ANALYTICAL METHODOLOGY

FOR

POLYCHLORINATED BIPHENYLS

FEBRUARY 1973

FOOD AND DRUG ADMINISTRATION
ROCKVILLE, MARYLAND

MCNS 078783

Determination of Polychlorinated Biphenyl Residues in Foods, Feeds, and Paper Products

The following outline lists the steps in the analysis of foods, feeds, and paper products currently employed by the Food and Drug Administration for residues of polychlorinated biphenyls (PCB). In general, methodology for foods and feeds is that used in analyses for organochlorine pesticides, with auxiliary steps as necessary to separate residues of PCB and pesticides. Extraction of paper products takes advantage of the stability of PCB to treatment with alkali; electron capture GLC determination is the same as specified for food and feed analysis.

Methodology for the analysis of food and feeds is described in the FDA Pesticide Analytical Manual Volume I (PAM I). This manual and its revisions are available on request from Food and Drug Administration, Office of the Associate Commissioner for Compliance, 5600 Fishers Lane, Rockville, Maryland 20852. Description of the method for paper products and other techniques necessary to analysis for PCB residues are attached.

Methodology for Analysis for PCB Residues

	Food and Feeds	Paper
Sample Preparation*	PAM I, 141.	Cut into about 1/4"x1/4" pieces; thoroughly mixed. Attachment I
Extraction	PAM I, 211.13 or 212.13, depending on sample type	Alcoholic alkali reflux Attachment I
Cleanup	CH_3CN-petr ether partitioning and/or Florisil column chromatography: PAM I, 211.14 or 212.14, depending on sample type	Florisil column chromatography when necessary Attachment I
Auxiliary Procedures	Separation from pesticide residues, when necessary: PAM I, 251. Supplemental cleanup, when necessary: PAM I, 211.15, Attachment II	
Determination	Electron capture GLC: PAM I, 251.15 and 311 Attachments III, IV	Electron capture GLC: PAM I, 311 Attachments III, IV

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Procedures Useful for Confirmation	GLC with halogen specific detection	GLC with halogen specific detection
	Micro scale alkali reflux: Attachment II	TLC - see PAM I, 251.16 for references
	TLC - See PAM I, 251.16 for references	Perchlorination: Attachment V
	Perchlorination: Attachment V	
General	Attachment VI	Attachment VI

*Care must be used to avoid contamination of a sample during sampling, storage, or laboratory operations. In particular, storage containers should be checked before using to assure that no PCB are present.

The multi - component nature of PCB residues challenges even an experienced residue analyst in the identification and quantitation of the residue. Quantitation of a residue (see attachment II) is made by comparing the magnitude of GLC response from the residue to the magnitude of response from reference Aroclor(s) (Monsanto Company). Judgment must be made as to which reference Aroclor most closely resembles the GLC peak pattern detected in the sample. In many cases, particularly in animal tissue samples, the residue pattern may not resemble any single Aroclor and a choice must be made as to which Aroclor(s) would provide the most accurate result. Since the choice of auxiliary procedures depends to some degree on the identity of the residue, as discussed in PAM I, 251.16, the analyst must develop a high degree of skill in the application of this type of methodology and an ability to interpret GLC chromatograms correctly.

The limits of detectability of the methods are dependent upon: (1) the sensitivity of the determinative step (GLC) to the particular PCB mixture (Aroclor); and (2) the weight equivalent of sample injected for GLC. The magnitude of electron capture response to a given quantity of Aroclor 1242 is about one third of the response to the same quantity of Aroclor 1254. Therefore in the determination of residues exhibiting GLC patterns similar to Aroclor 1242, injection of a larger weight equivalent of sample is required than for residues similar in GLC pattern to Aroclor 1254. When using the electron capture - GLC sensitivity fixed by the parameters defined in PAM I 311.2, the following sample weight equivalents should be injected in order to obtain GLC response sufficient for detection and quantitation of residues similar to Aroclor 1242 at the tolerance levels:

Products	Approximate milligrams sample equivalent for EC-GLC
Milk (fat)	8
Dairy Products (fat)	8
Poultry (fat)	4
Eggs	40
Complete and finished animal feeds	100
Animal feed components	10
Fish and shellfish	4
Infant and junior foods	100
Paper food packaging material	2

U.S. Food and Drug Administration

January 22, 1973

ATTACHMENT I

Extraction and Cleanup for Determination of PCB in Paper and Paperboard

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(To be submitted to Journal of Official Analytical Chemists.)

METHOD

Apparatus

(a) West condenser.--400 mm jacket length with ℊ inner drip joint to fit Erlenmeyer.

(b) Erlenmeyer flask.--250 ml with ℊ outer joint to fit West condenser.

(c) Separatory funnel.--250 ml with Teflon stopcock.

(d) Chromatographic column.--with Teflon stopcock and coarse fritted plate, 22 mm id x 300 mm (Kontes Glass Co. Vineland, N.J., No. K-420540, size 233 or equiv.)

(e) Chromatographic column.--plain, 22 mm id x 300 mm (Kontes Glass Co. Vineland, N.J., No. K-420300, size 21 or equiv.)

(f) Kuderna-Danish concentrator.--500 ml capacity with Snyder column and volumetric and graduated receiving tube. (Kontes Glass Co. Nos. K-621400 and K-570050 or equiv.)

(g) Gas chromatograph.--Equipped with electron capture detector and 6'x4 mm id glass column containing either (1) 10% DC-200 or (2) 1:1 mixture of 15% QF-1 + 10% DC-200 on 80-100 mesh Chromosorb W(HP).

Operating conditions: N_2 flow, 120 ml/min; temperatures, column and detector 200°C, injector 225°C; concentric design electron capture detector operated at dc voltage to cause 1/2 full scale recorder deflection for 1 ng heptachlor epoxide when full scale deflection is $1x10^{-9}$ amp.

Reagents

(a) Florisil.--60/100 PR Grade: Floridin Co, 3 Penn. Center Blvd., Pittsburgh, Pa. 15235. Transfer immediately from bulk container to glass containers with glass stoppered or foil lined screw top lids and store in the dark. Heat at least 5 hours but preferably overnight at 130°C before use. Store at 130°C in glass stoppered or foil covered bottles. Alternatively, store stoppered container in desiccator at room temperature and reheat at 130°C after two days.

(b) Potassium hydroxide.--Anhydrous pellets.

(c) Ethanol.--USP 95% (methanol, redistilled in glass may be substituted.)

(d) Alcoholic KOH reflux reagent.--Prepare a 2% (g/ml) solution of KOH in ethanol.

(e) Petroleum ether.--Suitable for use with electron capture gas chromatography (Burdick and Jackson Laboratories Inc., 1953, S. Harvey St., Muskegon, Mich. 49442 or equiv.)

(f) Polychlorinated biphenyls.--Commercial mixtures (Aroclors) for reference in gas chromatographic determination. (Monsanto Company, St. Louis, Mo.)

Extraction

Paper sample should be cut into pieces no larger than about 1/4"x1/4" and thoroughly mixed. Weigh 10g of the cut up and well mixed paper material into 125 ml Erlenmeyer flask. Do not pack sample tightly. (See note below if the volume of 10g of sample material is greater than about 50 ml.) Add 60 ml 2% ethanolic or methanolic KOH, and fit flask with a West condenser cooled with circulating cold tap water. Reflux gently on steambath for 30 minutes. When refluxing is complete, rinse down inside of condenser with a small volume of alcohol before removal from flask. Transfer the reflux solution using a small funnel with glass wool plug to a 250 ml separatory funnel, avoiding transfer of any paper material. Rinse paper and flask with three, 40 ml portions of petroleum ether, combining petroleum ether rinses in the separatory funnel. Add 60 ml water to the separatory funnel and shake vigorously for 30 seconds. Drain lower aqueous layer into a second 250 ml separatory funnel. Add 60 ml petroleum ether to the second separatory funnel and shake vigorously for 30 seconds. Discard aqueous layer and combine petroleum ether layers in first separatory funnel. Rinse second separatory funnel with a few small portions of petroleum ether,

collecting rinses in first separatory funnel. Wash petroleum ether with three, 40 ml portions of distilled water, discarding each wash. Dry petroleum ether through a 25 mm x 50 mm column of anhydrous Na_2SO_4, collecting eluate in a Kuderna-Danish concentrator. Rinse separatory funnel and then column with three, about 20 ml portions petroleum ether, collecting rinses. Concentrate combined petroleum ether extract and rinses on steam bath to about 5 ml. Extract is ready for cleanup on Florisil column. If experience with particular sample types indicates that Florisil column cleanup is not required, proceed to gas chromatographic determination.

Note: Adequate extraction of low density paper such as newspaper or tissue paper will require adjustment of either the amount of sample downward from 10 grams or the volume of reflux solution upward from 60 ml. If possible reduce the weight of the sample material below 10 grams to a quantity that is completely covered and wetted by 60 ml of the reflux reagent. If a larger amount of sample (not to exceed 10g) is required, use a volume of reflux reagent to completely cover and wet the sample. Increase in volume of reflux solution over 60 ml must be accompanied by proportional increases in volumes of petroleum ether rinses of sample, water diluent added to the alcoholic reagent in the separatory funnel, and size of Erlenmeyer flasks and separatory funnels.

Cleanup

Add 10g of Florisil to 22 mm id chromatographic tube. Settle column of Florisil by gentle tapping of chromatographic tube. Top column with about 1/2" anhydrous Na_2SO_4. Pre-wet column with 20 ml petroleum ether. Place Kuderna-Danish concentrator with volumetric collection vessel under column to receive eluate. Transfer petroleum ether extract to column letting it pass through column at about 5 ml/min. Rinse container with three, 5 ml portions petroleum ether transferring each to column, and rinse walls of chromatographic tube with additional small portions of petroleum ether. Elute column at about 5 ml/min with 150 ml of petroleum ether. Concentrate eluate to suitable definite volume in Kuderna-Danish evaporator. If volume less than 5 ml is required, use 2-ball micro Snyder or micro Vigreaux column during final evaporation. Eluate is suitable for analysis by gas chromatography with electron capture detection.

