

**METHODS FOR ORGANOCHLORINE PESTICIDES AND CHLOROPHENOXY
ACID HERBICIDES IN DRINKING WATER AND RAW SOURCE WATER**

**INTERIM
Pending Issuance of
Methods for Organic Analysis
of Water and Wastes**

**U. S. ENVIRONMENTAL PROTECTION AGENCY
ENVIRONMENTAL MONITORING AND SUPPORT LABORATORY
CINCINNATI, OHIO 45268**

July 1978

FOREWORD

The National Interim Primary Drinking Water Regulations promulgated on December 24, 1975, in accordance with the provisions of the Safe Drinking Water Act (Public Law 93-523), have set maximum contaminant levels for a variety of pollutants. The methods contained herein are provided to determine compliance with para. 141.12 (a) chlorinated hydrocarbon insecticides and 141.12 (b) chlorophenoxy herbicides. Endrin, lindane, methoxychlor and toxphene may be determined by the chlorinated hydrocarbon method while 2,4-D and 2,4,5-TP (Silvex) may be determined by the chlorophenoxy method.

These methods have been assembled by the staff of the Environmental Monitoring and Support Laboratory - Cincinnati (EMSL-CIN) and are provided only for the interim period until the manual, "Methods for Organic Analysis of Water and Wastes," becomes available.

Dwight G. Ballinger, Director
Environmental Monitoring and Support Laboratory - Cincinnati

DISCLAIMER

The mention of trade names or commercial products in this manual is for illustration purposes, and does not constitute endorsement or recommendation for use by the U. S. Environmental Protection Agency.

METHOD FOR ORGANOCHLORINE PESTICIDES IN DRINKING WATER

1. Scope and Application

- 1.1 This method covers the determination of organochlorine pesticides in drinking water and raw source water. Lindane, endrin, methoxychlor and toxaphene are determined by this procedure.
- 1.2 The method sensitivity is 0.001 to 0.010 ug/l for single component pesticides and 0.050 to 1.0 ug/l for multi-component pesticides when analyzing a 1 liter sample with the electron capture detector.
- 1.3 Other organochlorine pesticides, such as BHC, heptachlor, aldrin, heptachlor epoxide, dieldrin, Captan, DDE, DDD, DDT, endosulfan, dichloran, mirex, pentachloronitrobenzene, trifluralin, Strobane, chlordane (tech.) and others may also be determined by this method.

2. Summary

- 2.1 The method offers several analytical alternatives, dependent on the analyst's assessment of the nature and extent of interferences and/or the complexity of the pesticide mixtures found. Specifically, the procedure describes the use of an effective co-solvent for efficient sample extraction; provides, through use of column chromatography and liquid-liquid partition, methods for elimination of non-pesticide interferences and the pre-separation of pesticide mixtures.

Identification is made by selective gas chromatographic separations and may be corroborated through the use of two or more unlike columns. Detection and measurement is accomplished by electron capture, microcoulometric or electrolytic conductivity gas chromatography. Results are reported in micrograms per liter.

- 2.2 Confirmation of the identity of the compounds should be made by GC-MS when a new or undefined sample type is being analyzed and the concentration is adequate for such determination.
- 2.3 This method is recommended for use only by experienced pesticide analysts or under the close supervision of such qualified persons.

3. Interferences

- 3.1 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines, causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Refer to Part I, Sections 1.4 and 1.5 (1).
- 3.2 The interferences in drinking water should not pose any difficulty in obtaining accurate and precise measurement of organochlorine pesticides.

- 3.3 Polychlorinated Biphenyls (PCBs) - Special attention is called to industrial plasticizers and hydraulic fluids such as the PCBs, which are a potential source of interference in pesticide analysis. The presence of PCBs is indicated by a large number of partially resolved or unresolved peaks which may occur throughout the entire chromatogram. Particularly severe PCB interference will require special separation procedures (2,3).
- 3.4 Phthalate Esters - These compounds, widely used as plasticizers, respond to the electron capture detector and are a source of interference in the determination of organochlorine pesticides using this detector. Water leaches these materials from plastics, such as polyethylene bottles and tygon tubing. The presence of phthalate esters is implicated in samples that respond to electron capture but not to the microcoulometric or electrolytic conductivity halogen detectors.
- 3.5 Organophosphorus Pesticides - A number of organophosphorus pesticides, such as those containing a nitro group, e.g., parathion, respond to the electron capture detector and may interfere with the determination of the organochlorine pesticides. Such compounds can be identified by their response to the flame photometric detector (4).

4. Apparatus and Materials

- 4.1 Gas Chromatograph - Equipped with glass lined injection port.
- 4.2 Detector Options:
 - 4.2.1 Electron Capture - Radioactive (tritium or nickel-63)
 - 4.2.2 Microcoulometric Titration
 - 4.2.3 Electrolytic Conductivity
- 4.3 Recorder - Potentiometric strip chart (10 in.) compatible with the detector.
- 4.4 Gas Chromatographic Column Materials:
 - 4.4.1 Tubing - Pyrex (180 cm long X 4 mm ID)
 - 4.4.2 Glass Wool - Silanized
 - 4.4.3 Solid Support - Gas-Chrom-Q (100-120 mesh)
 - 4.4.4 Liquid Phases - Expressed as weight percent coated on solid support.
 - 4.4.4.1 OV-1, 3%
 - 4.4.4.2 OV-210, 5%
 - 4.4.4.3 OV-17, 1.5% plus QF-1 or OV-210, 1.95%
 - 4.4.4.4 QF-1, 6% plus SE-30, 4%
- 4.5 Kuderna-Danish (K-D) Glassware
 - 4.5.1 Snyder Column - three ball (macro) and two ball (micro)
 - 4.5.2 Evaporative Flasks - 500 ml
 - 4.5.3 Receiver Ampuls - 10 ml, graduated
 - 4.5.4 Ampul Stoppers

- 4.6 Chromatographic Column - Chromaflex (400 mm long x 19 mm ID) with coarse fritted plate on bottom and Teflon stopcock; 250 ml reservoir bulb at top of column with flared out funnel shape at top of bulb - a special order (Kontes K-420540-9011).
- 4.7 Chromatographic Column - pyrex (approximately 400 mm long x 20 mm ID) with coarse fritted plate on bottom.
- 4.8 Micro Syringes - 10, 25, 50 and 100 ul.
- 4.9 Separatory funnels - 125 ml, 1000 ml and 2000 ml with Teflon stopcock.
- 4.10 Graduated cylinders - 100 and 250 ml.

5. Reagents, Solvents, and Standards

- 5.1 Sodium Hydroxide - (ACS) 10 N in distilled water.
- 5.2 Sodium Sulfate - (ACS) Granular, anhydrous (conditioned at 400 C for 4 hrs.).
- 5.3 Sulfuric Acid - (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.
- 5.4 Florisil - PR Grade (60-100 mesh); purchase activated at 1250⁰F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use, activate each batch overnight at 130⁰C in foil-covered glass container. Determine lauric-acid value (See Section 13).
- 5.5 Diethyl Ether - Nanograde, redistilled in glass, if necessary.

