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METHODS FOR BENZIDINE, CHLORINATED ORGANIC COMPOUNDS, PENTACHLOROPHENOL AND PESTICIDES IN WATER AND WASTEWATER

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

U.S. ENVIRONMENTAL PROTECTION AGENCY
ENVIRONMENTAL MONITORING AND SUPPORT LABORATORY
CINCINNATI, OHIO 42568

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FOREWORD

This collection of methods for the determination of benzidine, chlorinated organic compounds, pentachlorophenol and pesticides has been assembled by the staff of the Environmental Monitoring and Support Laboratory - Cincinnati (EMSL-Cinti.) for use by the NPDES Permits Program.

These methods are as referenced in the Federal Register of December 1, 1976 and are being provided only for the interim period until the manual "Methods for Organic Analysis of Water and Wastes" becomes available.

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DISCLAIMER

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

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	Heptachloro epoxide	--	555	529	30	39420
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	BHC	7	555	529	--	--
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v

F.R.#	Parameter	EPA This Manual	14th ed. Std. Methods	ASTM (1975)	USGS*	STORET NUMBER
	DDD	7	555	529	30	39360
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	Demeton-O	25	--	--	--	39560
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	Dicamba	115	--	--	--	
	Dichlorofenthion	--	--	--	30	--
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	Dicofol	--	--	529	--	39780
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	Dioxathion	--	--	--	30	--
	Disulfoton	25	--	--	--	39010
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	Fenuron	104	--	--	--	--
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	Heptachlor	7	555	529	30	39410
	Isodrin	--	--	--	30	39430
	Lindane	7	555	529	30	39782
	Linuron	104	--	--	--	--
	Malathion	25	555	--	30	39530
	Methiocarb	94	--	--	--	--
	Methoxychlor	7	555	529	30	39489
	Mexacarbate	94	--	--	--	--
	Mirex	7	555	--	--	39755
	Monuron	104				

Mexacarbate	94	--	--	--	--
Mirex	7	555	--	--	39755
Monuron	104	--	--	--	--
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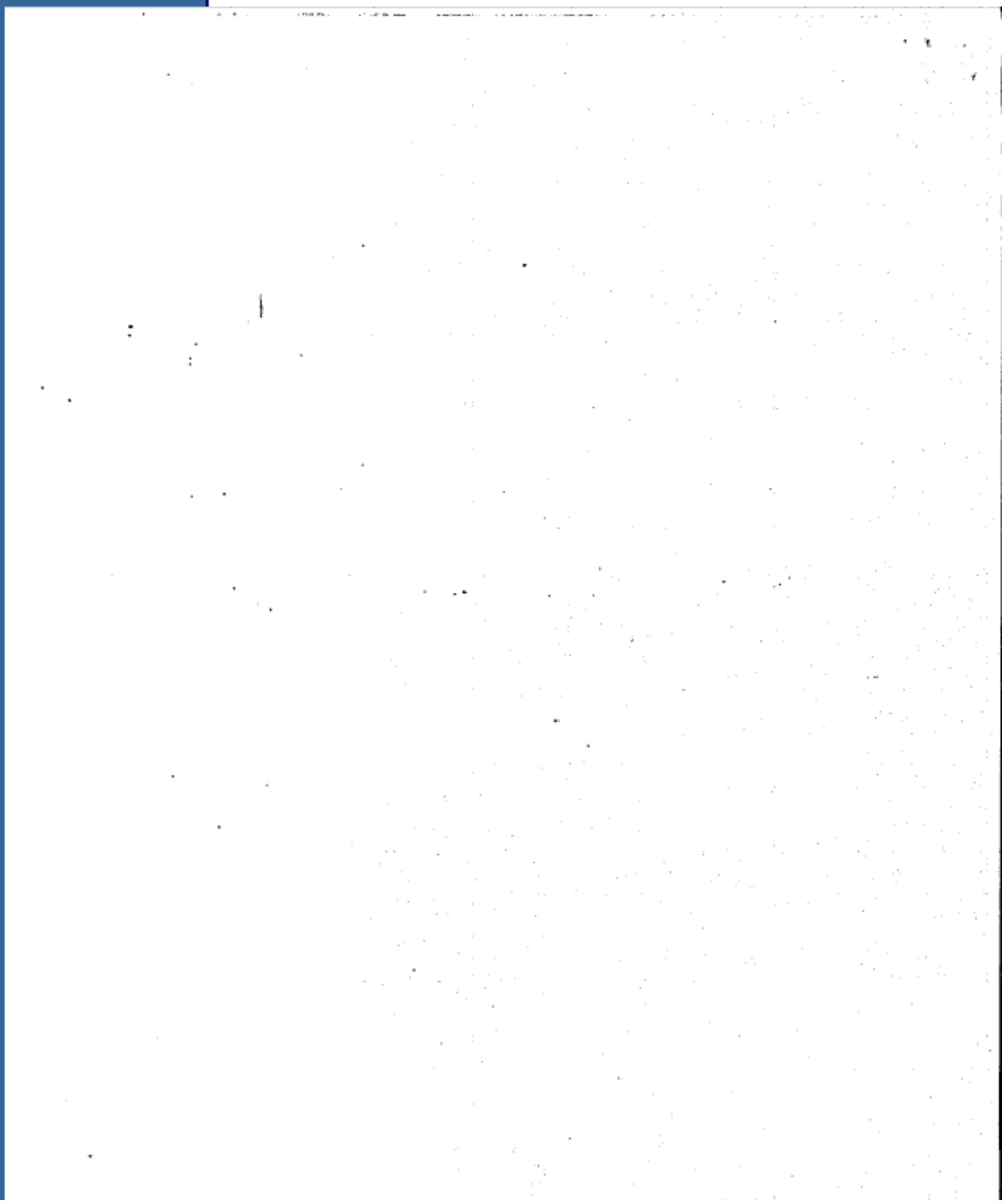
Terbuthylazine	83	--	--	--	--

F.R.#	Parameter	EPA This Manual	14th ed. Std. Methods	ASTM (1975)	USGS*	STORET NUMBER
	Toxaphene	7	555	529	30	39400
	Trifluraline	7	--	--	--	39030

*Goerlitz, D. & Brown, E. "Methods for Analysis of Organic Substances in Water," U.S. Geological Survey Techniques of Water-Resources Inv. Book 5, Ch. A3 (1972).



METHOD FOR BENZIDINE AND ITS SALTS IN WASTEWATERS

1. Scope and Application

1.1 This method covers the determination for benzidine and its salts in water and wastewaters. The method can be modified to apply also to the determination of closely related materials as described under Interferences (4.2).

1.2 The salts of benzidine, such as benzidine sulfate, are measured and reported as benzidine, STORET NO. 39120.

1.3 The method detection limit is 0.2 µg/l when analyzing 1 liter of sample.

2. Summary

2.1 The water sample is made basic and the benzidine is extracted with ethyl acetate. Cleanup is accomplished by extracting the benzidine from the ethyl acetate with hydrochloric acid. Chloramine-T is added to the acid solution to oxidize the benzidine. The yellow oxidation product is extracted with ethyl acetate and measured with a scanning spectrophotometer. The spectrum from 510 nm to 370 nm is used for qualitative identification.

3. Hazards

3. Hazards

3.1 Benzidine is a known carcinogen. All manipulations of this method should be carried out in a hood with protection

provided for the hands and arms of the analyst. Consult OSHA regulations (1) before working with benzidine.

4. Interferences

4.1 The multiple extractions effectively limit the interferences to organic bases. The oxidation with Chloramine-T to form a yellow product is very selective and has been described in detail (2,3). The use of the absorption spectrum for the identification of benzidine results in a highly specific procedure.

4.2 Some compounds having a structure very similar to benzidine will interfere with the quantification, if present. Examples of these interferring compounds are dichlorobenzidine, o-tolidine, and dianisidine.

4.3 A general yellow background color in the extract will limit the cell pathlength that can be employed and thus limit the sensitivity of the method.

5. Apparatus and Materials

5.1 Spectrophotometer-visible, scanning (510-370 nm).

5.2 Separatory Funnels - 125 ml, 250 ml, 2000 ml.

5.3 Cells - 1 to 5 cm pathlength, 20 ml volume maximum.

6. Reagents, Solvents and Standards

6.1 Ethyl acetate

6.2 Hydrochloric acid (1 N) - Add 83 ml conc. hydrochloric acid to water and dilute to one liter.

water and dilute to one liter.

6.3 Chloramine-T - 10% solution. Prepare fresh daily by dissolving 1.0g Chloramine-T in 10 ml distilled water.

6.4 Stock standard (0.2 µg/µl) - Dissolve 100.0 mg purified benzidine in about 30 ml 1 N HCl. Dilute to 500 ml with water.

7. Preparation of Calibration Curve

7.1 To a series of 125-ml separatory funnels, add 45 ml of hydrochloric acid and 10 ml of ethyl acetate. Shake for one minute to saturate the acid layers. Discard the solvent layers. Dose the series with volumes from 1.0 to 20.0 µl of stock standard, using syringes.

7.2 Treat standards according to the Procedure beginning with 8.5.

8. Quality Control

8.1 Duplicate and spiked sample analyses are recommended as quality control checks. Quality control charts 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.

8.2 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.

used to dilute the sample.

9. Procedure

9.1 Adjust the sample pH to 8.5 to 9.0 with dilute NaOH or HCl.

9.2 Transfer 1 liter of sample to a 2000-ml separatory funnel. Add 150 ml ethyl acetate and shake for two minutes. Allow the layers to separate, then drain the water layer into a

second 2-liter separatory funnel. Drain the solvent layer into a 250-ml separatory funnel.

9.3 Repeat the extraction of the water layer twice more with 50-ml portions of ethyl acetate. Combine all solvent layers, then discard the water layer.

9.4 Extract the solvent layer three times with 15-ml portions of hydrochloric acid by shaking 2 minutes and allowing the phases to separate. Combine the acid layers in a glass stoppered container for cold storage until time is available for analysis, or transfer the layers directly into a 125-ml separatory funnel.

9.5 Prepare the spectrophotometer so it is warmed and ready to use. The remaining steps of the procedure must be performed rapidly on one sample at a time.

9.6 To the hydrochloric acid solution in a 125 ml separatory funnel, add 1.0 ml chloramine-T solution and mix. Add 25.0-ml ethyl acetate with a pipet and shake for two minutes. Allow the layers to separate, then discard the aqueous phase.

Allow the layers to separate, then discard the aqueous phase.

9.7 Filter the solvent layer through coarse filter paper and fill a 5-cm cell with the filtrate.

9.8 Scan the solvent from 510 nm to 370 nm. Ethyl acetate is used for a blank with double beam instruments. Shorter pathlength cells should be used in cases where absorbance exceeds 0.8.

10. Calculation of Results

10.1 Benzidine is identified by its absorbance maximum at 436 nm. Dichlorobenzidine gives similar response but has its absorbance maximum at 445 nm.

10.2 Construct a baseline from the absorbance minimum at about 470 nm to the minimum at 390 nm (or 420 nm minimum for samples with a high background). Record the absorbance ofthe peak maximum and the absorbance of the constructed baseline at the 436 nm. Treat samples and standards in the same fashion.

10.3 Using the net absorbance values, prepare a calibration plot from the standards. Determine the total micrograms in each sample from this plot.

10.4 Divide the total micrograms by the sample volume, in liters, to determine µg/l. Correct results for cell pathlength if necessary.

11. Reporting Results

11.1 Report results in micrograms per liter as benzidine without correction for recovery data. When duplicate and spike samples are analyzed all data obtained should be reported.

12. Accuracy and Precision

12.1 When 1 liter samples of river water were dosed with 1.80 µg of benzidine, an average of 1.24 µg was recovered. The standard deviation was 0.092 µg/l (n=8).

REFERENCES:

1. Federal Register, Volume 39, Page 3779, Paragraph 1910.93; (January 29, 1974).

2. Glassman, J. M., and Meigs, J. W., "Benzidine (4,4'-Diaminobiphenyl) and Substituted Benzidines", Arch. Industr. Hyg., 4, 519, (1951).

3. Butt, L. T. and Strafford, N., "Papilloma of the Bladder in the Chemical Industry. Anlaytical Methods for the Determination of Benzidine and B-Naphtylamine, Recommended by A.B.C.M. Sub-Committee", J. Appl. Chem., 6, 525 (1956).

METHOD FOR CHLORINATED HYDROCARBONS IN WATER AND WASTEWATER

1. Scope and Application

1.1 This method covers the determination of various organochlorine pesticides and heptachlor epoxide in water and wastewater.

1.2 The following pesticides may be determined individually by this method:

Parameter	Storet No.
Aldrin	39330
BHC	----
Captan	39640
Chlordane	39350
DDD	39360
DDE	39365
DDT	39370
Dichloran	----
Dieldrin	39380
Endosulfan	39388
Endrin	39390
Heptachlor	39410
Lindane	39782
Methoxychlor	39480
Mirex	39755
PCNB	39029
Strobane	----
Toxaphene	39400
Trifluralin	39030

1.3 The following chlorinated organic compound may be determined individually by this method:

individually by this method:

Compound	Storet No.
Heptachlor epoxide	----

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

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 Appendix I.

3.2 The interferences in industrial effluents are high and varied and often pose great difficulty in obtaining accurate and precise measurement of organochlorine pesticides. Sample clean-up procedures are generally required and may result in the loss of certain organochlorine pesticides. Therefore, great care should be exercised in the selection and use of methods for eliminating or minimizing interferences. It is not possible to describe procedures for overcoming all of the interferences that may be encountered in industrial effluents.

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

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 (1, 2).

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 or to the flame photometric detector.

3.5 Organophosphorus Pesticides - A number of organophosphorus pesticides, such as those containing a nitro group, e.g., parathion, also 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 (3).

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

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 µl.

4.9 Separatory funnels - 125 ml, 1000 ml and 2000 ml with Teflon stopcock.

4.10 Blender - High speed, glass or stainless steel cup.

4.11 Graduated cylinders - 100 and 250 ml.

4.12 Florisil - PR Grade (60-100 mesh); purchase activated at 1250°F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use, activate each batch overnight at 130°C in foil-covered glass container. Determine lauric-acid value (See Appendix II).

5. Reagents, Solvents, and Standards

5.1 Sodium Chloride - (ACS) Saturated solution in distilled water

(pre-rinse NaCl with hexane).

5.2 Sodium Hydroxide - (ACS) 10 N in distilled water.

5.3 Sodium Sulfate - (ACS) Granular, anhydrous (conditioned at 400 C for 4 hrs.).

5.4 Sulfuric Acid - (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.

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, N.Y. 10523.)

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

5.6 Acetonitrile, 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 $\gtrsim$ 0.06 ng is injected for electron capture detection and $\gtrsim$ 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.

7. Quality Control

7.1 Duplicate and spiked sample analyses are recommended as quality control checks. Quality control charts (4) 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.

7.2 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.

8. Sample Preparation

8.1 The sample size taken for analysis is dependent on the type of sample and the sensitivity required for the purpose at hand. Background information on the pesticide levels previously detected at a given sampling site will assist in determining the sample size required, as well as the final volume to which the extract needs to be concentrated. A 1-liter sample is usually taken for drinking water and ambient water analysis to provide a detection limit of 0.050to 0.100 µg/l. One-hundred milliliters is usually adequate to provide a detection limit of 1 µg/l for industrial effluents.

1 µg/l for industrial effluents.