Note: Waxes, if present in the extract, can be removed prior to Florisil chromatography by partitioning between petroleum ether and acetonitrile, Pesticide Analytical Manual, Vol. I., 211.14.

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ATTACHMENT III

Gas Chromatographic Determination of PCB

The following is taken from changes in AOAC official multi-pesticide residue methodology, Chapter 29.001, made to include PCB in the gas chromatographic determination. To appear in March, 1973, Journal of the AOAC, "Changes in Official Methods of Analysis of the Association of Official Analytical Chemists Made at the Eighty-sixth Annual Meeting, October 9-12, 1972".

"Sec. 29.017 Gas Chromatography, introductory par., was revised as follows":

"(Applicable to chlorinated pesticides, phosphated pesticides, and polychlorinated biphenyls (PCB). Method is applicable to PCB residues when present alone in sample. If pesticidal or other compds are detected in chromatogram of the PCB residue, other chemical or physical operations must be applied to eliminate or miminize their interference before PCB quantitation.)

Inject suitable aliquot (3-8 µl) of concd eluate from Florisil or MgO-Celite column contg amt compd within linear range into gas chromatograph, 29.008, using 10 µl syringe. Tentatively identify residue peaks on basis of retention times. Measure area or ht of residue peak(s) and det. residue amt by comparison to peak area or ht obtained from known amt appropriate ref. material(s). To insure valid measurement of residue quantity, size of peaks from residue and ref. std should be within ±25%. Chromatograph ref. material(s) immediately after sample.

Measure PCB residues by comparing total area or ht of residue peaks to total area or ht of peaks from appropiate Aroclor(s)® (Monsanto Co., 800 N. Lindbergh Blvd, St. Louis, MO 63166) ref. materials. Measure total area or ht response from common baseline under all peaks. Use only those peaks from sample that can be attributed to chlorobiphenyls. These peaks must also be present in chromatogram of ref. material. Mixt. of Aroclors may be required to provide best match of GLC patterns of sample and ref."

ATTACHMENT II

Micro Scale Alkali Treatment for Use in Pesticide Residue Confirmation and Sample Cleanup

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Procedures involving alkali treatment for dehydrochlorination of certain organochlorine pesticides and saponification of fats have long been employed in pesticide residue analysis. In 1942 Brand and Busse-Sunderman (1), and in 1946 Soloway et al. (2), studied rates of dehydrochlorination of DDT. Mills (3) used refluxing alcoholic KOH in the cleanup of fatty foods for paper chromatographic detection of alkali-stable organochlorine pesticides. Klein and Watts (4) used alcoholic NaOH to dehydrochlorinate o,p'- and p,p'-DDT, p,p'-TDE, and Perthane prior to gas chromatographic separation of the respective olefins. These investigators called attention to several earlier uses of alkali dehydrochlorination in pesticide residue chemistry. Recent literature contains numerous references to application of this treatment in pesticide residue analyses, including an adaptation for use in a pre-gas chromatographic (GLC) column (5).

In spite of the knowledge of this reaction we have observed that it is not fully and effectively utilized by residue laboratories for forming derivatives, gaining information for identity confirmation, or obtaining better cleanup of troublesome extracts. This is probably because the procedure has not been described in detail for simple micro scale application in multiresidue analysis.

The purpose of this work was (1) to arrive at optimum and convenient parameters of the alkali treatment in order to obtain rapid dehydrochlorination resulting in product solutions suitable for analysis by GLC; (2) to obtain complete yield and recovery of olefins from a number of bis(phenyl) chloroethane pesticides; (3) to determine the effect of the treatment on several important pesticide and industrial chemicals; (4) to describe in detail the procedure for routine application in the residue analytical laboratory.

METHOD

Reagents and Apparatus

(a) Potassium hydroxide - Anhydrous pellets.
(b) Ethanol - USP 95%.
(c) Hexane - Suitable for use with electron capture gas chromatography (Burdick and Jackson Laboratories, Inc. 1953 S. Harvey St., Muskegon, Mich. 49442).

Bulletin of Environmental Contamination & Toxicology, Vol. 7, No. 3/3, 1972 © Springer-Verlag New York Inc.

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(d) Micro condenser - 19/22 $\bar{s}$; K-569250 (Kontes Glass Company, Vineland, N. J. 08360).

(e) Concentrator tube - Mills type, 19/22 $\bar{s}$ with stopper, 10 ml graduated in 0.1 ml up to 1.0 ml; K-570050 (Kontes Glass Company).

(f) Alkali dehydrochlorination reagent - Dissolve 2 g KOH in 100 ml ethanol.

(g) Ethanol - water, 1 + 1 - Combine equal parts by volume of distilled water and ethanol.

(h) Gas chromatograph - Equipped with electron capture detector and 6' x 4 mm id glass column containing either (1) 10% DC-200 or (2) 1:1 mixture of 15% QF-1 + 10% DC-200 on 80-100 mesh Chromosorb W(HP). Operating conditions: N_2 flow 120 ml/min; temperatures, column and detector 200°C, injector 225°C; concentric design electron capture detector operated at dc voltage to cause 1/2 full scale recorder deflection for 1 ng heptachlor epoxide when full scale deflection is 1 x 10^{-9} amp.

Procedure

Accurately pipet, into a 10 ml Mills tube, 2 ml of a petroleum ether solution of sample extract [6% or 15% Florisil eluate (6)] containing concentrations of pesticides suitable for subsequent GLC analysis. Add 1 ml of 2% ethanolic KOH and a few carborundum chips and fit the tube with a micro condenser. [Note: Avoid getting alkali on the ground glass joint; light greasing of joint with silicone lubricant may prevent sticking.] With a test tube clamp, hold the tube over an opening in the steam bath in such a manner that gentle boiling occurs. When the volume has been reduced to about 1 ml, insert the tube completely into the steam bath opening and heat vigorously for 15 minutes or until the volume reaches 0.2 ml. Remove tube from the steam. If a precipitate has formed, as is often the case with extracts containing fatty substances, add a few drops of 2% ethanolic KOH and warm gently in steam with swirling until the precipitate dissolves. After the solution has cooled slightly, add about 2 ml ethanol-H_2O (1 + 1). Allow solution to reach room temperature and pipet 1 or 2 ml hexane into tube. Stopper tube with ground glass, invert, shake vigorously for about 30 seconds, and allow solvent layer to separate sharply. With microliter syringe, carefully withdraw aliquot of upper layer for determination by GLC. [Note: Separation of phases should be sharp so that solution withdrawn for GLC analysis will be free from alkali.]

Discussion

Development of Method

Initial experimentation was performed to establish the reaction conditions which would give complete and rapid dehydrochlorination of p,p'-DDT, o,p'-DDT, p,p'-TDE, o,p'-TDE, methoxychlor, and Perthane and which would permit complete recovery of the respective olefins. Work was done to determine the effect on olefin formation of fatty substances not removed during sample cleanup and the capacity of the reaction to eliminate fatty substances.

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Several considerations found necessary for practical and reliable use of the alkali treatment have been incorporated into the method and are briefly discussed. The steam bath was chosen as a source of heat because of its ready availability and convenience. The Mills reaction tube was fitted with a micro condenser to eliminate losses due to volatilization, which often occurred in the absence of the condenser. Both KOH and NaOH have been used to the satisfaction of previous investigators. The more frequent use of KOH by other workers and its higher solubility in ethanol made it the choice for this work. An alkali concentration of 2% has been widely used, and was found ideal for treatment of aliquots of cleaned-up sample extracts containing quantities of bis(phenyl) chloroethane pesticides ranging from a few nanograms to 100 µg. In order to provide sufficient reflux time and temperature it was necessary that the initial volume of ethanol be in excess of 0.5 ml. When smaller volumes were used, dehydrochlorination was usually incomplete. Emulsions often occurred during extraction of the olefin into hexane after saponification of fatty substances. The use of ethanol + water (1 + 1) instead of water as the diluent resulted in a sharp separation of hexane and aqueous layers. Recoveries of olefins were not adversely affected if the ethanol content was less than about 70%. Less than 30% ethanol did not adequately enhance separation of the two layers. The non-volatile fatty substance transferred to acetonitrile by partitioning a petroleum ether solution of butterfat with acetonitrile was used in tests to evaluate the effects of fatty substances on the reaction. Experiments in which varying volumes of 2% ethanolic KOH were used to saponify 350 mg portions of this butterfat showed that each 1 ml of 2% KOH would saponify about 50 mg of the fat. When the weight of fatty substances exceeded about 50 mg, complete dehydrochlorination of Perthane (40 µg) and methoxychlor (4.0 µg) was not obtained. However, p,p'-DDT (8.0 µg) was completely dehydrochlorinated in the presence of 100-120 mg of fat. Additional experimentation showed that complete dehydrochlorination of methoxychlor and Perthane did not occur until the fat was completely saponified; these were the bis(phenyl) chloroethanes most resistent to dehydrochlorination. Quantities of Perthane and p,p'-DDT up to 100 µg in the presence of not more than 50 mg butterfat were readily dehydrochlorinated with 1 ml of 2% KOH at steam bath temperature; dehydrochlorination of larger amounts of pesticide was not attempted. Hexane, because of its higher boiling point and greater ease of drawing into a microsyringe, was used instead of petroleum ether, to extract the olefin after reaction in order to avoid possible errors in quantitation.

Effect on Selected Chemicals

The dehydrohalogenation reaction, as described under "Method", was applied to p,p'-DDT (0.8 µg), o,p'-DDT (0.8 µg), p,p'-TDE (2.0 µg), o,p'-TDE (0.4 µg), methoxychlor (4.0 µg), and Perthane (40 µg). Quantities shown in parentheses were chosen for ease in GLC determination. The pesticides were treated individually in petroleum ether, in 6% ethyl ether/petroleum ether Florisil eluates (6) containing the equivalent of 6 g of kale, and in

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petroleum ether containing 30-60 mg of fatty substances extracted from butter by partitioning (6) between petroleum ether and acetonitrile. Gas chromatography with electron capture detection, operated as described under "Method", was used for all determinations.

