PRELIMINARY
SUBJECT TO REVIEW

ADDENDUM TO
METHODS FOR ORGANIC PESTICIDES
IN WATER AND WASTEWATER
B. METHOD FOR POLYCHLORINATED
BIPHENYLS IN WATER

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B. METHOD FOR POLYCHLORINATED BIPHENYLS IN WATER

1. Scope and Application

1.1 This method covers the determination of certain polychlorinated biphenyls (PCB's, Aroclors[1]), including: 1221, 1232, 1242, 1248, 1254 and 1260.

1.2 The method is an extension of the Environmental Protection Agency's "Method for Organochlorine Pesticides" (1). It is designed so that determination of both the PCB's and the organochlorine pesticides may be made on the same sample.

2. Summary

2.1 The PCB's and the organochlorine pesticides are co-extracted by liquid-liquid extraction and, insofar as possible, the two classes of compounds separated from one another prior to gas chromatographic determination. A combination of the standard Florisil column procedure and the silica gel microcolumn procedure of Leoni (2) are employed. Identification is made from gas chromatographic patterns obtained through the use of two or more unlike columns. Detection and measurement is accomplished using an electron capture, microcoulometric, or electrolytic conductivity detector. Techniques for confirming qualitative identification are suggested.

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[1]Trade name of the Monsanto Co., St. Louis, Missouri

3. Significance

3.1 The extensive and widespread use of PCB's has resulted in their presence in all parts of the environment. Like the organochlorine pesticides the PCB's are very persistent. While they are generally less toxic than the organochlorine pesticides they do have lethal effects on fish and other aquatic animals. Thus, it is imperative that we identify and quantitate the PCB's present in the environment.

3.2 Because of their cumulative nature, we need to detect small quantitaties (µg/l and less) of the PCB's in water.

4. Interferences

4.1 Refer to EPA Method for Organochlorine Pesticides. Phthalate esters, certain organophosphorus pesticides, and elemental sulfur will interfere when using electron capture for detection.

4.2 Organochlorine pesticides and other halogenated compounds constitute interferences in the determination of PCB's. Most of these are separated by the method described below. However, certain compounds, if present in the sample, will occur with the PCB's. Included are: Sulfur, Heptachlor, aldrin, chlordane, and to some extent o, p'-DDT and p,p'-DDT.

5. Method of Analysis

5.1 Extraction of Sample

5.1.1 Transfer a measured volume of sample (1-liter) to a 2-liter separatory funnel equipped with a Teflon stopcock. Extract the sample twice with 60 ml of 15% ethyl ether in hexane and once with 60 ml of hexane only according to

Part II, A, 5.1.

5.1.2 Dry the combined extracts by pouring over a column of anhydrous sodium sulfate and concentrate to 10 ml in a Kuderna-Danish evaporator. Qualitatively analyze the sample by gas chromatography using the non-polar column described in Table 2, with an electron capture detector. From the response obtained determine:

a. If there are any organochlorine pesticides present,

b. If there are any PCB's present.

c. If there is a combination of a and b,

d. If the response is too complex to determine a, b, or c.

If no response, concentrate to 1.0 ml or less, as required, according to Part II, A, 5.1.4 and repeat the analysis looking for a, b, c, and d.

5.1.3 If condition a exists, quantitatively determine the organochlorines pesticides according to Part II, A, 5.2 and 5.3.

5.1.4 If condition b exists, PCB's only are present, no further separation or clean-up is necessary. Quantitatively determine the PCB's according to 5.6.1.

5.1.5 If condition c exists, compare peaks obtained from sample to those of standard Aroclors and make a judgment as to which Aroclors may be present. To separate the PCB's from the organochlorine pesticides, continue as outlined below.

5.1.6 If condition d exists, apply standard Florisil column procedure, Part II, A, 5.2.2. The first eluate from the Florisil column will contain certain of the organochlorine

pesticides and all of Aroclors 1221, 1232, 1242, 1248, 1254, and 1260, if they are present in the sample. Analyze the first eluate as indicated in 5.1.2 above. Analyze the second eluate according to Part II, A, 5.3.