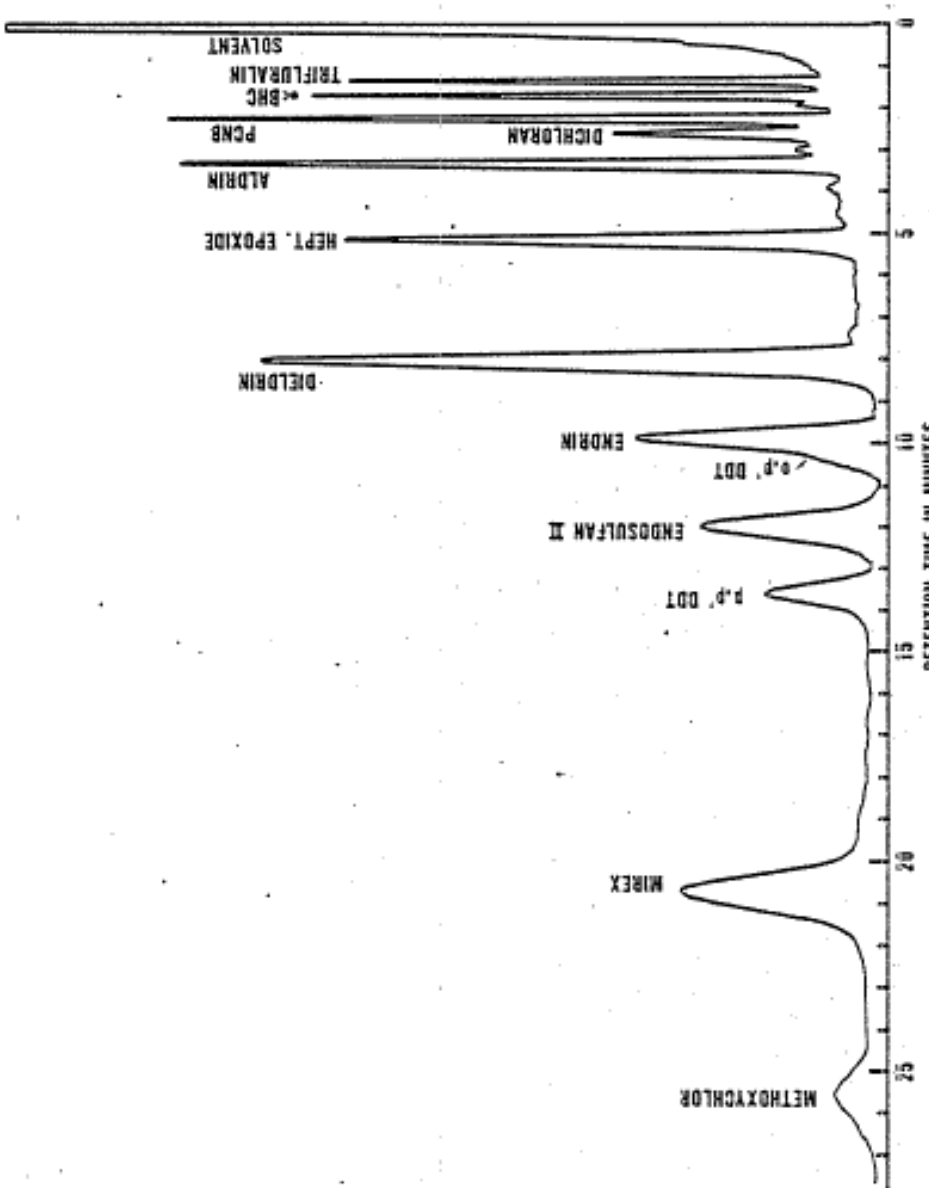
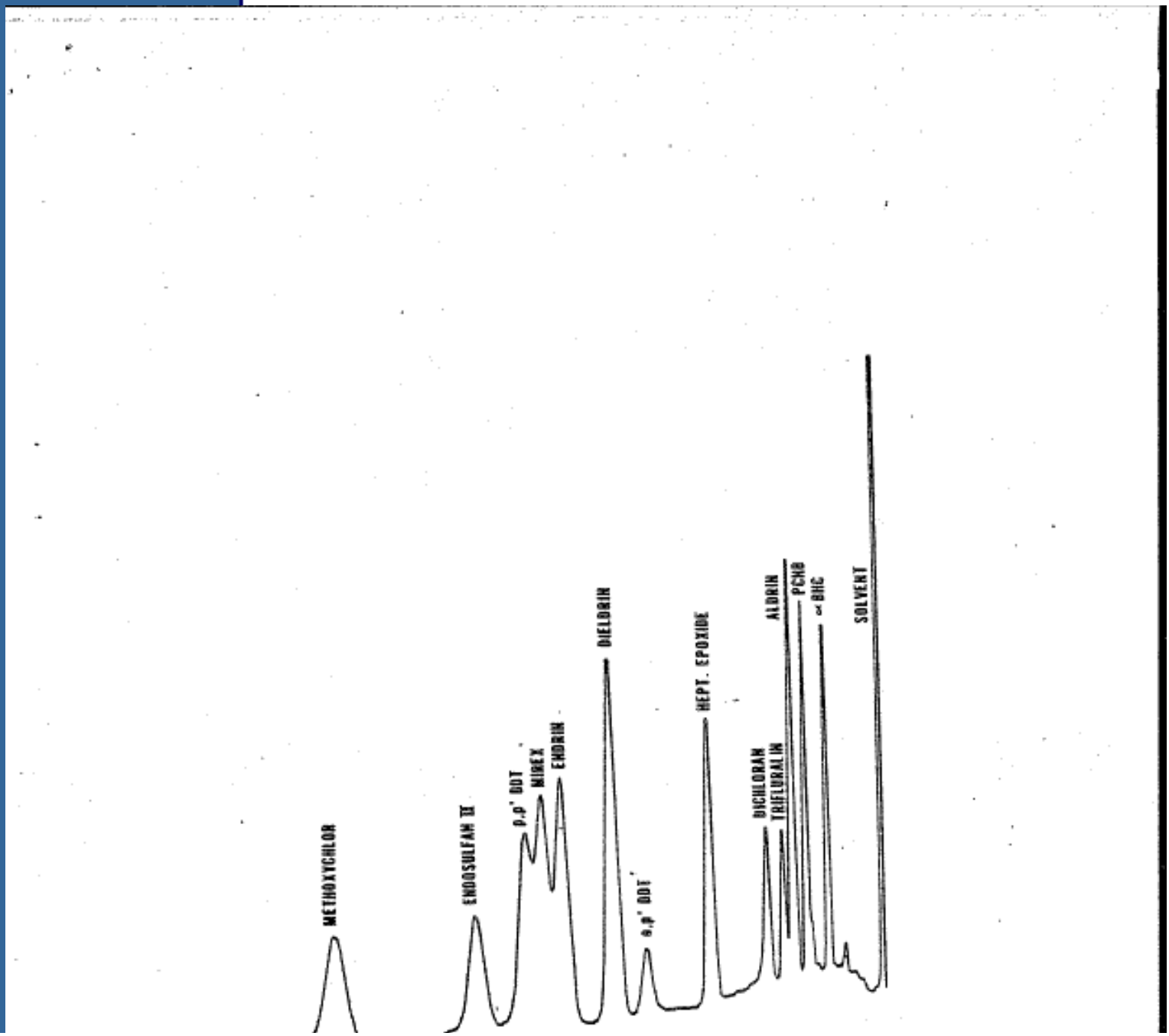


Figure 1. Column Packing: 1.5% OV-17 + 1.95% QF-1, Carrier Gas: Argon/Methane at 60 ml/min, Column Temperature: 200 C, Detector: Electron Capture.

Figur


METHOXYCHLOR
ENDOSULFAN II
p,p' DDT
MIREX
ENDRIN
DIELDRIN
o,p' DDT
HEPT. EPOXIDE
DICHLORAN
TRIFLURALIN
ALDRIN
PCNB
BHC
SOLVENT

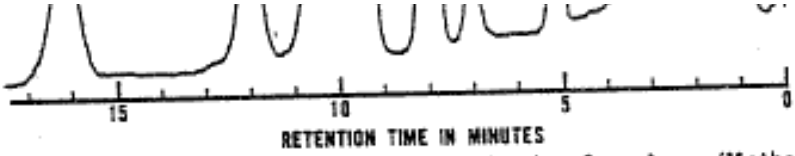
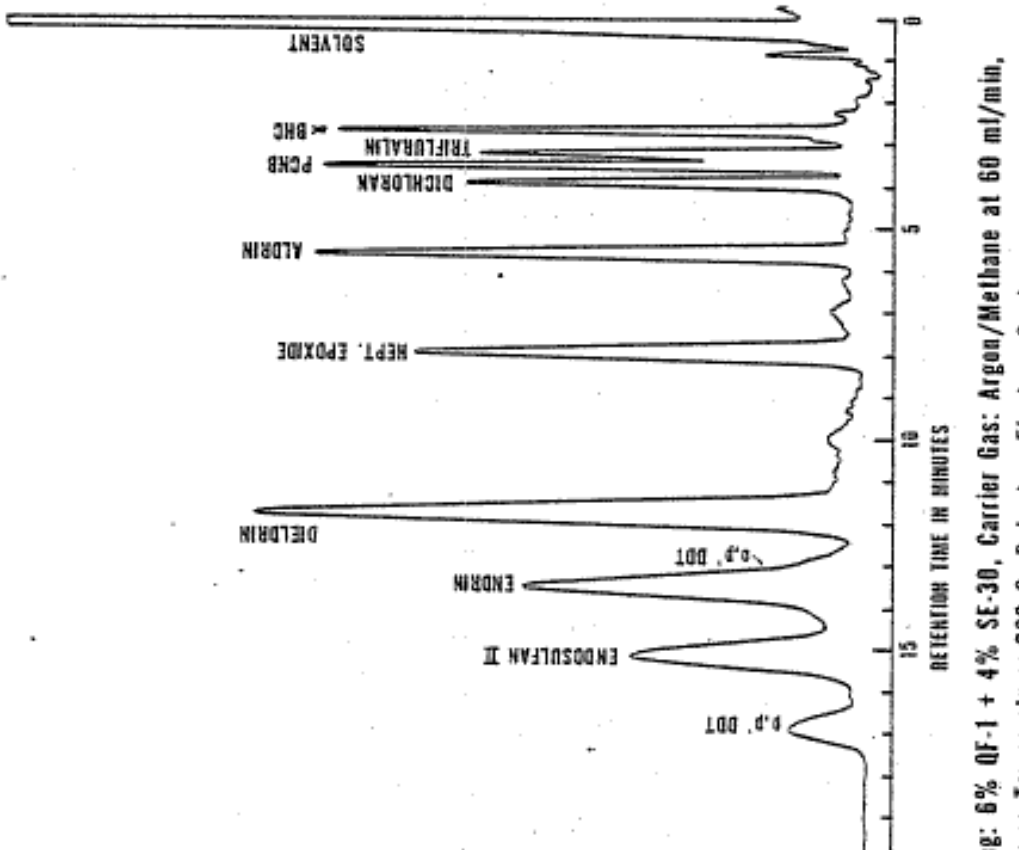


Figure 2. Column Packing: 5% OV-210, Carrier Gas: Argon/Methane at 70 ml/min, Column Temperature: 180 C, Detector: Electron Capture.



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

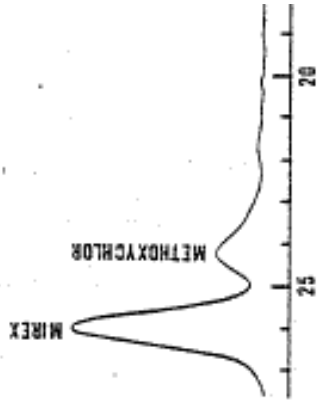


Figure 3. Column Packing: 6%
Column 1

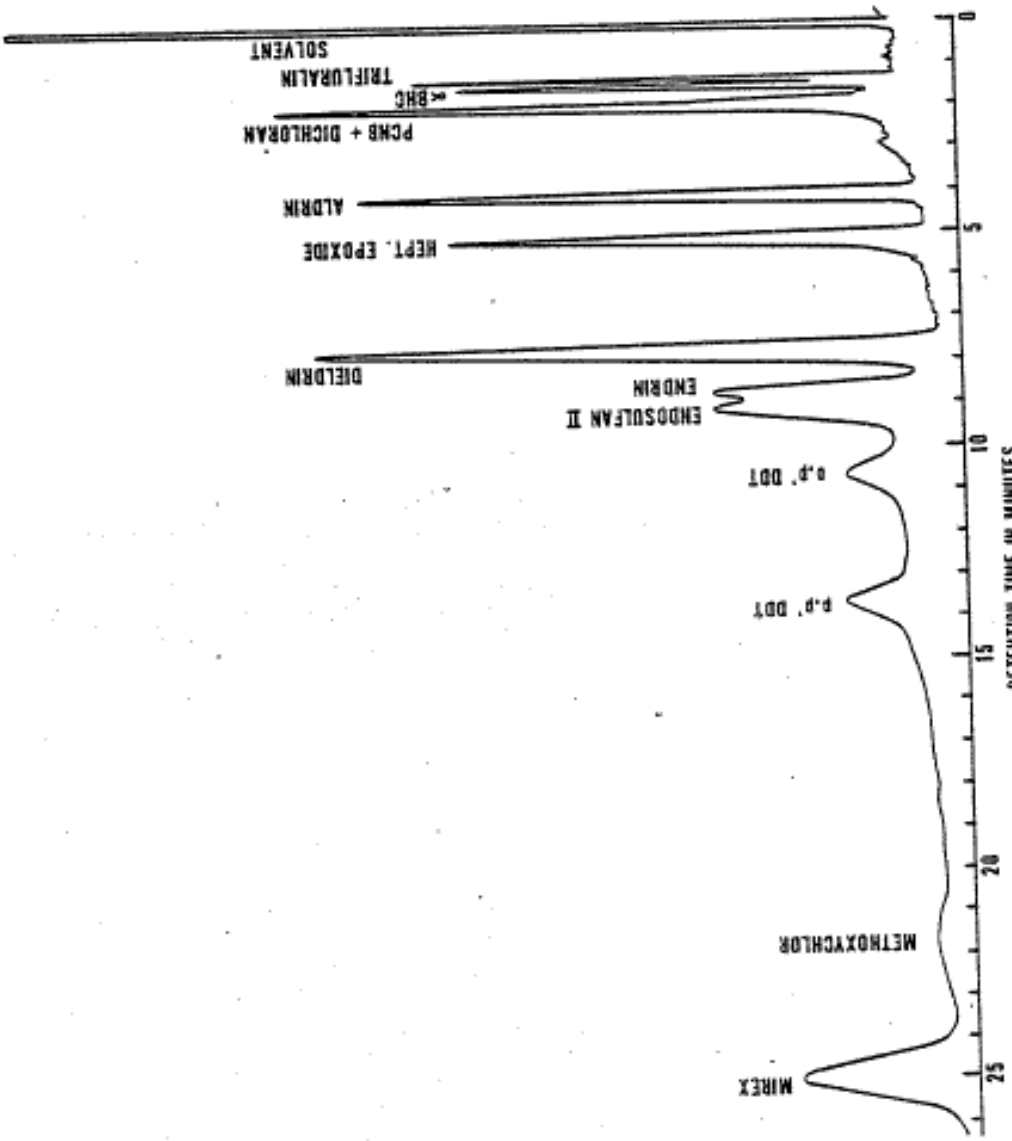


Figure 4. Column Packing: 3% OV-1, Carrier Gas: Argon/Methane at 70 ml/min, Column Temperature: 180 C, Detector: Electron Capture.

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

[1]All columns glass, 180 cm x 4 mm ID, solid support Gas-Chrom Q (100/120 mesh)
[2]OV-210 also may be used

8.2 Quantitatively transfer the proper aliquot of sample from the sample container into a two-liter separatory funnel. If less than 800 ml is analyzed, dilute to one liter with interference free distilled water.

9. Extraction

9.1 Add 60 ml of 15% methylene chloride in hexane (v:v) to the sample in the separatory funnel and shake vigorously for two minutes.

9.2 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 3-4 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 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.

9.3 Concentrate the extract in the K-D evaporator on a hot water bath.

9.4 Analyze by gas chromatography unless a need for cleanup is indicated (See Section 10).

10. Clean-up and Separation Procedures

10.1 Interferences in the form of distinct peaks and/or high background in the initial gas chromatographic analysis, as well as

ground 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.

10.2 Acetonitrile Partition - This procedure is used to isolate fats and oils from the sample extracts. It should be noted that not all pesticides are quantitatively recovered by this procedure. The analyst must be aware of this and demonstrate the efficiency of the partitioning for specific pesticides. All of the pesticides listed in Scope (1.2) with the exception of mirex are efficiently recovered.

10.2.1 Quantitatively transfer the previously concentrated extract to a 125-ml separatory funnel with enough hexane to bring the final volume to 15 ml. Extract the sample four times by shaking vigorously for one minute with 30-ml portions of hexane-saturated acetonitrile.

10.2.2 Combine and transfer the acetonitrile phases to a one-liter separatory funnel and add 650 ml of distilled water and 40 ml of saturated sodium chloride solution. Mix thoroughly for 30-45 seconds. Extract with two 100-ml portions of hexane by vigorously shaking about 15 seconds.

10.2.3 Combine the hexane extracts in a one-liter separatory funnel and wash with two 100-ml portions of distilled

funnel and wash with two 100-ml portions of distilled water. Discard the water layer and pour the hexane layer through a 3-4 inch anhydrous sodium sulfate column into a 500-ml K-D flask equipped with a 10-ml

ampul. Rinse the separatory funnel and column with three 10-ml portions of hexane.

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

10.2.5 Analyze by gas chromatography unless a need for further cleanup is indicated.

10.3 Florisil Column Adsorption Chromatography

10.3.1 Adjust the sample extract volume to 10 ml.

10.3.2 Place a charge of activated Florisil (weight determined by lauric-acid value, see Appendix II) 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.

10.3.3 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 wash- ings. Adjust the elution rate to about 5 ml per minute and,

Adjust the elution rate to about 5 ml per minute and, separately, collect up to three eluates in 500-ml K-D flasks equipped with 10-ml ampuls (see Eluate Composition 10.4.). 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. Perform the third elution with 200 ml of 50% ethyl ether - petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

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

10.3.5 Analyze by gas chromatography.

10.4 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	Mirex
BHC	Heptachlor	PCNB
Chlordane	Heptachlor Epoxide	Strobane
DDD	Lindane	Toxaphene
DDE	Methoxychlor	Trifluralin

15% Eluate	50% Eluate
Endosulfan I	Endosulfan II
Endrin	Captan
Dieldrin	
Dichloran	

Certain thiophosphate pesticides will occur in each of the above fractions as well as the 100% fraction. For additional

above fractions as well as the 100% fraction. For additional information regarding eluate composition, refer to the FDA Pesticide Analytical Manual (5).

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 Appendix III.

(1) 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 (µl)
V_t = Volume of total extract (µl)
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.

REFERENCES:

1. Monsanto Methodology for Aroclors - Analysis of Environmental Materials for Biphenyls, Analytical Chemistry Method 71-35, Monsanto Company, St. Louis, Missouri, 63166, 1970.

2. "Method for Polychlorinated Biphenyls in Water and Wastewater", this manual, p. 43.

3. "Method for Organophosphorus Pesticides in Water and Wastewater", this manual, p. 25.

4. "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.

5. "Pesticide Analytical Manual", U. S. Dept. of Health, Education and Welfare, Food and Drug Administration, Washington, D. C.

METHOD FOR ORGANOPHOSPHORUS PESTICIDES IN WATER AND WASTEWATER

1. Scope and Application

1.1 This method covers the determination of various organophosphorus pesticides in water and wastewater.

1.2 The following pesticides may be determined individually by this method:

Parameter	Storet No.
Azinphos methyl	---
Demeton-O	39560
Demeton-S	---
Diazinon	39570
Disulfoton	39010
Malathion	39530
Parathion methyl	39600
Parathion ethyl	39540

2. Summary

2.1 The method offers several analytical alternatives, dependent on the analyst's assessment of the nature and extent of interferences and 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 the column chromatography and liquid-liquid partition, methods for the elimination of non-pesticide interferences and the preseparation of pesticide mixtures. Identification is made by selective gas chromatographic separation and may be corroborated through the use

of pesticide mixtures. Identification is made by selective gas chromatographic separation and may be corroborated through the use of two or more unlike columns. Detection and measurement are best accomplished by flame photometric gas chromatography using a

phosphorus specific filter. The electron capture detector, though non-specific, may also be used for those compounds to which it responds. 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 Appendix I.

3.2 The interferences in industrial effluents are high and varied and often pose great difficulty in obtaining accurate and precise measurement of organophosphorus pesticides. Sample clean-up procedures are generally required and may result in the loss of certain organophosphorus pesticides. Therefore, great care should be exercised in the selection and use of methods for eliminating or minimizing interferences. It is not possible to describe

or minimizing interferences. It is not possible to describe procedures for overcoming all of the interferences that may be encountered in industrial effluents.

3.3 Compounds such as organochlorine pesticides, polychlorinated biphenyls and phthalate esters interfere with the analysis of organophosphorus pesticides by electron capture gas chromatography. When encountered, these interferences are overcome by the use of the phosphorus specific flame photometric detector. If such a detector is not available, these interferences may be removed from the sample by using the clean-up procedures described in the EPA methods for those compounds (1, 2).