Each pesticide was completely altered in each of the solution types, i.e., none of the parent compound remained after treatment as described under "Method". Percent recoveries of the respective olefins were calculated according to the following equation:

$$\frac{\text{wt. olefin compound determined by GLC}}{\text{wt. parent compound represented in aliquot to GLC}} \times \frac{\text{wt. parent compound/mol. wt.}}{\text{wt. olefin compound/mol. wt.}} \times 100$$

Recoveries of olefins approximated 100% and ranged from 86% for p,p'-DDE and o,p'-TDE olefin in petroleum ether to 110% for p,p'-DDE in the extract from butter. Two olefin derivatives, the cis and trans isomers, are formed from o,p'-TDE (7). These have identical retention times on the two GLC columns used in this work and were quantitated as a single compound.

Several additional common organochlorine pesticides and polychlorinated biphenyls (PCB) were subjected to the described alkali treatment. All tests were made with petroleum ether solutions of the chemical under study. The quantity of each chemical was chosen for ease of determination by GLC and is given in parentheses.

Polychlorinated biphenyls ranging from 21 to 60% average chlorine content were stable to this treatment. Complete recoveries were obtained for the commercial PCB mixtures, Aroclors 1221 (16 µg), 1232 (16 µg), 1242 (16 µg), 1254 (8 µg), and 1260 (8 µg). Recoveries of unchanged aldrin (0.4 µg), dieldrin (0.4 µg), and endrin (0.4 µg) ranged from 70 to 90%. No alteration products were detected.

Heptachlor (0.4 µg) and heptachlor epoxide (0.4 µg) were markedly affected; recoveries of the original compound ranged from 30 to 50%. Minor GLC peaks were observed on the 10% DC-200 column at retention times relative to aldrin of 1.63 after treatment of heptachlor epoxide and 0.59 and 0.93 after treatment of heptachlor.

The alkali treatment completely eliminated lindane (0.2 µg) and the alpha (0.2 µg), beta (0.2 µg), and delta (0.2 µg) isomers of BHC. Following the reaction, only small early eluting gas chromatographic peaks, presumably from trichlorobenzenes, were observed.

About 40% of mirex (4.0 µg) remained unchanged after reaction; sometimes a minor GLC peak appeared at a retention time relative to aldrin of 1.83 on the 10% DC-200 column.

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Endosulfan I and II, treated separately, were completely eliminated. Each isomer gave a single alteration product with retention time relative to aldrin of 1.82 on the 10% DC-200 column and 2.23 on the 1:1 10% DC-200/15% QF-1 column. The peak height of the alteration product was approximately one-tenth the peak height of the parent compound. A structure for this derivative has been proposed (8).

Endosulfan sulfate also was completely eliminated. Two alteration products were obtained with retention times relative to aldrin of 0.28 and 0.38 on the 10% DC-200 column.

Dicofol (1.0 µg) was completely eliminated but only 65% of the major alteration product, 4,4'-dichlorobenzophenone, was recovered. A minor peak was observed in the chromatogram at a retention time relative to aldrin of 1.71 on the 10% DC-200 column. The 4,4'-dichlorobenzophenone (2.0 µg) was not affected by treatment with alkali.

The products resulting from alkali treatment of toxaphene (10.0 µg) gave a multicomponent chromatogram but consisting of components with earlier retention times than toxaphene itself.

The electron capture GLC responses to sulfur (20 µg), frequently encountered in residue analysis at retention times relative to aldrin of 0.23, 0.55, and 1.13 on the 10% DC-200 column, were eliminated by the alkali treatment.

Application of Method

The most obvious use of the alkali treatment is to form the olefins of bis(phenyl) chloroethane pesticides for confirmation of residue identity. The complete yield and recovery of the olefin derivative makes possible quantitative confirmation of a residue of the parent pesticide. For example, a residue of p,p'-DDT can be quantitated before alkali treatment and verified by quantitation as p,p'-DDE after treatment.

In addition the GLC retention time region of the reacted compound can be examined for presence of peaks from unreacted and presumably interfering substances. We have found the alkali treatment especially useful in connection with the real or suspected presence of residues of PCB. In this case the characteristic olefin derivatives of p,p'-DDT, o,p'-DDT, and p,p'-TDE can be formed and the GLC retention time region underlying the parent compounds can be examined. The stability of PCB to alkali, with no change in the GLC pattern, is a characteristic which can be readily utilized in confirmation of the identity of this complex residue. The high recovery of unreacted dieldrin, endrin, and aldrin following alkali treatment likewise can be of value in the confirmation of identity of these pesticides.

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TABLE 1

Compounds Treated with Alkali According to the Described Procedure

Compound	Approximate Amount Altered, %	Major Alteration Products Detectable by GLC	Approximate Amount of Parent Compound Changed to Alteration Product, %
p,p'-DDT	100	p,p'-DDE	100
o,p'-DDT	100	o,p'-DDE	100
p,p'-TDE	100	p,p'-TDE olefin	100
o,p'-TDE	100	cis o,p'-TDE olefin trans o,p'-TDE olefin	100
Perthane	100	Perthane olefin	100
methoxychlor	100	methoxychlor olefin	100
dicofol	100	4,4'-dichlorobenzophenone	65
4,4'-dichlorobenzophenone	0	----	---
lindane	100	presumably trichlorobenzenes	---
α,β,δ isomers of BHC	100	presumably trichlorobenzenes	---
endosulfan I	100	1.82 DC-200[a,b] 2.23 mixed[c]	---
endosulfan II	100	1.82 DC-200[a,b] 2.23 mixed[c]	---
endosulfan sulfate	100	0.28, 0.38 DC-200[a]	---
mirex	60	1.83 DC-200[a]	---
heptachlor	50-70	0.59, 0.93 DC-200[a]	---
heptachlor epoxide	50-70	1.63 DC-200[a]	---
aldrin	10-30	----	
dieldrin	10-30	----	
endrin	10-30	----	
toxaphene	apparently greatly altered	substances with earlier RRT_A than toxaphene	---

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TABLE I (continued)

Compound	Approximate Amount Altered, %	Major Alteration Products Detectable by GLC	Approximate Amount of Parent Compound Changed to Alteration Product, %
polychlorinated biphenyls: Arochlors 1221, 1232, 1242, 1254, and 1260	0	----	---
sulfur: 0.23, 0.55, 1.13 DC-200[a]	100	----	---

[a] Retention time relative to aldrin on the 10% DC-200 column, described under "Reagents and Apparatus (h)".
[b] Structure elucidated by Greve and Wit (8).
[c] Retention time relative to aldrin on the 1:1 QF-1 + 10% DC-200 column, described under "Reagents and Apparatus (h)".

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Cleaned-up extracts of some samples may contain non-pesticidal substances which give rise to electron capture response. Other extracts may contain fatty substances, not removed by the clean-up, which can prohibit application of some tests, e.g., thin layer chromatography. This is particularly true of the 15% ethyl ether/petroleum ether Florisil column eluate (6) for non-fatty samples such as carrots and fatty samples such as some fish. Electron capturing substances present in carrots must be eliminated before determination of dieldrin and/or endrin residues. Treatment with alkali serves well for this purpose. Extracts of fatty samples may require treatment to eliminate both electron capturing substances and non-volatile fatty substances. In many instances thin layer chromatography or microcoulometric GLC cannot be accomplished prior to alkali treatment. Electron capture responses to sulfur, often a source of annoyance to the residue chemist, are also eliminated by this treatment.

These common uses of the alkali treatment serve to illustrate its value to the residue chemist. The directions for laboratory application outlined here should enhance the use of this procedure of long standing.



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MONS 078798

ATTACHMENT IV

Reprinted from

Journal of Chromatography
Elsevier Publishing Company, Amsterdam - Printed in The Netherlands
CHROM. 6243

GAS CHROMATOGRAPHIC DATA FOR POLYCHLORINATED BIPHENYL COMPONENTS IN SIX AROCLORS®

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(First received April 11th, 1972; revised manuscript received July 5th, 1972)

SUMMARY

Illustrations of gas chromatographic curves, relative retention times, and response data from 10% DC-200 and 1:1 15% QF-1/10% DC-200 on 80–100 mesh Chromosorb W HP columns with electron capture detection are compiled for six Aroclors®. Aroclors® are commercial mixtures of polychlorinated biphenyls commonly used as analytical references for polychlorinated biphenyl residue determination.

INTRODUCTION

The industrial chemicals known as polychlorinated biphenyls (PCB), which have become widespread environmental contaminants[1-3], are generally determined by essentially the same techniques used for organochlorine pesticides[4] and may act as interferences in the gas–liquid chromatographic (GLC) determination of pesticide residues. Because there is no standard PCB and individual chlorinated biphenyl compounds are not readily available, it is necessary to rely on Aroclors® as analytical references for PCB residue determinations.

Aroclor® is the general tradename for commercial mixtures of PCB manufactured in the United States by Monsanto Company. 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 two digits of the identifying number indicate the percentage weight of chlorine, *e.g.*, Aroclors 1254 and 1260 are biphenyls containing 54 and 60% chlorine, respectively[5]. GLC patterns of PCB residues in environmental samples have generally resembled Aroclors 1254 and 1260, although the possibility exists that residues may derive from any of the Aroclors.

The GLC retention times, relative peak sizes, peak shapes, and overall peak pattern of PCB residues must be carefully compared to the corresponding data for the Aroclors in order to determine the Aroclor(s) that most closely resemble the residue. Because the magnitude of total area varies significantly for the same weight of different Aroclors, the quantitative value determined for the PCB residue depends

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upon which Aroclor(s) is chosen for quantitation reference. The interpretation and evaluation of GLC chromatograms in PCB determinations is augmented by ready access to relative retention times, response data, and reference chromatograms of the various Aroclors.