5.5.1 Must be free of peroxides as indicated by EM Quant test strips. (Test strips are available from EM Laboratories, Inc., 500 Executive Blvd., Elmsford, NY 10523.)

5.5.2 Procedures recommended for removal of peroxides are provided with the test strips.

5.6 Hexane, Methanol, Methylene Chloride, Petroleum Ether (boiling range 30-60 C) - nanograde, redistill in glass if necessary.

5.7 Pesticide Standards - Reference grade.

6. Calibration

6.1 Gas chromatographic operating conditions are considered acceptable if the response to dicapthon is at least 50% of full scale when ≤ 0.06 ng is injected for electron capture detection and ≤ 100 ng is injected for microcoulometric or electrolytic conductivity detection. For all quantitative measurements, the detector must be operated within its linear response range and the detector noise level should be less than 2% of full scale.

6.2 Standards are injected frequently as a check on the stability of operating conditions. Gas chromatograms of several standard pesticides are shown in Figures 1, 2, 3 and 4 and provide reference operating conditions for the four recommended columns.

6.3 The elution order and retention ratios of various organochlorine pesticides are provided in Table 1, as a guide.

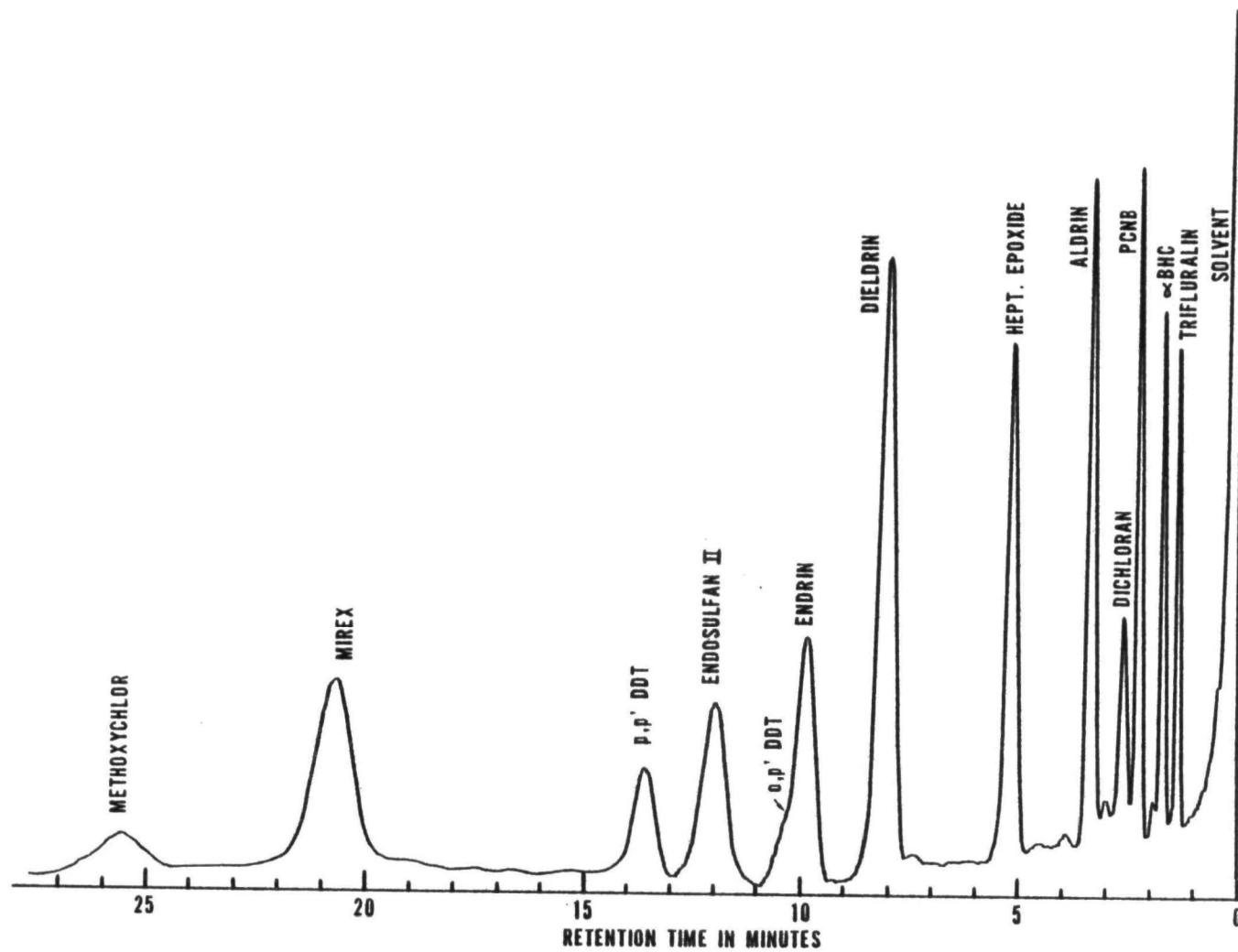


Figure 1. Column Packing: 1.5% OV-17 + 1.95% QF-1, Carrier Gas: Argon/Methane at 60 ml/min, Column Temperature: 200 C, Detector: Electron Capture.