3.4 Elemental sulfur will interfere with the determination of organophosphorus pesticides by flame photometric and electron capture gas chromatography. The elimination of elemental sulfur as an interference is described in Section 10.5, Clean-up and Separation Procedures.

4. Apparatus and Materials

4.1 Gas Crhomatograph - Equipped with glass lined injection port.

4.2 Detector options:

4.2.1 Flame Photometric - 526 mu phosphorus filter.

4.2.2 Electron Capture - Radioactive (tritium or nickel-63).

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

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 or OV-210, 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 x 19 mm ID) with coarse fritted plate and Teflon stopcock on bottom; 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 µl.

4.9 Separatory funnels - 125 ml, 1000 ml and 2000 ml with Teflon

4.9 Separatory funnels - 125 ml, 1000 ml and 2000 ml with Teflon stopcock.

4.10 Micro-pipets - disposable (140 mm long x 5 mm ID).

4.11 Blender - High speed, glass or stainless steel cup.

4.12 Graduated cylinders - 100 and 250 ml.

4.13 Florisil - PR Grade (60-100 mesh); purchase activated at 1250°F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use, activate each batch overnight at 130°C in foil-covered glass container. Determine lauric-acid value (See Appendix II).

4.14 Alumina - Woelm, neutral; deactivate by pipeting 1 ml of distilled water into 125 ml ground glass-stoppered Erlenmeyer flask. Rotate flask to distribute water over surface of glass. Immediately add 19.0 g fresh alumina through small powder funnel. Shake flask containing mixture for two hours on a mechanical shaker (3).

5. Reagents, Solvents, and Standards

5.1 Sodium Chloride - (ACS) Saturated solution in distilled water (pre-rinse NaCl with hexane).

5.2 Sodium Hydroxide - (ACS) 10 N in distilled water.

5.3 Sodium Sulfate - (ACS) Granular, anhydrous (conditioned at 400°C for 4 hrs.).

5.4 Sulfuric Acid - (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.

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., Emslford, N.Y. 10523.)

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

5.6 Acetonitrile, 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 ≤1.5 ng is injected for flame photometric detection and ≤ 0.06 ng is injected for electron capture 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 organophosphorus pesticides are provided in Table 1, as a guide.

7. Quality Control

7.1 Duplicate and spiked sample analyses are recommended as quality control checks. Quality control charts (4) 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.

7.2 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.

8. Sample Preparation

8.1 The sample size taken for analysis is dependent on the type of sample and the sensitivity required for the purpose at hand.

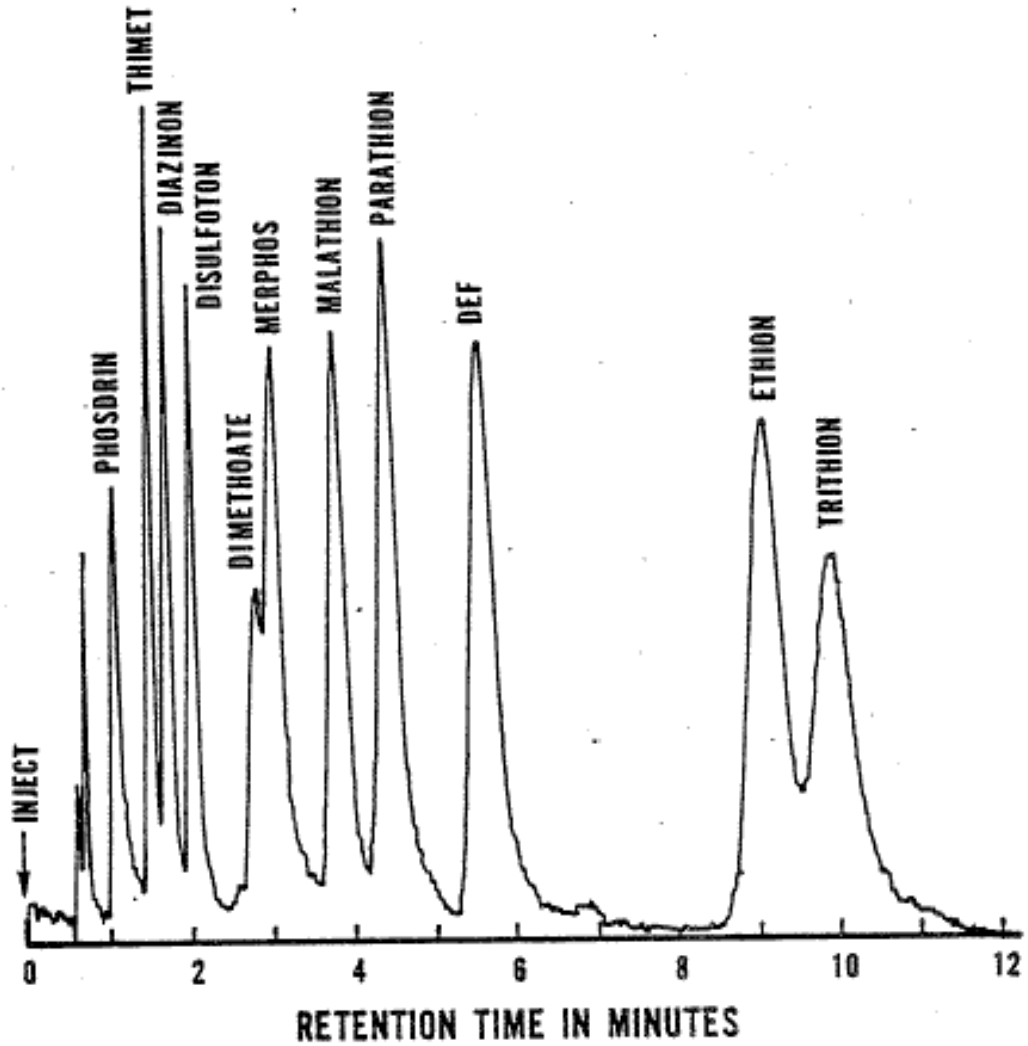
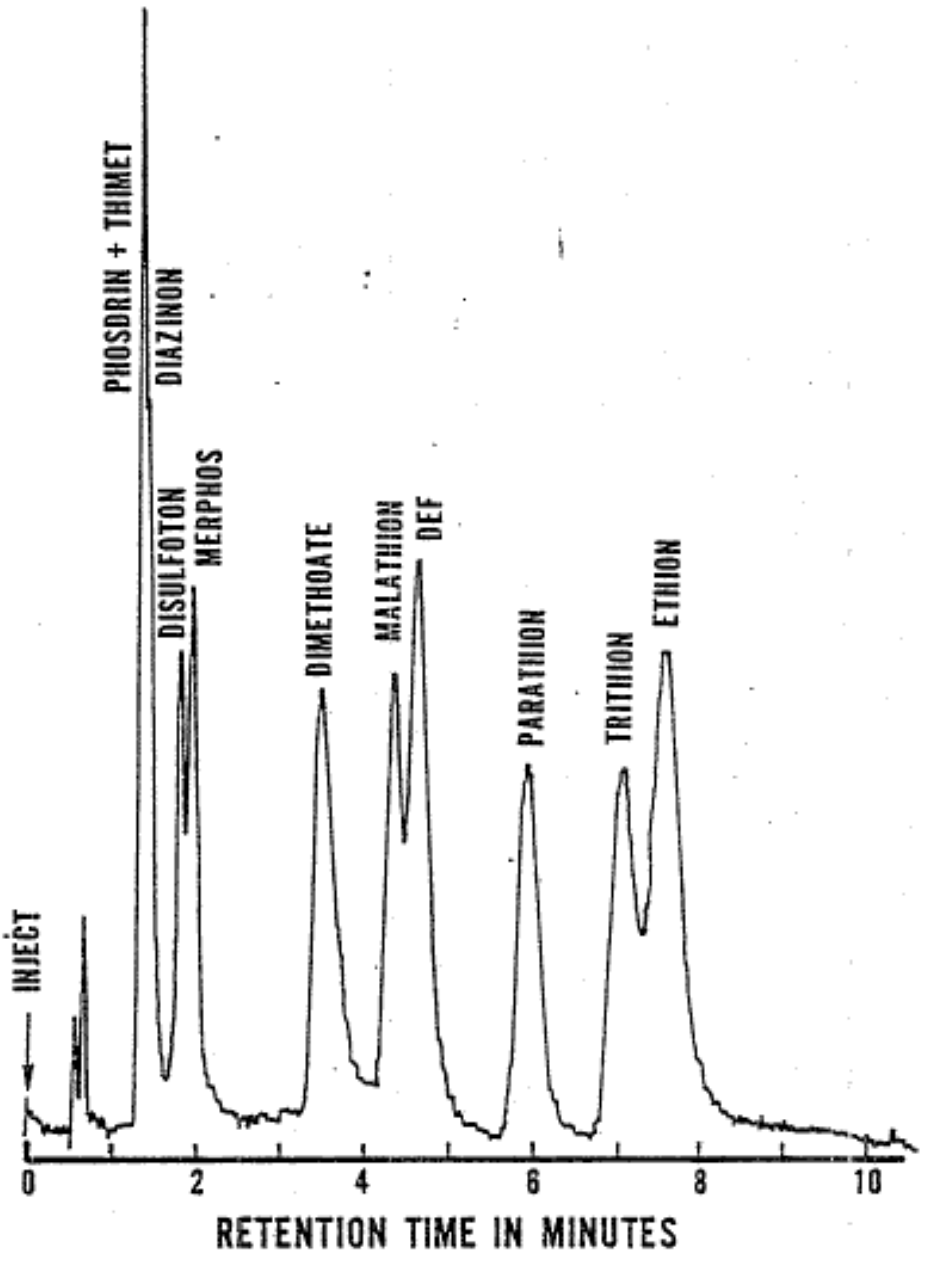


Figure 1. Column Packing: 1.5% OV-17 + 1.95 % QF-1, Carrier Gas: Nitrogen at 70 ml/min, Column Temperature: 215 C, Detector: Flame Photometric (Phosphorus).


INJECT
PHOSDRIN + THIMET
DIAZINON
DISULFOTON
MERPHOS
DIMETHOATE
MALATHION
DEF
PARATHION
TRITHION
ETHION
0
2
4
6
8
10
RETENTION TIME IN MINUTES

RETENTION TIME IN MINUTES

Figure 2. Column Packing: 5% OV-210, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200 C, Detector: Flame Photometric (Phosphorus).

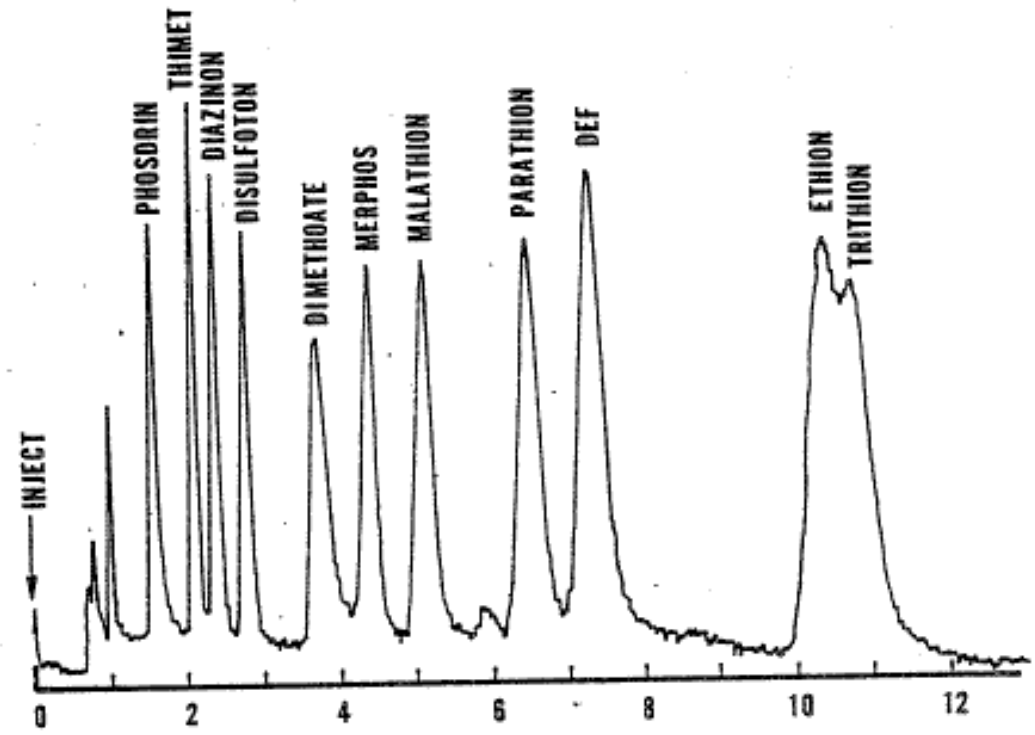


Figure 3. Column Packing: 6% QF-1 + 4% SE-30, Carrier Gas: Nitrogen at 70 ml/min, Column Temperature: 215 C, Detector: Flame Photometric (Phosphorus)

at 70 ml/min, Column Temperature: 215°C, Detector: Flame
Photometric (Phosphorus).

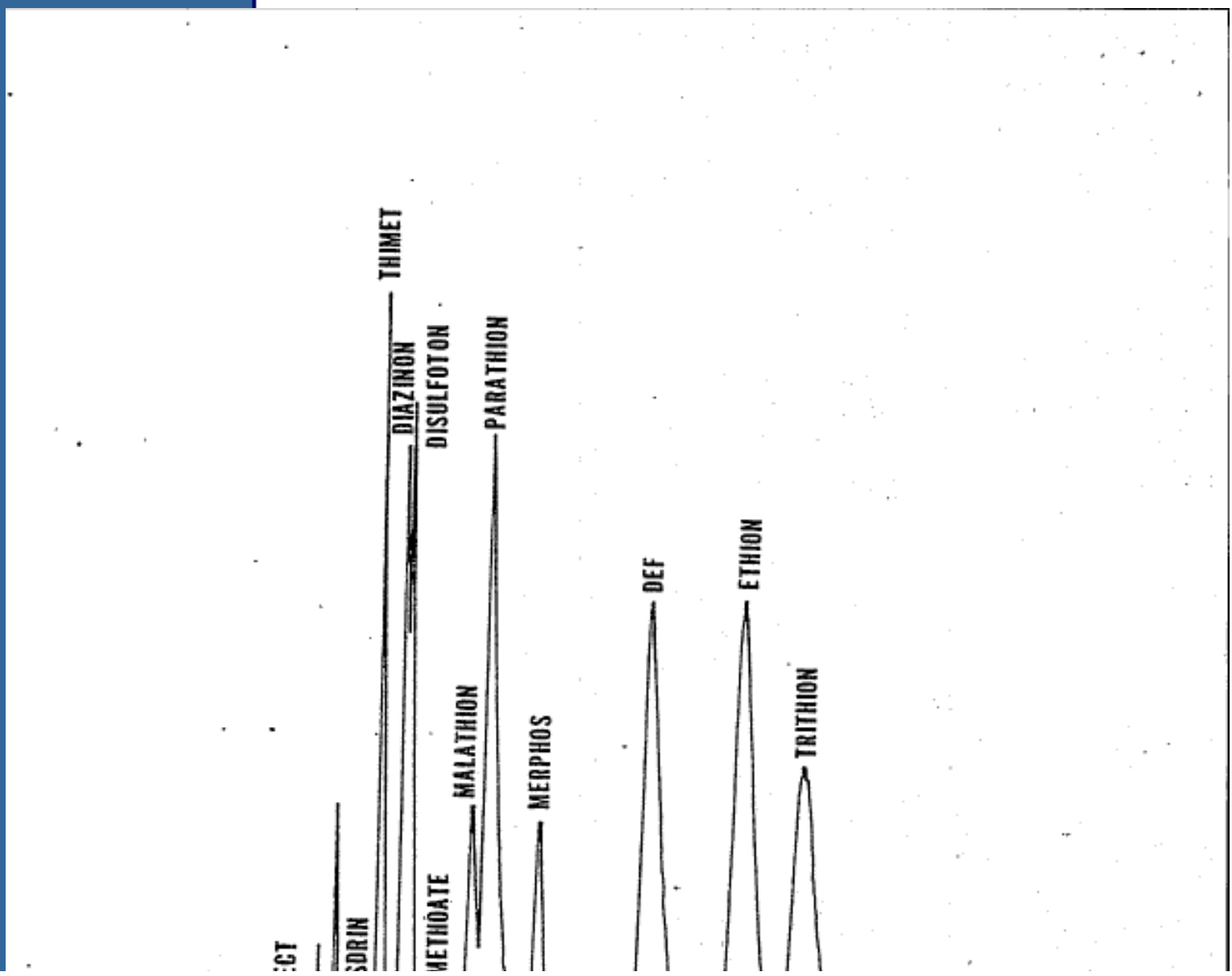


Figure 4. Column Packing: 3% OV-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200 C, Detector: Flame Photometric (Phosphorus).

TABLE 1

RETENTION TIMES OF SOME ORGANOPHOSPHOROUS PESTICIDES
RELATIVE TO PARATHION

Liquid Phase[1]	1.5% OV-17 + 1.95% QF-1[2]	6% QF-1[2] + 4% SE-30	5% OV-210	7% OV-1
Column Temp.	215 C	215 C	200 C	200 C
Nitrogen Carrier Flow	70 ml/min	70 ml/min	60 ml/min	60 ml/min
Pesticide	RR	RR	RR	RR
Demeton[3]	0.46	0.26 0.43	0.20 0.38	0.74
Diazinon	0.40	0.38	0.25	0.59
Disulfoton	0.46	0.45	0.31	0.62
Malathion	0.86	0.78	0.73	0.92
Parathion methyl	0.82	0.80	0.81	0.79
Parathion ethyl	1.00	1.00	1.00	1.00
Azinphos methyl	6.65	4.15	4.44	4.68
Parathion (min absolute)	4.5	6.6	5.7	3.1

[1]All columns glass, 180 xm x 4 mm ID, solid support Gas-Chrom Q, 100/120 mesh.
[2]May substitute OV-210 for QF-1.
[3]Anomalous, multipeak response often encountered.

Background information on the pesticide levels previously detected at a given sampling site will assist in determining the sample size required, as well as the final volume to which the extract needs to be concen- trated. A 1-liter sample is usually taken for drinking water and ambient water analysis to provide a detection limit of 0.050 to 0.100 µg/l. One-hundred milliliters is usually adequate to provide a detection limit of 1 µg/l for industrial effluents.

