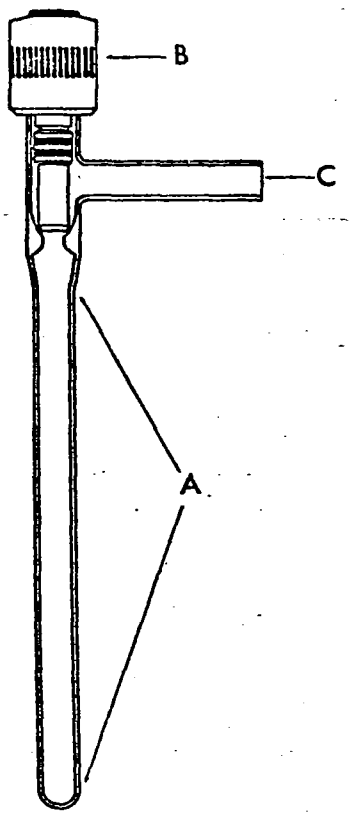


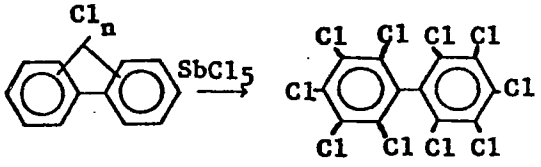
FIG. 1—Vacuum hydrolysis tube recommended for sealed system reaction: A, dimensions 10 mm od × 150 mm; B, tight Teflon sealing valve; C, venting side arm.

GLC systems suggested for DCB determination are those originally developed for the detection of polychlorinated terphenyls (W. W. Wiencke, 1972). At GLC conditions given in the *Method*, DCB elutes in 3–4 min on the 1% OV-101 column and in 12 min on the 3% Dexsil 300 column. These fast eluting systems produce increased GLC sensitivity to allow easy detection and quantitation of as little as 0.2 ng DCB.

Quantitation

Although the perchlorination procedure has practical applications in PCB quantitation, it is not meant to completely replace procedures normally used for this purpose. The analyst should still be aware of the identity of the contaminating

Aroclor(s), the presence of other residues which could interfere, and any alteration in Aroclor pattern which may be due to metabolism and/or environmental exposure. This information may be useful in tracing the source of contamination and may have toxicological significance. Some laboratories may wish to express residues in terms of ppm DCB in order to establish a uniformity in their reporting. However, when the procedure is used for quantitative confirmation, DCB is expressed in terms of a specific Aroclor by using a factor for mathematical conversion. The factors used for converting DCB to the equivalent concentration of a specific Aroclor are derived by assuming the reaction to proceed as follows:



where n = number of chlorines = approximate whole number of chlorines calculated from per cent chlorine substitution for a specific Aroclor (*see* Table 1). The approximate whole number of chlorines in a specific Aroclor was chosen for calculations, since Aroclors are commercial preparations and the actual per cent chlorine may vary from the theoretical for each lot. The factor for each Aroclor (*see* Table 1) is the ratio of amount of Aroclor necessary to yield one unit of DCB:

X = factor for expressing DCB as a specific Aroclor

X = molecular wt Aroclor/molecular wt DCB (499)

Calculations for measuring PCB residues by GLC based on the DCB derivative formed and converting mathematically to express the residue in terms of a specific Aroclor are:

(DCB peak ht sample/DCB peak ht std) × ng std injected = ng DCB in sample injection

ng DCB in sample injection × factor for specific Aroclor (X) = ng Aroclor in sample injection

(ng Aroclor in sample injection/wt sample injected (mg)) = ppm Aroclor in sample

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Table 1. Factors to mathematically convert decachlorobiphenyl to an equivalent amount of Aroclor

Aroclor	Av. No. Cl[a]	MW[b]	X[c]
1221	1	188.5	0.38
1232	2	223	0.45
1242	3	257.5	0.52
1016	3	257.5	0.52
1248	4	292	0.59
1254	5	326.4	0.65
1260	6	361	0.72
1262	7	395.3	0.79
DCB	10	499	1

[a] Average whole number of chlorines calculated from per cent chlorine substitution for a specific Aroclor.