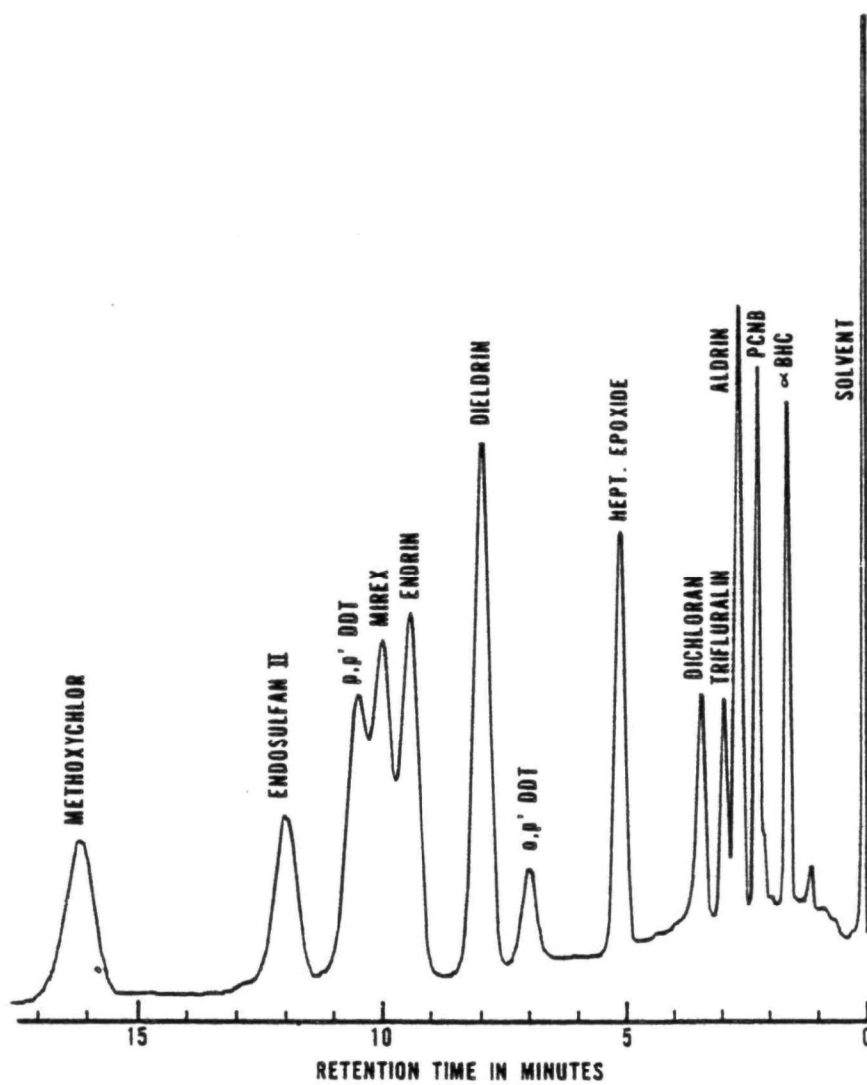


Figure 2. Column Packing: 5% OV-210, Carrier Gas: Argon/Methane at 70 ml/min, Column Temperature: 180 C, Detector: Electron Capture.

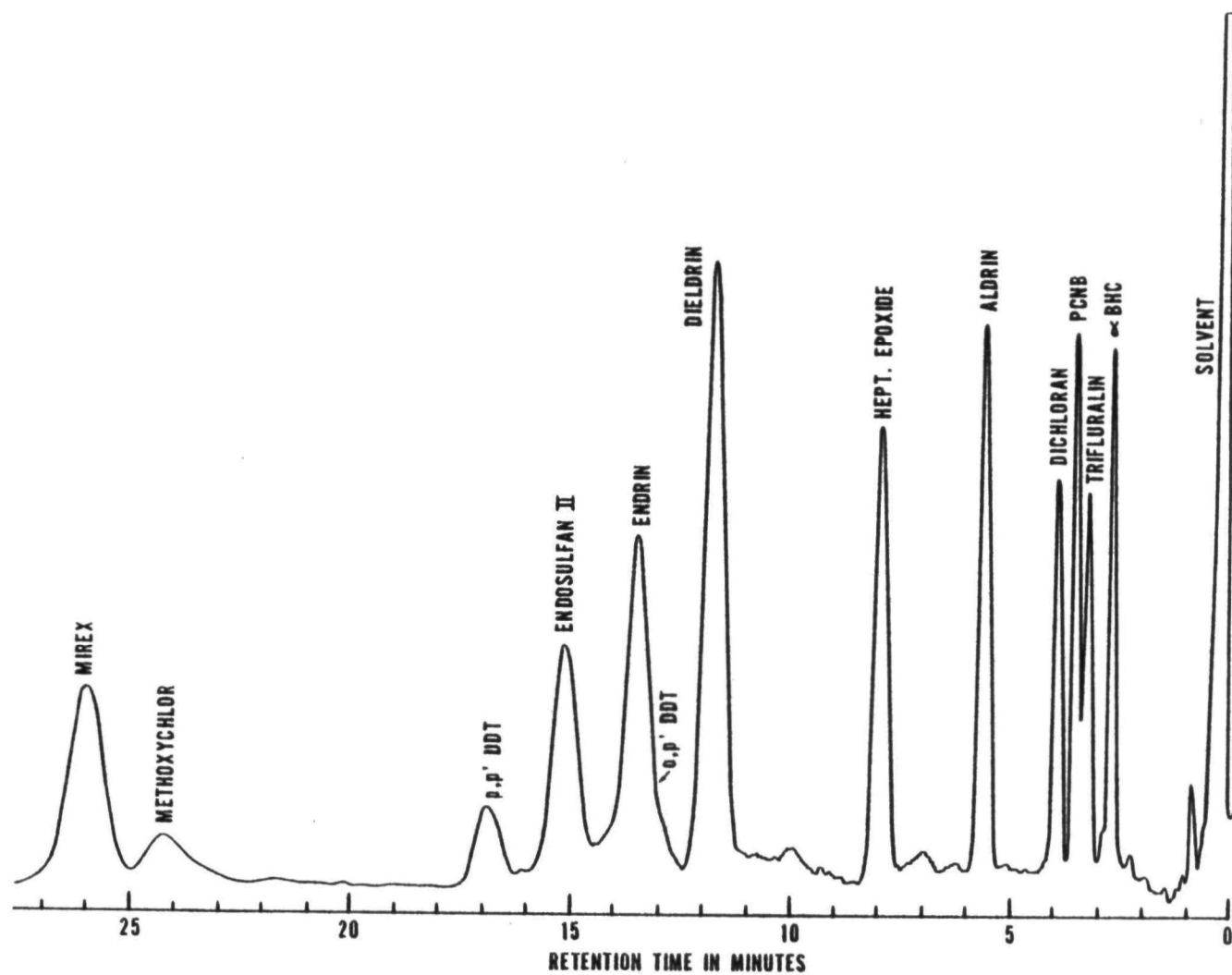


Figure 3. Column Packing: 6% QF-1 + 4% SE-30, Carrier Gas: Argon/Methane at 60 ml/min, Column Temperature: 200 C, Detector: Electron Capture.