8.2 Quantitatively transfer the proper aliquot of sample from the sample container into a two-liter separatory funnel. If less than a 800 ml is analyzed, dilute to one liter with interference free distilled water.

9. Extraction

9.1 Add 60 ml of 15% methylene chloride in hexane (v:v) to the sample in the separatory funnel and shake vigorously for two minutes.

9.2 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 3-4 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 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.

9.3 Concentrate the extract in the K-D evaporator on a hot water bath.

9.4 Analyze by gas chromatography unless a need for cleanup is indicated. (See Section 10).

10. Clean-up and Separation Procedures

10.1 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 source 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. The use of these procedures is not required for samples free of interferences. They are provided as options to the analyst to be used when needed.

10.2 Acetonitrile Partition - This procedure is used to separate fats and oils from the sample extracts. It should be noted that not all pesticides are quantitatively recovered by this procedure. The analyst must be aware of this and demonstrate the efficiency of the partitioning for specific pesticides.

10.2.1 Quantitatively transfer the previously concentrated extract to a 125-ml separatory funnel with enough hexane to bring the final volume to 15 ml. Extract the sample four times by shaking vigorously for one minute with 30 ml portions of hexane-saturated acetonitrile.

10.2.2 Combine and transfer the acetonitrile phases to a one-liter separatory funnel and add 650 ml of distilled

one-liter separatory funnel and add 650 ml of distilled water and 40 ml of saturated sodium chloride solution. Mix thoroughly for 30-45 seconds. Extract with two

100 ml portions of hexane by vigorously shaking about 15 seconds.

10.2.3 Combine the hexane extracts in a one-liter separatory funnel and wash with two 100 ml portions of distilled water. Discard the water layer and pour the hexane layer through a 3-4 inch anhydrous sodium sulfate column into a 500-ml K-D flask equipped with a 10-ml ampul. Rinse the separatory funnel and column with three 10 ml portions of hexane.

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

10.2.5 Analyze by gas chromatography unless a need for further clean-up is indicated.

10.3 Florisil Column Adsorption Chromatography

10.3.1 Adjust the sample extract volume to 10 ml.

10.3.2 Place a charge of activated Florisil (weight determined by lauric-acid value, see Appendix II) 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.

10.3.3 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

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 petro- leum ether washings. Adjust the elution rate to about 5 ml per minute and,

separately, collect up to four eluates in 500-ml K-D flasks equipped with 10-ml ampuls. (See Eluate Composition, 10.4.) 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. Perform the third elution with 200 ml of 50% ethyl ether - petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

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

10.3.5 Analyze by gas chromatography.

10.4 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	15% Eluate
Demeton	Diazinon
Disulfoton	Malathion (trace)
	Parathion Methyl
50% Eluate	**100% Eluate**
Malathion	Azinphos methyl (80%)

30% Eluate	100% Eluate
Malathion Azinphos methyl (20%)	Azinphos methyl (80%)

For additional information regarding eluate composition, refer to the FDA Pesticide Analytical Manual (5).

10.5 Removal of Sulfur - If elemental sulfur interferes with the gas chromatographic analysis, it can be removed by the use of an alumina microcolumn.

10.5.1 Adjust the sample extract volume to 0.5 ml in a K-D

apparatus, using a two-ball Snyder microcolumn.

10.5.2 Plug a disposable pipet with a small quantity of glass wool. Add enough alumina to produce a 3-cm column after settling. Top the alumina with a 0.5-cm layer of anhydrous sodium sulfate.

10.5.3 Quantitatively transfer the concentrated extract to the alumina microcolumn using a 100 µl syringe. Rinse the ampul with 200 µl of hexane and add to the microcolumn.

10.5.4 Elute the microcolumn with 3 ml of hexane and discard the first eluate which contains the elemental sulfur.

10.5.5 Next elute the column with 5 ml of 10% hexane in methylene chloride. Collect the eluate in a 10 ml graduated ampul.

10.5.6 Analyze by gas chromatography.

NOTE: If the electron capture detector is to be used methylene chloride must be removed. To do this, attach the ampul to a K-D apparatus (500-ml flask and 3-ball Snyder

to a K-D apparatus (500-ml flask and 3-ball Snyder column) and concentrate to about 0.5 ml. Adjust volume as required prior to analysis.

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 Appendix III.

(1) 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 (µl)

V_t = Volume of total extract (µl)

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.

REFERENCES:

1. "Method for Chlorinated Hydrocarbons in Water and Wastewater", this manual, p. 7.

2. "Method for Polychlorinated Biphenyls (PCBs) in Water and Wastewater", this manual, p. 43.

3. Law, L. M. and Georlitz, D. F., "Microcolumn Chromatographic Clean-up for the Analysis of Pesticides in Water", Journal of the Association for Analytical Chemists, 53, 1276 (1970).

4. "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.

5. "Pesticide Analytical Manual", U. S. Dept. of Health, Education and Welfare, Food and Drug Administration, Washington, D. C.

METHOD FOR POLYCHLORINATED BIPHENYLS (PCBs) IN WATER AND WASTEWATER

1. Scope and Application

1.1 This method covers the determination of various polychlorinated biphenyl (PCB) mixtures in water and wastewater.

1.2 The following mixtures of chlorinated biphenyls (Aroclors) may be determined by this method:

Parameter	Storet No.
PCB-1016	34671
PCB-1221	39488
PCB-1232	39492
PCB-1242	39496
PCB-1248	39500
PCB-1254	39504
PCB-1260	39508

1.3 The method is an extension of the Method for Chlorinated Hydrocarbons in Water and Wastewater (1). It is designed so that determination of both the PCBs and the organochlorine pesticides may be made on the same sample.

2. Summary

2.1 The PCBs 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 cleanup procedure and a silica gel microcolumn

chromatographic determination. A combination of the standard Florisil column cleanup procedure and a silica gel microcolumn separation procedure (2)(3) 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.

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 the purification of solvents by distillation in all-glass systems may be required. Refer to Appendix I.

3.2 The interferences in industrial effluents are high and varied and pose great difficulty in obtaining accurate and precise measurement of PCBs and organochlorine pesticides. Separation and clean-up procedures are generally required and may result in the loss of certain organochlorine compounds. Therefore, great care should be exercised in the selection and use of methods for eliminating or minimizing interferences. It is not possible to describe procedures for overcoming all of the interferences that may be encountered in industrial effluents.

3.3 Phthalate esters, certain organophosphorus pesticides, and

3.3 Phthalate esters, certain organophosphorus pesticides, and elemental sulfur will interfere when using electron capture for detection. These materials do not interfere when the

microcoulometric or electrolytic conductivity detectors are used in the halogen mode.

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

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)

4.4.1 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 SE-30 or OV-1, 3%

4.4.4.2 OV-17, 1.5% + QF-1 or OV-210, 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 - 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 Column Pyrex - constructed according to Figure 1.

4.9 Capillary pipets disposable (5-3/4 in.) with rubber bulb (Scientific Products P5205-1).

4.10 Low pressure regulator - 0 to 5 PSIG - with low-flow needle valve (see Figure 1, Matheson Model 70).

4.11 Beaker - 100 ml

4.11 Beaker - 100 ml

4.12 Micro Syringes - 10, 25, 50 and 100 ul.

4.13 Separatory funnels - 125 ml, 1000 ml and 2000 ml with Teflon stopcock.

4.14 Blender - High speed, glass or stainless steel cup.

4.15 Graduated cylinders - 100 and 250 ml.

4.16 Florisil - PR Grade (60-100 mesh); purchase activated at 1250°F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use, activate each

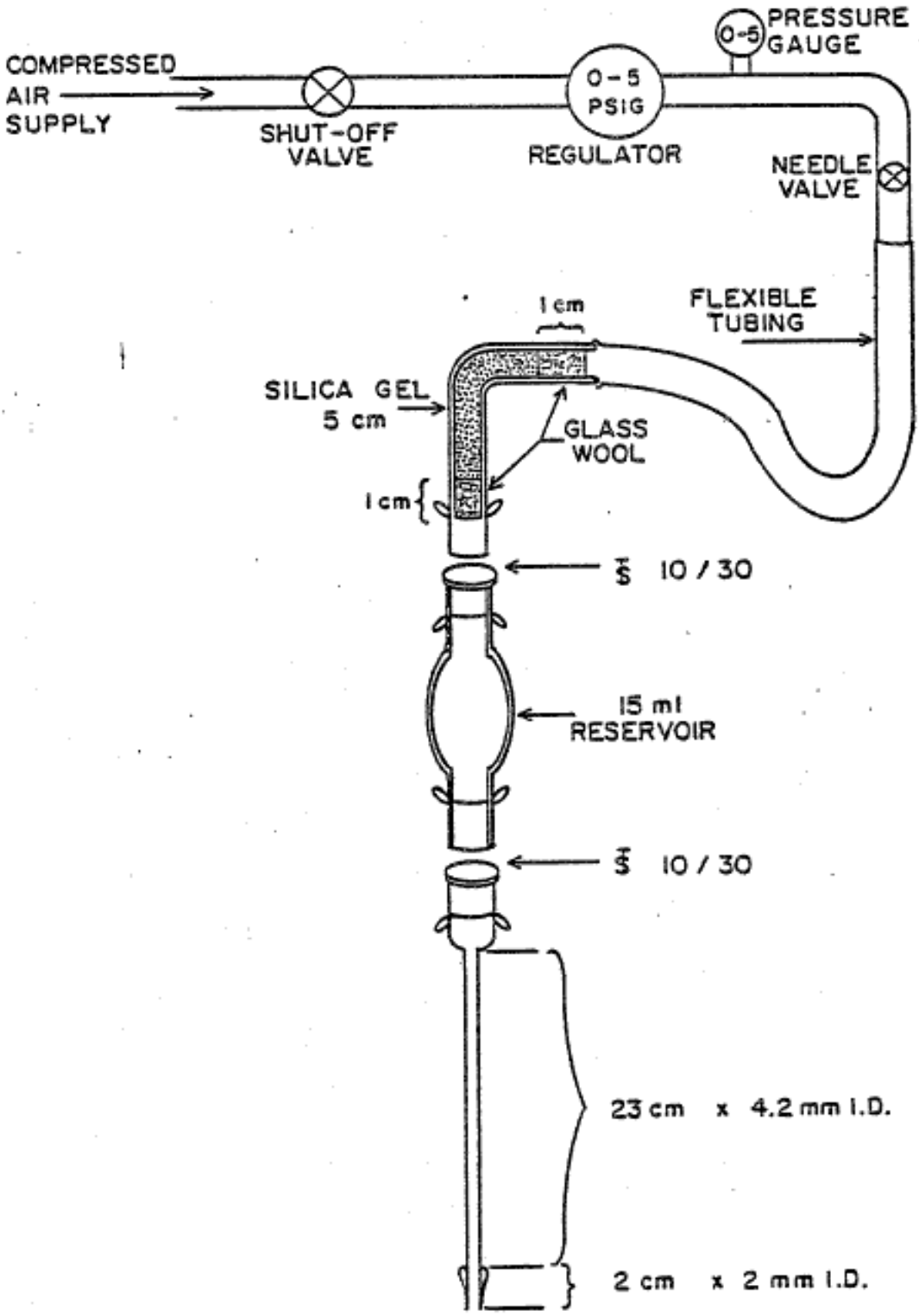


FIGURE 1. MICROCOLUMN SYSTEM

batch overnight at 130°C in foil-covered glass container. Determine lauric-acid value (See Appendix II).

4.17 Silica gel - Davison code 950-08008-226 (60/200 mesh).

4.18 Glass Wool - Hexane extracted.

4.19 Centrifuge Tubes - Pyrex calibrated (15 ml).

5. Reagents, Solvents, and Standards

5.1 Sodium Chloride - (ACS) Saturated solution in distilled water (pre-rinse NaCl with hexane).

5.2 Sodium Hydroxide - (ACS) 10 N in distilled water.

5.3 Sodium Sulfate - (ACS) Granular, anhydrous (conditioned at 400° C for 4 hrs.).

5.4 Sulfuric Acid - (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.

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, N.Y. 10523).

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

5.6 n-Hexane - Pesticide quality (NOT MIXED HEXANES).

5.7 Acetonitrile, Hexane, Methanol, Methylene Chloride, Petroleum Ether (boiling range 30-60°C) - pesticide quality, redistill in glass if necessary.

5.8 Standards - Aroclors 1221, 1232, 1242, 1248, 1254, 1260, and 1016.

5.9 Anti-static Solution - STATNUL, Daystrom, Inc., Weston Instrument Division, Newark, N.J., 95212.

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, detector and column. Example chromatograms are shown in Figures 3 through 8 and provide reference operating conditions.

7. Quality Control

7.1 Duplicate and spiked sample analyses are recommended as quality control checks. Quality control charts (4) 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.

7.2 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.

8. Sample Preparation

8.1 Blend the sample if suspended matter is present and adjust pH

8. Sample Preparation

8.1 Blend the sample if suspended matter is present and adjust pH

to near neutral (pH 6.5-7.5) with 50% sulfuric acid or 10 N sodium hydroxide.

8.2 For sensitivity requirement of 1 µg/l, when using microcoulometric or electrolytic conductivity methods for detection take 1000 ml of sample for analysis. If interferences pose no problem, the sensitivity of the electron capture detector should permit as little as 100 ml of sample to be used. Background information on the extent and nature of interferences will assist the analyst in choosing the required sample size and preferred detector.

8.3 Quantitatively transfer the proper aliquot into a two-liter separatory funnel and dilute to one liter.

9. Extraction

9.1 Add 60 ml of 15% methylene chloride in hexane (v:v) to the sample in the separatory funnel and shake vigorously for two minutes.

9.2 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 3-4 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 Erlenmeyer flask with a second 60-ml volume of solvent; add the solvent to

the water phase to the separatory funnel. Rinse 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.

9.3 Concentrate the extract in the K-D evaporator on a hot water bath.

9.4 Qualitatively analyze the sample by gas chromatography with an electron capture detector. From the response obtained decide:

a. If there are any organochlorine pesticides present.

b. If there are any PCBs present.

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

d. If elemental sulfur is present.

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

f. If no response, concentrate to 1.0 ml or less, as required, and repeat the analysis looking for a, b, c, d, and e. Samples containing Aroclors with a low percentage of chlorine, e.g., 1221 and 1232, may require this concentration in order to achieve the detection limit of 1 µg/l. Trace quantities of PCBs are often masked by background which usually occur in samples.

9.5 If condition a exists, quantitatively determine the organochlorine pesticides according to (1).

chlorine pesticides according to (1).

9.6 If condition b exists, PCBs only are present; no further separation or cleanup is necessary. Quantitatively determine the PCBs according to step 11.

9.7 If condition c exists, compare peaks obtained from the sample to those of standard Aroclors and make a judgment as to which Aroclors may be present. To separate the PCBs from the organochlorine pesticides, continue as outlined in 10.4.

9.8 If condition d exists, separate the sulfur from the sample using the method outlined in 10.3 followed by the method in 10.5.

9.9 If condition e exists, the following macro cleanup and separation procedures (10.2 and 10.3) should be employed and, if necessary, followed by the micro separation procedures (10.4 and 10.5).

10. Cleanup and Separation Procedures

10.1 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 PCBs, or affect column life or detector sensitivity, proceed as directed below.

10.2 Acetonitrile Partition - This procedure is used to remove fats and oils from the sample extracts. It should be noted that not

and oils from the sample extracts. It should be noted that not all pesticides are quantitatively recovered by this procedure. The analyst must be aware of this and demonstrate the efficiency of the partitioning for the compounds of interest.

10.2.1 Quantitatively transfer the previously concentrated extract to a 125-ml separatory funnel with enough hexane to bring the final volume to 15 ml. Extract the sample four times by shaking vigorously for one minute with 30-ml portions of hexane-saturated acetonitrile.

10.2.2 Combine and transfer the acetonitrile phases to a one-liter separatory funnel and add 650 ml of distilled water and 40 ml of saturated sodium chloride solution. Mix thoroughly for 30-45 seconds. Extract with two 100-ml portions of hexane by vigorously shaking about 15 seconds.

10.2.3 Combine the hexane extracts in a one-liter separatory funnel and wash with two 100-ml portions of distilled water. Discard the water layer and pour the hexane layer through a 3-4 inch anhydrous sodium sulfate column into a 500-ml K-D flask equipped with a 10-ml ampul. Rinse the separatory funnel and column with three 10-ml portions of hexane.

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

10.2.5 Analyze by gas chromatography unless a need for further cleanup is indicated.

10.3 Florisil Column Adsorption Chromatography

10.3.1 Adjust the sample extract volume to 10 ml.

10.3.2 Place a charge of activated Florisil (weight determined by lauric-acid value, see Appendix II) 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.

10.3.3 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 up to three eluates in 500-ml K-D flasks equipped with 10-ml ampuls (see Eluate Composition 10.4.). 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. Perform the third elution with 200 ml of 50% ethyl ether - petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

10.3.3.1 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 as follows.

6% Eluate

Aldrin	DDT	Pentachloro-nitrobenzene
BHC	Heptachlor	
Chlordane	Heptachlor Epoxide	Strobane
DDD	Lindane	Toxaphene
DDE	Methoxychlor	Trifluralin
	Mirex	PCBs

15% Eluate

Endosulfan I
Endrin
Dieldrin
Dichloran
Phthalate esters

50% Eluate

Endosulfan II
Captan

Certain thiophosphate pesticides will 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 (5).

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

10.3.5 Analyze by gas chromatography.

10.4 Silica Gel Micro-Column Separation Procedure (6)

10.4.1 Activation for Silica Gel

10.4.1.1 Place about 20 gm of silica gel in a 100-ml beaker. Activate at 180°C for approximately 16 hours. Transfer the silica gel to a 100-ml glass-stoppered bottle. When cool, cover with about 35 ml of 0.50% diethyl ether in benzene (volume:volume). Keep bottle well sealed. If silica gel collects on the ground glass surfaces, wash off with the above solvent before resealing. Always maintain an excess of the mixed solvent in bottle (aproximately 1/2 in. above silica gel). Silica gel can be effectively stored in this manner for several days.