5.2 Silica Gel Micro Column Separation Procedure

5.2.1 Apparatus

5.2.1.1 Micro Column - Constructed of borosilicate glass tubing, according to Figure 1.

5.2.1.2 Erlenmeyer Flask - 250 ml, glass stoppered (24/40)

5.2.1.3 Desiccator - Vacuum, bobosilicate glass, 160 mm diameter x 255 mm high.

5.2.2 Standards, Reagent and Solvents

5.2.2.1 Standards - Aroclors 1221, 1232, 1242, 1248, 1254 and 1260, Monsanto Co., St. Louis, MO and polychlorinated biphenyl isomers (99% pure), Analabs, 80 Republic Drive, North Haven, CT 06473.

5.2.2.2 n-Hexane - Pesticide quality (not mixed hexanes).

5.2.2.3 Benzene - Pesticide quality

5.2.2.4 Silica Gel - Davison Code 950-08-08-226 (60-200 mesh).

5.2.2.5 Glass Wool - Pre-extracted with Hexane.

5.2.2.6 Sodium Sulfate - Anhydrous, granular, conditioned for 2 hours @ 400°C.

5.2.2.7 Gas Chromatographic Column - liquid phase OV-1

5.3 Activation for Silica Gel

5.3.1 Place about 20 g of silica gel in an open, tared, glass stoppered, 250 ml Erlenmeyer flask and heat at 130°C for

8 hours. Remove from the oven and weigh, while still hot, on a triple beam balance. Then seal with ground glass stopper and store in vented desiccator, containing no desiccant, until cool. Then deactivate with 5% distilled water. Add the water dropwise down the side of the flask and shake until all lumps are broken and the silica gel is free flowing. Store for about 8 hours in a sealed desiccator, containing no desiccant, prior to use to insure equal distribution of the water. The proper activation state will be maintained for about 10 days, if stored in a well sealed flask in the desiccator.

5.4 Preparation of the Chromatographic Column

5.4.1 Pack the lower, 2mm I.D. section of the micro column with glass wool and add 1 g ± 20 mg of activated silica gel to the column. Tap the column to pack the silica gel. This should result in a column of adsorbent ~10 cm high. Mark this level, and for future work fill to the mark. Add 1 cm of sodium sulfate to the top of the column and tap to settle.

5.4.2 Pipet a 1.0 ml aliquot of the concentrated sample extract (previously reduced to a total volume of 2.0 ml) on to the column (do not prewash the column). As the last of the sample passes into the sodium sulfate layer, rinse down the internal wall of the column twice with a minimum of n-hexane. Then assemble the upper section of the column. As the last of the n-hexane rinse reaches the surface of the sodium sulfate, add enough n-hexane (volume predetermined, see 5.5

below) to just elute all of the PCB's present in the sample. Apply air pressure until the effluent flow is 1 ml/min. Collect the desired volume of eluate (predetermined, see 5.5 below) in an accurately calibrated ampul. As the last of the n-hexane reaches the surface of the sodium sulfate, release the air pressure and change the collection ampul.

5.4.3 Fill the column with benzene, again apply air pressure and adjust flow to 1 ml/min. Collect the eluate until all of the organochlorine pesticides of interest have been eluted (volume predetermined, see 5.5 below).

5.5 Determination of Elution Volumes

5.5.1 The elution volumes for the PCB's and the pesticides depend upon a number of parameters which are difficult to control. These include variation in:

a. Mesh size of the silica gel

b. Adsorption properties of the silica gel.

c. Polar contaminants present in the eluting solvent.

d. Polar materials present in the sample and sample solvent.

e. The dimensions of the microcolumns.

Therefore, the optimum elution volume must be experimentally determined each time a parameter is changed. To determine the elution volumes, add standard mixtures of Aroclors and pesticides to the column and collect 1 ml elution volumes.

Analyze the individual eluates by gas chromatography and determine the cut-off volume for n-hexane and for benzene. Table 1 lists the retention order of the various Aroclor components and of the pesticides. Using the data in this table, prepare the proper standard mixtures required for analysis of the n-hexane and benzene eluates.