[b] Molecular weight of Aroclor based on the average whole number of chlorines calculated from per cent chlorine substitution.

[c] X = molecular wt Aroclor/molecular wt DCB (499). To convert ppm DCB to ppm of a specific Aroclor, multiply ppm DCB by X for the Aroclor.

Table 2. Per cent conversion of 1 μg PCB to decachlorobiphenyl at overnight conditions

Aroclor	Trials	Range, %	Average, %
1242	10	90–105	97
1016	5	87–105	95
1248	5	98–102	99
1254	5	95–104	100
1260	5	90–95	93
1262	3	89–103	95

Recoveries

To determine the per cent conversion of specific Aroclors to DCB, a series of 1 μg of 6 different Aroclors was allowed to react at the given overnight conditions and was extracted as described. The 1% OV-101 column was used for the gas chromatographic determinations. Results are given in Table 2. The method demonstrated good reproducibility for quantitative conversion of the 6 Aroclors to DCB with results ranging from 87 to 105% and an average recovery of 97%.

In the experiments with shorter reaction times, 10 trials with 4 hr reactions using Aroclor 1242 gave a wide range of results, although the average conversion to DCB was 90%. Trials with 6 hr reaction gave results falling in a much narrower range and averaging 102% conversion. Results from these and experiments using the 6 Aroclors at 6 hr conditions are given in Table 3. The perchlorination is quantitative (99% average conversion for 6 Aroclors) and reproducible (range 83–110%) for 6 hr reaction with iron catalyst but offers little advantage over the convenient overnight conditions.

In order to determine the behavior of the perchlorination procedure in the presence of extracts of fatty samples, 3 g samples of chicken fat and corn oil were extracted by FDA multipesticide residue methodology (9). The 6% ethyl ether-petroleum ether fractions from Florisil cleanup were fortified with Aroclor 1242 at 6.7 ppm. Three aliquots of the cleaned up chicken fat, each equivalent to 0.15 g fat, and 2 samples of the cleaned up corn oil, each equivalent to 3 g, were perchlorinated at overnight conditions with average recoveries of 107 and 96%, respectively. These results show that the method can tolerate cleaned up extracts of up to 3 g samples of fat and can handle 1–20 μg PCB.

Applications

Samples of fresh chub, fresh sturgeon, and eggs environmentally contaminated with PCBs, and chicken fat fortified with PCBs were extracted and cleaned up by FDA multipesticide residue methodology (9). Where necessary, PCBs were separated from pesticides by chromatography on silicic acid. The PCB residues were quantitated on electron capture GLC with a 10% DC-200 column (9, 10) by comparing the total area of the residue with total area of the Aroclor reference with most similar GLC pattern. Aliquots of the 6% ethyl ether-petroleum ether Florisil eluate equivalent to 3 g chicken fat, 0.2 g sturgeon fat, and 0.2 g egg were perchlorinated for qualitative and quantitative confirmation. The residue level reported for each sample after perchlorination was determined by measuring the DCB derivative (1% OV-101 column) and mathematically converting it to the equivalent amount of the Aroclor used as reference in the total area quantitation. Comparison of the residue levels obtained by the 2 determinative procedures is presented in Table 4. Results obtained by the 2 methods of quantitation are in close agreement, demonstrating the practical application of the perchlorination procedure for quantitative confirmation. Gas chromatograms of an egg sample (Aroclor 1242) quantitated for PCBs as a multicomponent residue before perchlorination and as the DCB derivative after perchlorination are shown in Fig. 2. In the case of the chub samples, the extracts containing residues of both PCB and DDT analogs were separated on silicic acid prior to quantitation of the multicomponent PCB residues. Aliquots equivalent to 0.3 g chub from the silicic acid petroleum ether (PCB) fraction were perchlorinated and results are compared (*see* Table 4). Gas chromatograms of a chub sample quantitated for PCBs (Aroclor 1254) as a multicomponent residue before perchlorination and as the DCB derivative after perchlorination are shown in Fig. 3. Figures 2 and 3 also illustrate the increase in sensitivity obtained by perchlorination. For approximately equivalent peak heights on GLC only about $\frac{1}{16}$ as much of the egg sample (Aroclor 1242) and $\frac{1}{10}$ as much of the chub sample (Aroclor 1254) were injected after perchlorination as was needed before perchlorination.

