

*on pilot  
method*

Rec'd 6/28/79

ANALYTICAL METHODOLOGY  
FOR  
POLYCHLORINATED BIPHENYLS  
JUNE 1979

FOOD AND DRUG ADMINISTRATION  
ROCKVILLE, MARYLAND

REC 1

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 to government agencies on request from Food and Drug Administration, Public Records and Documents Center, 5600 Fishers Lane, Rockville, Maryland 20857 or to non-government requesters from The National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161, as *item NTISUB/C/118*. Description of the method for paper products and other techniques necessary to analysis for PCB residues are attached.

Methodology for Analysis for PCB Residues

	<u>Food and Feeds</u>	<u>Paper</u>
Sample Preparation*	PAM I, 141.	Cut into about 1/4" x 1/4" pieces; thoroughly mixed. Attachment I
Extraction	PAM I, 211.13 or 212.13, depending on sample type	Alcoholic alkali <b>reflux</b> Attachment I
Cleanup	<b>CH<sub>3</sub>CN-petr</b> 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, 651.1	

Determination	Electron capture GLC: PAM I, 251.15 <b>300.64d(6/79)</b> , and 311 Attachment II	Electron capture GLC: PAM I, <b>300.64d(6/79)</b> and 311 Attachment II
Quantitation	PAM I 143, <b>300.64d(6/79)</b>	PAM I 143, <b>300.64d(6/79)</b>
Procedures Useful for Confirmation	GLC with halogen specific detection, PAM I, 312 or 315	GLC with halogen specific detection, PAM I, 312 or 315
	Micro scale alkali reflux, <b>PAM I 651.1</b>	TLC - see PAM I, 251.02 for references
	TLC - See PAM I, 251.02 for references	Perchlorination: Attachments III, IV
	Perchlorination: Attachments III, IV	
General	Attachment V	Attachment V

\*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 **PCBs** 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 PAM I **300.64d (6/79)**) 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. Once a choice has been made concerning which reference material(s) to use, the technique by which the GLC responses of the residue and reference material will be quantitatively compared must also be chosen (**PAM I, 300.64d (6/79)**). Since the choice of auxiliary procedures depends to some degree on the identity of the residue, 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 143.21, 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:

<u>Products</u>	<u>Approximate milligrams sample equivalent for <sup>H</sup> EC-GLC (PAM I, Table 331-A)</u>
Milk (fat)	12
Dairy Products (fat)	12
Poultry (fat)	6
<b>Eggs</b>	60
Complete and finished animal feeds	100
Animal feed components	10
Fish and shellfish	10
Infant and junior foods	100
Paper food packaging material	2

As pointed out in PAM I 143.21, the amount of sample equivalent injected should be adjusted to **accomodate** changes in detector sensitivity in order to maintain a constant limit of quantitation.

NOTE: The following references describe the successful interlaboratory collaborative studies which were performed on methods for PCB under the auspices of the Association of Official Analytical Chemists (AOAC); the methods thus tested have been granted AOAC official status:

Sawyer, L. D., Collaborative Study of the Recovery and Gas Chromatographic Quantitation of Polychlorinated Biphenyls in Chicken Fat and **Polychlorinated Biphenyl-DDT** Combinations in Fish, 3. Assoc. Offic. Anal. Chem. **56**, 1015-1023 (1973).

**Finsterwalder**, C.E., Collaborative Study of the Determination of **Polychlorinated Biphenyls** in Paperboard, J. Assoc. Offic. Anal. Chem. **57**, 518-524 (1974).

Sawyer, L.D., Quantitation of Polychlorinated **Biphenyl** Residues by Electron Capture Gas-Liquid Chromatography: Collaborative Study, J. Assoc. Offic. Anal. Chem., **61**, 282-291 (1978).

## Extraction and Cleanup for Determination of Polychlorinated Biphenyls in Paper and Paperboard

SUSAN J. V. YOUNG, CHARLES FINSTERWALDER,\* and JERRY A. BURKE  
*Division of Chemistry and Physics, Food and Drug Administration, Washington, D.C. 20204*  
*\* Food and Drug Administration, 1141 Central Parkway, Cincinnati, Ohio 45202*

A procedure is described for the determination of polychlorinated biphenyls (PCBs) in paper and paperboard. PCBs are extracted from the paper sample by reflux with alcoholic KOH. Column chromatography on Florisil is used for cleanup, if needed. Compounds are determined by GLC with electron capture detection, using conditions previously devised for organochlorine pesticides. An exhaustive extraction utilizing sulfuric acid was developed in order to obtain comparative data on extraction effectiveness. Recoveries of Aroclors 1242 and 1254 from fortified paper samples averaged 95.5 and 96.8% with the alcoholic KOH reflux and sulfuric acid digestion extractions, respectively. Aroclor 1242 determined after alcoholic KOH extraction of paperboard was 96% of the amount found after sulfuric acid digestion of comparable samples. Aroclor 1242 had been incorporated into the paperboard during manufacture.

Polychlorinated biphenyls (PCBs) are now well known as chemical contaminants. Their general uses, chemistry, and widespread residue occurrence have been thoroughly discussed (1-3). In 1971 the Food and Drug Administration discovered the presence of PCB in paper food packaging and in some foods packaged in this paperboard. The packaging containing PCB had been manufactured from recycled waste paper, including waste carbonless copying paper. Prior to discontinuance of its use in 1971, Aroclor 1242, a commercial PCB, had been an ingredient in carbonless copying paper. It is anticipated, however, that the presence of PCB in paper destined for recycling will continue for some time. To effectively control the disposition and use of paper containing PCBs, an analytical method is required to determine PCBs in paper and paperboard. Our objective was to make maximum utilization of existing analytical techniques for PCBs in foods (4, 5) in the analysis of paper and paperboard. Most attention was directed to ex-

traction of PCBs from the sample and optimization of the parameters of the procedure. The 2 extraction procedures presented here take advantage of the stability of PCBs to alkali and acid. Because PCBs are the only chemicals sought by the analysis, the use of a harsh chemical treatment and an abbreviated cleanup is possible. The extraction by reflux with alcoholic KOH was designed for use with routine samples. The more tedious sulfuric acid digestion was developed as an extraction for comparative purposes on selected samples.

### METHOD

#### Apparatus for Alcoholic Alkali Extraction

(a) *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: nitrogen, 120 ml/min; 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  $1 \times 10^{-9}$  amp.

(b) *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 equivalent).

(c) *Chromatographic column*.—Plain, 22 mm id X 300 mm (Kontes Glass Co., No. K-420300, size 21, or equivalent).

(d) *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 equivalent).

(e) *West condenser*.—400 mm jacket length with  $\overline{\text{F}}$  inner drip joint to fit 250 and 500 ml Erlenmeyer flasks.

(f) *Erlenmeyer flasks*.—250 ml, with  $\overline{\text{F}}$  outer joint to fit West condenser.

(g) *Separatory funnel*.—250 ml, with Teflon stopcock.



#### Apparatus for Sulfuric Acid Digestion Extraction

See (a)–(e), (f) (except 500 ml), and (g) (except 1 L), above, plus the following:

- (a) *Buchner funnel*.—Porcelain, 125 mm id.
- (b) *Filter paper*.—11 cm diameter.

#### Reagents for Alcoholic Alkali Extraction

(a) *Florisil*.—60–100 mesh 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 dark. Heat  $\geq 5$  hr but preferably overnight at 130°C before use. Store at 130°C in glass-stoppered or foil-covered bottle. Alternatively, store stoppered container in desiccator at room temperature and reheat at 130°C after 2 days.

(b) *Potassium hydroxide*.—Anhydrous pellets.

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

(d) *Alcoholic KOH reflux reagent*.—2% (g/ml) KOH in ethanol.

(e) *Petroleum &*.—Suitable for use with electron capture gas chromatography (Burdick and Jackson Laboratories Inc., 1953 S. Harvey St., Muskegon, Mich. 49442, or equivalent).

(f) *Polychlorinated biphenyls*.—Commercial mixtures (*Aroclors*) for reference in gas chromatographic determination (Monsanto Co., St. Louis, Mo. 63186).

#### Reagents for Sulfuric Acid Digestion Extraction

See (a) and (a) above plus the following:

(a) *Concentrated H<sub>2</sub>SO<sub>4</sub>*.—ACS grade.

(b) *Hexane*.—Suitable for use with electron capture gas chromatography (Burdick and Jackson Laboratories, Inc., or equivalent).

(c) *Ethanol*.—95%, USP.

#### Alcoholic Alkali Extraction

Paper sample representative of lot in question should be cut into pieces no larger than  $\frac{1}{4} \times \frac{1}{4}$ " and thoroughly mixed.

Weigh 10 g cut up and well mixed paper material into 125 ml Erlenmeyer flask. Do not pack sample tightly. (See note below if volume of 10 g sample is  $> \text{ca. } 50$  ml.) Add 60 ml 2% ethanolic or methanolic KOH, and fit flask with West condenser cooled with circulating cold tap water. Reflux gently on steam bath 30 min. When refluxing is complete, rinse down inside of condenser with a small volume of alcohol before removal from flask. Transfer reflux solution, using a small funnel with glass wool plug, to 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 separator-y funnel. Add 60 ml water to separator-y funnel and shake vigorously 30 sec. Drain



lower aqueous layer into second 250 ml separatory funnel. Add 60 ml petroleum ether to second separatory funnel and shake vigorously 30 sec. Discard aqueous layer and combine petroleum ether layers in first separatory funnel. Rinse second separatory funnel with 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 25 x 50 mm column of anhydrous Na<sub>2</sub>SO<sub>4</sub>, collecting eluate in Kuderna-Danish concentrator. Rinse separatory funnel and then column with three ca 20 ml portions of petroleum ether, collecting rinses. Concentrate combined petroleum ether extract and rinses on steam bath to ca 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 g or the volume of reflux solution upward from 60 ml. If possible, reduce the weight of the sample material below 10 g 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 10 g) 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.