5.5.2 In determining the volume of hexane required to elute the PCB's, the sample volume (1 ml), the volume of hexane retained by the silica gel (~0.5 ml), and the volume of n-hexane used to rinse the column wall must be considered. Thus, if it is determined that a 10.0 ml elution volume is required to elute the PCB's, the volume of hexane to be added in addition to the sample volume but including the rinse volume should be 9.5 ml.

5.5.3 Figure 2 shows that as the average chlorine content of a PCB mixture decreases the solvent volume for complete elution increases. Qualitative determination (5.1.2) indicates which Aroclors are present and provides the basis for selection of the ideal elution volume. This helps to minimize the quantity of organochlorine pesticides which will elute along with the low percent chlorine PCB's and insures the most efficient separations possible for accurate analysis.

5.6 Quantitative Determination

5.6.1 Measure the volume of the n-hexane eluate, containing the PCB's, and inject 1 to 5 µl into the gas chromatograph.

(Conditions are listed in Table 2). If necessary, adjust the volume of the eluate to give linear response to the electron capture detector (Detection limit approximately 0.01 ng/µl). The microcoulometric or the electrolytic detector may be employed to improve specificity for samples having higher concentrations of PCB's (Detection limit approximately 10 ng/µl).

5.6.2 Calculations

5.6.2.1 Since polychlorinated biphenyls occur in the environment in varying degrees of complexity:

a. As the original mixture, unchanged, eg., Aroclor 1242.

b. As a combination of Aroclors, eg., 1242 + 1260.

c. As metabolized or biodegraded products of the original Aroclor or combination of Aroclors.

d. As a combination of a, b, and c, it is impossible to prescribe a simple method for quantitative determination.

5.6.2.2 For the least complicated situation, a , compare quantitative Aroclor reference standards (eg. 1242, 1248) to the unknown. Measure and sum the areas of the unknown and the reference Aroclor and calculate the result as follows:

$$\text{Micrograms/liter} = \frac{[A]\ [B]\ [Vt]}{[Vi]\ [Vs]} \times 2$$

$$A = \frac{\text{ng of Standard Injected}}{\Sigma \text{ of Peak Areas}} = \frac{ng}{mm^2}$$

$$B = \Sigma \text{ of Sample Peak Areas} = mm^2$$

Vi = Volume of sample injected (µl)

Vt = Volume of total extract (µl)

Vs = Volume of water sample extracted (ml).

5.6.2.3 For situation b, combination of intact Aroclors, prepare mixtures of Aroclor reference standards and compare them to the unknown until similar chromatograms are obtained. Calculate the result as in 5.6.2. At best, this is a time consuming procedure and the analyst may choose to use the approach described below.

5.6.2.4 The most practical calibration procedure for complex mixtures of PCB's involves:

a. Identification of the individual isomers,

b. Grouping them according to the number of chlorine atoms present in the molecule,

c. Reporting the amount of the various isomeric groups present based on the average response factor obtained for each group.

Since the response factors for PCB isomers, containing the same number of chlorine atoms, may vary, the average response factor for the isomers in the group should be used for calculation. To obtain the average response factor for each isomeric group to the electron capture detector, prepare standard mixtures of the individual pure isomers and determine gas chromatographic response. This operation is not necessary when using the microcoulometric detector, since its response is directly proportional to the amount of chlorine entering the cell per unit time, ie, the number of chlorine atoms in the molecule.

The various isomeric groups are located by experimentally duplicating the chromatogram shown in Figure 3. Obtain the chromatogram on the OV-1 column by injecting a mixture of Aroclors 1221, 1242, 1248, 1254 and 1260 (1232 is not included because it is a mixture of 1221 and 1242), 10 ng each in a total of 100 ml of hexane. There are 209 possible isomers of PCB's (5). Many of them overlap and it is impossible to resolve them by common gas chromatographic techniques. In the case of the above mixtures, the isomers have been identified as accurately as possible using GC-MS. Minor amounts of various isomers in the mixture do overlap and are unavoidably included in certain isomeric groups.

Calculate the results by summing the areas of the peaks in each isomeric group and applying the appropriate response factor as indicated below:

$$\text{microgram/liter}_{Cl_x} = \frac{[A]\,[B]\,[Vt]}{[Vi]\,[Vs]}$$

$$A = \frac{\Sigma \text{ ng } Cl_x \text{ Standard}}{\Sigma \text{ Areas } Cl_x \text{ Standard}}$$

B = Σ Sample Areas of Cl_x

Vt = Volume of total extract (µl)

Vi = Volume of extract injected (µl)

Vs = Volume of water sample extracted (ml)

Cl_x = Isomeric group, where x = 1 thru 8.