Data for these GLC characteristics of six Aroclors, obtained by using two GLC columns regularly employed in our laboratories[8], are presented here. Data are not presented for Aroclors 1232, 1268, 5442, 5460, and 4465. Aroclor 1232 has been commercially prepared[7] by blending appropriate quantities of Aroclors 1221 and 1242 to obtain 32% total chlorine; chromatograms of material obtained from different lots revealed substantial differences between some lots. Aroclor 1268 was not available at the time of this work. GLC at the described conditions of Aroclors 5442 and 5460 (chlorinated terphenyls) and 4465 (mixture of PCB and chlorinated terphenyls) resulted in multicomponent chromatograms with peaks emerging at retention times extending to several hours; several hundred nanograms were required for detection of late-eluting constituents. These GLC parameters are considered unsuitable for detection and measurement of the polychlorinated terphenyls. A system utilizing parallel columns with liquid phases of 1% OV-101 and 3% Dexsil 300 operated at 240° has been devised for GLC of both the polychlorinated terphenyls and PCB[8].

EXPERIMENTAL

GLC data were obtained with a gas chromatograph equipped with an electron capture detector and 4-mm × 6-ft. glass columns, packed both with 10% DC-200 on 80–100 mesh Chromosorb W HP and a 1:1 mixture of 15% QF-1 plus 10% DC-200 on 80–100 mesh Chromosorb W HP[8]. Operating conditions: nitrogen, 120 ml/min; column and detector temperature, 200°; injection temperature, 225°. The concentric design electron capture detector was operated at a d.c. voltage to produce half full scale recorder deflection for 1 ng heptachlor epoxide when full scale deflection is 1×10^{-9} A. Recorder speed was $\frac{1}{4}$ in./min.

Different concentrations of Aroclors were injected at the conditions given to determine the quantity necessary for approximately half full scale recorder response or enough response to illustrate all the characteristic constituents of a particular Aroclor. Retention times were measured in millimeters from the leading edge of the response to the solvent and reported relative to the retention time of the pesticide aldrin. These conditions and manner of reporting GLC data for Aroclors were chosen to conform to GLC data compiled in the Food and Drug Administration *Pesticide Analytical Manual*[9] for a large number of pesticides.

RESULTS

The following information has been compiled in the tables and figures to be a source of reference and comparison for the GLC behavior of PCB: retention times, response data and illustrations of chromatograms for six Aroclors from two GLC columns. Table I lists the quantities necessary for approximately half full scale

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TABLE I

QUANTITIES OF AROCLORS NECESSARY FOR APPROXIMATELY HALF FULL SCALE RECORDER RESPONSE[a]

Aroclor	*Nanograms for 1/2 f.s.r.*	
	DC-200[b]	*QF-1/DC-200*[b]
1221	80	60
1242	40	40
1248	40	30
1254	30	30
1260	20	20
1262	20	20

[a] Quantities of Aroclor mixtures to produce approximately half full scale recorder response (1/2 f.s.r.) for major Aroclor components or sufficient response to detect all the characteristic Aroclor constituents.

[b] GLC column and detector conditions are given under EXPERIMENTAL.

TABLE II

GLC RETENTION TIME DATA FOR SIX AROCLORS

Support material: Chromosorb W HP, 80–100 mesh; stationary phase: 10% DC-200 (6 ft. × 4 mm I.D. column); carrier gas: nitrogen, 120 ml/min; temperature: 200°; detector: electron capture (tritium); sample size: quantities necessary for approximately half full scale response (see Table I); data given in: retention sequence relative to aldrin from solvent peak.

Aroclor 1221	*Aroclor 1242*	*Aroclor 1248*	*Aroclor 1254*	*Aroclor 1260*	*Aroclor 1262*
0.21					
0.26					
0.31					
0.36					
0.39	0.39				
0.52	0.51	0.51			
0.59	0.57	0.57			
0.64					
0.69	0.67	0.67			
0.75	0.72				
		0.80			
0.88	0.86	0.85	0.87		
0.99	0.96	0.96	0.98		
	1.03	1.03	1.05		
1.27	1.22	1.23	1.27	1.28	1.26
1.42	1.39	1.39			
1.52	1.49	1.49	1.52	1.50	1.50
1.76	1.74	1.74	1.78		
1.86	1.83	1.84	1.88	1.86	1.85
2.08				2.07	2.07
2.22	2.20	2.20	2.20	2.21	2.22
	2.56	2.54			
2.65			2.63	2.63	2.61
				2.84	2.82
3.10		3.04	3.08	3.08	3.06
			3.60	3.50	3.50
			4.10	4.10	4.10
			4.30		
			4.90	4.90	4.90
			5.80	5.80	5.80
					6.40
				6.50	6.60
				7.80	7.80
				9.10	9.10

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recorder response or for enough response to illustrate all the characteristic constituents of a particular Aroclor. Tables II and III give the retention times relative to aldrin for all the peaks present in six different Aroclors. These tables are arranged to readily show similarity and differences in the GLC elution pattern of the various Aroclors. Peaks with similar relative retention times in the different Aroclors are not necessarily responses to the same compound. Figs. 1–3 are gas chromatograms of six Aroclors from the 10% DC-200 column; Figs. 4–6 are gas chromatograms of six Aroclors from the 1:1 15% QF-1/10% DC-200 column at the specified conditions.

The GLC data for PCB presented here should be a useful reference for evaluation and interpretation of GLC chromatograms for PCB residue determinations.

TABLE III

GLC RETENTION TIME DATA FOR SIX AROCLORS

Support material: Chromosorb W HP, 80–100 mesh; stationary phase: 1:1 15% QF-1/10% DC-200 (6 ft. × 4 mm I.D. column); carrier gas: nitrogen, 120 ml/min; temperature: 200°; detector: electron capture (tritium); sample size: quantities necessary for approximately half full scale recorder response (see Table I); data given in: retention sequence relative to aldrin from solvent peak.

Aroclor 1221	*Aroclor 1242*	*Aroclor 1248*	*Aroclor 1254*	*Aroclor 1260*	*Aroclor 1262*
0.20					
0.29					
0.36					
0.39	0.41				
0.53	0.53	0.53			
0.57	0.61	0.61			
0.71	0.72	0.72			
0.78					
	0.81	*0.81*			
0.88	0.90	0.90	0.87		
1.02	1.02	1.02	1.02		
1.10	1.09	1.09			
1.31	1.34	1.34	1.32	1.31	1.31
1.53	1.52	1.52	1.52	1.53	1.53
1.82	1.82	1.82	1.82		1.84
				1.86	
1.96	1.97	1.97	1.96		
2.08					2.08
				2.14	
2.26				2.26	2.24
2.34	2.36	2.36	2.34		
2.68			2.68	2.66	2.66
2.84	2.80	2.80	2.80	2.88	2.88
3.22	3.24	3.24	3.20	3.22	3.22
			3.50	3.50	3.50
			3.90	3.90	3.90
			4.20	4.20	4.20
			5.00	5.00	5.00
			6.10	6.10	6.10
				6.50	6.50
					7.40
				8.00	
				9.50	9.30
					11.9

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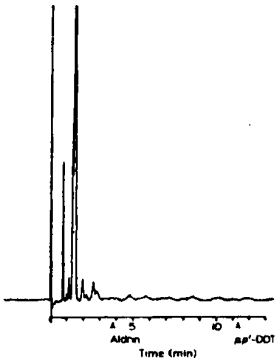

Fig. 1. GLC separation on 10% DC-200 column of 120 ng Aroclor 1221. GLC conditions are given under EXPERIMENTAL.

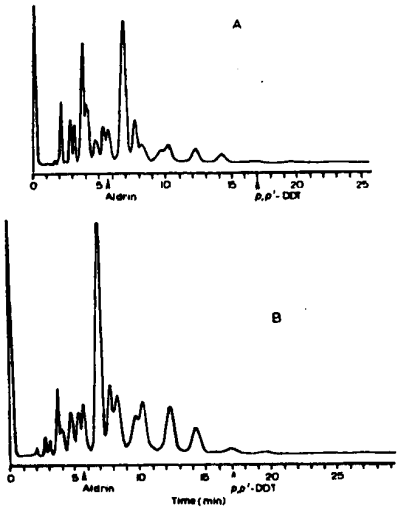

Fig. 2. GLC separation on 10% DC-200 column of (A) 50 ng Aroclor 1242 and (B) 50 ng Aroclor 1248. GLC conditions are given under EXPERIMENTAL.

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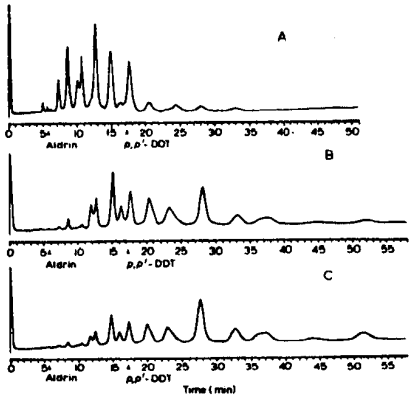

Fig. 3. GLC separation on 10% DC-200 column of (A) 32 ng Aroclor 1254, (B) 20 ng Aroclor 1260 and (C) 20 ng Aroclor 1262. GLC conditions are given under EXPERIMENTAL.

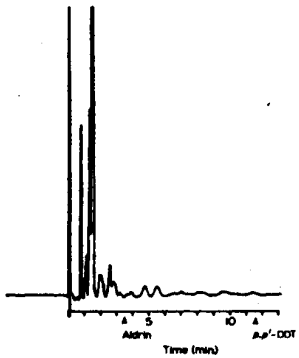

Fig. 4. Gas chromatographic separation on 1:1 15% QF-1/10% DC-200 of 120 ng Aroclor 1221. GLC conditions are given under EXPERIMENTAL.

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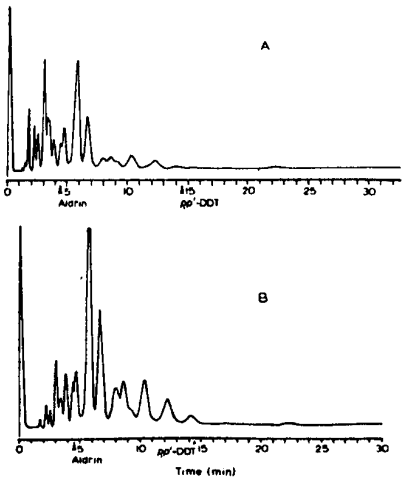

Fig. 5. Gas chromatographic separation on 1:1 15% QF-1/10% DC-200 of (A) 50 ng Aroclor 1242 and (B) 50 ng Aroclor 1248. GLC conditions are given under EXPERIMENTAL.