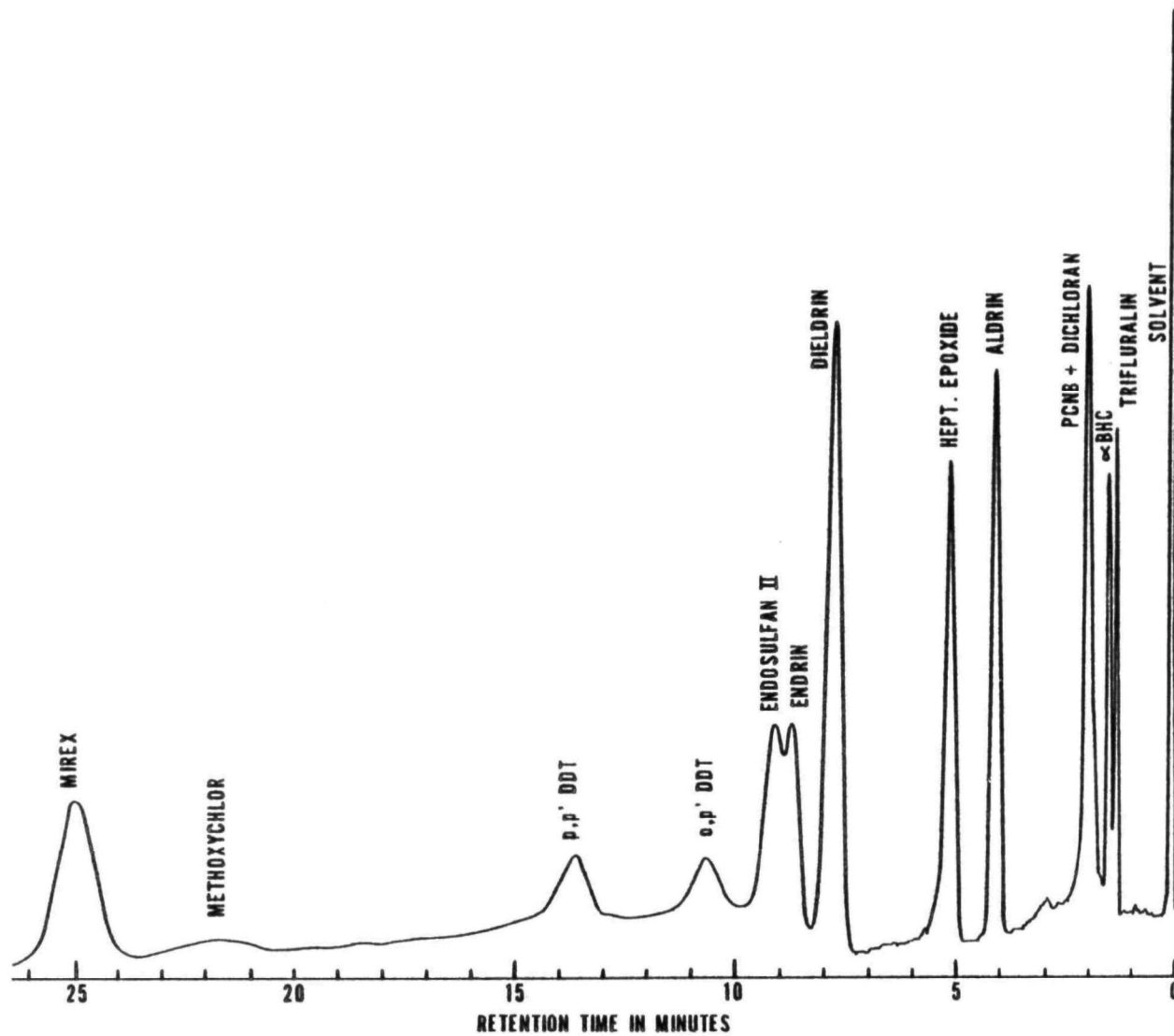


Figure 4. Column Packing: 3% OV-1, Carrier Gas: Argon/Methane at 70 ml/min, Column Temperature: 180 C, Detector: Electron Capture.

Table 1
RETENTION RATIOS OF VARIOUS ORGANOCHLORINE PESTICIDES RELATIVE TO ALDRIN

Liquid Phase ¹	1.5% OV-17 + 1.95% QF-1 ²	5% OV-210	3% OV-1	6% QF-1 + 4% SE-30
Column Temp.	200 C	180 C	180 C	200 C
Argon/Methane Carrier Flow	60 ml/min	70 ml/min	70 ml/min	60 ml/min
Pesticide	RR	RR	RR	RR
Trifluralin	0.39	1.11	0.33	0.57
-BHC	0.54	0.64	0.35	0.49
PCNB	0.68	0.85	0.49	0.63
Lindane	0.69	0.81	0.44	0.60
Dichloran	0.77	1.29	0.49	0.70
Heptachlor	0.82	0.87	0.78	0.83
Aldrin	1.00	1.00	1.00	1.00
Heptachlor Epoxide	1.54	1.93	1.28	1.43
Endosulfan I	1.95	2.48	1.62	1.79
p,p'-DDE	2.23	2.10	2.00	1.82
Dieldrin	2.40	3.00	1.93	2.12
Captan	2.59	4.09	1.22	1.94
Endrin	2.93	3.56	2.18	2.42
o,p'-DDT	3.16	2.70	2.69	2.39
p,p'-DDD	3.48	3.75	2.61	2.55
Endosulfan II	3.59	4.59	2.25	2.72
p,p'-DDT	4.18	4.07	3.50	3.12
Mirex	6.1	3.78	6.6	4.79
Methoxychlor	7.6	6.5	5.7	4.60
Aldrin (Min. absolute)	3.5	2.6	4.0	5.6

¹All columns glass, 180 cm x 4 mm ID, solid support Gas-Chrom Q (100/120 mesh)

²OV-210 also may be used

7. Sample Collection and Handling

- 7.1 Wash all bottles and cap liners in detergent water. Rinse with tap water and finally distilled water.
- 7.2 Allow bottles and cap liners to air-dry. Muffle the sample bottles at 400⁰C for 1 hour.
- 7.3 Rinse the cap liners with pesticide-grade hexane.
- 7.4 When cool, seal the bottles and store in a dust-free environment.
- 7.5 Collect samples in 1 quart narrow-mouth bottles with a Teflon lined screw cap (option 1 quart widemouth screw-cap bottles with Teflon lid liner). Collect all samples in duplicate.
- 7.6 Sampling from a water tap: Turn on water and allow system to flush. When the temperature has stabilized, adjust the flow to about 1 l/min. Fill the bottle about 90% full and seal.
- 7.7 The sample should be maintained near 4⁰C until analysis and should be extracted as soon as possible after collection.

8. Sample Procedure

- 8.1 Quantitatively transfer the entire sample into a two-liter separatory funnel. Rinse the container with 60 ml of 15% methylene chloride in hexane (v:v) and add to the separatory funnel. Also rinse the sample bottle with each succeeding volume of extracting solvent.

- 8.2 Shake the sample in the separatory funnel vigorously for two minutes. Allow the mixed solvent to separate from the sample, then draw the water into a one-liter Erlenmeyer flask. Pour the organic layer into a 100-ml beaker and then pass it through a column containing 3 to 4 inches of anhydrous sodium sulfate, and collect it in a 500-ml K-D flask equipped with a 10 ml ampul. Return the water phase to the separatory funnel. Rinse the sample bottle and the Erlenmeyer flask with a second 60 ml volume of solvent; add the solvent to the separatory funnel and complete the extraction procedure a second time. Perform a third extraction in the same manner.
- 8.3 Combine the extracts and concentrate in the K-D evaporator on a hot water bath.
- 8.4 Adjust the ampul volume to 10.0 ml with hexane.
- 8.5 Analyze by gas chromatography. If interferences are noted, proceed to Section 9.
- 8.6 The sample extract can be further concentrated using a micro Snider column if greater sensitivity is required.

9. Clean-up and Separation Procedures

- 9.1 Interferences in the form of distinct peaks and/or high background in the initial gas chromatographic analysis, as well as the physical characteristics of the extract (color, cloudiness, viscosity) and background knowledge of the sample will indicate whether clean-up is required. When

these interfere with measurement of the pesticides, or affect column life or detector sensitivity, proceed as directed below.

9.2 Florisil Column Adsorption Chromatography

9.2.1 Adjust the sample extract volume to 10 ml.

9.2.2 Place a charge of activated Florisil (weight determined by lauric-acid value, see Section 13) in a Chromaflex column. After settling the Florisil by tapping the column, add about one-half inch layer of anhydrous granular sodium sulfate to the top.