5.6.3 Confirmatory Techniques

5.6.3.1 Unequivocal identification of PCB's can be made by GC-MS when present in sufficient concentration (approximately 20 ng/µl in the final extract). The methods described by Bonelli (6) and Budde (7) are useful for this purpose. When GC-MS is not available, separate GC analysis using both non-polar and polar columns will give added confidence in the qualitative determination. The use of specific halogen detectors, such as the microcoulometric and electrolytic conductivity, will eliminate non-halogen interferences and further support the identification. The concentration of PCB's required is about 10 ng/µl in the final extract.

5.6.3.2 The Monsanto method (8) for PCB's which incorporates a two-step chemical treatment - saponification with alcoholic potassium hydroxide followed by sulfuric acid effectively eliminates many interferences while the PCB's are retained intact.

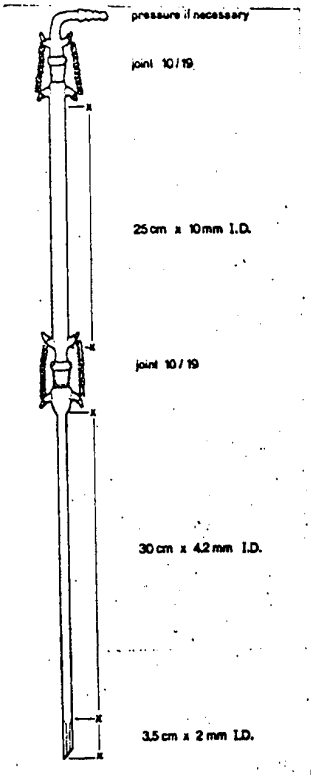

Fig. 1. The microcolumn.

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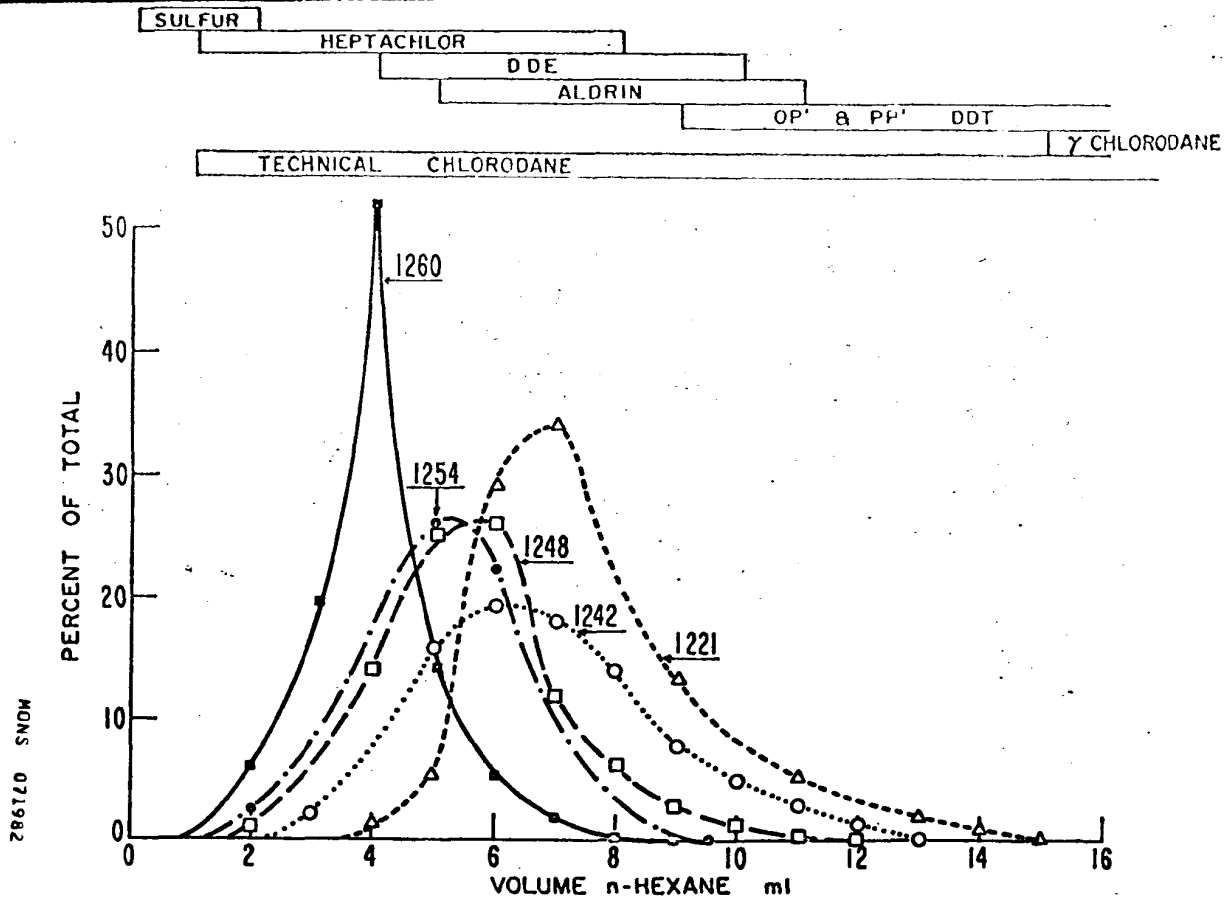