10.4.2 Preparation of the Chromatographic Column

10.4.2 Preparation of the Chromatographic Column

10.4.2.1 Pack the lower 2 mm ID section of the micro-column with glass wool. Permanently mark

the column 120 mm above the glass wool. Using a clean rubber bulb from a disposable pipet seal the lower end of the microcolumn. Fill the microcolumn with 0.50% ether in benzene (v:v) to the bottom of the 10/30 joint (Figure 1). Using a disposable capillary pipet, transfer several aliquots of the silica gel slurry into the microcolumn. After approximately 1 cm of silica gel collects in the bottom of the microcolumn, remove the rubber bulb seal, tap the column to insure that the silica gel reaches the 120 ± 2 mm mark. Be sure that there are no air bubbles in the column. Add about 10 mm of sodium sulfate to the top of the silica gel. Under low humidity conditions, the silica gel may coat the sides of the column and not settle properly. This can be minimized by wiping the outside of the column with an anti-static solution.

10.4.2.2 Deactivation of the Silica Gel

a. Fill the microcolumn to the base of the 10/30 joint with the 0.50% ether-benzene

10/30 joint with the 0.50% ether-benzene mixture, assemble reservoir (using spring clamps) and fill with approximately 15 ml of the 0.50% ether-benzene mixture. Attach the air pressure device (using spring

clamps) and adjust the elution rate to approximately 1 ml/min. with the air pressure control. Release the air pressure and detach reservoir just as the last of the solvent enters the sodium sulfate. Fill the column with n-hexane (not mixed hexanes) to the base of the 10/30 fitting. Evaporate all residual benzene from the reservoir, assemble the reservoir section and fill with 5 ml of n-hexane. Apply air pressure and remove the reservoir just as the n-hexane enters the sodium sulfate. The column is now ready for use.

b. 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. As the last of the sample passes into the sodium sulfate layer, rinse down the internal wall of the column twice with 0.25

internal wall of the column twice with 0.25 ml 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 10.4.3) to just elute all of the PCBs present in the sample. Apply air pressure and adjust until the

flow is 1 ml/min. Collect the desired volume of eluate (predetermined, see 10.4.3) 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.

c. Fill the column with 0.50% diethyl ether in 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 10.4.3).

d. Analyze the eluates by gas chromatography.

10.4.3 Determination of Elution Volumes

10.4.3.1 The elution volumes for the PCBs and the pesticides depend upon a number of factors

pesticides depend upon a number of factors 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 factor is changed. To determine the elution volumes, add standard mixtures of Aroclors and pesticides to the column and serially collect 1-ml elution volumes. Analyze the individual eluates by gas chromatography and determine the cut-off volume for n-hexane and for ether-benzene. Figure 2 shows the retention order of the various PCB components and of the pesticides. Using this information, prepare the mixtures required for calibraton of the microcolumn.

10.4.3.2 In determining the volume of hexane required to elute the PCBs the sample volume (1 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 PCBs, the volume of hexane to be added in addition to the sample volume but including the rinse volume should be 9.5 ml.

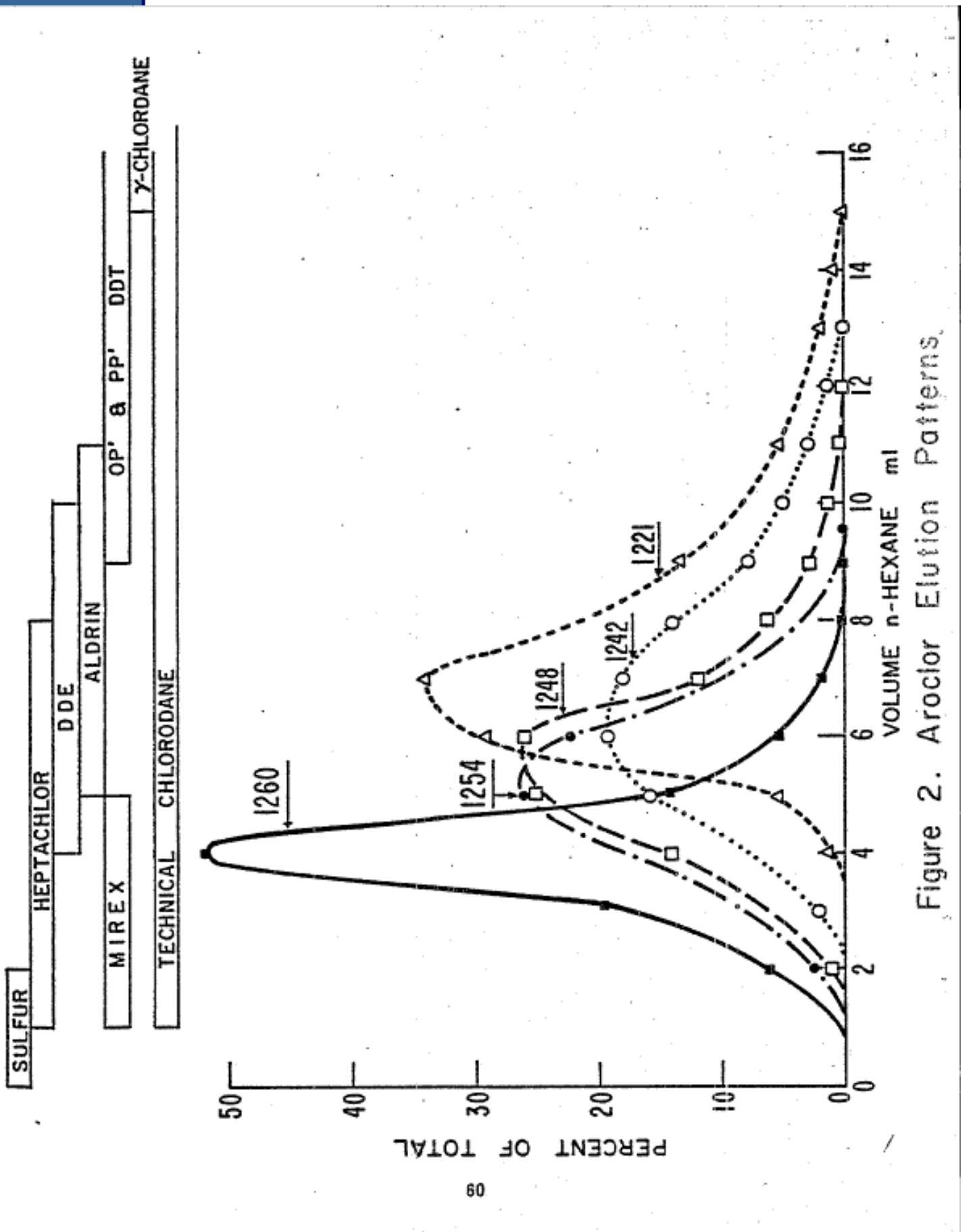


Figure 2. Aroclor Elution Patterns

10.4.3.3 Figure 2 shows that as the average chlorine content of a PCB mixture decreases the solvent volume for complete elution increases. Qualitative determination (9.4) 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 PCBs and insures the most efficient separations possible for accurate analysis.

10.4.3.4 For critical analysis where the PCBs and pesticides are not separated completely, the column should be accurately calibrated according to (10.4.3.1) to determine the percent of material of interest that elutes in each fraction. Then flush the column with an additional 15 ml of 0.50% ether in benzene followed by 5 ml of n-hexane and use this reconditioned column for the sample separation. Using this technique one can accurately predict the amount (%) of materials in each micro column fraction.

10.5 Micro Column Separation of Sulfur, PCBs, and Pesticides

10.5.1 See procedure for preparation and packing micro column

10.5.1 See procedure for preparation and packing micro column in PCB analysis section (10.4.1 and 10.4.2).

10.5.2 Microcolumn Calibration

10.5.2.1 Calibrate the microcolumn for sulfur and PCB separation by collecting 1.0-ml fractions and analyzing them by gas chromatography to determine the following:

1) The fraction with the first eluting PCBs (those present in 1260),
2) The fraction with the last eluting PCBs (those present in 1221),
3) The elution volume for sulfur,
4) The elution volume for the pesticides of interest in the 0.50% ether-benzene fraction.

From these data determine the following:

1) The eluting volume containing only sulfur (Fraction I),
2) The eluting volume containing the last of the sulfur and the early eluting PCBs (Fraction II),
3) The eluting volume containing the remaining PCBs (Fraction III),
4) The ether-benzene eluting volume containing the pesticides of interest (Fraction IV)

4) The ether-benzene eluting volume containing the pesticides of interest (Fraction IV).

10.5.3 Separation Procedure

10.5.3.1 Carefully concentrate the 6% eluate from the

florisil column to 2.0 ml in the graduated ampul on a warm water bath.

10.5.3.2 Place 1.0 ml (50%) of the concentrate into the microcolumn with a 1-ml pipet. Be careful not to get any sulfur crystals into the pipet.

10.5.3.3 Collect Fractions I and II in calibrated centrifuge tubes. Collect Fractions III and IV in calibrated ground glass stoppered ampuls.

10.5.3.4 Sulfur Removal (7) - Add 1 to 2 drops of mercury to Fraction II stopper and place on a wrist-action shaker. A black precipitate indicates the presence of sulfur. After approximately 20 minutes the mercury may become entirely reacted or deactivated by the precipitate. The sample should be quantitatively transferred to a clean centrifuge tube and additional mercury added. When crystals are present in the sample, three treatments may be necessary to remove all the sulfur. After

be necessary to remove all the sulfur. After all the sulfur has been removedfrom Fraction II (check using gas chromatography) combine Fractions II and III. Adjust the volume to 10 ml and analyze by gas chromatography. Be sure no mercury is transferred to the combined Fractions II and III, since it can react with certain pesticides.

By combining Fractions II and III, if PCBs are present, it is possible to identify the Aroclor(s) present and a quantitative analysis can be performed accordingly. Fraction I can be discarded since it only contains the bulk of the sulfur. Analyze Fractions III and IV for the PCBs and pesticides. If DDT and its homologs, aldrin, heptachlor, or technical chlordane are present along with the PCBs, an additional microcolumn separation can be performed which may help to further separate the PCBs from the pesticides (See 10.4).

11. Quantitative Determination

11.1 Measure the volume of n-hexane eluate containing the PCBs and inject 1 to 5 μl into the gas chromatograph. If necessary, adjust the volume of the eluate to give linear response to the electron capture detector. The microcoulometric or the

electron capture detector. The microcoulometric or the electrolytic detector may be employed to improve specificity for samples having higher concentrations of PCBs.

11.2 Calculations

11.2.1 When a single Aroclor is present, compare quantitative Aroclor reference standards (e.g., 1242, 1260) to the unknown. Measure and sum the areas of the unknown and the reference Aroclor and calculate the result as follows:

$$\text{Microgram/liter} = \frac{[A]\,[B]\,[V_t]}{[(V_i)\ \ (Vs)]} \times [N]$$

$$A = \frac{\text{ng of Standard Injected}}{\text{of Sample Peak Areas - } (mm^2)} = \frac{ng_2}{mm}$$

V_i = Volume of sample injected (µl)

V_t = Volume of Extract (µl) from which sample is injected into gas chromatograph

V_s = Volume of water sample extracted (ml)

N = 2 when micro column used
1 when micro column not used

Peak Area = Peak height (mm x Peak Width at 1/2 height

11.2.2 For complex situatons, use the calibration method described below (8). Small variations in components between different Aroclor batches make it necessary to obtain samples of several specific Aroclors. These reference Aroclors can be obtained from the Southeast Environmental Research Laboratory, EPA, Athens, Georgia, 30601. The procedure is as follows:

11.2.2.1 Using the OV-1 column, chromatograph a known quantity of each Aroclor reference standard. Also chromatograph a sample of p,p'-DDE. Suggested concentration of each standard is 0.1 ng/µl for the Aroclors and 0.02 ng/µl for the p,p'-DDE.

11.2.2.2 Determine the relative retention time (RRT) of each PCB peak in the resulting chromatograms using p,p'-DDE as 100.

$$RRT = \frac{RT \times 100}{RT_{DDE}}$$

RRT = Relative Retention Time

RT = Retention time of peak of interest

RT_{DDE} = Retention time of p,p'-DDE

Retention time is measured as that distance in mm between the first appearance of the solvent peak and the maximum for the compound.

11.2.2.3 To calibrate the instrument for each PCB measure the area of each peak.

Area = Peak height (mm) x Peak width at 1/2 height. Using Tables 1 through 6 obtain the proper mean weight factor, then determine the response factor ng/mm^2.

$$ng/mm^2 = \frac{(ng_f) \quad \frac{(\text{mean weight percent})}{100}}{(\text{Area})}$$

ng_f = ng of Aroclor Standard Injected

Mean weight percent - obtained from Tables 1 through 6.

11.2.2.4 Calculate the RRT value and the area for each PCB peak in the sample chromatogram. Compare the sample chromatogram to those obtained for each reference Aroclor standard. If it is

Table 1

Composition of Aroclor 1221 (8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]	
11	31.8	15.8	1	
14	19.3	9.1	1	
16	10.1	9.7	2	
19	2.8	9.7	2	
21	20.8	9.3	2	
28	5.4	13.9	2	85%
			3	15%
32	1.4	30.1	2	10%
			3	90%
37	1.7	48.8	3	
40				

[a]Retention time relative to p,p'-DDE=100. Measured from first appearance of solvent. Overlapping peaks that are quantitated as one peak are bracketed.

[b]Standard deviation of seventeen results as a percentage of the mean of the results.

[c]From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

Table 2

Composition of Aroclor 1232 (8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]	
11	16.2	3.4	1	
14	9.9	2.5	1	
16	7.1	6.8	2	
20	17.8	2.4	2	
21				
28	9.6	3.4	2	40%
			3	60%
32	3.9	4.7	3	
37	6.8	2.5	3	
40	6.4	2.7	3	
47	4.2	4.1	4	
54	3.4	3.4	3	33%
			4	67%
58	2.6	3.7	4	
70	4.6	3.1	4	90%
			5	10%
78	1.7	7.5	4	
Total	94.2			

[a]Retention time relative to p,p'-DDE=100. Measured from first appearance of solvent. Overlapping peaks that are quantitated as one peak are bracketed.

[b]Standard deviation of four results as a mean of the results.

[c]From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

[c]From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

Table 3

Composition of Aroclor 1242 (8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]	
11	1.1	35.7	1	
16	2.9	4.2	2	
21	11.3	3.0	2	
28	11.0	5.0	2	25%
			3	75%
32	6.1	4.7	3	
37	11.5	5.7	3	
40	11.1	6.2	3	
47	8.8	4.3	4	
54	6.8	2.9	3	33%
			4	67%
58	5.6	3.3	4	
70	10.3	2.8	4	90%
			5	10%
78	3.6	4.2	4	
84	2.7	9.7	5	
98	1.5	9.4	5	
104	2.3	16.4	5	
125	1.6	20.4	5	85%
			6	15%
146	1.0	19.9	5	75%
			6	25%

[a]Retention time relative to p,p'-DDE=100. Measured from first appearance of solvent.

[b]Standard deviation of six results as a percentage of the mean of the results.

[c]From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

Table 4

Composition of Aroclor 1248 (8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]	
21	1.2	23.9	2	
28	5.2	3.3	3	
32	3.2	3.8	3	
47	8.3	3.6	3	
40	8.3	3.9	3	85%
			4	15%
47	15.6	1.1	4	
54	9.7	6.0	3	10%
			4	90%
58	9.3	5.8	4	
70	19.0	1.4	4	80%
			5	20%
78	6.6	2.7	4	
84	4.9	2.6	5	
98	3.2	3.2	5	
104	3.3	3.6	4	10%
			5	90%
112	1.2	6.6	5	
125	2.6	5.9	5	90%
			6	10%

125	2.6	5.9	5	90%
			6	10%
146	1.5	10.0	5	85%
			6	15%
Total	103.1			

[a]Retention time relative to p,p'-DDE=100. Measured from first appearance of solvent.

[b]Standard deviation of six results as a percentage of the mean of the results.

[c]From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

Table 5

Composition of Aroclor 1254 (8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]	
47	6.2	3.7	4	
54	2.9	2.6	4	
58	1.4	2.8	4	
70	13.2	2.7	4	25%
			5	75%
84	17.3	1.9	5	
98	7.5	5.3	5	
104	13.6	3.8	5	
125	15.0	2.4	5	70%
			6	80%
146	10.4	2.7	5	30%
			6	70%
160	1.3	8.4	6	
174	8.4	5.5	6	
203	1.8	18.6	6	
232	1.0	26.1	7	
Total	100.0			

[a]Retention time relative to p,p'-DDE=100. Measured from first appearance of solvent.

[b]Standard deviation of six results as a percentage of the mean of the results.

[c]From GC-MS data. Peaks containing mixtures of isomers are bracketed.

Table 6

Composition of Aroclor 1260 (8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]	
70	2.7	6.3	5	
84	4.7	1.6	5	
98	3.8	3.5	[d]	
104			5	60%
			6	40%
117	3.3	6.7	6	
125	12.3	3.3	5	15%
			6	85%
146	14.1	3.6	6	
160	4.9	2.2	6	50%
			7	50%
174	12.4	2.7	6	
203	9.3	4.0	6	10%
			7	90%
232			[e]	
244	9.8	3.4	6	10%
			7	90%
280	11.0	2.4	7	
332	4.2	5.0	7	
372	4.0	8.6	8	
448	.6	25.3	8	
528	1.5	10.2	8	
Total	98.6			

[a]Retention time relative to p,p'-DDE=100. Measured from first appearance of solvent. Overlapping peaks that are quantitated as one peak are bracketed.

[b]Standard deviation of six results as a mean of the results.

[c]From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

[d]Composition determined at the center of peak 104.

[e]Composition determined at the center of peak 232.

apparent that the PCB peaks present are due to only one Aroclor, then calculate the concentration of each PCB using the following formula:

ng PCB = ng/mm^2 x Area

Where Area = Area (mm^2) of sample peak

ng/mm^2 = Response factor for that peak measured.

Then add the nanograms of PCBs present in the injection to get the total number of nanograms of PCBs present. Use the following formula to calculate the concentration of PCBs in the sample:

Micrograms/Liter =

V_s = volume of water extracted (ml)

V_t = volume of extract (µl)

V_i = volume of sample injected (µl)

ng = sum of all the PCBs in nanograms for that Aroclor identified

N = 2 when microcolumn used

N = 1 when microcolumn not used

The value can then be reported as micrograms/liter PCBs or as the Aroclor. For samples containing more than one Aroclor, use Figure 9 chromatogram divisional flow chart to assign a proper response factor to each peak

assign a proper response factor to each peak and also identify the "most likely" Aroclors

present. Calculate the ng of each PCB isomer present and sum them according to the divisional flow chart. Using the formula above, calculate the concentration of the various Aroclors present in the sample.