9.2.3 Pre-elute the column, after cooling, with 50-60 ml of petroleum ether. Discard the eluate and just prior to exposure of the sulfate layer to air, quantitatively transfer the sample extract into the column by decantation and subsequent petroleum ether washings. Adjust the elution rate to about 5 ml per minute and, separately, collect the two eluates in 500 ml K-D flasks equipped with 10 ml ampuls (see Eluate Composition 10.3.). Perform the first elution with 200 ml of 6% ethyl ether in petroleum ether, and the second elution with 200 ml of 15% ethyl ether in petroleum ether.

9.2.4 Concentrate the eluates to 6-10 ml in the K-D evaporator in a hot water bath.

9.2.5 Analyze by gas chromatography.

9.3 Eluate Composition - By using an equivalent quantity of any

batch of Florisil, as determined by its lauric acid value, the pesticides will be separated into the eluates indicated below:

6% Eluate

Aldrin	DDT	Pentachloronitrobenzene
BHC	Heptachlor	Strobane
Chlordane	Heptachlor Epoxide	Toxaphene
DDD	Lindane	Trifluralin
DDE	Methoxychlor	PCB's
	Mirex	

15% Eluate

Endosulfan I
Endrin
Dieldrin
Dichloran
Phthalate esters

50% Eluate

Endosulfan II
Captan

Certain thiophosphate pesticides can occur in each of the above fractions as well as the 100% fraction. For additional information regarding eluate composition, refer to the FDA Pesticide Analytical Manual (6).

10. Quality Control

10.1 Duplicate and spiked sample analyses are recommended as quality control checks. Quality control charts (5) should be developed and used as a check on the analytical system. Quality control check samples and performance evaluation samples should be analyzed on a regular basis.

10.2 Each time a set of samples is extracted, a method blank is determined on a volume of distilled water equivalent to that used to dilute the sample.

11. Calculation of Results

11.1 Determine the pesticide concentration by using the absolute

calibration procedure described below or the relative calibration procedure described in Part I, Section 3.4.2 (1).

$$(1) \quad \text{Micrograms/liter} = \frac{(A) (B) (V_t)}{(V_i) (V_s)}$$

A = $\frac{\text{ng standard}}{\text{Standard area}}$
B = Sample aliquot area
 V_i = Volume of extract injected (ul)
 V_t = Volume of total extract (ul)
 V_s = Volume of water extracted (ml)

12. Reporting Results

12.1 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, all data obtained should be reported.

13. Standardization of Florisil column by weight adjustment based on adsorption of lauric acid

13.1 A rapid method for determining adsorptive capacity of Florisil is based on adsorption of lauric acid from hexane solution (6) (8). An excess of lauric acid is used and the amount not adsorbed is measured by alkali titration. The weight of lauric acid adsorbed is used to calculate, by simple proportion, equivalent quantities of Florisil for batches having different adsorptive capacities.

13.2 Apparatus

13.2.1 Buret - 25 ml with 1/10 ml graduations.

13.2.2 Erlenmeyer flasks - 125 ml narrow mouth and 25 ml glass stoppered.

13.2.3 Pipet - 10 and 20 ml transfer.

13.2.4 Volumetric flasks - 500 ml.

13.3 Reagents and Solvents

- 13.3.1 Alcohol, ethyl - USP or absolute, neutralized to phenolphthalein.
- 13.3.2 Hexane - Distilled from all glass apparatus.
- 13.3.3 Lauric acid - Purified, CP.
- 13.3.4 Lauric acid solution - Transfer 10.000 g lauric acid to 500 ml volumetric flask, dissolve in hexane, and dilute to 500 ml (1 ml = 20 mg).
- 13.3.5 Phenolphthalein Indicator - Dissolve 1 g in alcohol and dilute to 100 ml.
- 13.3.6 Sodium hydroxide - Dissolve 20 g NaOH (pellets, reagent grade) in water and dilute to 500 ml (1N). Dilute 25 ml 1N NaOH to 500 ml with water (0.05N). Standardize as follows: Weigh 100-200 mg lauric acid into 125 ml Erlenmeyer flask. Add 50 ml neutralized ethyl alcohol and 3 drops phenolphthalein indicator; titrate to permanent end point. Calculate mg lauric acid/ml 0.05N NaOH (about 10 mg/ml).

13.4 Procedure

- 13.4.1 Transfer 2.000 g Florisil to 25-ml glass-stoppered Erlenmeyer flasks. Cover loosely with aluminum foil and heat overnight at 130°C. Stopper, cool to room temperature, add 20.0 ml lauric acid solution (400 mg), stopper, and shake occasionally for 15 minutes. Let the adsorbent settle and

pipet 10.0 ml of supernatant into 125 ml
Erlenmeyer flask. Avoid inclusion of any Florisil.

13.4.2 Add 50 ml of neutral alcohol and 3 drops of
indicator solution; titrate with 0.05N to a
permanent end point.

13.5 Calculation of lauric acid value and adjustment of column
weight

13.5.1 Calculate amount of lauric acid adsorbed on
Florisil as follows:
$$\text{Lauric acid value} = \text{mg lauric acid/g Florisil} =$$
$$200 - (\text{ml required for titration} \times \text{mg lauric}$$
$$\text{acid/ml } 0.05\text{N NaOH}).$$

13.5.2 To obtain an equivalent quantity of any batch of
Florisil, divide 110 by lauric acid value for that
batch and multiply by 20 g. Verify proper elution
of pesticides by 13.6.

13.6 Test for proper elution pattern and recovery of pesticides

13.6.1 Prepare a test mixture containing aldrin,
heptachlor epoxide, p,p'-DDE, dieldrin, Parathion
and malathion. Dieldrin and Parathion should
elute in the 15% eluate; all but a trace of
malathion in the 50% eluate and the others in the
6% eluate.

REFERENCES

1. "Method for Organic Pesticides in Water and Wastewater", Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio, 45268, 1971.
2. Monsanto Methodology for Aroclors - Analysis of Environmental Materials for Biphenyls, Analytical Chemistry Method 71-35, Monsanto Company, St. Louis, Missouri, 63166, 1970.
3. "Method for Polychlorinated Biphenyls in Industrial Effluents," Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio, 45268, 1973.
4. "Method for Organophosphorus Pesticides in Industrial Effluents," Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio, 45268, 1973.
5. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," Chapter 6, Section 6.4, U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio, 45268, 1973.
6. "Pesticide Analytical Manual," U.S. Dept. of Health, Education and Welfare, Food and Drug Administration, Washington, D.C.
7. "Analysis of Pesticide Residues in Human and Environmental Samples," U.S. Environmental Protection Agency, Perrine Primate Research Laboratories, Perrine, Florida, 33157, 1971.
8. Mills, P.A., "Variation of Florisil Activity: Simple Method for Measuring Adsorbent Capacity and Its Use in Standardizing Florisil Columns," Journal of the Association of Official Analytical Chemists, 51, 29 (1968).
9. Goerlitz, D.F. and Brown, E., "Methods for Analysis of Organic Substances in Water," Techniques of Water Resources Investigations of the United States Geological Survey, Book 5, Chapter A3, U.S. Department of the Interior, Geological Survey, Washington, D.C. 20242, 1972, pp. 24-40.
10. Steere, N.V., editor, "Handbook of Laboratory Safety," Chemical Rubber Company, 18901 Cranwood Parkway, Cleveland, Ohio, 44128, 1971, pp. 250-254.