Organochlorine pesticides, if present in a sample, should be separated from PCBs prior to derivatizing the residue to DCB. Pesticides such as DDT may be chlorinated to derivatives other than DCB. Preliminary studies show that perchlorination of DDT itself results in the formation of several derivatives which are separated from DCB on the 3% Dexsil 300 column at the specified conditions and do not interfere.

Table 3. Per cent conversion of 1 μg PCB to decachlorobiphenyl with iron catalyst and 6 hr reaction

Aroclor	Trials	Range, %	Average, %
1242[a]	10	78–98	90
1242	10	96–110	102
1016	3	87–95	92
1248	3	95–100	98
1254	3	98–106	103
1260	3	91–108	98
1262	3	83–107	98

[a] 4 hr reaction.

Table 4. PCB residue levels in environmentally contaminated samples obtained by 2 different methods of quantitation

		Residue, ppm		
		By total area	By DCB[a]	
Sample	Ref. Aroclor		Trial 1	Trial 2
Chub	1254	3.5	4.2	
Chub	1254	3.4	4.2	
Chub	1254	4.2	5.1	
Chub	1254	3.4	4.5	
Sturgeon[b]	1248	28.4	28.6	28.4
Eggs	1242	7.1	6.8	7.1
Chicken fat[c]	1242	4.6	5.0	
Chicken fat[c]	1248	6.9	6.6	

[a] Expressed as reference Aroclor.
[b] Residue is reported on a fat basis.
[c] Samples fortified with Aroclor for an interlaboratory study.