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.

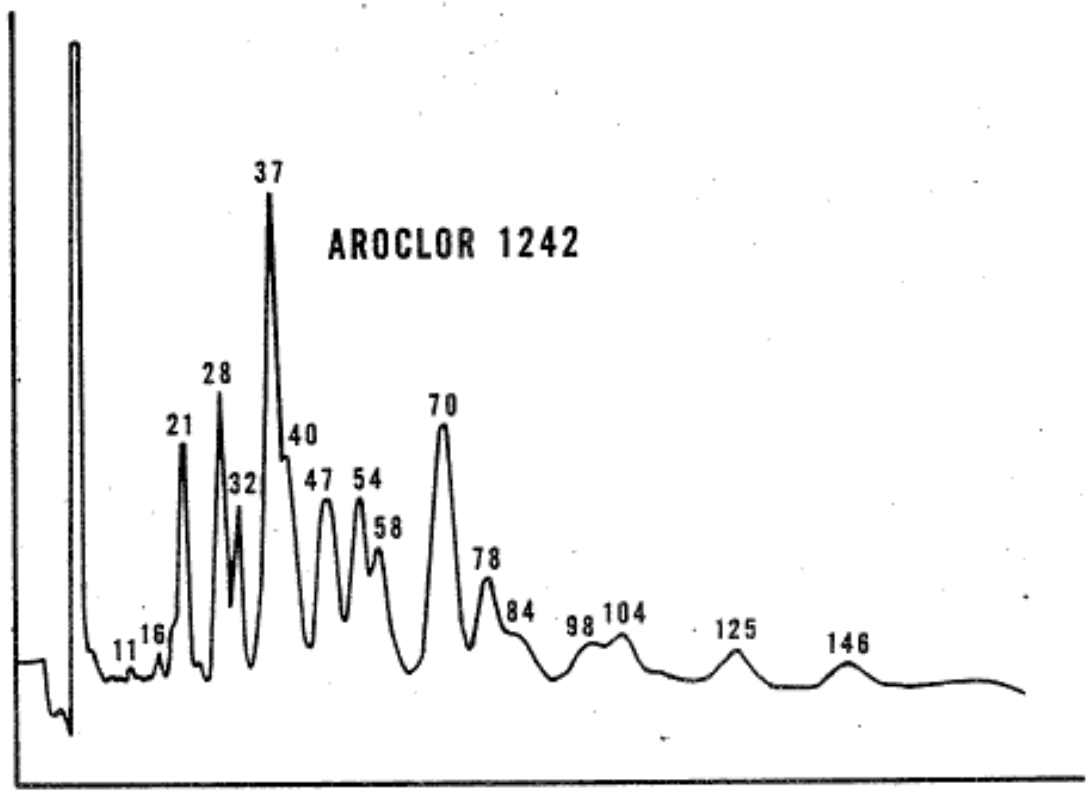


Figure 3. Column: 3% OV-1, Carrier Gas: Nitrogen at 60 ml/min,
Column Temperature: 170 C, Detector: Electron Capture

Figure 3. Column: 3% OV-1, Carrier Gas: Nitrogen at 60 ml/min,
Column Temperature: 170 C, Detector: Electron Capture

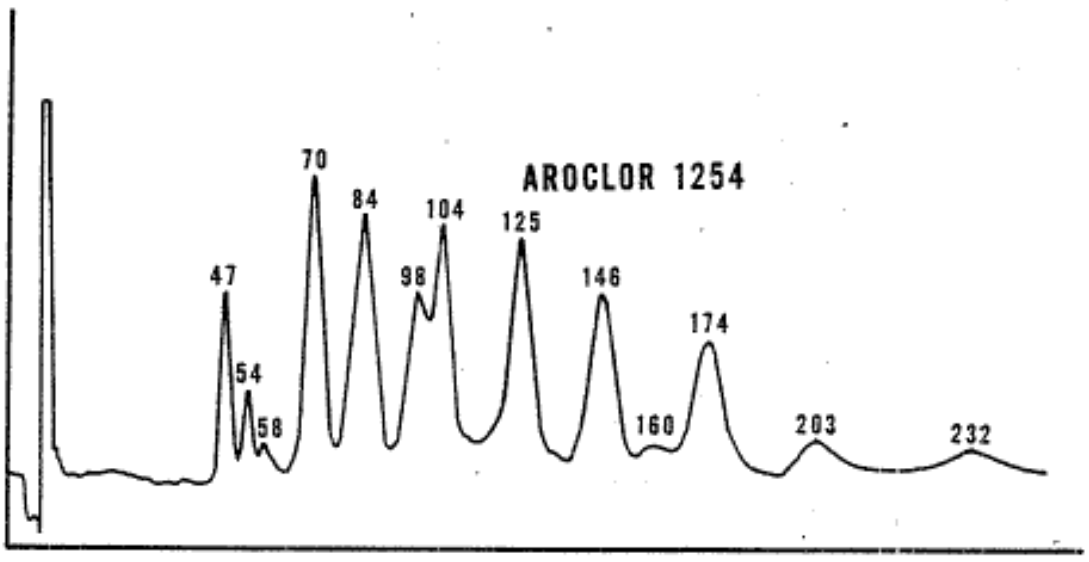


Figure 4. Column: 3% OV-1, Carrier Gas: Nitrogen at 60 ml/min,

Figure 4. Column: 3% OV-1, Carrier Gas: Nitrogen at 60 ml/min,
Column Temperature: 170 C, Detector: Electron Capture.

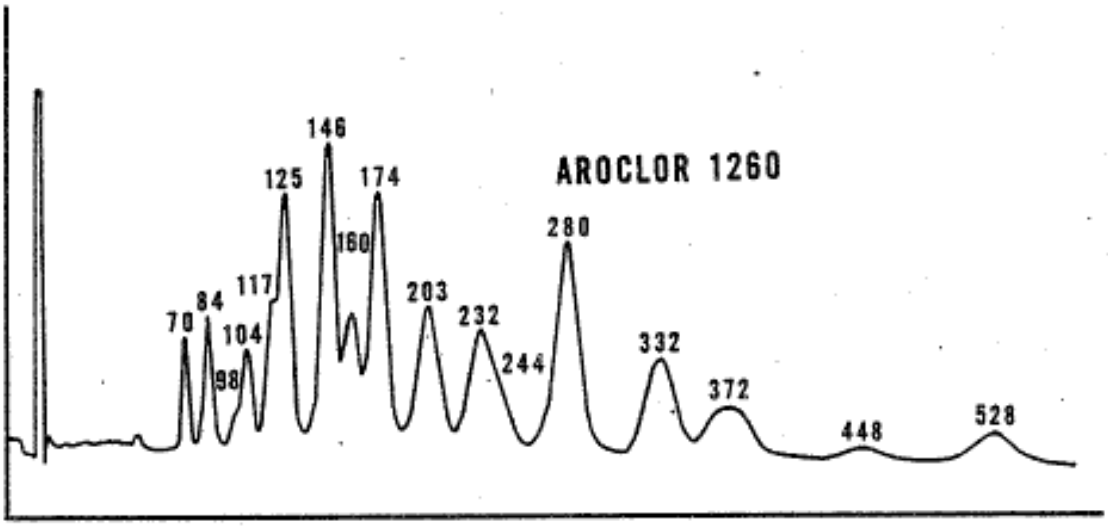


Figure 5. Column: 3% OV-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 170 C, Detector: Electron Capture.

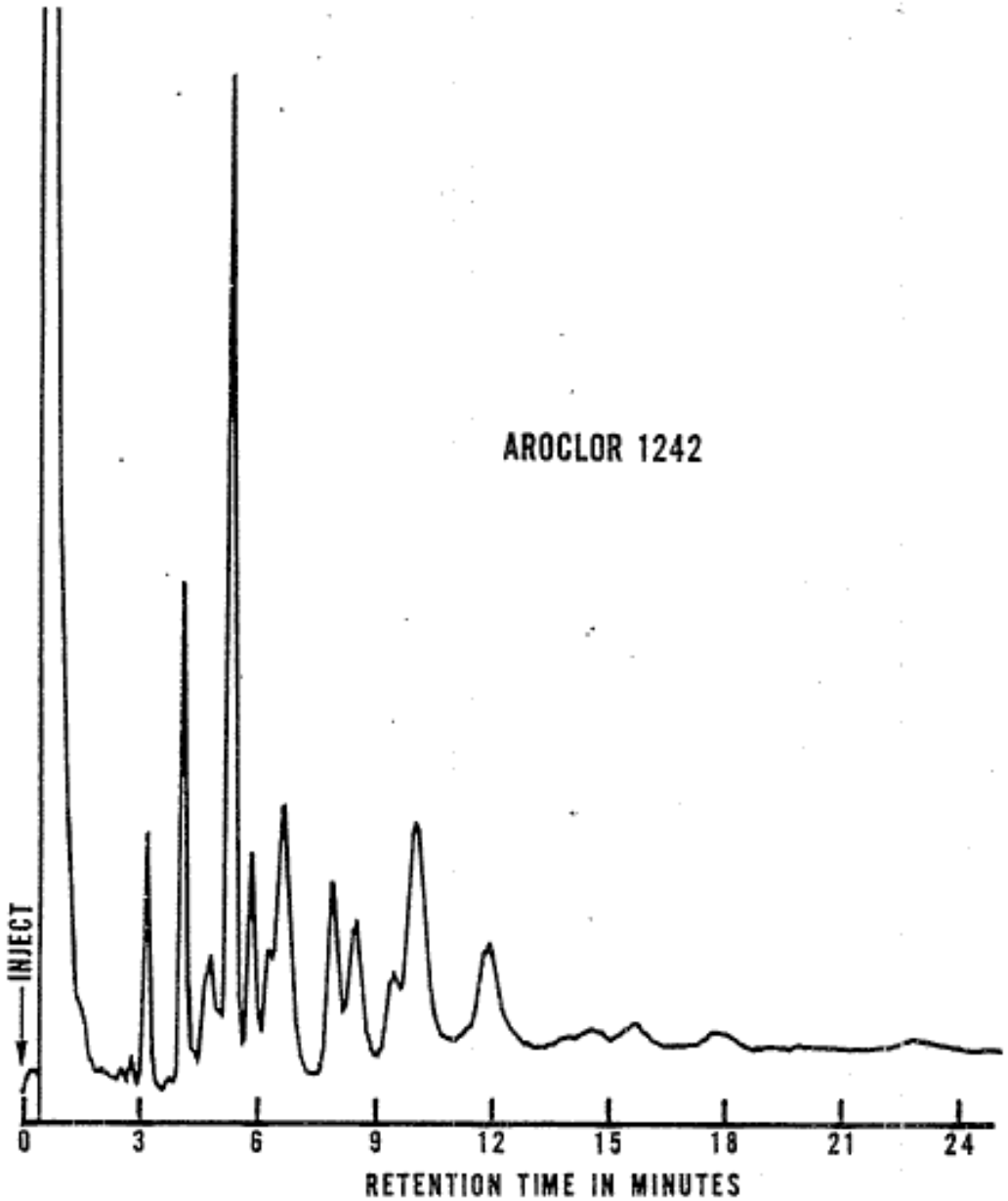


Figure 6. Column: 1.5% OV-17 + 1.95% QF-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200 C, Detector: Electron Capture.

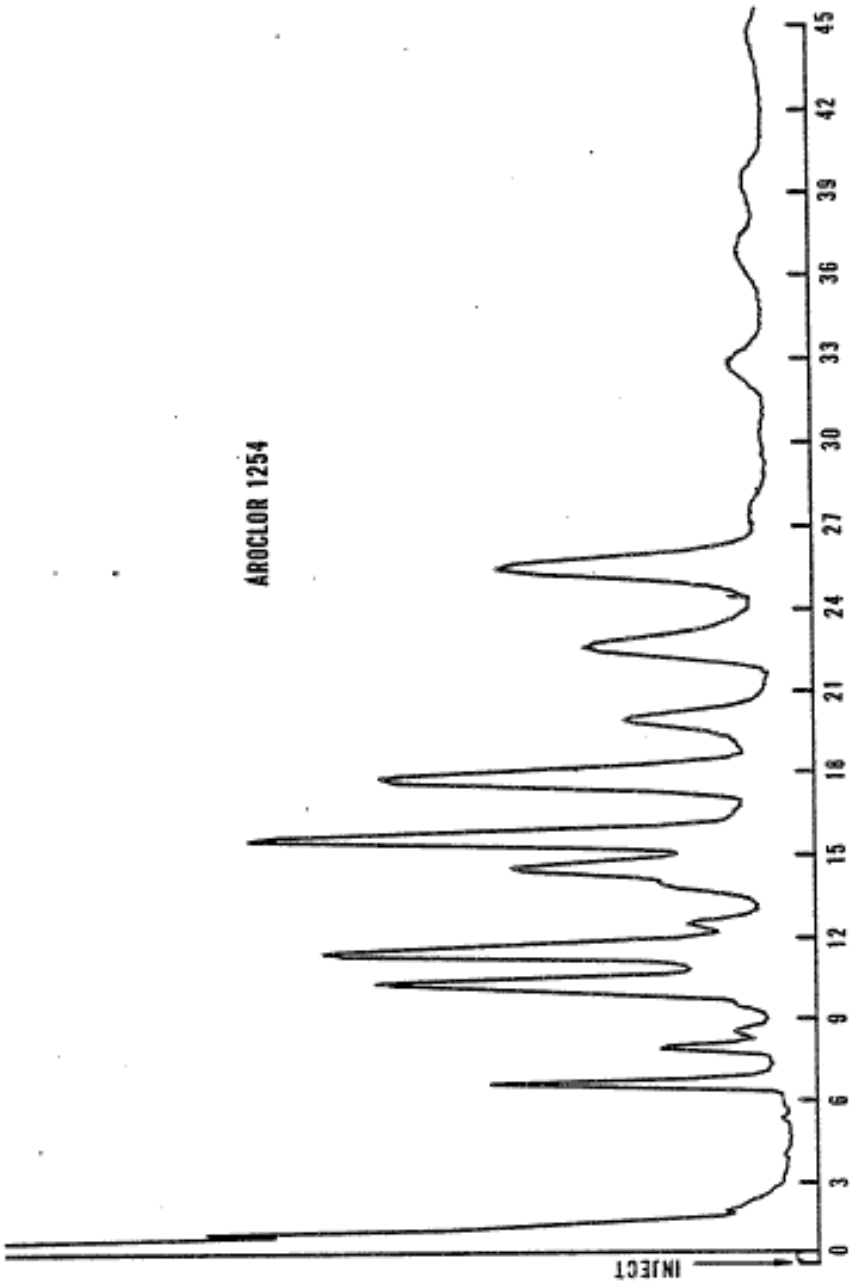


Figure 7. Column: 1.5% OV-17 + 1.95% QF-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200 C, Detector: Electron Capture.

INJECT
0
79
AROCLOR 1260
9
12
15
18
21
24
27
30
33
36
39
42
45
48
51
54
RETENTION TIME IN MINUTES
: 1.5% OV-17 + 1.95% QF-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200C, Detector: Electron Capture.

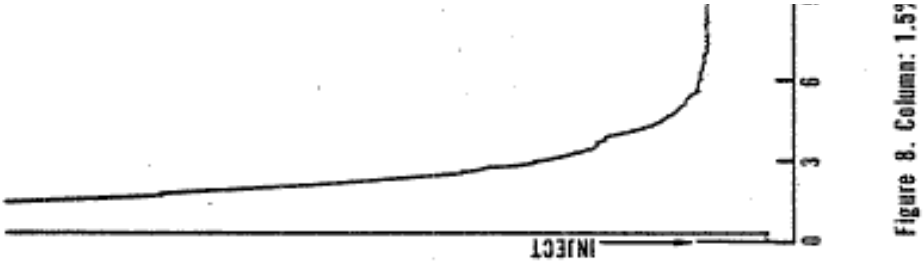


Figure 8. Column: 1.5%

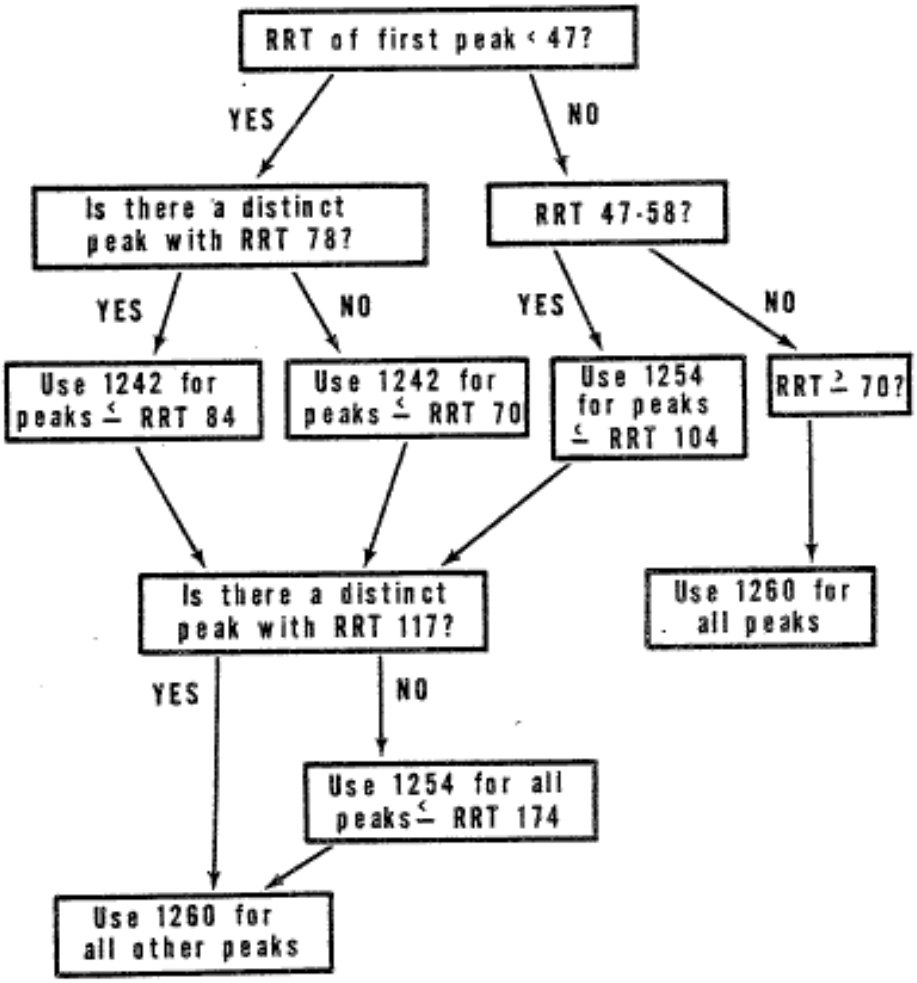


Figure 9. Chromatogram Division Flowchart (8).