#### Sulfuric Acid Digestion Extraction (Optional)

Prepare representative paper sample as above and weigh 5–10 g cut up and mixed sample into 500 ml Erlenmeyer flask. Add distilled water and mix until paper material is completely saturated and small amount of unabsorbed water is present. (Three g water/g sample is usually adequate; very low density paper material may require a larger amount of water.) Fit flask with West condenser cooled with circulating cold tap water. Immerse flask about  $\frac{3}{4}$  of its height into bath of cold tap water. Slowly add fresh concentrated sulfuric acid, equivalent to  $\frac{1}{3}$  volume water added, through top of the condenser, with frequent gentle swirling of flask. Let reaction mixture cool in water bath ca 5 min. Slowly and cautiously add 75 ml distilled water through top of condenser. Swirl flask until contents are well mixed. Let reaction mixture cool in water bath ca 5 min. Add 75 ml ethanol through top of condenser. Swirl flask until contents are well mixed. Let reaction mixture cool to room temperature while standing in water bath 20 min. Filter reaction mixture with vacuum thre-



125 mm Büchner funnel fitted with 11 cm **Sarkis** paper into 500 ml Erlenmeyer flask. Keep vacuum on throughout entire filtering and rinsing. Rinse reaction flask and then Büchner funnel with three 25 ml portions of ethanol. Rinse reaction flask and then Büchner with four 25 ml portions of hexane. Carefully and completely transfer entire filtrate (two Layers, hexane and aqueous) to 1 L separatory funnel, rinsing filter flask a few times with small portions of ethanol followed by hexane. Add 75 ml distilled water to separatory funnel and shake vigorously 1 min. Drain lower aqueous layer into second 1 L separatory funnel. Add 100 ml hexane to second separatory funnel and shake vigorously 1 min. Discard aqueous layer and combine hexane layers in first separatory funnel. Rinse second separatory funnel with few small portions of hexane, collecting rinses in first separatory funnel. Wash hexane with three 100 ml portions of distilled water, discarding each wash. Dry hexane through 25 × 50 mm column of anhydrous Na<sub>2</sub>SO<sub>4</sub>, collecting eluate in Kuderna-Danish concentrator. Rinse separatory funnel and column with 3 ca 20 ml portions of hexane, collecting rinses. Concentrate combined hexane extract and rinses on steam bath to ca 5 ml. Extract is ready for cleanup on Florisil column.

#### Cleanup

Add 10 g Florisil to 22 mm id chromatographic tube. Settle column of Florisil by gentle tapping of chromatographic tube. Top column with ca 1/2" anhydrous Na<sub>2</sub>SO<sub>4</sub>. 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 ca 5 ml/min. Rinse container with three 5 ml portions of petroleum ether, transferring each to column, and rinse walls of chromatographic tube with additional small portions of petroleum ether. Elute column at ca 5 ml/min with 150 ml 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-Vigreux 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* (1972) Vol. 1, Food and Drug Administration, Washington, D.C., 211.14.)

#### Results and Discussion



Our objective of an extraction and cleanup procedure specifically for the analysis of paper material for PCBs was met by the alcoholic alkali

reflux extraction and Florisil column chromatographic cleanup described above. An extraction procedure based on the digestion of paper with sulfuric acid was developed in order to evaluate the extraction efficiency of the alcoholic KOH reflux.

Preliminary experiments indicated that ethanol or methanol was a good extractant for PCBs from paperboard and that hexane did not remove as much PCBs as did alcohol. The stability of PCBs to alkali permitted the incorporation of alkali treatment into the extraction operation. This had the advantages of eliminating electron-capturing substances and of providing additional evidence for the identity of PCB residues. Column chromatography on Florisil with petroleum ether eluant provided a generally effective cleanup procedure. Numerous experiments with paperboard fortified with Aroclors 1242 or 1254 were conducted to arrive at optimum physical parameters for the alcoholic KOH extraction and cleanup. The ratio 60 ml extractant to 10 g sample was found to accommodate paperboard of the density usually present in food containers. Analysis of lower density paper, such as newspaper, requires reduction in sample size or proportional scaling up of all extraction reagents. Adequate rinsing of the reflux flask and residual sample during transfer of the extract to the separatory funnel is essential for good recovery. The 3 rinses with 40 ml portions of petroleum ether as directed in the Method are necessary to transfer all PCBs. Aroclors 1242 and 1254 are completely eluted from the Florisil column with the first 75 ml of the 150 ml eluant. Waxes encountered in heavily waxed papers may not be completely removed from the extract by Florisil column chromatography. Waxes can be removed from samples of this type by partitioning a petroleum ether solution of the extract with acetonitrile (4) prior to Florisil column chromatography. Recoveries of PCB through the partitioning decreased with increasing chlorination, ranging from 94% for Aroclor 1221 to 74% for Aroclor 1262. Recoveries of Aroclors 1242 and 1254 were 97 and 87%, respectively. Extracts of certain paper material will not require cleanup. With experience, the analyst can decide which extracts can be analyzed by gas chromatography (GLC) without prior cleanup.

GLC with electron capture detection as described under Apparatus was used throughout this investigation and is recommended for sample

Table 1. Recovery of added PCB from paperboard

Level added <sup>a</sup>	Recovery	
	Alc. KOH reflux	H <sub>2</sub> SO <sub>4</sub> digestion
Aroclor 1242—8 ppm	96.1	95.9
	94.0	92.5
	95.2	99.0
	97.9	99.0
Aroclor 1254—8.2 ppm	93.4	97.0
	55.7	98.3
	95.0	96.0
	—	—
Average	95.5	96.8

<sup>a</sup> Aroclor added to sample in laboratory.

analyses. This is the same GLC system and parameters used by our laboratories for determination of organochlorine pesticide residues (5). In general under these GLC conditions, 20–40 ng Aroclor 1242 and 10–20 ng Aroclor 1254 produce a response suitable for quantitation. With this magnitude of response, injection of 5 mg equivalent of paper is desirable for determination of 4–5 ppm Aroclor 1242. To achieve this with a 10 g sample of paper requires that the prepared extract be concentrated to 10 ml, and that 5  $\mu$ l be injected for GLC. Sample examination and residue measurement are made at different PCB levels with appropriate adjustment of extract volume. All quantitative measurements in the work reported here were made by comparing the total area of the sample chromatogram with the total area of the chromatogram of either Aroclor 1242 or 1254.

Table 1 gives recoveries of Aroclors 1242 and 1254 from paperboard fortified prior to extraction by alcoholic KOH reflux and cleanup by Florisil chromatography as described in the *Method*. These results, averaging 95.5% for the 2 Aroclors, demonstrate essentially complete recovery of these representative PCBs.

The completeness of extraction of PCBs from paper by the alcoholic KOH reflux was determined in comparison with the extraction based on digestion of the sample with sulfuric acid. The more rigorous sulfuric acid digestion resulted in disintegration of the paper into a fine particulate suspension. PCBs were then readily extracted from the diluted aqueous digest into hexane. Parameters of the sulfuric acid digestion were developed in order to achieve nearly complete recovery of Aroclors 1242 and 1254. Table 1 gives recoveries of these representative PCBs added to

Table 2. Extraction of PCB (Aroclor 1242) from paperboard prepared to contain 2 levels of PCBs during manufacture

	Alcoholic KOH reflux		H <sub>2</sub> SO <sub>4</sub> digestion	
	Low	High	Low	High
	8.49	67.5	8.80	75.1
	8.02	73.9	8.98	77.3
	8.61	72.4	8.13	70.8
	8.53	74.1	—	—
	8.60	—	—	—
Av.	8.45	72.0	8.86	74.4
KOH/H <sub>2</sub> SO <sub>4</sub>	95.4%	96.8%	—	—

paper, determined with the sulfuric acid digestion and Florisil column cleanup as described in the *Method*. Recoveries were essentially complete, averaging 96 and 98% for Aroclors 1242 and 1254, respectively.

The comparison of extraction procedures was conducted on samples of paperboard containing Aroclor 1242 incorporated during manufacture. One lot of the *paperboard* had been prepared to contain about 10 times the level of Aroclor 1242 as the other lot. All comparisons were conducted by using the alcoholic KOH reflux and sulfuric acid digestion extractions as described above. The Florisil column cleanup was used with both extraction procedures. Results of the extraction comparison are given in Table 2. There is consistent good agreement on the levels of Aroclor 1242 determined following either extraction procedure. Aroclor 1242 determined at the 8 or 70 ppm level after alcoholic KOH reflux extraction averaged 96% of the average level determined after sulfuric acid digestion. Sulfuric acid digestion or additional alcoholic KOH reflux of the paper residue remaining after the initial alcoholic KOH reflux resulted in determination of only 1–3% additional Aroclor 1242. No additional PCBs were found on re-extraction of the particulate residue remaining after sulfuric acid digestion. These results show that extraction by alcoholic KOH reflux removes essentially all PCBs in paper.

The good recoveries, high extraction efficiency, and the relative ease of laboratory application recommend the alcoholic KOH reflux extraction and Florisil column chromatography cleanup for use in analysis of paperboard and paper for determination of PCBs. Because of the length of time and attention required and the inconvenience of working with sulfuric acid, this proce-

Image: A small, dark, rectangular, textured area, possibly a scan artifact or a small photograph, located on the left side of the page.

Image: A small, dark, rectangular, textured area, possibly a scan artifact or a small photograph, located on the left side of the page.

Image: A small, dark, rectangular, textured area, possibly a scan artifact or a small photograph, located on the left side of the page.

dure is not recommended for general use but only for comparative purposes on selected samples.

**Acknowledgment**

The authors wish to thank Richard Stanovick, Hazleton Laboratories, Inc., Falls Church, Va., for the generous contribution of paperboard specially prepared to contain Aroclor 1242.