METHOD FOR CHLOROPHENOXY ACID HERBICIDES IN DRINKING WATER

1. Scope and Application

- 1.1 This method covers the determination of chlorinated phenoxy acid herbicides in drinking water and raw source water. The compounds 2,4-dichlorophenoxy-acetic acid (2,4-D) and 2-(2,4,5-trichlorophenoxy) propionic acid (silvex) are determined by this procedure.
- 1.2 The detection limits are 20 ng/l for 2,4-D and 5 ng/l for Silvex and 2,4,5-T.
- 1.3 Since these compounds may occur in water in various forms (i.e., acid, salt, ester, etc.) a hydrolysis step is included to permit the determination of the active part of the herbicide. The method may be applied to additional phenoxy acids such as 2,3-dichloro-o-anisic acid (dicamba) and 2,4,5-trichlorophenoxy-acetic acid (2,4,5-T) and certain phenols.

2. Summary

- 2.1 Chlorinated phenoxy acids and their esters are extracted from the acidified water sample with ethyl ether. The esters are hydrolyzed to acids and extraneous organic material is removed by a solvent wash. The acids are converted to methyl esters which are extracted from the aqueous phase. Identification of the esters is made by selective gas chromatographic separations and may be corroborated through the use of two or more unlike columns. Detection and measurement is accomplished by electron

capture, microcoulometric or electrolytic conductivity gas chromatography (1). Results are reported in micrograms per liter.

2.2 This method is recommended for use only by experienced pesticide analysts or under the close supervision of such qualified persons.

3. Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interference under the conditions of the analysis. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Refer to Part 1, Section 1.4 and 1.5, (2).

3.2 The interferences encountered in drinking water should not pose great difficulty in obtaining accurate and precise measurement of chlorinated phenoxy acid herbicides.

3.3 Organic acids, especially chlorinated acids, cause the most direct interference with the determination. Phenols including chlorophenols will also interfere with this procedure.

3.4 Alkaline hydrolysis and subsequent extraction eliminates many of the predominant chlorinated insecticides which might otherwise interfere with the test.

3.5 The herbicides, being strong organic acids, react readily with alkaline substances and may be lost during analysis. Glassware and glass wool should be acid-rinsed and sodium sulfate should be acidified with sulfuric acid to avoid this possibility.

4. Apparatus and Materials

4.1 Gas Chromatograph - Equipped with glass lined injection port.

4.2 Detector Options:

4.2.1 Electron Capture - Radioactive (tritium or nickel-63)

4.2.2 Microcoulometric Titration

4.2.3 Electrolytic Conductivity

4.3 Recorder - Potentiometric strip chart (10 in.) compatible with the detector.

4.4 Gas Chromatographic Column Materials:

4.4.1 Tubing - Pyrex (180 cm long X 4 mm ID)

4.4.2 Glass Wool - Silanized

4.4.3 Solid Support - Gas-Chrom-Q (100-120 mesh)

4.4.4 Liquid Phases - Expressed as weight percent coated on solid support.

4.4.4.1 OV-210, 5%

4.4.4.2 OV-17, 1.5% plus QF-1, 1.95%

4.5 Kuderna-Danish (K-D) Glassware

4.5.1 Snyder Column - three ball (macro) and two ball (micro)

4.5.2 Evaporative Flasks - 250 ml

4.5.3 Receiver Ampuls - 10 ml, graduated

4.5.4 Ampul Stoppers

- 4.6 Graduated cylinders - 100 and 250 ml.
- 4.7 Erlenmeyer flasks - 125 ml, 250 ml ground glass T 24/40 with stopper
- 4.8 Micro Syringes - 10, 25, 50 and 100 μ l.
- 4.9 Pipets - Pasteur, glass disposable (140 mm long X 5 mm ID).
- 4.10 Separatory Funnels - 60 ml and 2000 ml with Teflon stopcock.
- 4.11 Glass wool - Filtering grade, acid washed.
- 4.12 Diazald Kit - Recommended for the generation of diazomethane (available from Aldrich Chemical Co., Cat. #210,025-2)
- 4.13 Florisil - PR grade (60-100 mesh) purchased activated at 1250F and stored at 130 C.

5. Reagents, Solvents and Standards

- 5.1 Boron Trifluoride-Methanol-esterification-reagent, 14 percent boron trifluoride by weight.
- 5.2 N-methyl-N-nitroso-p-toluenesulfonamide (Diazald) - High purity, melting point range 60-62 C. Precursor for the generation of diazomethane (see Appendix I).
- 5.3 Potassium Hydroxide Solution - A 37 percent (w:v) aqueous solution prepared from reagent grade potassium hydroxide pellets and reagent water.
- 5.4 Sodium Sulfate, Acidified - (ACS) granular sodium sulfate, treated as follows: Add 0.1 ml of conc. sulfuric acid to 100g of sodium sulfate slurried with enough ethyl ether to just cover the solid. Remove the ether with the vacuum. Mix 1 g of the resulting solid with 5 ml of reagent water and ensure the mixture to have a pH below 4. Store at 130 C.

- 5.5 Sulfuric acid - (ACS) concentrated, Sp. Gr. 1.84.
- 5.5.1 Sulfuric Acid (1:1) - Carefully mix equal volumes of H_2SO_4 (5.5) with distilled water. Cool while adding acid.
- 5.5.2 Sulfuric Acid (1+3) - Carefully mix 1 volume of H_2SO_4 (5.5) with 3 volumes of distilled water. Cool while adding acid.
- 5.6 Carbitol (diethylene glycol monoethyl ether).
- 5.7 Diethyl Ether - Nanograde, redistilled in glass, if necessary.
- 5.7.1 Must be free of peroxides as indicated by EM Quant test strips (available from EM Laboratories, Inc., 500 Executive Blvd., Elmsford, N.Y., 10523).
- 5.7.2 Procedures recommended for removal of peroxides are provided with the test strips.
- 5.8 Benzene Hexane - Nanograde, redistilled in glass, if necessary.
- 5.9 Pesticide Standards - Acids and Methyl Esters, reference grade.
- 5.9.1 Stock standard solutions - Dissolve 100 mg of each herbicide in 60 ml ethyl ether; then make to 100 ml with redistilled hexane. Solution contains 1 mg/ml.
- 5.9.2 Working standard - Pipet 1.0 ml of each stock solution into a single 100 ml volumetric flask. Make to volume with a mixture of ethyl ether and hexane (1:1). Solution contains 10 ug/ml of each standard.
- 5.9.3 Standard for Chromatography (Diazomethane Procedure) - Pipet 1.0 ml of the working standard into a glass stoppered test tube and evaporate off the solvent

using steam bath. Add 2 ml diazomethane to the residue. Let stand 10 minutes with occasional shaking, then allow the solvent to evaporate spontaneously. Dissolve the residue in 200 ul of hexane for gas chromatography.