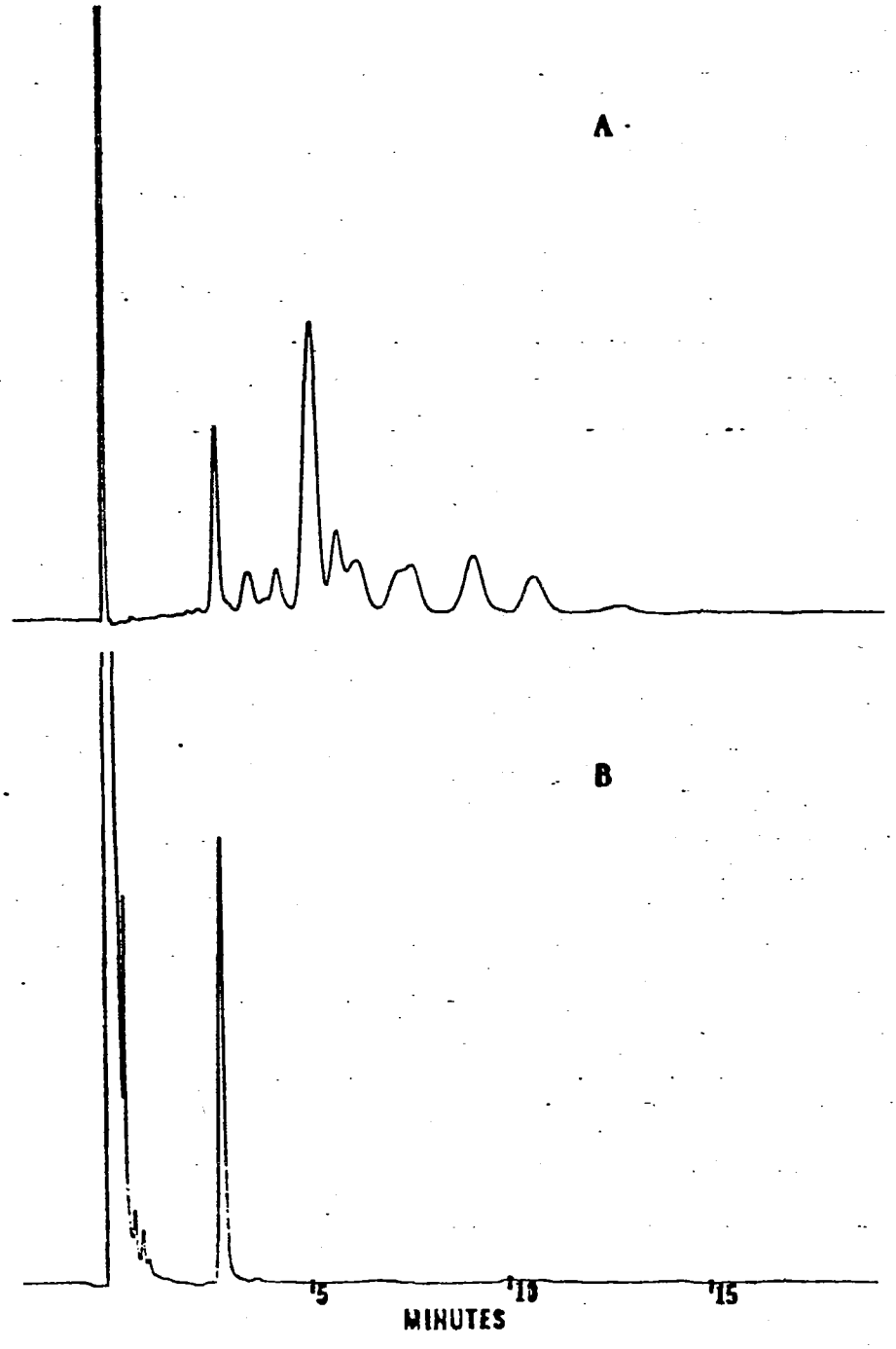
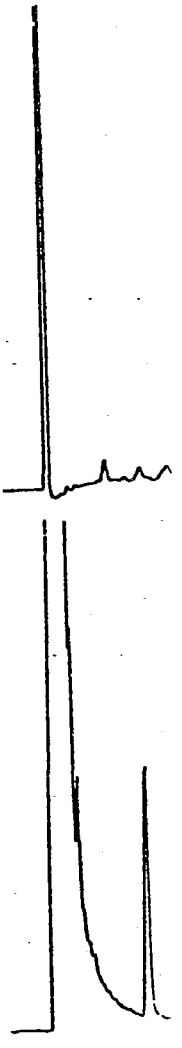


FIG. 2—GLC curves of egg samples containing 7.1 ppm PCBs (as Aroclor 1242) before and after perchlorination: A, 1.11 mg sample (7.9 ng Aroclor 1242), 6% Florisil eluate before perchlorination, 10% DC-200 column (9,10); B, 0.07 mg sample (0.5 ng equivalent Aroclor 1242), 6% Florisil eluate after perchlorination, 1% OV-101 column. GLC conditions given in *Method*.