**REFERENCES**

- (1) Peakall, D. B., & Lincer, J. L. (1970) *BioScience* 20, 958-964
- (2) Zitko, V., & Choi, P. M. K. (1971) *Fish. Res. Board Can. Tech. Rep.* 272, pp. 1-55
- (3) Interdepartmental Task Force on PCB's (May, 1972) National Technical Information Service, Springfield, Va. 22151, Com-72-10419
- (4) Young, S. J. V., & Burke, J. A. (1972) *Bull Environ. Contam. Toxicol.* 7, 160-167
- (5) *Pesticide Analytical Manual* (1968) Vol. 1, 2nd Ed., Food and Drug Administration, sec. 211.14(a) and 211.14(d); Rev. Apr. 1971, sec. 251; Rev. Jan. 1972, sec. 311.5A and 311.5B



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<sup>®</sup>

JUDITH A. ARMOUR

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

(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<sup>®</sup>. Aroclors<sup>®</sup> 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<sup>1-3</sup>, are generally determined by essentially the same techniques used for organochlorine pesticides<sup>4</sup> 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<sup>®</sup> as analytical references for PCB residue determinations.

Aroclor<sup>®</sup> 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<sup>5</sup>. 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

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<sup>6</sup>, are presented here. Data are not presented for Aroclors 1232, 1268, 5442, 5460, and 4465. Aroclor 1232 has been commercially prepared<sup>7</sup> 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<sup>8</sup>.

**EXPERIMENTAL**

GLC data were obtained with a gas chromatograph equipped with an electron capture detector and 4-mm x 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<sup>9</sup>. 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  $1 \times 10^{-9}$  A. Recorder speed was  $\frac{1}{2}$  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*<sup>6</sup> 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

<sup>6</sup>*Chromatogr.*, 72 (1972) 175-182

TABLE I

--B-s-

QUANTITIES OF AROCLORS NECESSARY FOR APPROXIMATELY HALF FULL SCALE RECORDER RESPONSE\*

Aroclor	Nanograms for 1/2 f.s.r.	
	DC-200 <sup>b</sup>	QF-r/DC-200 <sup>c</sup>
1221	80	60
1242	40	40
1248	40	30
1254	30	30
1260	20	20
1262	20	20

\* 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.

<sup>b</sup> 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. X 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	Aroclor	Aroclor	Aroclor	Aroclor	Aroclor
1221	1242	1248	1254	1260	1262
0.21					
0.26					
0.31					
0.36					
0.39	Q-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	1.36
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	2.86	1.85
2.08				2.07	2.07
2.22	2.20	2.20	2.20	2.21	2.22
	2.36	2.54			
2.65			2.63	2.63	2.61
				2.84	2.82
3.10		3.04	3.06	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

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 H<sub>2</sub> 80-100 mesh; stationary phase: 1:1 15% QF-1/10% DC-200 (6 ft. x 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	5.82		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 - Y	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

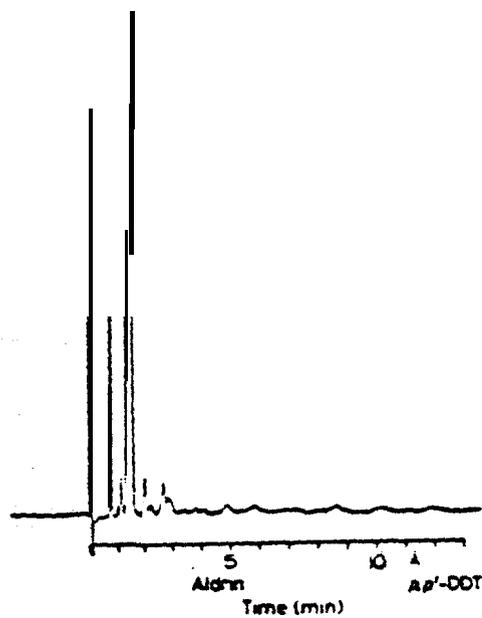


Fig. 1. CLC separation on 10% DC-200 column of 20 ng Aroclor 1221. CLC conditions are given under EXPERIMENTAL.

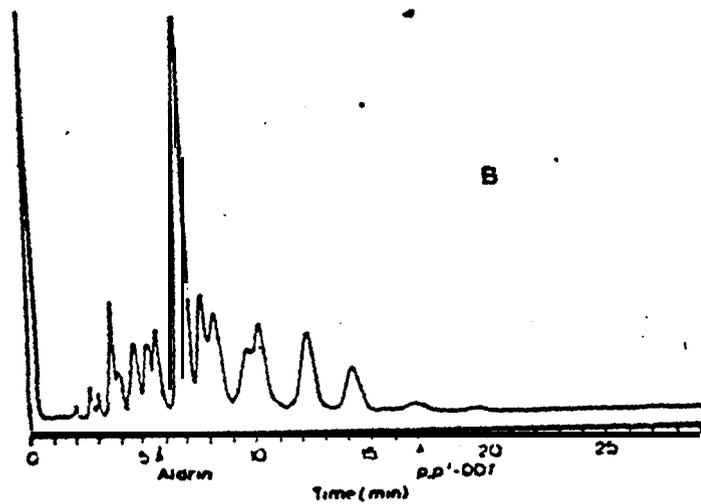
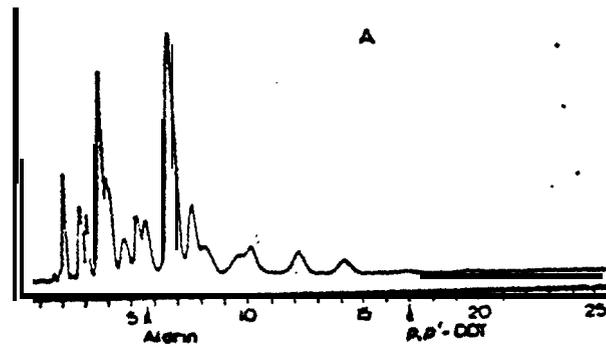


Fig. 2. GLC separation on 10% DC-200 column of (A) 50 ng Aroclor 1242 and (B) 50 ng Aroclor 1248. GLC conditions are given under EXPERIMENTAL.

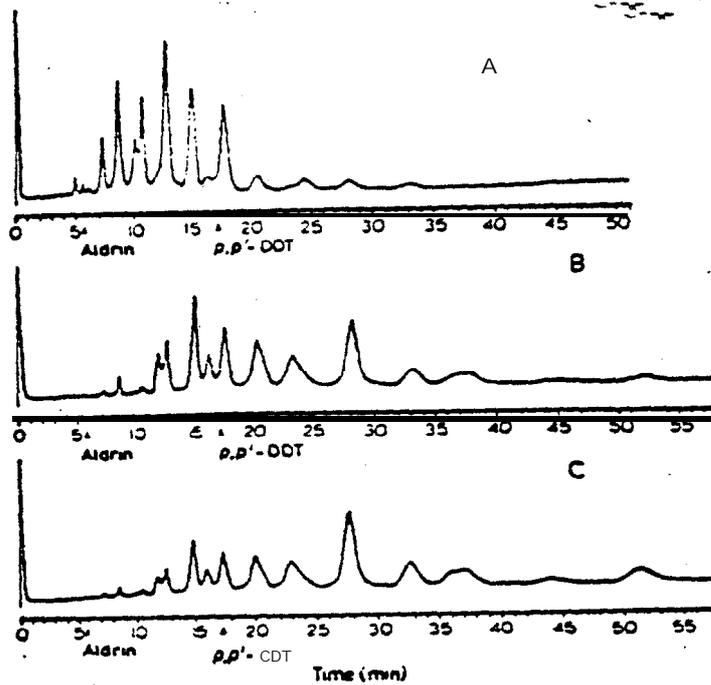


Fig 3. GLC separation on 20% DC-200 column of (A) 32 ng Aroclor 1254, (B) 20 ng Aroclor 1260 and (C) 20 ng Aroclor 1262. GLC conditions are given under EXPERIMENTAL.

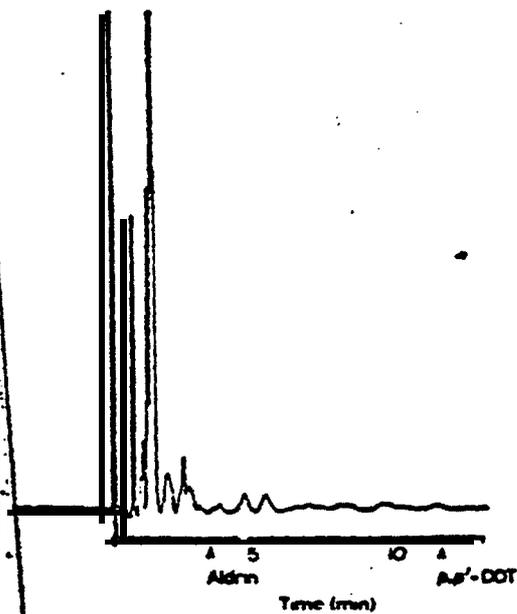


Fig 4. Gas chromatographic separation on 1:1 15% QF-1/10% DC-200 of 120 ng Aroclor 1221. GLC conditions are given under EXPERIMENTAL.