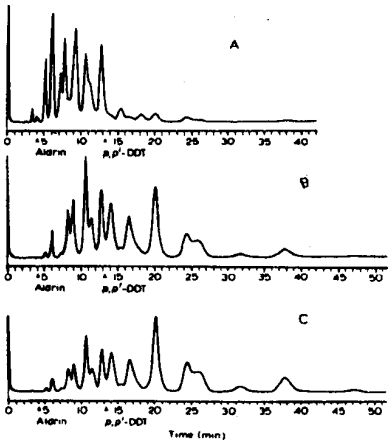

Fig. 6. Gas chromatographic separation on 1:1 15% QF-1/10% DC-200 of (A) 32 ng Aroclor 1254, (B) 20 ng Aroclor 1260, and (C) 20 ng Aroclor 1262. GLC conditions are given under EXPERIMENTAL.

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ATTACHMENT V

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

By J. A. ARMOUR (Division of Chemical Technology, U. S. Food and Drug Administration, Washington, D. C. 20204)

To be published: Journal Association Official Analytical Chemists, July, 1973

Abstract

The perchlorination procedure for derivatization of PCB 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 µg-20 µg) are reacted with antimony pentachloride in the presence of chloroform to form decachlorobiphenyl. This procedure converts a multicomponent mixture to a single derivative detectable by EC-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 measuring the total area of the EC-GLC response for the residue against the Aroclor it most closely resembles to results obtained by measuring the single peak of the decachlorobiphenyl derivative and expressing the results in terms of the particular Aroclor.

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

By J. A. ARMOUR (Division of Chemical Technology, U. S. Food and Drug Administration, Washington, D. C. 20204)

Introduction

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 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 lack of sensitivity, the multicomponent nature of PCB residues, alterations in residue pattern due to 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 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 microscale 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 PCB 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.

Methodology

Reagents

Antimony pentachloride - Allied Chemical Reagent Code 1365.

Sodium Sulfate - anhydrous, granular; it may be necessary to wash with acetone and ethyl ether to remove electron capturing substances.

HCl - 6N solution (1 + 1 dilution of concentrated HCl in water).

Sodium Bicarbonate - 10% weight/volume aqueous solution.

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 mm X 10 mm o.d. with No. 4 Teflon valve (Kontes Glass Co. K-896860-0004) or Carius pyrex combustion tube 200 mm X 8 mm i.d. X 10 mm o.d. (Corning 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. K5470000, or equivalent).

(d) Micro-Snyder column - ₿ 19/22 column size 2-19 (Kontes Glass Co. K569001, or equivalent).

(e) Gas chromatograph - Equipped with concentric design electron capture detector operated at a dc voltage to produce 1/2 full scale recorder deflection for 1 ng decachlorobiphenyl when full scale deflection is 1 X 10^{-9} amps, and 6' X 4 mm i.d. glass column of either

(1) 1% OV-101 on 100-120 mesh Gas Chrom Q (9)

Gas flow - 120 ml/min N_2

Column temperature - 220°C

Injector temperature - 230°C

Detector temperature - 200°C

(2) 3% Dexsil 300 on 100-120 mesh Gas Chrom Q (9)

Gas flow - 60 ml/min N_2

Column temperature - 240°C

Injector temperature - 250°C

Detector temperature - 200°C

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Perchlorination Procedure

The sample to be perchlorinated should be extracted and cleaned up by procedures (such as the FDA multiresidue pesticide procedure (10)) 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 a 1 to 2 ml volume of cleaned up extract containing 1 to 20 µg PCB residue to a vacuum hydrolysis tube (a), (Carius tube may be substituted). Add several drops chloroform, 1-2,20 mesh carborundum chips, and reduce volume to 0.1 ml by carefully heating tube in a steambath. Allow tube to cool to room temperature, add 2 ml chloroform, 1-2 carborundum chips, and reduce volume to 0.1 ml on a steambath. Repeat the addition of chloroform, concentrate to 0.1 ml, and cool to room temperature. Working in a well ventilated hood, carefully add 0.2 ml antimony pentachloride to tube. Immediately seal tube tightly to ensure a closed system reaction. (Note: it may be necessary to cool Teflon valves in ice before fitting into vacuum hydrolysis tube. The narrowed bottom of the valve should fit flush against the constriction in the glass when sealed. Carius tubes should be sealed in an oxygen flame). Immerse tube to a depth of 2 to 3 inches in an oil bath heated to 165-175°C and react overnight (about 15 hours). Remove tube from bath and cool to room temperature. In a hood, and while pointing the venting side arm of vacuum hydrolysis tube away from face, open by slowly unscrewing the valve and pulling it out of the tube. (Carius tube should be scored with a file and snapped open in a hood at

arms length away from face; use extreme precaution since there is a rapid release of pressure and sometimes splintered glass). Slowly add 1 ml 6N 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, using a total volume of 5 ml 6N 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 volume of 15 ml hexane and quantitatively transfer rinsings to the 30 ml separatory funnel containing the HCl. Shake funnel vigorously for 1 minute; allow layers to separate. Drain the lower HCl layer into a second 30 ml separatory funnel containing 15 ml hexane, and shake one minute. Drain the HCl layer into a third separatory funnel containing another 15 ml hexane and repeat extraction. Discard HCl layer and combine the three hexane extracts in a 125 ml separatory funnel. Wash hexane extract successively with two 20 ml portions H_2O, one 20 ml volume 10% $NaHCO_3$ solution, followed by two additional 20 ml portions H_2O. Discard all washes. Dry the washed hexane extract by passing through a 2 inch column of anhydrous sodium sulfate and rinse column with 100 ml hexane, collecting hexane extract and rinse in a Kuderna-Danish concentrator. Add a few drops of methanol and concentrate on steambath to about 3 ml. Separate the volumetric receiver from concentrator, attach a micro-Snyder column to receiver and concentrate to a final volume of less than 0.5 ml to remove traces of chloroform. Dilute to a suitable definite volume for GLC determination. Perform GLC determination and calculations as described under "Discussion."

Discussion

Berg, Diosady and Rees' microscale perchlorination procedure 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 Berg and needed to be established prior to testing the procedure for reproducibility. Even after we developed a method of extraction that would recover over 95% of DCB standard from the reaction mixture, perchlorination using Berg's reaction conditions 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 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 PCB were evaporated to dryness before reaction with antimony pentachloride. The observed non reproducible 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 with $SbCl_5$ exothermically 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 Figure 1).

Parameters for extraction of DCB from the reaction mixture were developed to completely recover (over 95%) DCB standard. Traces of chloroform present in the extract need to be removed before EC-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.

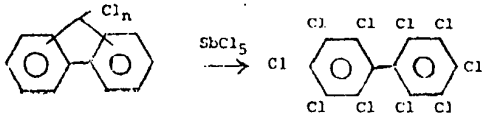
In experiments designed to determine the feasibility of a shorter reaction time, iron was tested as a catalyst. One microgram Aroclor 1242 samples were reacted and extracted as described for overnight conditions except that two milligram iron powder were added after the addition of antimony pentachloride,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

By-products that would interfere with quantitative derivatization were not evident in our perchlorination experiments, however, Stalling (11) 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 $SbCl_5$.

GLC systems suggested for DCB determination are those originally developed for the detection of polychlorinated terphenyls (9). At GLC conditions given under "Methodology", DCB elutes in 3-4 minutes on the 1% OV-101 column and in 12 minutes 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 can have 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 parts per million 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 is the approximate whole number of chlorines calculated from percent 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 percent chlorine may vary from the theoretical for each lot. The factor for each Aroclor (See Table 1) is the ratio of amount Aroclor necessary to yield one unit of DCB:

X = factor for expressing DCB as a specific Aroclor

$$X = \frac{\text{molecular wt. Aroclor}}{\text{molecular wt. DCB (499)}}$$

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:

$$\frac{\text{DCB peak height sample}}{\text{DCB peak height Std}} \times \text{Nanograms of standard injected} = \text{Nanograms DCB in sample injection}$$

$$\text{Nanograms DCB in sample injection} \times \text{factor for specific Aroclor (X)} = \text{Nanogram Aroclor sample injection}$$

$$\frac{\text{Nanograms Aroclor in sample injection}}{\text{wt. sample injected (mg)}} = \text{ppm Aroclor in sample}$$

Recoveries: To determine the percent conversion of specific Aroclors to DCB, a series of one microgram quantities of six different Aroclors were reacted at the given overnight conditions and 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 six Aroclors to DCB with results ranging from 87-105% and an average recovery of 97%.

In the experiments with shorter reaction times, 10 trials with 4 hour reactions using Aroclor 1242 gave a wide range of results although the average conversion to DCB was 90%. Trials with 6 hour reaction times gave results falling in a much narrower range and averaging 102% conversion. Results from these and experiments using the six Aroclors at 6 hour conditions are given in Table 3. The perchlorination is quantitative (99% average conversion for six Aroclors) and reproducible (range 83-110%) when reacted for 6 hours with iron catylst 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, three gram samples of chicken fat and corn oil were extracted by FDA multipesticide residue methodology (10). The 6% ethyl ether/petroleum ether fractions from Florisil cleanup were fortified with Aroclor 1242 at the 6.7 ppm level. Three aliquots of the cleaned up chicken fat each equivalent to 0.15 gram of fat and two samples of the cleaned up corn oil each equivalent to 3 grams 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 gram samples of fat and can handle 1 µg-20 µg quantities of PCB.