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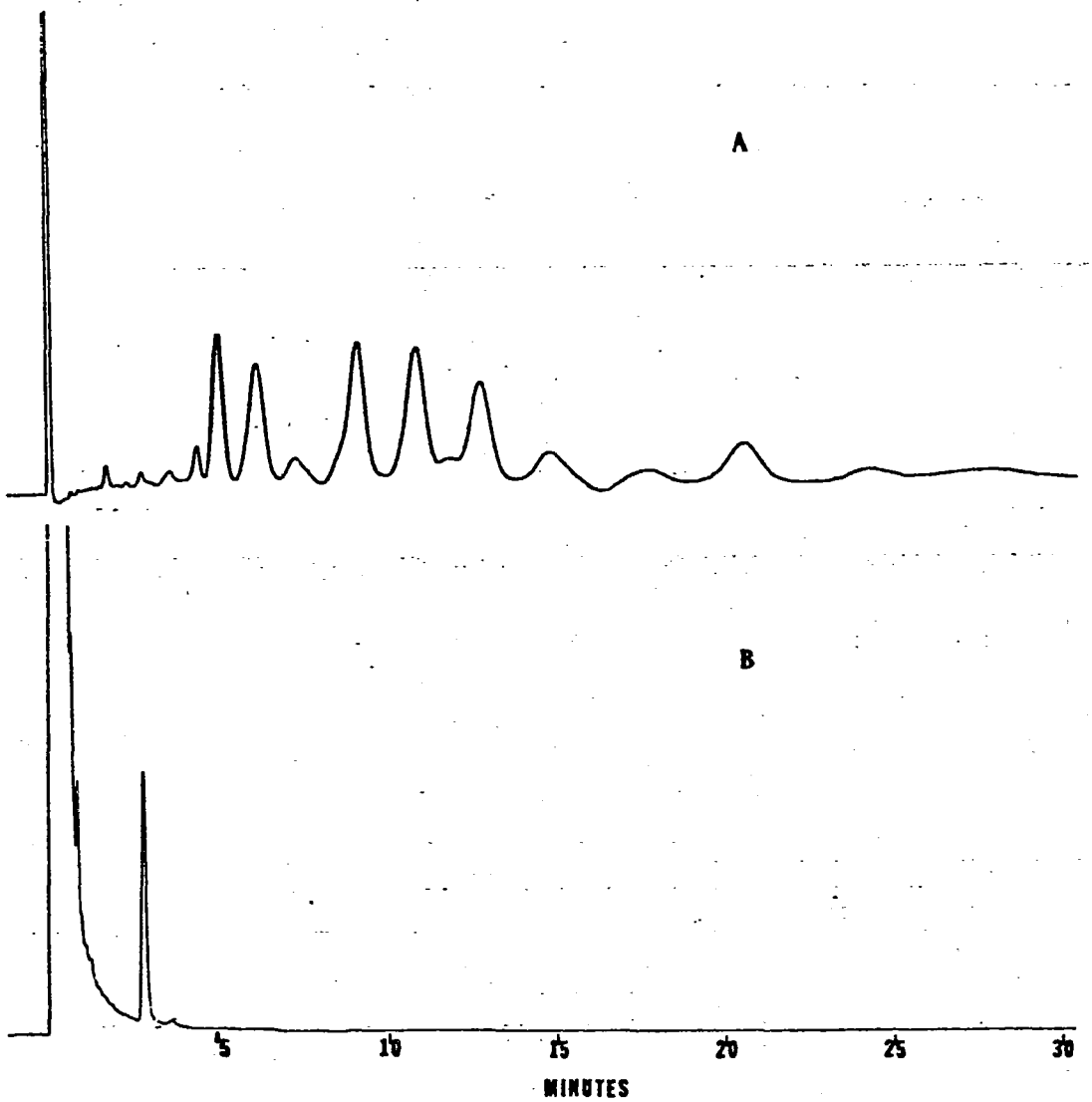

FIG. 3—GLC curves of chub sample calculated as 3.4 ppm PCBs (as Aroclor 1254) before perchlorination and calculated as 4.2 PCBs (as Aroclor 1254) after perchlorination: A, 1.5 mg sample (5.1 ng Aroclor 1254), petroleum ether eluate from silicic acid before perchlorination, 10% DC-200 column (9,10); B, 0.15 mg sample (0.63 ng equivalent Aroclor 1254), petroleum ether eluate from silicic acid after perchlorination, 1% OV-101 column. GLC conditions given in *Method*.

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