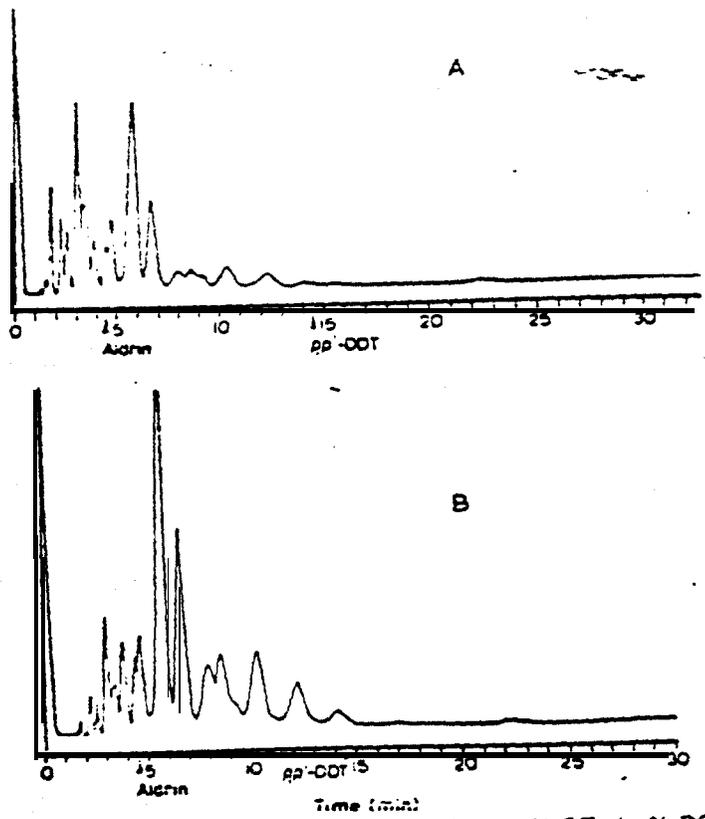


Fig. 5. Gas chromatographic separation on 1:1 15% QF-1/10% DC-200 of (A) 50 ng Aroclor 1242 and (B) 50 ng Aroclor 1248. GLC conditions are given under EXPERIMENTAL.

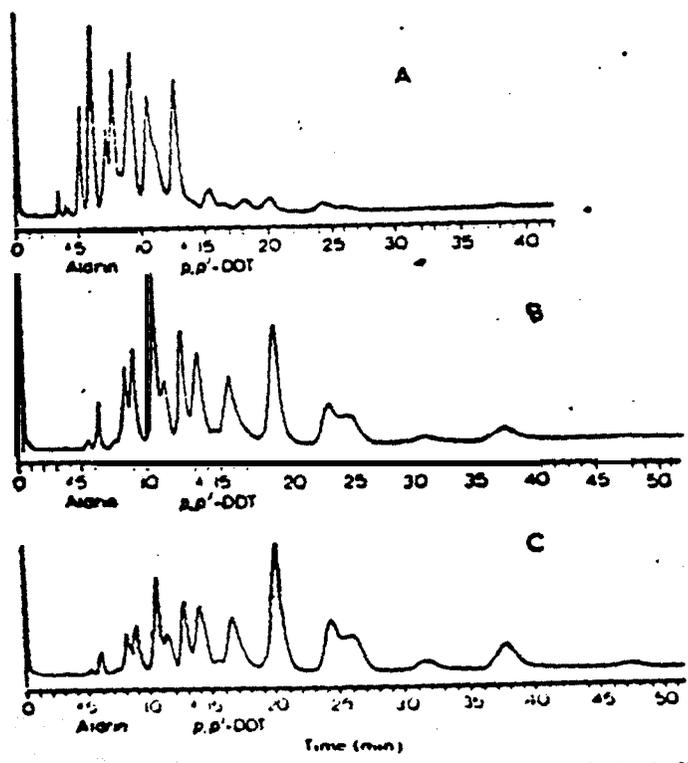


Fig. 6. Gas chromatographic separation on 1:1 15% QF-1/10% DC-200 of (A) 32 ng Aroclor 1251, (B) 20 ng Aroclor 1261, and (C) 20 ng Aroclor 1262. GLC conditions are given under EXPERIMENTAL.

## REFERENCES

- 1 D. B. PEAKALL AND J. L. LINGER, *Bioscience*, 20 (1970) 958.
  - 2 L. M. REYNOLDS, *Residue Rev.*, 34 (1970) 27.
  - 3 R. W. RISEBROUGH, P. REICHE AND H. S. OLCOTT, *Bull. Environ. Contam. Toxicol.*, 4 (2969) 192.
  - 4 J. A. ARMOUR AND J. A. BURKE, *J. Ass. Offic. Anal. Chrm.*, 53 (1970) 761.
  - 5 *Aroclor Plasticizers*, Technical Bulletin O/PL-306, Monsanto Co., St. Louis, Mo.
  - 6 *Pesticide Analytical Manual, Vol. I*, Food and Drug Administration, Washington, D.C., 1968.
  - 7 Monsanto Chemical Company, private communication, November, 1971.
  - 8 W. W. WIENCKE, U.S. Food and Drug Administration, private communication, January, 1972.
  - 9 J. BURKE AND W. HOLSWADE, *J. Ass. Offic. Anal. Chrm.*, 49 (1966) 374.
- J. Chromatogr.*, 7: (1972) 275-282

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

JUDITH A. ARMOUR

Division of Chemical Technology, Food and Drug Administration, Washington, D.C. 20204

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  $\mu\text{g}$ ) 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<sup>®</sup>) (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 decachloro-

biphenyl (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.

### 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 x 10 mm od with No. 4 Teflon valve (Kontes Glass Co. No. K896860-0004) or Carius Pyrex combustion tube, 200 x 8 mm id, 10 mm od (timing 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, 300 ml capacity (Kontes Glass Co. No. K5470000, or equivalent).

(d) *Micro-Snyder column*.—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  $\frac{1}{2}$  full scale recorder deflection for 1 ng decachlorobiphenyl when full scale deflection is  $1 \times 10^{-9}$  amp, and 6' X 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 I-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 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: use a total 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 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<sub>3</sub> 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 multi-pesticide residue methodology. In the Berg *et al.* procedure, cleaned up extracts containing PCBs were evaporated to dryness before reaction with

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 clean-up 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-

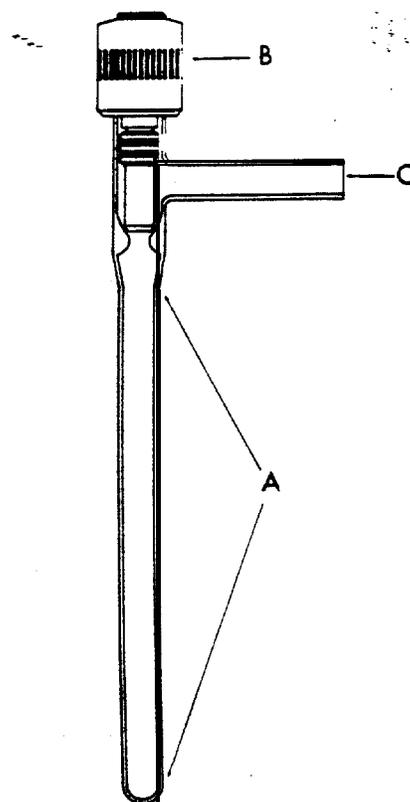


FIG. 1.—Vacuum hydrolysis tube recommended for sealed system reaction: A, dimensions: 13 mm od  $\times$  150 mm; B, tight Teflon sealing valve; C, venting side arm.

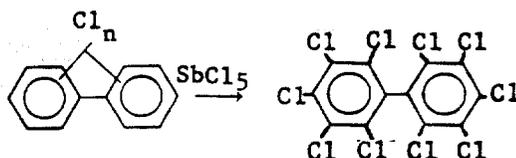
product is formed when the antimony pentachloride is contaminated with bromine. Care should be taken to avoid use of contaminated antimony pentachloride.

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:

$(\text{DCB peak ht sample} / \text{DCB peak ht std}) \times \text{ng std injected} = \text{ng DCB in sample injection}$   
 $\text{ng DCB in sample injection} \times \text{factor for specific Aroclor } (X) = \text{ng Aroclor in sample injection}$

$(\text{ng Aroclor in sample injection} / \text{wt sample injected (mg)}) = \text{ppm Aroclor in sample}$

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

Aroclor	Av. No. Cl <sup>a</sup>	MW <sup>b</sup>	X <sup>c</sup>
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

<sup>a</sup> Average whole number of chlorines calculated from per cent chlorine substitution for a specific Aroclor.

<sup>b</sup> Molecular weight of Aroclor based on the average whole number of chlorines calculated from per cent chlorine substitution.

<sup>c</sup>  $X$  = 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  $\mu\text{g}$  PC6 to dodechlorobiphenyl st overnight conditions

Aroclor	Trials	Range, %	Average, %
1242	10	90-105	97
1016	5	87-105	95
1248	5	98-102	99
1254			
1260	5	990-95	100
1262	3	89-103	95

### Recoveries

To determine the per cent conversion of specific Aroclors to DCB, a series of 1  $\mu\text{g}$  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 (89% 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  $\mu\text{g}$  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

Table 3. Per cent conversion of 1  $\mu\text{g}$  PCB to decachlorobiphenyl with iron catalyst and 6 hr reaction

Aroclor	Trials	Range, %	Average, %
1242 <sup>a</sup>	10	78-98	90
1242	10	96-110	102
1016	3	87-95	92
1248	3	95-100	98
1254			
1260	1	91-108	198
1262	3	83-107	98

<sup>a</sup> 4 hr reaction.

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

Sample	Ref. Aroclor	Residue, ppm		
		By total area	By DCB <sup>a</sup>	
			Trial 1	Trial 2
Chub	1254	3.5	4.2	
Chub			4.2	
Chub	1254	3.2	5.1	
Chub			4.5	
Sturgeon <sup>b</sup>	1248	28.4	28.6	28.4
Eggs	1242	1.1	6.8	7.1
Chicken fat <sup>c</sup>	1242	4.6	5.0	
Chicken fat <sup>c</sup>	1248	5.9	6.6	

<sup>a</sup> Expressed as reference Aroclor.

<sup>b</sup> Residue is reported on a fat basis.

<sup>c</sup> Samples fortified with Aroclor for an interlaboratory study.

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  $\frac{1}{15}$  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.