Figure 2. Aroclor Elution Patterns

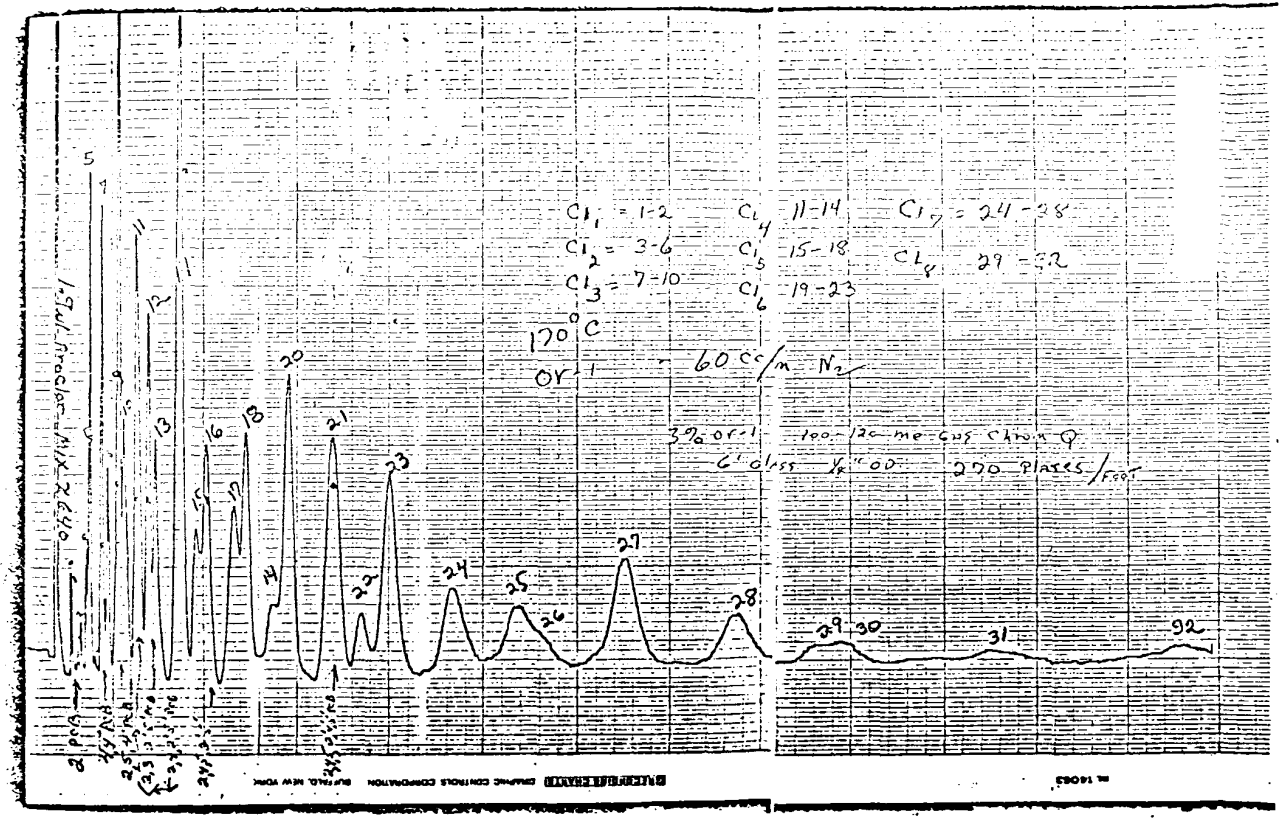
Cl_1 = 1-2
Cl_2 = 3-6
Cl_3 = 7-10
Cl_4 11-14
Cl_5 15-18
Cl_6 19-23
Cl_7 = 24-28
Cl_8 29-32
120°C
OV-1 - 60 cc/m N_2
3% OV-1 100-120 mesh Chrom Q
6' Glass 1/8" OD 270 Plates/Foot
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32