- 5.9.4 Standard for Chromatography (Boron Trifluoride Procedure) - Pipet 1.0 ml of the working standard into a glass stoppered test tube. Add 0.5 ml of benzene and evaporate to 0.4 ml using a two-ball Snyder microcolumn and a steam bath. Proceed as in 10.3.1. Esters are then ready for gas chromatography.

6. Calibration

- 6.1 Gas chromatographic operating conditions are considered acceptable if the response to dicapthon is at least 50% of full scale when 0.06 ng is injected for electron capture detection and 100 ng is injected for microcoulometric or electrolytic conductivity detection. For all quantitative measurements, the detector must be operated within its linear response range and the detector noise level should be less than 2% of full scale.
- 6.2 Standards, prepared from methyl esters of phenoxy acid herbicides calculated as the acid equivalent, are injected frequently as a check on the stability of operating conditions. Gas chromatograms of several chlorophenoxys are shown in Figure 1.
- 6.3 The elution order and retention ratios of methyl esters of

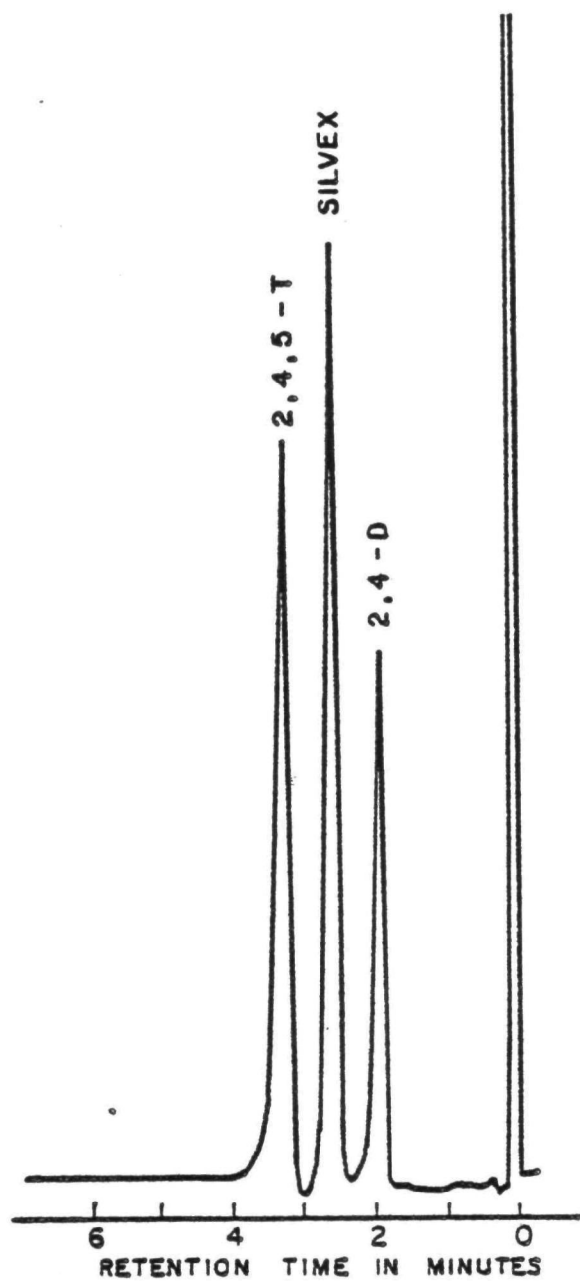


Fig. 1 Column: 1.5 % OV-17 + 1.95 % QF-1,
Carrier Gas: Argon (5%) / Methane: 70ml/min.,
Column Temp. 185 C, Detector: Electron Capture.

Table 1
RETENTION RATIOS FOR METHYL ESTERS OF SOME CHLORINATED
PHENOXY ACID HERBICIDES RELATIVE TO 2,4-D

Liquid Phase ¹	1.5% OV-17 + 1.95% QF-1	5% OV-210
Column Temp.	185 C	185 C
Argon/Methane Carrier Flow	70 ml/min	70 ml/min
Herbicide	RR	RR
dicamba	0.60	0.61
2,4-D	1.00	1.00
silvex	1.34	1.22
2,4,5-T	1.72	1.51
2,4-D (minutes absolute)	2.00	1.62

¹All columns glass, 180 cm x 4 mm ID, solid support
Gas Chrom Q (100/120 mesh)

chlorinated phenoxy acid herbicides are provided in Table 1, as a guide.

7. Sample Collection and Handling

- 7.1 Wash all bottles and cap liners in detergent water. Rinse with tap water and finally distilled water.
- 7.2 Allow bottles and cap liners to air-dry. Muffle the sample bottles at 400°C for 1 hour.
- 7.3 Rinse the cap liners with pesticide-grade hexane.
- 7.4 When cool, seal the bottles and store in a dust-free environment.
- 7.5 Collect samples in 1 quart narrow-mouth bottles with a Teflon lined screw cap (option 1 quart widemouth screw-cap bottles with Teflon lid liner). Collect all samples in duplicate.
- 7.6 Sampling from a water tap: Turn on water and allow system to flush. When the temperature has stabilized, adjust the flow to about 1 l/min. Fill the bottle about 90% full and seal.
- 7.7 The sample should be maintained near 4°C until analysis and should be extracted as soon as possible after collection.

8. Sample Procedure

- 8.1 Quantitatively transfer 1 liter of sample into a two-liter separatory funnel, and acidify to approximately pH 2 with concentrated sulfuric acid. Check pH with indicator paper.
- 8.2 Add 150 ml of ether to the sample in the separatory funnel and shake vigorously for one minute. Allow the contents to separate for at least ten minutes. After the layers have separated, drain the water phase into a one-liter Erlenmeyer flask. Then

collect the extract in a 250 ml ground-glass Erlenmeyer flask containing 2 ml of 37 percent aqueous potassium hydroxide.

- 8.3 Extract the sample two more times using 50 ml of ether each time, and combine the extracts in the Erlenmeyer flask. (Rinse the one-liter flask with each additional aliquot of extracting solvent.)

9. Hydrolysis

- 9.1 Add 15 ml of distilled water and a small boiling stone to the flask containing the ether extract, and fit the flask with a 3-ball Snyder column. Evaporate the ether on a steam bath and continue heating for a total of 60 minutes.
- 9.2 Transfer the concentrate to a 60 ml separatory funnel. Extract the basic solution two times with 20 ml of ether and discard the ether layers. The herbicides remain in the aqueous phase.
- 9.3 Acidify the contents of the separatory funnel by adding 2 ml of cold (4°C) sulfuric acid (1+3). Extract the herbicides once with 20 ml of ether and then two more times with 10 ml of ether. Collect the extracts in a 125 ml Erlenmeyer flask containing about 0.5 g of acidified anhydrous sodium sulfate (5.4). Allow the extract to remain in contact with the sodium sulfate for approximately two hours.

10. Esterification (4,5)

- 10.1 Transfer the ether extract into a Kuderna-Danish flask equipped with a 10-ml graduated ampul. Use liberal washings of ether. Using a glass rod, crush any caked sodium sulfate during the washing.