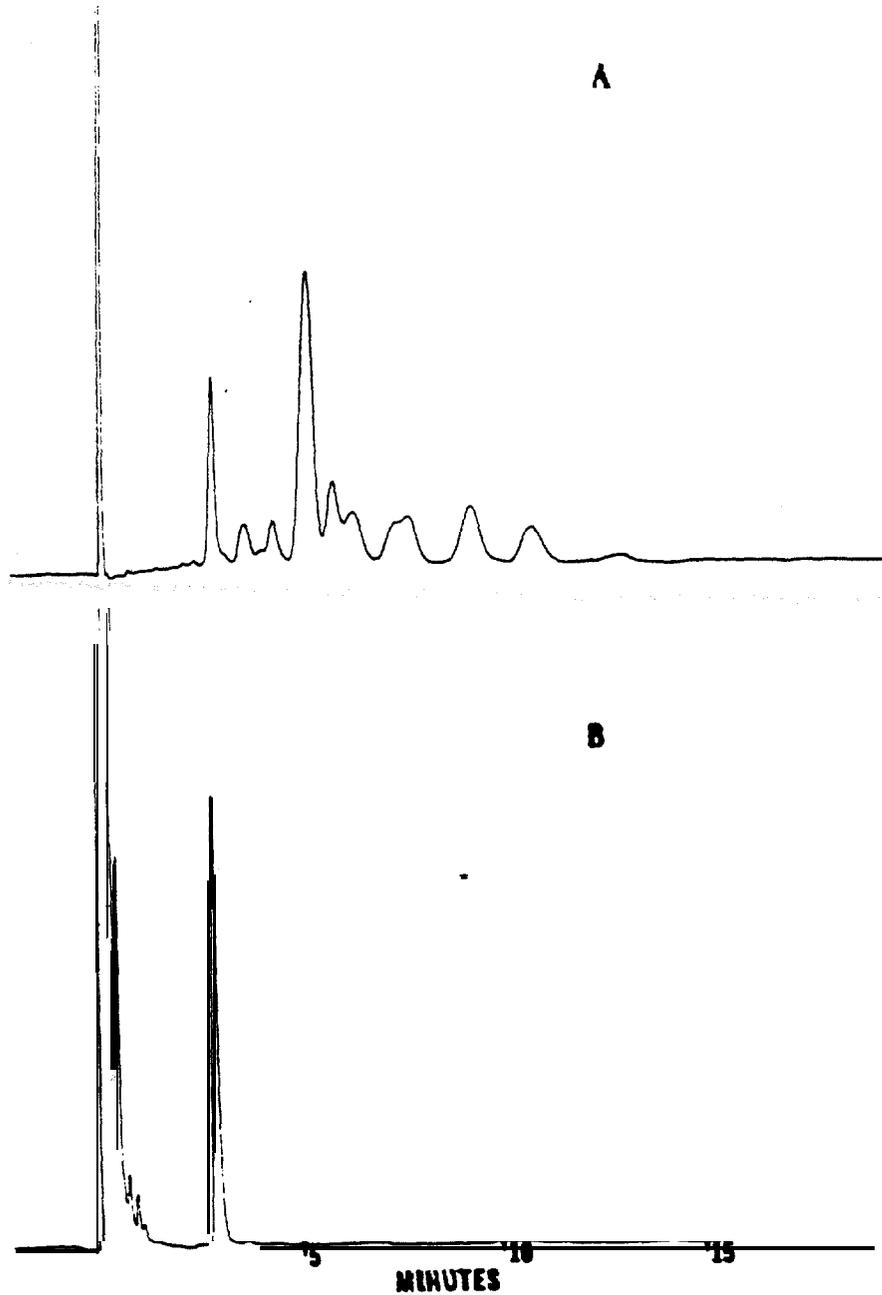


FIG. 2—GLC curves of egg samples containing 7.1 ppm PCBs (as Aroclor 1242) before and after perchlorination: A, 1.11 mg sample (7.9 ng Aroclor 1242), 6% Florisil eluate before perchlorination, 10% DC-200 column (9,10); B, 0.07 mg sample (0.5 ng equivalent Aroclor 1242), 6% Florisil eluate after perchlorination,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

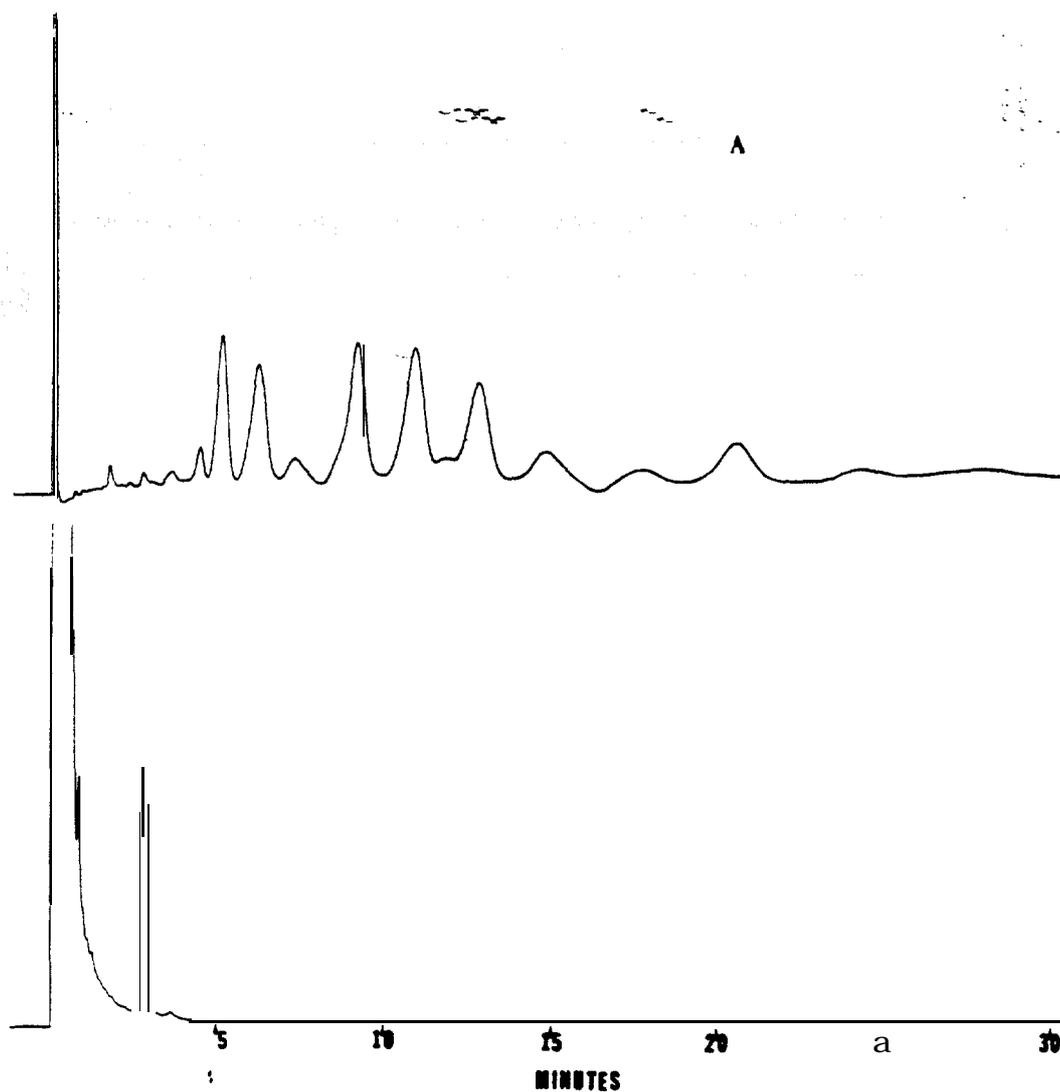


FIG. 3—GLC curves of chub sample calculated as 3.4 ppm PCBs (as Aroclor 1254) before perchlorination and calculated as 4.2 PCBs (as Aroclor 1254) after perchlorination: A, 1.5 mg sample (5.1 ng Aroclor 1254), petroleum ether eluate; □□□● illic acid before perchlorination, 10% DC-200 column (9,10); B,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

## REFERENCES

- (1) Peakall, D. B., & Lincer, J. L. (1970) *Bio-Science* **20**, 958-964
- (2) Reynolds, L. M. (1970) *Residue Rev.* **34**, 25-57
- (3) Risebrough, R. W., Reiche, P., & Olcott, H. S. (1969) *Bull. Environ. Contam. Toxicol.* **4**, 192-201
- (4) Armour, J. A., & Burke, J. A. (1970) *JAOAC* **53**, 761-768
- (5) *Aroclor Plasticizers*, Technical Bulletin O/PL306, Monsanto Co., St. Louis, Mo.
- (6) Armour, J. A. (1972) *J. Chromatogr.* **72**, 275-282
- (7) Berg, O. W., Diosady, P. L., & Rees, G. A. (1972) *Bull. Environ. Contam. Toxicol.* **7**, 338-347
- (8) Hutzinger, O., Safe, S. H., & Zitka, J. (1972) *Int. J. Environ. Anal. Chem.* **2**, 95-106
- (9) *Pesticide Analytical Manual* (1972) Vol. I, Food and Drug Administration, Washington, D.C.
- (10) *Official Methods of Analysis* (1970) 11th Ed., AOAC, Washington, D.C.

## Limitation on the Use of Antimony Pentachloride for Perchlorination of Polychlorinated Biphenyls

WILLIAM J. TROTTER and SUSAN J. V. YOUNG

Division of Chemistry and Physics, Food and Drug Administration, Washington, DC 20204

Two contaminants are present in commercially available antimony pentachloride ( $\text{SbCl}_5$ ) used to perchlorinate polychlorinated biphenyls (PCBs) to decachlorobiphenyl (DCB). DCB is found in the  $\text{SbCl}_5$  perchlorination reaction blank in which no PCBs were added. Bromononachlorobiphenyl (BNCB) is found after use of  $\text{SbCl}_5$  to perchlorinate PCBs. Levels of DCB found in the  $\text{SbCl}_5$  reaction blanks from various distributors ranged from 8 to 972 ng DCB/ml  $\text{SbCl}_5$ . The relationship of the formation of BNCB to amounts of various PCB Aroclors perchlorinated is examined.