Application

Samples of fresh chub, fresh sturgeon, and eggs environmentally contaminated with PCB, and chicken fat fortified with PCB were extracted and cleaned up by FDA multipesticide residue methodology (10). Where necessary, PCB were seperated from pesticides by chromatography on silicic acid. The PCB residues were quantitated on electron capture GLC with a 10% DC-200 column (10,12) 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 grams chicken fat, 0.2 grams sturgeon fat, and 0.2 grams 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 two determinative procedures are presented in Table 4. Results obtained by the two 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

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1242) quantitated for PCB as a multicomponent residue before perchlorination and as the DCB derivative after perchlorination are shown in Figure 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 grams chub from the silicic acid petroleum ether (PCB) fraction were perchlorinated and results compared (see Table 4). Gas chromatograms of a chub sample quantitated for PCB (Aroclor 1254) as a multicomponent residue before perchlorination and as the DCB derivative after perchlorination are shown in Figure 3. Figures 2 and 3 also illustrate the increase in sensitivity obtained by perchlorination. For approximately equivalent peak heights on GLC only about 1/16 as much of the egg sample (Aroclor 1242) and 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 PCB 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.

Conclusions

The perchlorination procedure developed for microscale qualitative and quantitative confirmation of PCB is routinely applicable to samples prepared for GLC residue analysis. It reinforces judgments made by the

analyst when dealing with a multicomponent residue. The derivatization procedure coupled with GLC parameters used for chromatographing PCB also greatly increases the sensitivity of PCB detection.

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Table 1. Factors to Mathematically Convert Decachlorobiphenyl to an Equivalent Amount Aroclor.

Aroclor	Average 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 percent chlorine substitution for a specific Aroclor.

b Molecular weight of Aroclor based on the average whole number of chlorines calculated from percent chlorine substitution.

c $X = \frac{\text{molecular wt. Aroclor}}{\text{molecular wt. DCB (499)}}$

To convert ppm DCB to ppm of a specific Aroclor multiply ppm DCB by "X" for the Aroclor.

Table 2. Percent 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

Table 3. Percent Conversion of 1 µg PCB to Decachlorobiphenyl with Iron Catalyst and Six Hour Reaction Time.

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 hour reaction.

Table 4. PCB Residue Levels in Environmentally Contaminated Samples Obtained by Two Different Methods of Quantitation.

Sample	Reference Aroclor	PPM Residue by Total Area	by DCB[c]	
			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[a]	1248	28.4	28.6	28.4
Eggs	1242	7.1	6.8	7.1
Chicken fat[b]	1242	4.6	5.0	
Chicken fat[b]	1248	6.9	6.6	

a Residue is reported on a fat basis.

b Samples fortified with Aroclor for an interlaboratory study.

c Expressed as reference Aroclor.

Figure 1. Vacuum hydrolysis tube recommended for sealed system reaction A. Dimensions 10 mm o.d. X 150 mm length. B. Tight Teflon sealing valve. C. Venting side arm.

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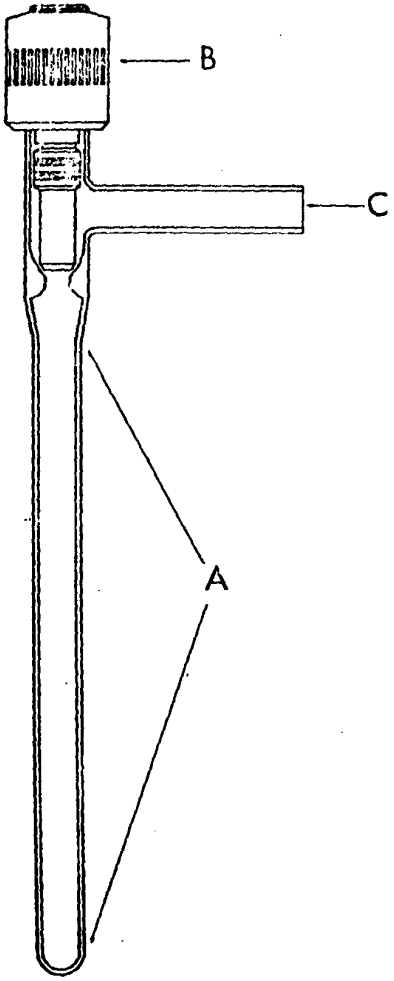

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Figure 2. GLC curves of egg samples 7.1 ppm PCB (as Aroclor 1242) before and after perchlorination. A. 1.11 mg sample (7.9 ng Aroclor 1242) 6% Florisil eluate before perchlorination, 10% DC 200 column (10,12). B. 0.07 mg sample (0.5 ng equivalent Aroclor 1242) 6% Florisil eluate after perchlorination, 1% OV-101 column. GLC conditions given under "Methodology".

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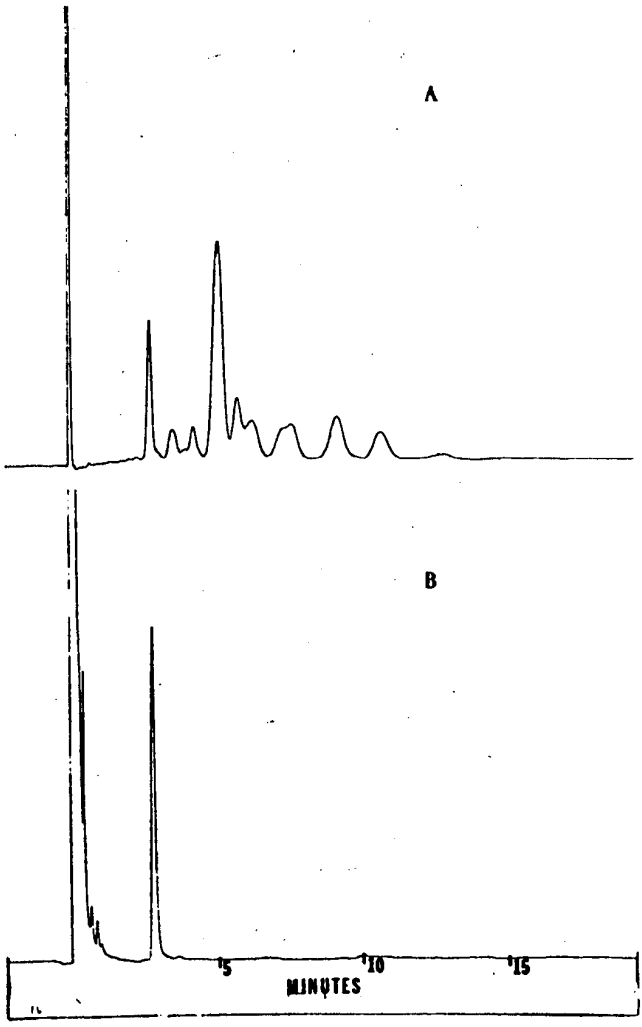

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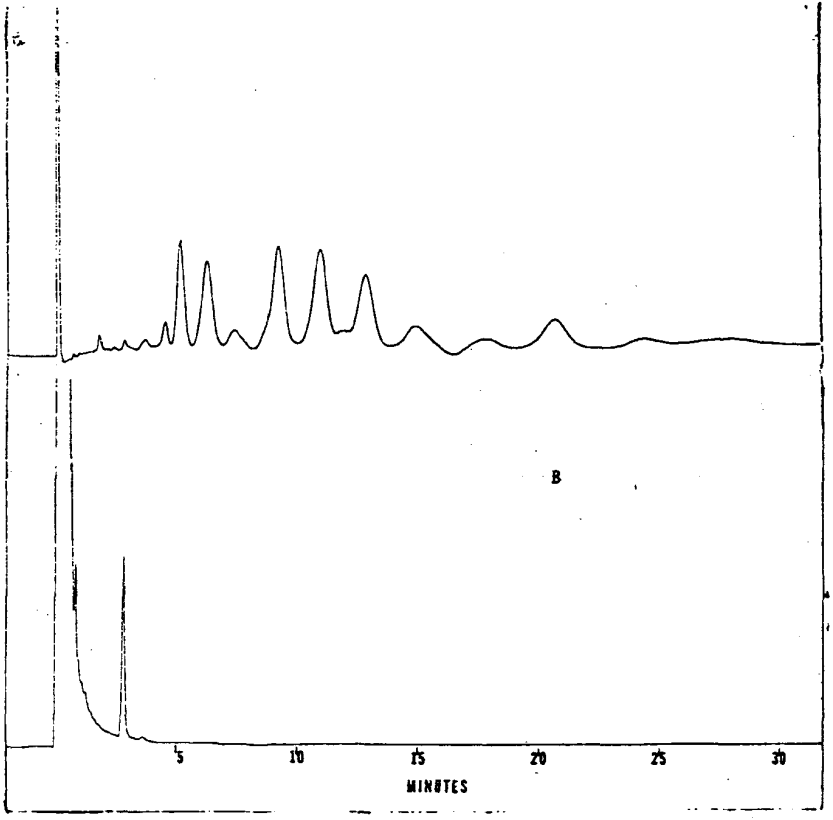
Figure 3. GLC curves of chub sample calculated as 3.4 ppm PCB (as Aroclor 1254) before perchlorination and calculated as 4.2 PCB (as Aroclor 1254) after perchlorination. A. 1.5 mg sample (5.1 ng Aroclor 1254) petroleum ether eluate from silicic acid before perchlorination, 10% DC 200 column (10,12). 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 under "Methodology."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ATTACHMENT VI

LABORATORY INFORMATION BULLETIN

EDRO
March 23, 1972

No. 918H
Pesticides

ADDITIONAL INFORMATION ON THE BEHAVIOR OF POLYCHLORINATED BIPHENYLS AND POLYCHLORINATED TERPHENYLS IN RESIDUE METHODOLOGY

By

Los Angeles District
Buffalo District
Division of Chemical Technology

Interest has continued to grow in the analytical aspects of polychlorinated biphenyls and terphenyls (PCB/PCT). Emphasis on the identification and measurement of the residues themselves has surpassed the original concern of preventing their interference in pesticide residue analysis.

Experimental investigation and industry contact has led to elucidation of composition of several Aroclor reference materials. In addition, a number of short term studies were done to determine the behavior of Aroclors in various analytical procedures, and the responses of several GLC detectors to the chlorine content of Aroclors were compared.