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TABLE 1

ELUTION ORDER OF PCB'S, CHLORINATED HYDROCARBON PESTICIDES AND SULFUR FROM SILICA GEL MICROCOLUMN

SOLVENT	COMPOUND
n-Hexane	Sulfur
	Hexachlorobenzene
	Heptachlor
	DDE
	Aldrin
	DDT
	Polychlorinated biphenyls
	γ - Chlordane
Benzene	α - BHC
	β - BHC
	Lindane
	Perthane
	Heptachlor Epoxide
	Endosulfan I
	Endrin
	Dieldrin
	Endosulfan II
	Methoxychlor

TABLE 2

CONDITIONS FOR GAS CHROMATOGRAPHIC DETERMINATION OF PCB'S

NON-POLAR COLUMN

Packing:	3% OV-1 on 100-120 Mesh Gas Chrom-Q
Column:	Borosilicate Glass 1/4" O.D. (~ 4 mm I.D.) 6' Long
Carrier:	Nitrogen
Flow:	60 ml/min.
Oven Temp:	170°C

POLAR COLUMN

Packing:	1.5% OV-17 + 1.95% QF-1 on 100-120 Mesh Gas Chrom Q
Column:	Borosilicate Glass 1/4" O.D. (~ 4 mm I.D.) 6' Long
Carrier:	Nitrogen
Flow:	60 ml/min.
Oven Temp.:	190°C
Inlet Temp.	200°C (On column Injection)
Detector:	Nickel - 63 Electron capture
	Operating Temp.: - 260 C
	Scavenger Flow - Nitrogen, 20 ml/min.

REFERENCES

(1) "Methods for Organic Pesticides in Water and Wastewater", U.S. Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio 45268, 1971.

(2) Leoni, V., "The Separation of Fifty Pesticides and Related Compounds and Polychlorinated Biphenyls into Four Groups by Silica Gel Microcolumn Chromatography", Journal of Chromatography, 62, 63 (1971).

(3) Rote, J.W. and Murphy, P.G., "A Method for the Quantitation of Polychlorinated Biphenyl (PCB) Isomers", Bulletin of Environmental Contamination and Toxicology, 6, 377 (1971)

(4) Zitko, V., Hutzinger, O., and Safe, S., "Retention Times and Electron Capture Detector Response of Some Individual Chlorobiphenyls", Bulletin of Environmental Contamination and Toxicology, 6, 160 (1971).

(5) Hayes, H. and Riseborough, R.W., Natural History, 80, 38 (1971)

(6) Bonelli, E.J., "Gas Chromatographic/Mass Spectrometer Techniques for Determination of Interferences in Pesticide Analysis", Analytical Chemistry, 40, 603 (1972).

(7) Budde, W.L., Private Communication, May, 197[illegible].

(8) Monsanto Methodology for Aroclors - Analysis of Water & Sediment for Polychlorinated Biphenyls, Analytical Method 69-13, Monsanto Co., 800 North Lindbergh Blvd., St. Louis, MO 63166.