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1. "Method for Chlorinated Hydrocarbons in Water and Wastewater", this manual, p. 7.

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).

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4. "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, 1972.

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6. Bellar, T. A. and Lichtenberg, J. J., "Method for the Determination of Polychlorinated Biphenyls in Water and Sediment", U. S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio, 45268, 1973.

7. Goerlitz, D. F. and Law, L. M., "Note on Removal of Sulfur Interferences from Sediment Extracts for Pesticide Analysis", Bulletin of Environmental Contamination and Toxicology, 6, 9 (1971).

8. Webb, R. G. and McCall, A. C., "Quantitative PCB Standards for Electron Capture Gas Chromatography", Journal of Chromatographic Science, 11, 366 (1973).

Capture Gas Chromatography", Journal of Chromatographic Science, 11, 366 (1973).

METHOD FOR TRIAZINE PESTICIDES IN WATER AND WASTEWATER

1. Scope and Application

1.1 This method covers the determination of various symmetrical triazine pesticides in water and wastewaters.

1.2 The following pesticides may be determined individually by this method:

Parameter	Storet No.
Ametryn	---
Altraton	---
Atrazine	39033
Prometon	39056
Prometryn	39057
Propazine	39024
Secbumeton	---
Simazine	39055
Terbuthylazine	---

2. Summary

2.1 The method describes an efficient sample extraction procedure and provides, through use of column chromatography, a method for the elimination of non-pesticide interferences and the pre-separation of pesticide mixtures. Identification is made by selective gas chromatographic separation, and measurement is accomplished by the use of an electroytic conductivity detector (CCD) in the nitrogen mode or a nitrogen specific thermionic detector. 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 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 Appendix I.

3.2 The interferences in industrial effluents are high and varied and often pose great difficulty in obtaining accurate and precise measurement of triazine pesticides. The use of a specific detector supported by an optional column cleanup procedure will eliminate many of these interferences.

3.3 Nitrogen containing compounds other than the triazines may interfere.

4. Apparatus and Materials

4.1 Gas Chromatograph - Equipped with glass lined injection port.

4.2 Detector Options

4.2.1 Electrolytic Conductivity.

4.2.2 Nitrogen specific thermionic

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 Carbowax 20M, 1%

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 x 19 mm ID) with coarse fritted plate and Teflon stopcock on bottom; 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 µl.

4.9 Separatory funnels - 2000 ml with Teflon stopcock.

4.10 Blender - High speed, glass or stainless steel cup.

4.11 Graduated Cylinders - 1000 ml.

4.12 Florisil - PR Grade (60-100 mesh); purchase activated at 1250°F and store in the dark in glass containers with glass

1250°F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use, activate each

batch overnight at 130°C in foil-covered glass container. Determine lauric acid value (See Appendix II).

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 Diethyl Ether - Pesticide Quality, redistilled in glass, if necessary

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

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

5.5 Hexane, Methanol, Methylene Chloride, Petroleum Ether (boiling range 30-60°C) - pesticide quality, redistill in glass if necessary.

5.6 Pesticide Standards - Reference grade.

6. Calibration

6.1 Gas chromatographic operating conditions are considered optimum when an injection of ≠ 20 ng of each triazine will

optimum when an injection of ≥ 20 ng of each triazine will yield a peak at least 50% of full scale deflection with the modified Coulson detector (1). 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 Inject standards frequently as a check on the stability of operating conditions. A chromatogram of a mixture of several pesticides is shown in Figure 1 and provides reference operating conditions for the recommended column.

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

7. Quality Control

7.1 Duplicate and spiked sample analyses are recommended as quality control checks. Quality control charts (2) 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.

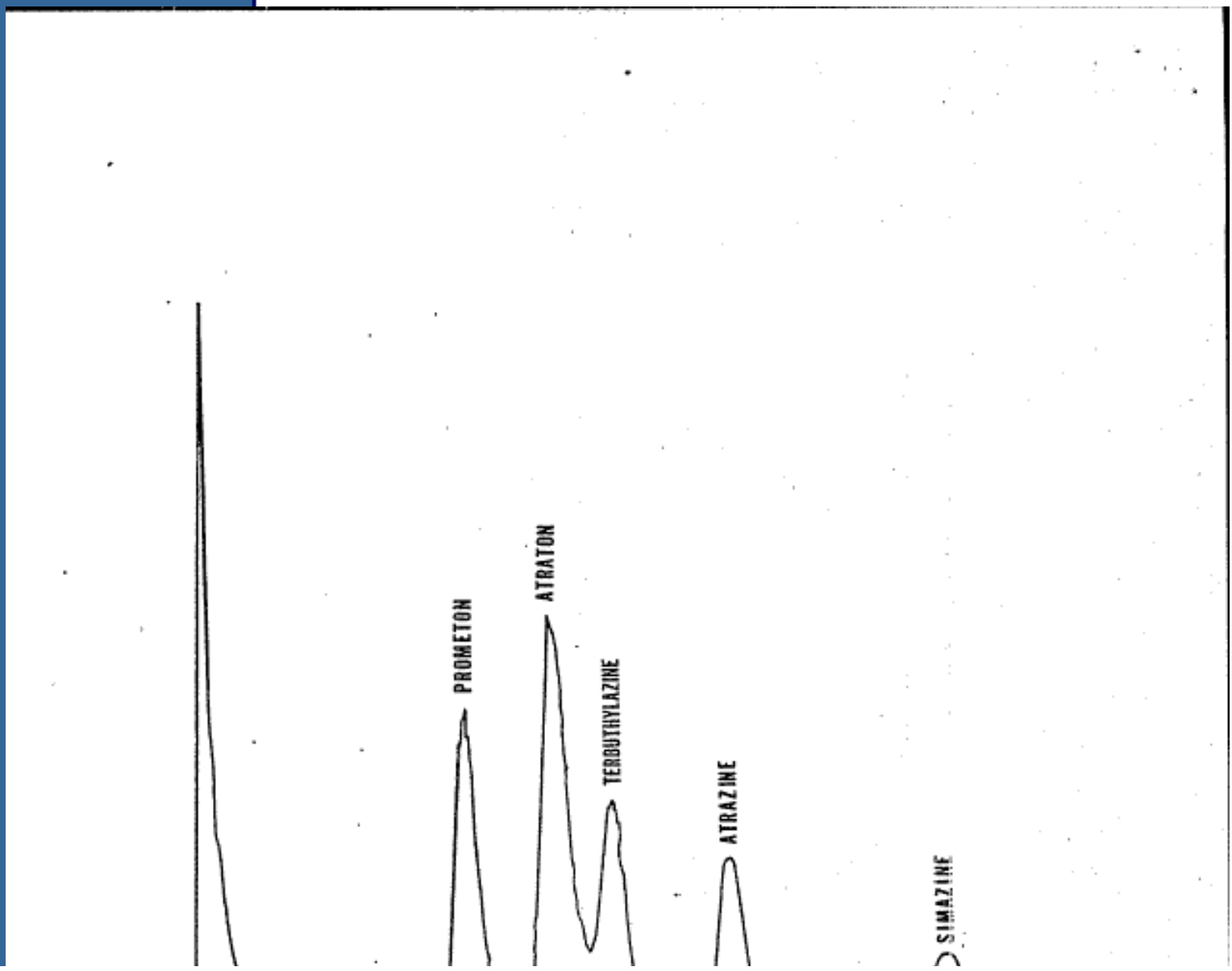
7.2 Each time a set of samples is extracted, a method blank is determined on a volume of distilled water equivalent to that used to dilute the sample.

used to dilute the sample.

8. Sample Preparation

8.1 Blend the sample if suspended matter is present and adjust pH to near neutral (pH 6.5-7.5) with 50% sulfuric acid or 10N sodium hydroxide.

8.2 Quantitatively transfer a 1000 ml aliquot into a two-liter separatory funnel.



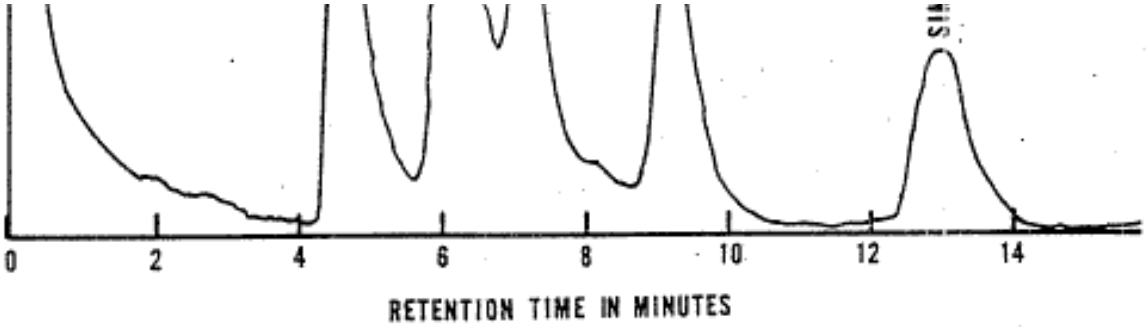


Figure 1. Column Packing: 1% Carbowax 20M on Gas-Chrom Q (100/120 mesh), Column Temperature : 155 C, Carrier Gas: Helium at 80 ml/min, Detector: Electrolytic Conductivity.

TABLE 1

RETENTION RATIOS OF VARIOUS TRIAZINE

PESTICIDES RELATIVE TO ATRAZINE

Pesticide	Retention Ratio
Prometon	0.52
Atraton	0.67
Propazine	0.71
Terbuthylazine	0.78
Secbumeton	0.88
Atrazine	1.00
Prometryne	1.10
Simazine	1.35
Ametryne	1.48

Absolute retention time of atrazine = 10.1 minutes

9. Extraction

9.1 Add 60 ml methylene chloride to the sample in the separatory funnel and shake vigorously for two minutes.

9.2 Allow the solvent to separate from the sample, draw the organic layer into a 100-ml beaker, then pass the organic layer through a chromatographic column containing 3-4 inches anhydrous sodium sulfate, and collect it in a 500-ml K-D flask equipped with a 10 ml ampul. Add a second 60-ml volume of solvent to the separatory funnel and complete the extraction procedure a second time. Perform a third extraction in the same manner.

9.3 Concentrate the extract to 10 ml in a K-D evaporator on a hot water bath. Disconnect the Snyder column just long enough to add 10 ml hexane to the K-D flask and then continue the concentration to about 5-6 ml. This operation is to displace methylene chloride and give a final hexane solution. If the need for cleanup is indicated, continue to Florisil Column Cleanup (10 below).

9.4 If further cleanup is not required, replace the Snyder column and flask with a micro-Snyder column and continue the concentration to 0.5-1.0 ml. Analyze this final concentrate by gas chromatography.

10. Florisil Column Adsorption Chromatography

10.1 Adjust the sample extract volume to 10 ml.

10.2 Place a charge of activated Florisil (weight determined by lauric acid value, see Appendix II) 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.

10.3 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 up to four eluates in 500-ml K-D flasks equipped with 10-ml ampuls. (See Eluate Composition, 10.4.) 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. Perform the third elution with 200 ml of 50% ethyl ether - petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

10.4 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 as follows:

15% Eluate	50% Eluate	100% Eluate
Propazine (90%)	Propazine (10%)	Atraton
Terbuthylazine (30%)	Terbuthylazine(70%)	Secbumeton
Atrazine (20%)	Atrazine (80%)	Prometon
	Ametryne	
	Prometryne	
	Simazine	

10.5 Concentrate the eluates to 6-10 ml in the K-D evaporator in a hot water bath. Change to the micro-Snyder column and continue concentration to 0.5-1.0 ml.

10.6 Analyze by gas chromatography.

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 Appendix III.

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

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

B = Sample aliquot area

V_i = Volume of extract injected (μl)

V_t = Volume of total extract (μl)

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

recovery data. When duplicate and spiked samples are analyzed all data obtained should be reported.

REFERENCES:

1. Patchett, G. G., "Evaluation of the Electrolytic Conductivity Detector for Residue Analyses of Nitrogen-Containing Pesticides", Journal of Chromatographic Science, 8, 155 (1970).

2. "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, 1972. (Revised edition to be available soon.)

METHOD FOR O-ARYL CARBAMATE PESTICIDES IN WATER AND WASTEWATER

1. Scope and Application

1.1 This method covers the determination of various O-aryl carbamate pesticides in water and wastewater.

1.2 The following pesticides may be determined individually by this method:

Parameter	Storet No.
Aminocarb	---
Carbaryl	39750
Methiocarb	---
Mexacarbate	---
Propoxur	---

2. Summary

2.1 A measured volume of water is extracted with methylene chloride. The concentrated extract is cleaned up with a Florisil column. Appropriate fractions from the column are concentrated and portions are separated by thin-layer

concentrated and portions are separated by thin-layer chromatography. The carbamates are hydrolyzed on the layer and the hydrolysis products are reacted with 2,6-dibromoquinone chlorimide to yield specific colored products. Quantitative measurement is achieved by visually comparing the responses of sample extracts to the responses of standards on the same thin-layer. Identifications are confirmed by changing the pH of the layer and observing color changes of the reaction products. 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 Direct interferences may be encountered from phenols that may be present in the sample. These materials react with the chromogenic reagent and yield reaction products similar to those of the carbamates. In cases where phenols are suspected of interfering with a determination, a different solvent system should be used to attempt to isolate the carbamates.

3.2 Indirect interferences may be encountered from naturally colored materials whose presence masks the chromogenic reaction.

4. Apparatus and Materials

4.1 Thin-layer plates - Glass plates (200 x 200 mm) coated with 0.25 mm layer of Silica Gel G (gypsum binder).

4.2 Spotting Template

4.3 Developing Chamber

4.4 Sprayer - 20 ml capacity

4.5 Kuderna-Danish (K-D) Glassware (Kontes)

4.5.1 Snyder Column - three ball (K-503000)

4.5.2 Micro-Snyder Column - two ball (K-569001)

4.5.3 Evaporative Flasks - 500 ml (K-570001)

4.5.4 Receiver Ampuls - 10 ml graduated (K-570050)

4.5.5 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 µl.

4.9 Separatory Funnel - 2000 ml, with Teflon stopcock.

4.10 Blender - High speed, glass or stainless steel cup.

4.11 Florisil - PR Grade (60-80 mesh); purchase activated at 1250°F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use activate each batch overnight at 130°C in foil-covered glass container. Determine lauric acid value (see Appendix II).

5. Reagents, Solvents, and Standards

5.1 Sodium Hydroxide - (ACS) 10 N in distilled water.

5.2 Sodium Sulfate - (ACS) Granular, anhydrous.

5.3 Sulfuric Acid - (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.

5.4 Diethyl Ether - Nanograde, redistilled in glass, if necessary.

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

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

5.5 Hexane, Methanol, Methylene Chloride, Petroleum Ether - nanograde, redistill in glass if necessary.

5.6 Pesticide Standards - Reference grade.

5.8.1 TLC Standards - 0.100 µg/ul in chloroform.

5.7 Chromogenic agent - Dissolve 0.2 g 2,6-dibromoquinone chlorimide in 20 ml chloroform.

5.8 Buffer solution - 0.1 N sodium borate in water.

6. Calibration

6.1 To insure even solvent travel up the layer, the tank used for layer development must be thoroughly saturated with developing solvent before it is used. This may be achieved by lining the inner walls of the tank with chromatography paper and introducing the solvent 1-2 hours before use.

6.2 Samples and standards should be introduced to the layer using a syringe, micropipet or other suitable device that permits all the spots to be about the same size and as small as possible. An air stream directed on the layer during spotting will speed solvent evaporation and help to maintain small spots.

6.3 For qualitative and quantitative work, spot a series representing 0.1-1.0 µg of a pesticide. Tables 1 and 2 present color responses and R_f values for several solvent systems.

7. Quality Control

7.1 Duplicate and spiked sample analyses are recommended as quality control checks. Quality control charts should be developed

control checks. Quality control charts should be developed and used as a check on the analytical system. Quality control

Table 1

R_f Values of O-Aryl Carbamate Pesticides in Several Solvent Systems

	A	B	C	D	E	F
Carbaryl	0.26	0.22	0.48	0.41	0.58	0.24
Aminocarb	0.26	0.02	0.46	0.52	0.54	0.04
Mexacarbate	0.34	0.22	0.54	0.53	0.60	0.24
Methiocarb	0.31	0.31	0.55	0.55	0.59	0.28
Propoxur	0.27	0.10	0.53	0.59	0.60	0.13

Solvent Systems:

A. Hexane/acetone (3:1)
B. Methylene chloride
C. Benzene/acetone (4:1)
D. Benzene/cyclohexane/diethylamine (5:2:2)
E. Ethyl acetate
F. Chloroform

Table 2

Color Responses and Detection Limit for O-Aryl Carbamates

	Colors:		Detection Limit (ug)
	Before Buffer	After Buffer	
Carbaryl	Brown	Red-Purple	0.1
Aminocarb	Gray	Green	0.1
Mexacarbate	Gray	Green	0.1
Methiocarb	Brown	Tan	0.2
Propoxur	Blue	Blue	0.1

check samples and performance evaluation samples should be analyzed on a regular basis.

7.2 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.

8. Sample Preparation

8.1 Blend the sample if suspended matter is present and adjust pH to near neutral (pH 6.5-7.5) with 50% sulfuric acid or 10 N sodium hydroxide.

8.2 Quantitatively transfer a one-liter aliquot into a two-liter separatory funnel.

9. Extraction

9.1 Add 60 ml of methylene chloride to the sample in the separatory funnel and shake vigorously for two minutes.

9.2 Allow the solvent to separate from the sample, draw the organic layer into a 100-ml beaker, then pass the organic layer through a chromatographic column containing 3-4 inches anhydrous sodium

chromatographic column containing 3-4 inches anhydrous sodium sulfate, and collect it in a 500-ml K-D flask equipped with a 10-ml ampul. Add a second 60-ml volume of solvent to the separatory funnel and complete the extraction procedure a second time. Perform a third extraction in the same manner.