Experimental data presented here were collected by: Herbert Masumoto and Milton Luke of Los Angeles District; Robert V. Hoffman of Buffalo District; Judith Armour, John Roach, Winfred Wiencke, and Susan Young of Division of Chemical Technology; and compiled by Bernadette McMahon of DCT.

Aroclor 1232 Reference Material

New information on the manufacturing process for Aroclor 1232 sheds lights on the reason for the differences among the chromatograms of several samples of 1232 obtained from Monsanto at different times. It has been revealed that when Aroclor 1232 was being manufactured for sale (it is no longer available), it was made by combining amounts of Aroclors 1221 and 1242 until the mixture contained 32% chlorine by weight. This information explains how it is possible that the gas chromatograms of some lots of Aroclor 1232 reference materials have resembled chromatograms of 1242, and others have more closely resembled chromatograms of 1221.

It is probable that the manufactured lots of Aroclor 1232 varied in composition from one to another. For this reason, residues of Aroclor 1232 that may be found would be unlikely to match any arbitrarily chosen 1232 reference material. It is therefore recommended that residues suspected of being "Aroclor 1232", but resembling Aroclor 1221 and /or 1242, be quantitated by comparison to Aroclor 1221 and /or 1242 reference rather than by comparison to an Aroclor 1232 reference.

From now on, Aroclor 1232 will not be considered a separate entity, and lists of data on the behavior of Aroclors in this LIB will not include Aroclor 1232.

Significance: Little Aroclor 1232 was ever sold (according to Monsanto) and none is available now, so residues of "1232" are not likely. The suggested means of quantitation is available only in case such a residue is found.

Toxicological and ecological reasons for identifying the residue will not be jeopardized by reporting the residue as 1221 and/or 1242 rather than as 1232.

Aroclors 4465, 5442, and 5460 Reference Materials

By definition of the manufacturer, the Aroclors named in the title are composed of mixtures of the following:

4465 - chlorinated biphenyls and terphenyls, with 65% chlorine by weight
5442 - chlorinated terphenyls, with 42% chlorine by weight
5460 - chlorinated terphenyls, with 60% chlorine by weight

Chromatograms of each of the above mixtures were presented in LIB 918F. These chromatograms were obtained at conditions normally used for the chromatography of the mixtures of chlorinated biphenyls. It has since been discovered that, at these conditions, the chlorinated terphenyls present in 4465, 5442, and 5460 were seen, at best, only as very late eluting peaks. In turn, this failure of the chromatographic system to visualize the terphenyls led to some inaccurate results in other experiments which used GLC as a determinative step.

Recent investigations from several sources have revealed new information about the Aroclors in question. In part, the evidence has been obtained by use of a GLC column which permits terphenyls to elute quickly enough to be easily seen and measured (LIB 918 G).

New findings on the Aroclors of interest follow.
4465: The reference material distributed to FDA Districts by the former Reference Standards Branch appears to be mostly biphenyl compounds, despite Monsanto's description of this mixture as 40:60 terphenyl: biphenyl. Chromatography of 4465 on a GLC column which permits visualization of terphenyls does not reveal the presence of additional significant compounds.

Because the 4465 reference material obtained from Monsanto by FDA does not match Monsanto's description of the product, it is suspected that the terphenyl: biphenyl proportion must vary among different manufactured lots. The description of the material as a combination of terphenyl and biphenyl also suggests that 4465 may have been made by combining amounts of other Aroclors until 65% chlorination was obtained. Therefore, it may be possible to handle residues of "4465" by comparing them to other Aroclor references which they resemble.

Monsanto no longer manufactures Aroclor 4465, so no additional samples of reference material are available. For this reason, data presented in other sections of this LIB were obtained using the reference material containing mostly chlorobiphenyls.

5442: The reference material of 5442 available in FDA contains some contamination with chlorinated biphenyls, as evidenced by the early eluting peaks in the chromatograms in LIB 918F. The contamination is relatively small, and the data for 5442 presented elsewhere in this LIB were obtained by measuring the recovery of the terphenyls with a GLC column which permits the terphenyls to elute quickly.

5460: The 5460 distributed as a reference material has now been shown to contain a relatively large (about 5%) contamination of chlorinated biphenyls. It is now apparent that only the biphenyl contaminant was being visualized by the GLC systems used in LIB 918F, which explains why the chromatograms of 5460 resembled 1260 so closely. The chlorinated terphenyl components of 5460 can be seen and measured when the alternate GLC column is used. Data on the behavior of 5460 presented elsewhere in this LIB were obtained using the alternate column to visualize the terphenyl components of 5460 for measurement.

Significance: Residues of chlorinated terphenyls are presumably of as much interest and importance as those of chlorinated biphenyls. Until recently, the capability for detecting and measuring chloroterphenyl residues was limited by the very long retention times and consequent poor sensitivity for the compounds on the GLC columns (PAM 311.2) normally used for analysis of chlorinated biphenyls.

The chlorinated terphenyls are recovered, at least partially, through the analytical procedures used for organochlorine pesticides and PCB (see other sections of this LIB for details). It is now possible, by means of the GLC column described by Wiencke (LIB 918G), to visualize residues of chlorinated terphenyls and to estimate their quantity against a known amount of

reference material. This is the recommended procedure for detection and measurement of residues in samples suspected of contamination with chlorinated terphenyls. In particular, samples whose preliminary GLC analysis shows peaks eluting beyond the retention times of chlorinated biphenyls should be injected on the alternate column to visualize any terphenyls present. Chloroterphenyl residues too low to be recognized in ordinary sample examination can be discovered by injections of the sample on the alternate column.

Variations in Partitioning Among PCB/PCT

It has been determined that the efficiency of transfer of PCB from petr ether to acetonitrile decreases with increasing chlorination of the compounds. In particular, the following recoveries of Aroclor standards were obtained when a known amount of each Aroclor in 15 ml petr ether was partitioned into four successive 30 ml portions acetonitrile, each acetonitrile portion being backwashed with an additional 15 ml petr ether (PAM I, 211.14b):

Aroclor	ug in 15 ml PE	% recovery
1221	40	94
1242	40	97
1248	40	93
1254	40	87
1260	40	78
1262	40	74
4465	20	71

Among the PCB components of any given Aroclor, those whose peaks eluted first in the normal gas chromatographic system showed the highest recoveries. Percentage recovery decreased with increasing retention times (which generally increases with increasing chlorination), again suggesting that the more highly chlorinated the isomer, the poorer its transfer from petr ether to acetonitrile. The two Aroclors made up of polychlorinated terphenyls (PCT) display a similar pattern. When Aroclors 5442 and 5460 were partitioned as described above, 92 and 77% of the original 20 ug were recovered, respectively. Recoveries of individual PCT in the Aroclors were not determined, because the GLC column for PCT does not resolve the components well enough for measurement of each.

Significance: The experiments performed in gathering this recovery data were designed to duplicate the partitioning step most often used in the analysis of fatty foods for

organochlorine pesticides and/or PCB. The residue analyst should be aware that recovery through PAM I method 211 (fatty foods) will be different for different Aroclors and should avoid attributing losses completely to the Florisil or silicic acid column chromatographic steps. In addition, the pattern of PCB isomers in a given Aroclor is altered to some degree by this phenomenom, and may cause confusion to the inexperienced analyst.

Recovery of Aroclors Through Florisil Column Chromatography

New information on the behavior of Aroclors in other parts of the methodology has prompted reassessment of the elution of Aroclors from the Florisil column (PAM 211.14d). Frequent use of pesticide residue methodology for the analysis for PCB has assured us that the chlorinated biphenyls elute in the 6% ethyl ether/petroleum ether eluate. Additional experiments have shown that Aroclors 4465, 5442, and 5460 are also eluted by the 6% eluant.

Recovery of Aroclors Through Silicic Acid Column Chromatography

Masumoto (in press, JAOAC) has pointed out that some of the chlorobiphenyl isomers present in Aroclors are not eluted from the silicic acid column by petroleum ether as originally reported. Instead, the chlorobiphenyl isomers with early GLC retention times (which, generally, are those with lowest chlorination) are likely to be held on the silicic acid column and eluted from it only by the second, polar eluant. Aroclors consisting mostly of lower chlorobiphenyl isomers will therefore show low recoveries in the petroleum ether eluate.

Recoveries of PCB in the following Aroclors were obtained from a column made of silicic acid and Celite (LIB 918C-E) which had been previously standardized by addition of enough H_2O to effect separation of pp'DDE and Aroclor 1254:

Aroclor	Recovery
1221	essentially all in polar eluate
1242	20-35% in polar eluate
1248	less than 20% in polar eluate
1254	completely in petroleum ether
1260	completely in petroleum ether
1262	completely in petroleum ether
4465*	completely in petroleum ether

*Reference material contained mostly polychlorinated biphenyl isomers, rather than both biphenyl and terphenyl isomers.

The silicic acid column chromatography of the two Aroclors consisting of PCT was also examined . All of the Aroclor 5442 and most of the Aroclor 5460 added to a silicic acid column was eluted by the polar eluant. Aroclor 5460 was partially eluted by the petroleum ether eluant.

Significance: Knowledge of the elution characteristics of the Aroclors from the silicic acid column will aid the analyst in his choice of the appropriate analytical treatment of samples containing both pesticides and PCB. If examination of the original GLC chromatogram shows the PCB to resemble an Aroclor known to be incompletely separated from pesticides by silicic acid, alternate treatment(s) can be chosen to permit quantitation of the residues.

Response of GLC Detectors to the Chlorine Content in Aroclors

Experiments were performed to determine whether the responses of the electron capture, microcoulometric and electrolytic conductivity detectors were proportional to the chlorine content of Aroclors regardless of the nature of the compounds comprising the Aroclor. Results indicate that the response of the halogen specific microcoulometric and electrolytic conductivity detectors to the same amount of chlorine in different Aroclors was essentially the same. In contrast, the response of the electron capture detector to the more highly chlorinated Aroclors was disproportionately high when compared to the response of the same detector to Aroclors with lower chlorine content.