Polychlorinated biphenyl (PCBs) residues are extracted, cleaned up, and detected by methods similar to those used for organochlorine pesticides. PCB residues are quantitatively determined by comparing the gas-liquid chromatographic (GLC) response of the multicomponent residue and commercial PCBs (Aroclor®) or a mixture of Aroclors producing a GLC response pattern similar to that of the residue (1). This approach is limited because the multicomponent PCB residue may not have the same proportional composition as the Aroclor or Aroclors used as the quantitation reference. Residues can be composed of mixtures of chlorobiphenyl components from more than 1 Aroclor. Metabolic and other environmental factors complicate the description of the PCB residue composition.

There has been considerable work to develop methods to convert the multicomponent PCBs to a single derivative on which to base the residue determination. Procedures have been reported to catalytically dechlorinate PCBs with hydrogen over palladium or platinum to biphenyl, cyclohexylbenzene, and bicyclohexyl (2, 3). A principal disadvantage with that procedure is that the hydrocarbon product is determined with a GLC flame ionization detector, resulting in low sensitivity. Attempts have been made to convert PCBs to the fully chlorinated decachlorobiphenyl (DCB) (3-5). Armour (6) reported optimum conditions for perchlorinating PCBs with antimony pentachloride

( $\text{SbCl}_5$ ). The method provides a qualitative confirmatory procedure for PCB determination. The GLC electron capture detector response is enhanced because total PCBs are manifested as a single peak for DCB. In measuring the single peak for DCB the analyst is not faced with analytical judgments such as baseline correction, method of integration, or discrimination between PCBs and non-PCB components. However, it is necessary to be aware that the various Aroclors give rise to different equivalents of DCB (6) and that the nonchlorinated biphenyl (also used as a fungicide) is perchlorinated by  $\text{SbCl}_5$  to DCB. Nonetheless, using the perchlorination derivatization can reinforce the residue value determined by measuring a multicomponent PCB residue.

During attempts to apply the perchlorination derivatization in determining low residue levels of PCB and make use of the increased electron capture response to DCB, 2 contaminants were indicated which led to erratic recoveries of DCB.

### Experimental

#### Reagents and Apparatus

(a) *Antimony pentachloride*.—Hooker Chemical, Niagara Falls, ST 14302 (received in glass bottle with lead-lined cap); Matheson Coleman & Bell (MCB), Norwood, OH 45212 (reagent grade); B&A (Allied Chemical), Morristown, NJ 07960 (reagent grade, 99%); Research Organic-Inorganic Chemical (ROC-RIC), Belleville, S J 07109 (99.99%); and J. T. Baker Chemical, Phillipsburg, NJ 08865 (Baker Analyzed Reagent).

(b) *Gas chromatograph*.—Searle-Analytic (Des Plaines, IL 60088) Model 5360 with 6' x 4 mm id glass column containing 1% OV-101 on 80-100 mesh Chromosorb W (HP). Operating conditions: column flow, 60 ml nitrogen/min; column, 202°C; detector, 202°C; injector, 225°C; pin-cup design electron capture detector with titanium  $^3\text{H}$  foil; detector voltage (constant dc) adjusted to cause one-half full scale recorder deflection for 0.7 ng DCB when full scale deflection is  $1 \times 10^{-8}$  amp.

(c) *Mass spectrometer*.—Varian MAT (25 Route 22, Springfield, NJ 07081) CH5-DF mass spectrometer (MS) coupled to Varian Aerograph 2740 gas

chromatograph via all-glass system using Watson-Biemann 2-stage separator. GLC operating conditions: 6' x 4 mm id glass column containing 3% OV-1 on 80-100 mesh Chromosorb W (HP); column flow, 60 ml helium/min; column 240°C. MS operating conditions: electron energy, 70 eV; emission current, 300  $\mu$ A; multiplier voltage, 2.2 kv.

### Results and Discussion

A peak identical to that of DCB was found in the reaction blank for the Armour perchlorination procedure (6) with the described GLC operating conditions. The identification of DCB was confirmed by GLC-MS of a hexane extract of a hydrolyzed sample of  $SbCl_5$  which had not been subjected to the perchlorination procedure. Various quantities (0.2-2.0 ml) of  $SbCl_5$  from the 5 commercial sources were examined to determine the presence of DCB.  $SbCl_5$  alone was carried through the perchlorination reaction (6) except that no  $CHCl_3$  was present with  $SbCl_5$ . In the reaction vessel DCB was determined by GLC. Table I lists the amounts of DCB found.

After perchlorinating PCBs with  $SbCl_5$ , a secondary peak with a GLC retention time relative to DCB of 1.31 was observed similar to that reported by Huckins *et al.* (7). This later eluting peak is seen in Fig. 1, the chromatogram from the 0.2 ml  $SbCl_5$  (Hooker Chemical) perchlorination of 0.50  $\mu$ g Aroclor 1221. This peak was found when  $SbCl_5$  from each supplier was used. The peak was determined by GLC-MS to be due to bromononachlorobiphenyl (BNCB). BNCB was assumed to be a competing product with DCB arising from a small amount of  $SbCl_5Br$  in  $SbCl_5$ , so parameters relating to possible limitations of the perchlorination procedure were studied. Various quantities (0.5-10  $\mu$ g) of Aroclors 1221, 1242, 1254, and 1260 in  $CHCl_3$  were perchlorinated. Recoveries of DCB and estimates of the relative amounts of BNCB formed are given in Table 2. Calculation of the relative

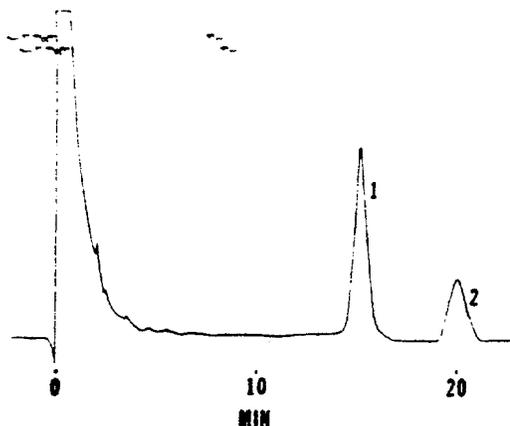


FIG. 1—Electron capture GLC curve from the 0.2 ml  $SbCl_5$  (Hooker Chemical) perchlorination of 0.50  $\mu$ g Aroclor 1221; 0.5 ng equivalent Aroclor 1221 injected. Peak 1 represents 0.8 ng DCB. Peak 2 represents 0.28 ng BNCB.

amounts of BNCB product formed was based on comparison of the electron capture GLC peak height of BSCB with that of a DCB reference.

The amount of DCB determined in the reaction blank was directly proportional to the amount of  $SbCl_5$  used (Table 1). This indicates  $SbCl_5$  was the source of the DCB and that contamination from other possible sources during the perchlorination was negligible. The procedure for perchlorinating PCBs specifies the use of 0.2 ml  $SbCl_5$ ,  $SbCl_5$  producing 8-972 ng  $SbCl_5$ /ml in the reaction blank would add 0.5-65 ppb, based on a 3 g sample. The amount of DCB produced in the reaction blank was assumed to come from PCB contamination of  $SbCl_5$ . In an effort to locate the origin of this contamination,  $SbCl_5$  bottle closures were investigated. GLC analysis of hexane, in which the plastic caps were soaked for 4 days, did not reveal PCBs. Hooker Chemical, the sole domestic source of  $SbCl_5$ , supplied  $SbCl_5$  in glass bottles with lead-lined caps. This bulk supplier of  $SbCl_5$  indicated that the production of chlorine in carbon anode half-cells with linseed oil or other organic binders forms certain organic compounds; however, the destructive oxidative environment in the electrolytic cells would make the production of PCB unlikely as a result of this pathway. On the other hand, antimony metal is commonly obtained as a metallurgical by-product by carbon reduction of its oxide; therefore,

Table 1. DCB (ng/ml) formed from various amounts of  $SbCl_5$

Supplier	$SbCl_5$ , ml			Av.
	0.2	1.0	2.0	
Hooker Chemical	37	U	47	43
MCB	35	42	38	38
B&A	960	1042	913	972
ROC-RIC	12	13	12	12
J. T. Baker	9	7	7	8

**Table 2.** DCB and BNCB from perchlorination of various Aroclors with 0.2 ml  $SbCl_5^a$

Aroclor	Amt. $\mu g$	DCB recd. %	BNCB <sup>b</sup> recd. %	DCB + BNCB <sup>b</sup> combined rec. %
1260	10	86	0	86
1254	10	84	0	84
124 <sup>c</sup>	10	88	4	92
1221	10	61	16	83
1260	a	81	0	81
1254	4	80	0	80
1242	4	78	8	86
1221	4	70	18	88
1260	0.5	09	2	11
1254	0.5	78	6	84
1242	0.5	72	10	82
1221	0.5	60	19	79

<sup>a</sup> Hooker Chemical  $SbCl_5$ .

<sup>b</sup> Quantity calculated by comparison of electron capture GLC response to BNCB vs. response to DCB reference standard.

It is conceivable that PCBs could be associated with the antimony metal employed in the  $SbCl_5$  process. Some heat transfer systems containing PCBs are used in either the chlorine or  $SbCl_5$  production facilities, and  $SbCl_5$  does not come into contact with plastics in the manufacturing operation or in shipping containers (Hooker Chemical and Plastics Corp., 1974, private communication).

Two parameters (various quantities and various Aroclors) were studied in relationship to the production of BNCB as a competing product of DCB during the perchlorination of PCBs. BNCB was calculated by comparison of the electron capture GLC response to BNCB vs. the response to DCB. Several factors are considered: (1) In this reaction bromination is kinetically favored over chlorination. With perchlorination

of lower amounts of PCBs the relative yield of BNCB to DCB is greater because the brominating agent is the limiting quantity in contaminated  $SbCl_5$ . (2) Bromination occurs to a larger degree for a given quantity of the less chlorinated PCBs such as Aroclors 1221 and 1242 rather than for 1254 and 1260. This likely is due to a greater number of reactive sites and less steric hindrance. (3) In the range of PCBs perchlorinated (0.5–10  $\mu g$ ) in the above study, it is likely that with lower amounts of PCBs and/or less chlorinated Aroclors the decrease in DCB recovery is principally due to the increase of BNCB formed.