- 10.1.1 If esterification is to be done with diazomethane, evaporate to approximately 4 ml on a steam bath (do not immerse the ampul in water) and proceed as directed in Section 10.2.
- 10.1.2 If esterification is to be done with boron trifluoride, add 0.5 ml benzene and evaporate to about 5 ml on a steam bath. Remove the ampul from the flask and further concentrate the extract to 0.4 ml using a two-ball Snyder microcolumn and proceed as in 10.3.

10.2 Diazomethane Esterification

- 10.2.1 Disconnect the ampul from the K-D flask and place in a hood away from steam bath. Adjust volume to 4 ml with ether, add 2 ml diazomethane, and let stand 10 minutes with occasional swirling.
- 10.2.2 Rinse inside wall of ampul with several hundred microliters of ethyl ether. Take sample to approximately 2 ml to remove excess diazomethane by allowing solvent to evaporate spontaneously (room temperature).
- 10.2.3 Dissolve residue in 5 ml of hexane. Analyze by gas chromatography.
- 10.2.4 If further clean-up of the sample is required, proceed as in 10.3.4.

10.3 Boron Trifluoride Esterification

- 10.3.1 After the benzene solution in the ampul has cooled,

add 0.5 ml of boron trifluoride-methanol reagent. Use the two-ball Snyder microcolumn as an air-cooled condenser and hold the contents of the ampul at 50°C for 30 minutes on the steam bath.

10.3.2 Cool and add about 4.5 ml of a neutral 5 percent aqueous sodium sulfate solution. Seal the flask with a ground glass stopper and shake vigorously for about one minute. Allow to stand for three minutes for phase separation. Using a pipet, withdraw the bottom water phase and discard.

10.3.4 Pipet the solvent layer from the ampul to the top of a small column prepared by plugging a disposable Pasteur pipet with glass wool and packing with 2.0 cm of sodium sulfate over 1.5 cm of Florisil adsorbent. Collect the eluate in a graduated ampul. Complete the transfer by repeatedly rinsing the ampul with small quantities of benzene and passing the rinses through the column until a final volume of 5.0 ml of eluate is obtained. Analyze by gas chromatography.

11. Quality Control

11.1 Duplicate and spiked sample analyses are recommended as quality control checks. Quality control charts (3) should be developed and used as a check on the analytical system. Quality control check samples and performance evaluation samples should be analyzed on a regular basis.

11.2 Each time a set of samples is extracted, a method blank is determined on a volume of distilled water equivalent to that used to dilute the sample.

12. Calculation of Results

12.1 Determine the methyl ester concentration by using the absolute calibration procedure described below or the relative calibration procedure described in Part I, Section 3.4.2 (2).

$$(1) \quad \text{Micrograms/liter} = \frac{(A)}{(V_i)} \frac{(B)}{(V_s)} \frac{(V_t)}{(V_s)}$$

A = $\frac{\text{ng standard}}{\text{standard area}}$

B = Sample aliquot area

V_i = Volume of extract injected (ul)

V_t = Volume of total extract (ul)

V_s = Volume of water extracted (ml)

12.2 Molecular weights for the calculation of methyl esters as the acid equivalents.

2,4-D	222.0	Dicamba	221.0
2,4-D methyl ester	236.0	Dicamba methyl ester	236.1
Silvex	269.5	2,4,5-T	255.5
Silvex methyl ester	283.5	2,4,5-T methyl ester	269.5

13. Reporting Results

13.1 Report results in micrograms per liter as the acid equivalent without correction for recovery data. When duplicate and spiked samples are analyzed all data obtained should be reported.

REFERENCES

- (1) Goerlitz, D. G., and Lamar, W. L., "Determination of Phenoxy and Herbicides in Water by Electron-Capture and Microcoulometric Gas Chromatography", U. S. Geol. Survey Water-Supply Paper 1817-C (1967).
- (2) "Methods for Organic Pesticides in Water and Wastewater", (1971), U. S. Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio, 45268.
- (3) "Handbook for Analytical Quality Control in Water and Wastewater Laboratories" (1972), U. S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio, 45268.
- (4) Metcalf, L. D., and Schmitz, A. A., "The Rapid Preparation of Fatty Acid Esters for Gas Chromatographic Analysis", Analytical Chemistry, 33, 363 (1961).
- (5) Schlenk, H. and Gellerman, J. L., "Esterification of Fatty Acids with Diazomethane on a Small Scale", Analytical Chemistry, 32, 1412 (1960).
- (6) "Pesticide Analytical Manual", U. S. Department of Health, Education and Welfare, Food and Drug Administration, Washington, D. C.
- (7) Steere, N. V., editor, "Handbook of Laboratory Safety", Chemical Rubber Company, 18901 Cranwood Parkway, Cleveland, Ohio, 44128, 1971, pp. 250-254.

APPENDIX I

Diazomethane in ether (6)

1. CAUTIONS:

Diazomethane is very toxic. It can explode under certain conditions.

The following precautions should be observed.

Use only in well-ventilated hood.

Use safety screen.

Do not pipette solution of diazomethane by mouth.

For pouring solutions of diazomethane, use of gloves is optional.

Do not heat solutions to 100°C (EXPLOSIONS).

Store solutions of gas at low temperatures (freezer compartment of explosion-proof refrigerators).

Avoid ground glass apparatus, glass stirrers and sleeve bearings where grinding may occur (EXPLOSIONS).

Keep solutions away from alkali metals (EXPLOSIONS).

Solutions of diazomethane decompose rapidly in presence of solid material such as copper powder, calcium chloride, boiling stones, etc. These solid materials cause polymethylene and nitrogen gas to form.

2. PREPARATION:

Use a well-ventilated hood and cork stoppers for all connections.

Fit a 125 ml long-neck distilling flask with a dropping funnel and an efficient condenser set downward for distillation. Connect the condenser to two receiving flasks in a series - a 500 ml Erlenmeyer followed by a 125 ml Erlenmeyer containing 30 ml ether. The inlet to the 125 ml Erlenmeyer should dip below the ether. Cool both receivers to 0°C.

As water bath for the distilling flask, set up a 2-liter beaker on a stirplate (hot plate and stirrer), maintaining temperature at 70°C.

Dissolve 6 g KOH in 10 ml water in the distilling flask (no heat). Add 35 ml Carbitol (diethylene glycol monoethyl ether), stirring bar, and another 10 ml ether. Connect the distilling flask to the condenser and immerse distilling flask in water bath. By means of the dropping funnel, add a solution of 21.5 g Diazald in 140 ml ether over a period of 20 minutes. After distillation is apparently complete, add another 20 ml ether and continue distilling until distillate is colorless. Combine the contents of the two receivers in a glass bottle (WITHOUT ground glass neck), stopper with cork, and freeze overnight. Decant the diazomethane from the ice crystals into a glass bottle, stopper with cork, and store in freezer until ready for use. The final solution may be stored up to six months without marked deterioration.

The 21.5 g of Diazald reacted in this manner produce about 3 g of Diazomethane.