The electron capture detector that was used was first shown to give reproducible results and to be linear in response to amounts of heptachlor epoxide injected. Then, injections of six Aroclors were made and the total area of response measured for each. Calculations were performed so that responses to the different Aroclors could be compared to one another, on the basis of response/weight chlorine. The following ratios were obtained in this way:

Aroclor	Relative Response/Weight Chlorine
1221	1
1242	3.02
1248	4.56

1254	9.64
1260	12.70
1262	10.35

It is obvious from these data that response of the EC detector is not proportional to the amount of chlorine present in the chlorobiphenyl molecule.

In contrast, the response of the microcoulometric detector to injections of the same amounts of chlorine in each of four different Aroclors was essentially the same for each Aroclor. Because detector sensitivity changed with time, ratios of response to Aroclor X / response to Aroclor 1242 are compared; the injection of Aroclor 1242 and the injection of the other Aroclor were always made within an hour of one another. Other special precautions were also utilized to improve reproducibility; e.g., the first two injections made after cell flushing and equilibration were not considered.

The following ratios were obtained:

	Relative Response (for same weight chlorine)	
Aroclor	Mean	Range (number of injections)
1242	1.00	(used as standard)
1248	0.97	0.90-1.03 (3)
1254	1.00	0.92-1.23 (5)
1262	1.10	0.86-1.26 (6)

Despite the problems encountered in dealing with the microcoulometric system, these data do present evidence that microcoulometric response is essentially proportional to the amount of chlorine present, regardless of the structure of the compounds.

Figures 1-5 demonstrate visually the contrast between EC and microcoulometric responses to chlorine content in the PCB contained in Aroclors. In particular, the lower chlorinated Aroclors and the early eluting (lower chlorinated) PCB within the Aroclors show the most marked difference in pattern between EC and microcoulometric responses. This effect is consistent with the data on EC and microcoulometric responses presented above. Note that the amounts of each Aroclor injected

vary and that no attempt has been made to compare instrument sensitivity in these chromatograms.

The electrolytic conductivity detector was also shown to respond to the chlorine content of PCB in a study using a Coulson electrolytic conductivity detector specific for halogens. The system was operated in the reductive mode and utilized a platinum catalyst. Two injections each of Aroclor 1221 through 1260 were made and compared to the response of Aroclor 1242 which was used as a reference. The reference was injected immediately prior to the Aroclor being checked in each case. Aroclor 1242 was also used as a means of checking the sensitivity of the detector which varied from day to day and even during the course of a day's operation.

For these calculations the response for Aroclor 1242 and the Aroclor being checked were measured by planimeter in square inches. In this regard it was noted that the linearity for this detector appeared to drop off sharply when the response fell below 0.09 $in.^2$/ng chlorine, which was due primarily to the inability to accurately measure all the peaks in the reference or Aroclor being tested. Results were as follows:

Aroclor	ng injected	Response in ^{2}/ng Cl	Response to Aroclor / Response to Aroclor 1242
1221	125	0.12	1.01
1221	112.5	0.12	1.00
1242	Used as Reference		1.00
1248	115	0.09	1.05
1248	125	0.09	0.99
1254	125	0.10	1.01
1254	100	0.10	0.99
1260	125	0.09	1.02
1260	150	0.09	1.08

Significance. The use of Aroclors as reference materials for the quantitation of PCB residues involves inherent problems because the residue is most often altered in composition from the original Aroclor. Alteration can occur in several ways; e.g., weathering and/or metabolism, preferential storage of certain components in animals, variations in recovery of components through the methodology (described in this LID), and variation in

the amount of loss through volatilization of the different components (to be described in a forthcoming report from LOS-DO). The accuracy of the quantitative result decreases with the increasing alteration of the PCB residue from the pattern of the Aroclor reference.

The disproportionate response of the EC detector to the more highly chlorinated components aggravates the problem of quantitation of an altered residue. The analyst should be aware of these limitations and, if possible, quantitate altered residues using one of the halogen-specific detectors and reference Aroclor(s) whose pattern matches that of the residue most closely. This should minimize the problem until such time when an improved method of quantitation is available.

Fig 1 Aroclor 1221

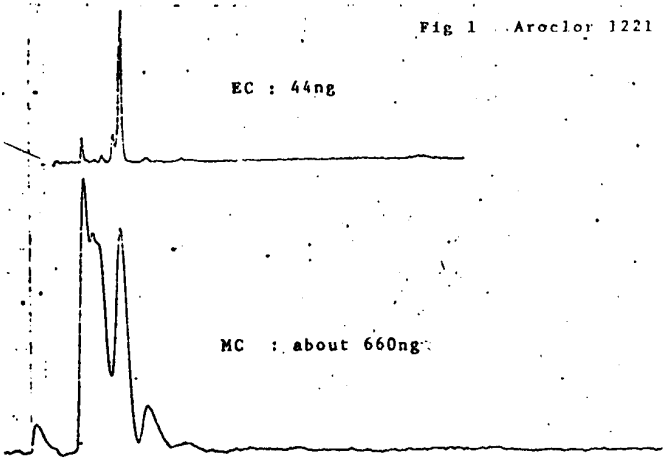


Fig. 2 Aroclor 1242

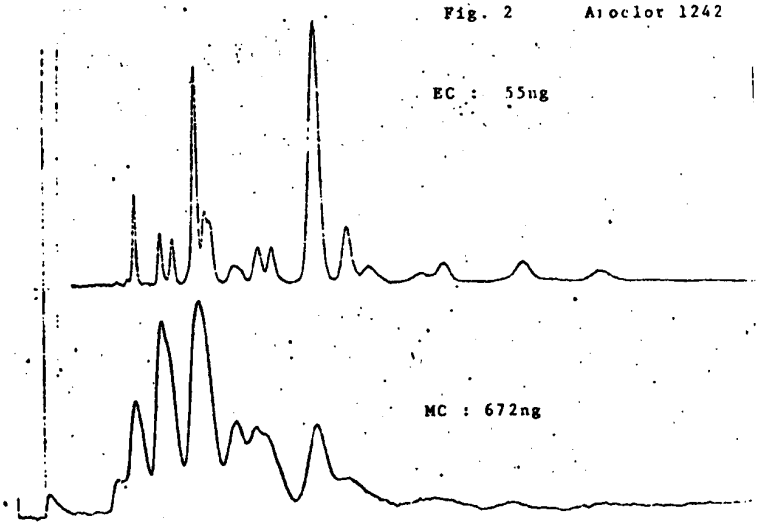


Fig. 3 Aroclor 1248

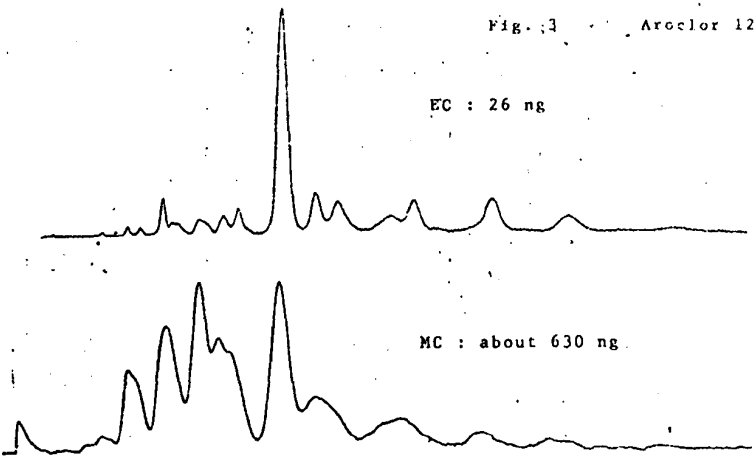


Fig. 4 Aroclor 1254

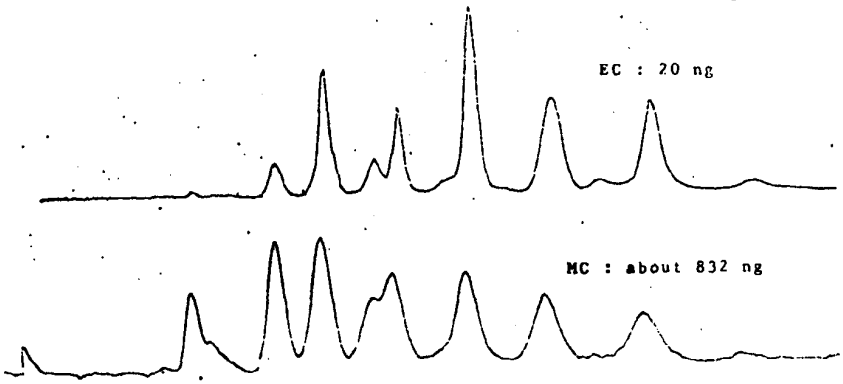
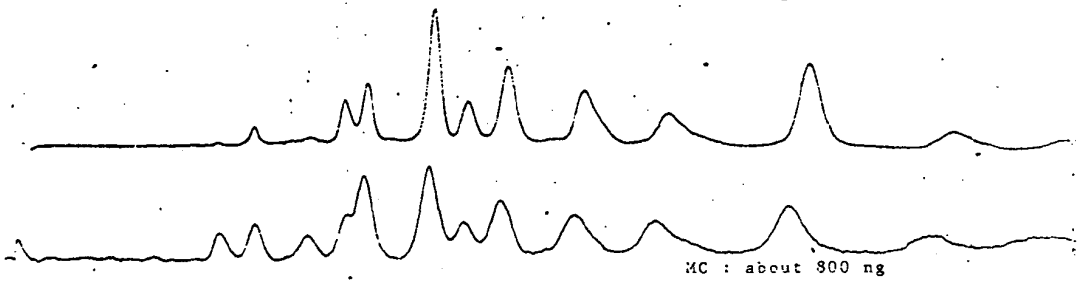


Fig. 5 Aroclor 1260



MCNS 078843

Instrument Conditions : Figures 1 - 5

EC : 10% DC 200 col, PAM I 311.2 Parameters; 70V; 1 x 10-9 afs

MC : 10% DC 200 col. at 200°C, 120 ml/min N_2; injector temp 225°C; halogen specific detector, 100X. gain 25.