One of the major advantages of perchlorination in determining minute quantities of PCBs is the inherent increase in effective GLC detector response. Contaminated  $SbCl_5$ , as described here, would preclude its use in many of these cases.

#### REFERENCES

- (1) *Official Methods of Analysis* (1975) 12th Ed., AOAC, Washington, DC, secs. 29.001–29.007
- (2) Asai, R., Gunther, R., Westlake, W., & Iwata, Y. (1971) *J. Apr. Fond Chem.* 19, 396–398
- (3) Berg, O. W., Diosady, P. L., & Rees, G. A. V. (1972) *Bull. Environ. Contam. Toxicol.* 7, 338–347
- (4) Hutzinger, O. W., Safe, S., & Zitko, V. (1972) *Int. J. Environ. Anal. Chem.* 2, 95–106
- (5) Hutzinger, O. W., Jamieson, D., Safe, S., & Zitko, V. (1973) *JAOAC* 56, 982–986
- (6) Armour, S. A. (1973) *JAOAC* 56, 987–993
- (7) Huckins, J. N., Swanson, J. E., & Stallings, D. L. (1974) *JAOAC* 57, 416–417

Received August 21, 1974.

This paper was presented at the 88th Annual Meeting of the AOAC, Oct. 14–17, 1974, at Washington, DC.

## LABORATORY INFORMATION BULLETIN

EDRO  
March 23, 1972

No. 918H  
Pesticide  
Page 1 of 1

ADDITIONAL INFORMATION ON THE BEHAVIOR OF POLYCHLORINATED BIPHENYL  
AND POLY CHLORINATED 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 material 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 CLC 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 52) 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 an 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):

<u>Aroclor</u>	<u>ug in 15 ml PE</u>	<u>% recovery</u>
1221	4 0	94
1242	40	9 7
1248	40	93
<b>1254</b>	40	87
1260	40	<b>78</b>
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 CLC 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 phenomenon, 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<sub>2</sub>O to effect separation of pp'DDE and Aroclor 1254:

<u>Aroclor</u>	<u>Recovery</u>
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

March 23, 1972

5  
No. 9181  
Pesticide  
Page 6 c

\*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:

<u>Aroclor</u>	<u>Relative Response/Weight Chlorine</u>
I.221	1
1242	3.02
1248	4.56

March 23, 1972

No. 918H  
Pesticides  
Page 7 of

1254	9.64
1260	1 2 . 7 0
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 are compared; the injection of Aroclor response to 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:

<u>Aroclor</u>	<u>Mean</u>	<u>Range (number of injections)</u>
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-S 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

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.<sup>2</sup>/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:

<u>Aroclor</u>	<u>ng injected</u>	<u>Response in <sup>2</sup>/ng Cl</u>	<u>Response to Aroclor Response to Aroclor 1242</u>
1221	125	0.12	1.01
1221	112.5	0.12	1.00
-1242	<b>Used</b>	as Reference	1.00.
1248	<b>115</b>	0.09	1.05
1248	<b>125</b>	0.09	0.99
1254	<b>125</b>	0.10	1.01
1254	100	0.10	0.99
1260	<b>125</b>	0.09	1.02
1260	<b>150</b>	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 LLE), and variation in

March 23, 1972

No. 918H  
Pesticides  
Page 9 of 12

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

EC : 44ng

MC : about 660ng

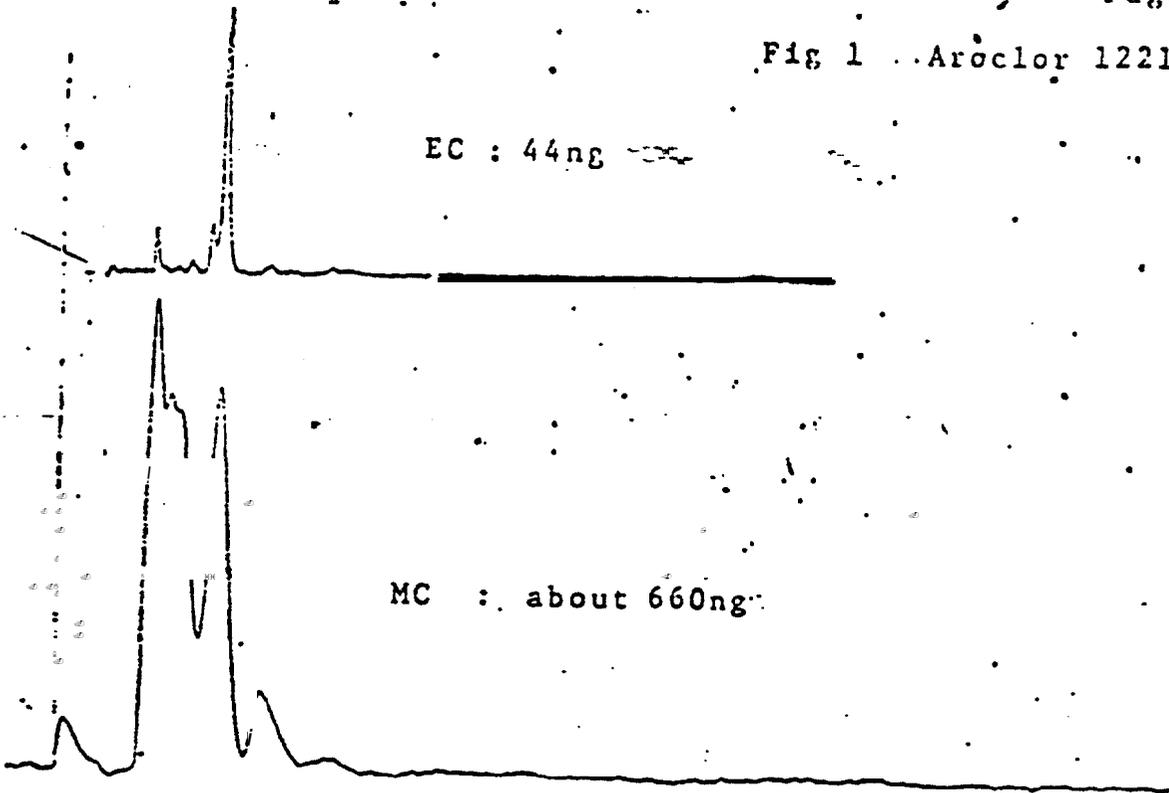
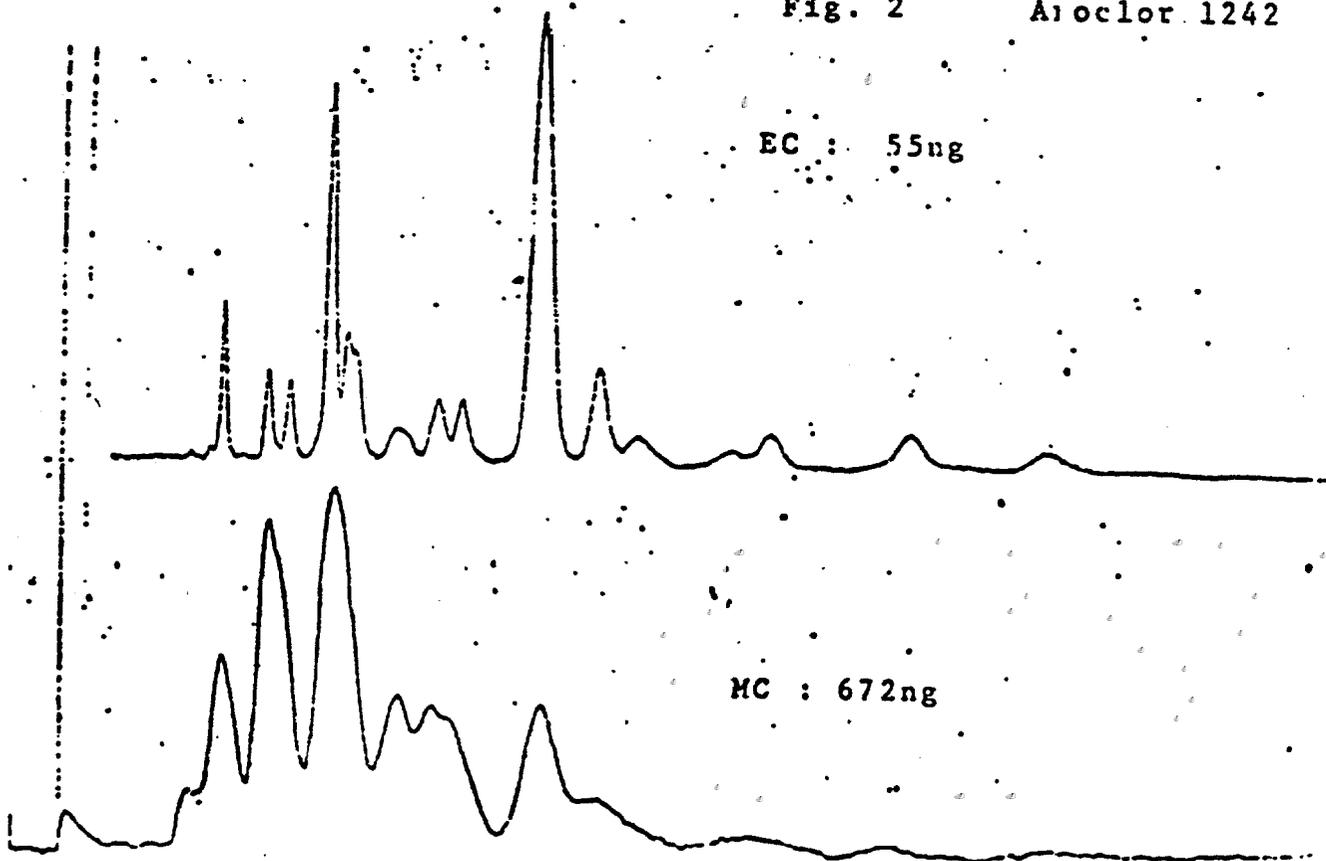


Fig. 2

Aroclor 1242

EC : 55ng

MC : 672ng

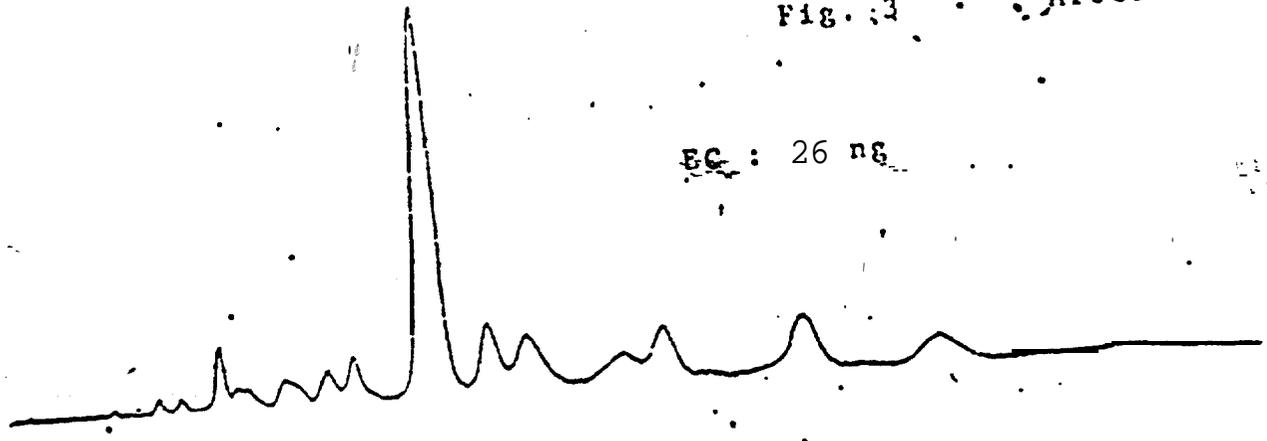


60

Fig. 3

Aroclor 1248

EC : 26 ng



MC : about 630 ng.

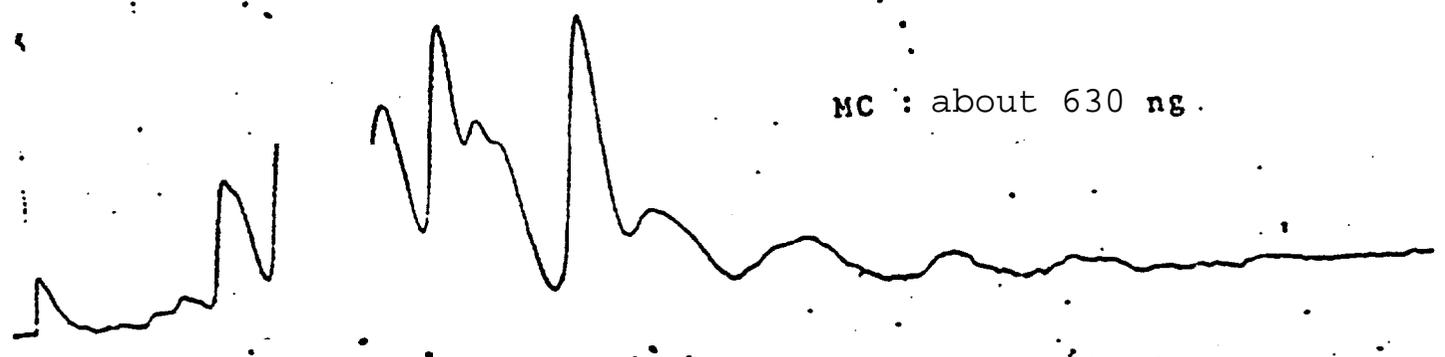
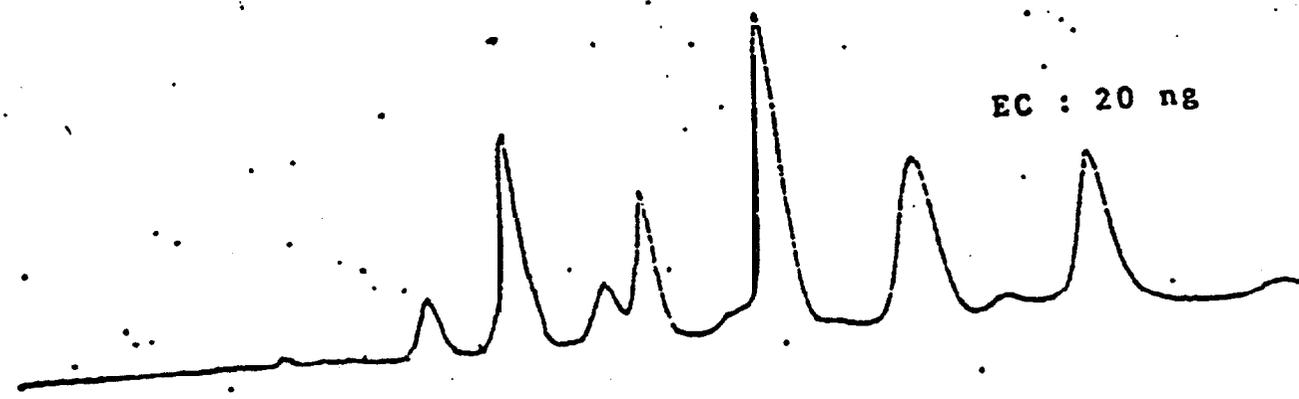
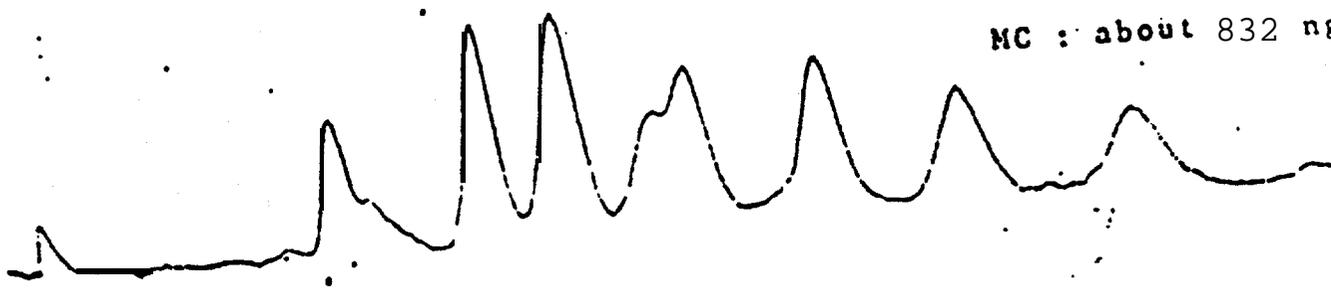


Fig. 4 Aroclor 1254

EC : 20 ng



MC : about 832 ng

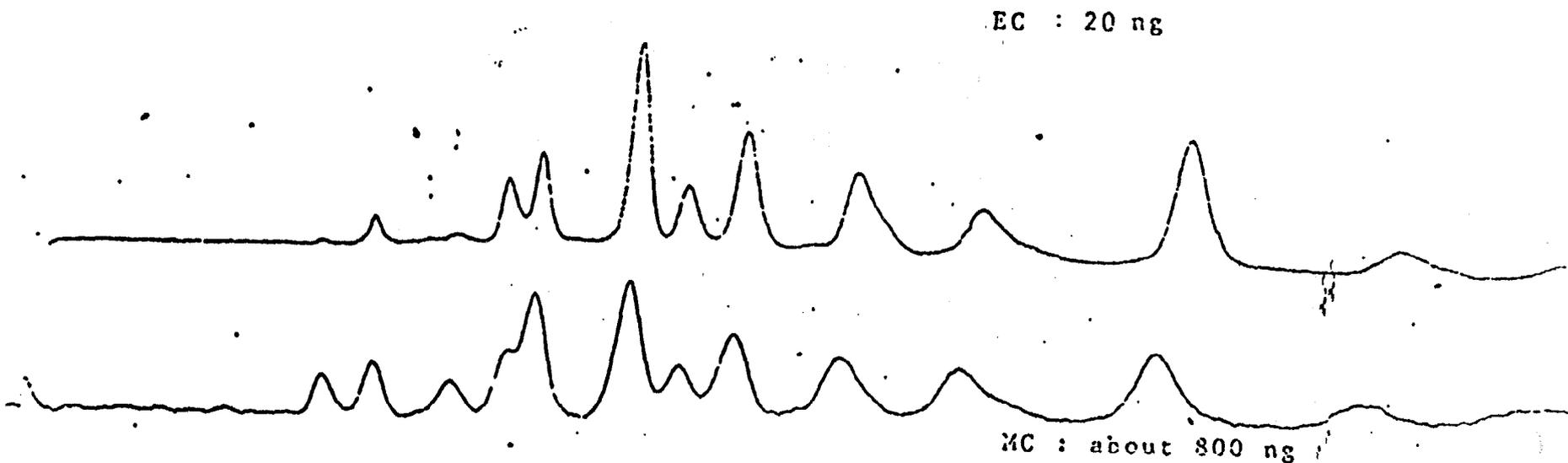


ATTACHMENT VI

No. 918H  
Pesticides  
Page 12 of 12

March 23, 1972

Fig. 5 Aroclor 1260



Instrument Conditions : Figures 1 - 5

EC / MC DC 200 col, PAM I 311.2 Parameters; 70V;  $1 \times 10^{-9}$  afs

MC : DC 200 col. at 200°C, 120 ml/min No: injector temp 225°C.

6