9.3 Concentrate the extract to 10 ml in a K-D evaporator on a hot water bath. Disconnect the Snyder column just long enough to add 10 ml of hexane to the K-D flask and then continue the concentration to about 5-6 ml. If the need for cleanup is indicated, continue to Florisil Column Cleanup (10 below).

9.4 If further cleanup is not required, replace the Snyder column and flask with a micro-Snyder column and continue the concentration to 0.5-1.0 ml. Analyze this final concentrate by thin-layer chromatography (Section 11).

10. Florisil Column Cleanup

10.1 Adjust the sample extract to 10 ml with hexane.

10.2 Place a charge of activated Florisil (weight determined by lauric-acid value, see Appendix II) 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.

10.3 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 four eluates in 500-ml K-D flasks equipped with 10-ml ampuls. 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. Perform the third elution with 200 ml of 50% ethyl ether - petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

10.3.1 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 as follows:

50% Eluate	100% Eluate
Carbaryl (70%)	Carbaryl (30%)
Mexacarbate	Aminocarb
	Propoxur

10.4 Concentrate the eluates to 6 - 10 ml in the K-D evaporator in a hot water bath. Change to the micro-Snyder column and continue concentration to 0.5-1.0 ml.

10.5 Analyze according to 11. below.

11. Separation and Detection

11.1 Carefully spot 10% of the extract on a thin layer. On the same plate spot several pesticides or mixtures for screening purposes, or a series of 1, 2, 4, 6, 8 and 10 µl of specific standards for quantitative analysis.

11.2 Develop the layers 10 cm in a tank saturated with solvent vapors. Remove the plate and allow it to dry.

11.3 Spray the layer rapidly and evenly with about 10-15 ml chromogenic reagent. Heat the layer in an oven at 110°C for 15 minutes. The pesticides will appear with colors as indicated in Table 2. Make quantitative estimates by visually comparing the intensity and size of the spots with those of the series of standards.

11.4 Spray the layer with sodium borate reagent and observe the color shift of the reaction products. The color shift must be the same for sample and standard for identification to be confirmed.

12. Calculation of Results

12.1 Determine the concentration of pesticide in a sample by comparing the response in a sample to that of a quantity of standard treated on the same layer. Divide the result, in micrograms, by the fraction of extract spotted to convert to micrograms per liter.

13. Reporting Results

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

METHOD FOR N-ARYL CARBAMATE AND UREA PESTICIDES IN WATER AND WASTEWATER

1. Scope and Application

1.1 This method covers the determination of various N-aryl carbamate and urea pesticides in water and wastewater.

1.2 The following pesticides may be determined individually by this method:

Parameter	Storet No.
Barban	---
Chlorpropham	---
Diuron	39650
Fenuron	---
Fenuron-TCA	---
Linuron	---
Monuron	---
Monuron-TCA	---
Neburon	---
Propham	39052
Siduron	---
Swep	---

2. Summary

2.1 A measured volume of water is extracted with methylene chloride and the concentrated extract is cleaned up with a Florisil column. Appropriate fractions from the column are concentrated and portions are separated by thin-layer chromatography. The pesticides are hydrolyzed to primary amines, which in turn are

pesticides are hydrolyzed to primary amines, which in turn are chemically converted to diazonium salts. The layer is sprayed with 1-naphthol and the products appear as colored spots. Quantitative measurement is achieved by visually comparing the

responses of sample extracts to the responses of standards on the same thin layer. 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 Direct interferences may be encountered from aromatic amines that may be present in the sample. These materials react with the chromogenic reagent and yield reaction products similar to those of the pesticides. In cases where amines are suspected of interfering with a determination, a different solvent system should be used to attempt to isolate the pesticides on the layer.

3.2 Indirect interferences may be encountered from naturally colored materials whose presence masks the chromogenic reaction.

4. Apparatus and Materials

4.1 Thin-layer plates - Glass plates (200 x 200 mm) coated with 0.25 mm layer of Silica Gel G (gypsum binder).

4.2 Spotting Template

4.3 Developing Chamber

4.4 Sprayer - 20 ml capacity

4.3 Developing Chamber

4.4 Sprayer - 20 ml capacity

4.5 Kuderna-Danish (K-D) Glassware (Kontes)

4.5.1 Snyder Column - three ball (K-503000)

4.5.2 Micro-Snyder Column - two ball (K-569001)

4.5.3 Evaporative Flasks - 500 ml (K-570001)

4.5.4 Receiver Ampuls - 10 ml graduated (K-570050)

4.5.5 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 µl.

4.9 Separatory Funnel - 2000 ml, with Teflon stopcock.

4.10 Blender - High speed, glass or stainless steel cup.

4.11 Florisil - PR Grade (60-80 mesh); purchase activated at 1250°F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use activate each batch overnight at 130°C in foil-covered glass container. Determine lauric acid value (see Appendix II).

5. Reagents, Solvents, and Standards

5.1 Sodium Chloride - (ACS) Saturated solution in distilled water (pre-rinse NaCl with hexane).

(pre-rinse NaCl with hexane).

5.2 Sodium Hydroxide - (ACS) 10 N in distilled water.

5.3 Sodium Sulfate - (ACS) Granular, anhydrous (conditioned at 400°C for 4 hrs.).

5.4 Sulfuric Acid - (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.

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, N.Y. 10523.)

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

5.6 Hexane, Methanol, Methylene Chloride, Petroleum Ether - nanograde, redistill in glass if necessary.

5.7 Pesticide Standards - Reference grade.

5.9.1 TLC Standards - 0.100 µg/µl in chloroform.

5.8 Nitrous acid - prepare just before use by mixing 1 g $NaNO_2$ with 20 ml 0.2 N HCl.

5.9 Chromogenic agent - dissolve 1.0 g 1-Naphthol in 20 ml ethanol. Prepare fresh daily.

6. Calibration

6.1 To insure even solvent travel up the layer, the tank used for layer development must be thoroughly saturated with developing solvent before it is used. This may be achieved by lining the inner walls of the tank with chromatography paper and introducing the solvent 1-2 hours before use.

6.2 Samples and standards should be introduced to the layer using a syringe, micropipet or other suitable device that permits all the spots to be about the same size and as small as possible. An air stream directed on the layer during spotting will speed solvent evaporation and help to maintain small spots.

6.3 For qualitative and quantitative work, spot a series representing 0.1-1.0 µg of a pesticide. Tables 1 and 2 present color responses and R_f values for several solvent systems.

TABLE 1

R_f VALUES OF N-ARYL CARBAMATE AND UREA PESTICIDES
IN SEVERAL SOLVENT SYSTEMS

Carbamates	A	B	C	D	E	F	G
Propham	0.49	0.54	0.73	0.48	0.36	0.68	0.69
Chloropropham	0.57	0.60	0.73	0.49	0.37	0.70	0.73
Barban	0.61	0.59	0.72	0.41	0.28	0.70	0.74
Swep	0.48	0.44	0.70	0.41	0.28	0.67	0.66
Urea							
Fenuron	0.03	0.04	0.38	0.22	0.10	0.41	0.30
Fenuron-TCA	0.03	0.04	0.36	0.22	0.10	0.41	0.30
Monuron	0.04	0.05	0.37	0.24	0.10	0.47	0.34
Monuron-TCA	0.04	0.06	0.34	0.24	0.10	0.46	0.34
Diuron	0.05	0.09	0.38	0.28	0.13	0.54	0.44
Linuron	0.40	0.43	0.62	0.39	0.24	0.66	0.64
Neburon	0.21	0.28	0.64	0.41	0.26	0.68	0.65
Siduron	0.02	0.07	0.68	0.39	0.25	0.62	0.55

Solvent Systems:

A. Methylene chloride
B. Chloroform
C. Ethyl Acetate
D. Hexane/acetone (2:1)
E. Hexane/acetone (4:1)
F. Chloroform/acetonitrile (2:1)
G. Chloroform/acetonitrile (5:1)

TABLE 2

COLOR RESPONSES AND DETECTION LIMIT FOR THE N-ARYL CARBAMATES AND UREAS

Carbamates	Color	Detection Limit (ug)
Propham	Red-purple	0.2
Chlorpropham	Purple	0.1
Barban	Purple	0.05
Swep	Blue-purple	0.2
Ureas		
Fenuron	Red-purple	0.05
Fenuron-TCA	Red-purple	0.1
Monuron	Pink-orange	0.05
Monuron-TCA	Pink-orange	0.1
Diuron	Blue-purple	0.1
Linuron	Blue-purple	0.1
Neburon	Blue-purple	0.1
Siduron	Red-purple	0.05

7. Quality Control

7.1 Duplicate and spiked sample analyses are recommended as quality control checks. Quality control charts (1) 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.

7.2 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.

8. Sample Preparation

8.1 Blend the sample if suspended matter is present and adjust pH to near neutral (pH 6.5-7.5) with 50% sulfuric acid or 10 N sodium hydroxide.

8.2 Quantitatively transfer a one-liter aliquot into a two-liter separatory funnel.

9. Extraction

9.1 Add 60 ml of methylene chloride to the sample in the separatory funnel and shake vigorously for two minutes.

9.2 Allow the solvent to separate from the sample, draw the organic layer into a 100-ml beaker, then pass the organic layer through a chromatographic column containing 3-4 inches anhydrous sodium sulfate, and collect it in a 500-ml K-D flask equipped with a 10-ml ampul. Add a second 60-ml volume of solvent to the

sulfate, and collect it in a 500-ml K-D flask equipped with a 10-ml ampul. Add a second 60-ml volume of solvent to the separatory funnel and complete the extraction procedure a second time. Perform a third extraction in the same manner.

9.3 Concentrate the extract to 10 ml in a K-D evaporator on a hot water bath. Disconnect the Snyder column just long enough to add 10-ml hexane to the K-D flask and then continue the concentration to about 5-6 ml. If the need for cleanup is indicated, continue to Florisil Column Cleanup (10 below).

9.4 If further cleanup is not required, replace the Snyder column and flask with a micro-Snyder column and continue the concentration to 0.5-1.0 ml. Analyze this final concentrate by thin-layer chromatography (Section 11).

10. Florisil Column Cleanup

10.1 Adjust the sample extract to 10 ml with hexane.

10.2 Place a charge of activated Florisil (weight determined by lauric acid value, see Appendix II) 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.

10.3 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

into the column by decantation and subsequent petroleum ether washings. Adjust the elution rate to about 5 ml per minute and, separately, collect up to four eluates in 500-ml K-D flasks equipped with 10-ml ampuls. (See Eluate Composition, 10.3.1.) 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. Perform the third elution with 200 ml

of 50% ethyl ether - petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

10.3.1 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:

15% Eluate	50% Eluate	100% Eluate
Chlorpropham	Barban (5%)	Neburon (92%)
Propham	Linuron	Diuron
Barban (95%)	Neburon (8%)	Monuron
		Siduron

CAUTION: Fenuron and Fenuron-TCA are not recovered from the Florisil column.

10.4 Concentrate the eluates to 6-10 ml in the K-D evaporator in a hot water bath. Change to the micro-Snyder column and continue concentration to 0.5-1.0 ml.

10.5 Analyze according to 11. below.

11. Separation and Detection

11.1 Carefully spot 10% of the extract on a thin layer. On the same

11. Separation and Detection

11.1 Carefully spot 10% of the extract on a thin layer. On the same plate spot several pesticides or mixtures for screening purposes, or a series of 1, 2, 4, 6, 8 and 10 µl of specific standards for quantitative analysis.

11.2 Develop the layers 10 cm in a tank saturated with solvent vapors. Remove the plate and allow it to dry.

11.3 Spray the layer rapidly and evenly with about 10-15 ml sulfuric acid solution. Heat the layer in an oven at 110°C for 15 minutes.

11.4 When the layer is cool, spray it with nitrous acid reagent and allow it to dry. Spray the layer with 1-naphthol reagent and allow it to dry again. The pesticides will appear as purple spots (see Table 2). Identifications are made by comparison of colors and R_f values. Quantitative estimates are made by visually comparing the intensity and size of the spots with those of the series of standard.

12. Calculation of Results

12.1 Determine the concentration of pesticide in a sample by comparing the response in a sample to that of a quantity of standard treated on the same layer. Divide the result, in micrograms, by the fraction of extract spotted to convert to micrograms per liter.

13. Reporting Results

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

REFERENCES:

1. "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, 1972.

METHOD FOR CHLOROPHENOXY ACID PESTICIDES IN WATER AND WASTEWATERS

1. Scope and Application

1.1 This method covers the determination of various chlorinated phenoxy acid pesticides in water and wastewater.

1.2 The following pesticides may be determined individually by this method:

Parameter	Storet No.
2,4-D	---
Dicamba	---
Silvex	39760
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.

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. The extract is cleaned by passing it through a micro-adsorption column. Identification of the esters is made by selective gas chromatographic separations and may be corroborated through the

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 Appendix I.

3.2 The interferences in industrial effluents are high and varied and often pose great difficulty in obtaining accurate and precise measurement of chlorinated phenoxy acid herbicides. Sample clean-up procedures are generally required and may result in loss of certain of these herbicides. It is not possible to describe procedures for overcoming all of the interferences that may be encountered in industrial effluents.

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

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%

4.4.4.1 OV-210, 5%

4.4.4 2 OV-17, 1.5% plus QF-1 or OV-210, 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 Blender - High speed, glass or stainless steel cup.

4.7 Graduated cylinders - 100 and 250 ml.

4.8 Erlenmeyer flasks - 125 ml, 250 ml ground glass ≋ 24/40

4.9 Microsyringes - 10, 25, 50 and 100 l.

4.10 Pipets - Pasteur, glass disposable (140 mm long X 5 mm ID).

4.11 Separatory Funnels - 60 ml and 2000 ml with Teflon stopcock.

4.12 Glass wool - Filtering grade, acid washed.

4.13 Diazald Kit - Recommended for the generation of diazomethane (available from Aldrich Chemical Co., Cat. #210,025-2).

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 IV).

5.3 Potassium Hydroxide Solution - A 37 percent aqueous solution

generation of diazomethane (see Appendix A).

5.3 Potassium Hydroxide Solution - A 37 percent aqueous solution prepared from reagent grade potassium hydroxide pellets and reagent water.

5.4 Sodium Chloride - (ACS) Saturated solution (pre-rinse NaCl with hexane) in distilled water.

5.5 Sodium Hydroxide - (ACS) 10 N in distilled water.

5.6 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.

5.7 Sulfuric acid - (ACS) concentrated, Sp. Gr. 1.84.

5.8 Florisil - PR grade (60-100 mesh) purchased activated at 1250°F and stored at 130°C.

5.9 Carbitol (diethylene glycol monoethyl ether).

5.10 Diethyl Ether - Nanograde, redistilled in glass, if necessary.

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

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

5.11 Benzene Hexane - Nanograde, redistilled in glass, if necessary.

5.12 Pesticide Standards - Acids and Methyl Esters, reference grade.

5.12.1 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.

5.12.2 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 µg/ml of each standard.

5.12.3 Standard for Chromatography (Diazomethane Procedure) - Pipet 1.0 ml of the working standard into a glass stoppered test tube and evaporate the solvent using a 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 µl of hexane for gas chromatography.

5.12.4 Standard for Chromatgraphy (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 11.3.1. 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.

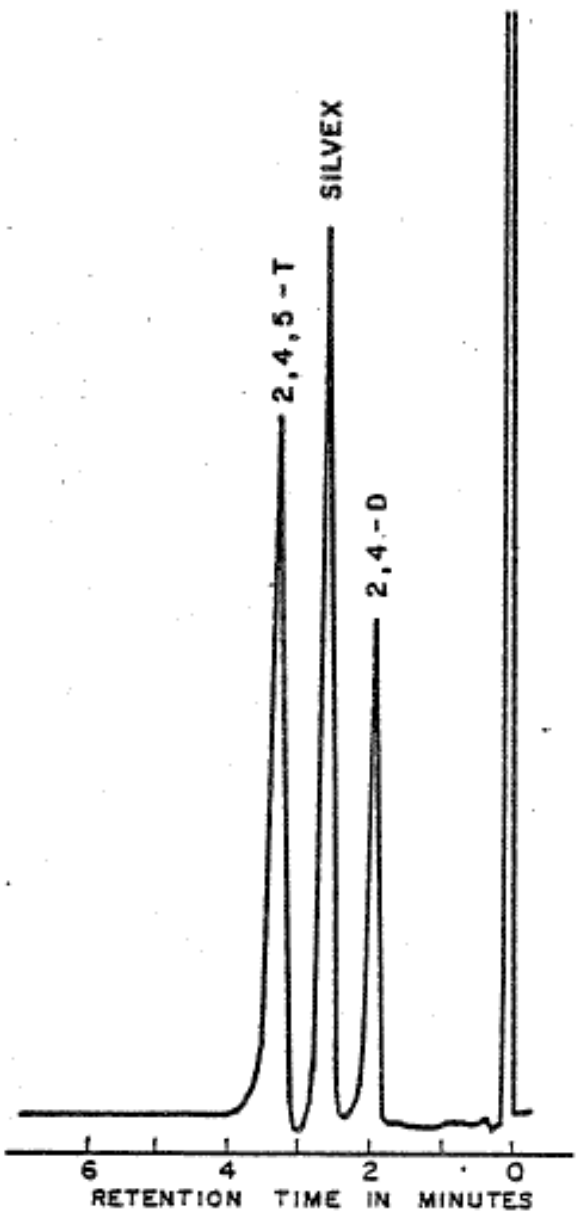


Fig. 1 Column: 1.5 % OV-17 + 1.95 % QF-1,

RETENTION TIME IN MINUTES

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

6.3 The elution order and retention ratios of methyl esters of chlorinated phenoxy acid herbicides are provided in Table 1, as a guide.

7. Quality Control

7.1 Duplicate and spiked sample analyses are recommended as quality control checks. Quality control charts (2) 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.

7.2 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.

8. Sample Preparation

8.1 The sample size taken for analysis is dependent on the type of sample and the sensitivity required for the purpose at hand. Background information on the pesticide levels previously detected at a given sampling site will assist in determining the sample size required, as well as the final volume to which the extract needs to be concentrated. A 1-liter sample is usually taken for drinking water and ambient water analysis to provide a detection limit of 0.050 to 0.100 µg/l. One-hundred milliliters is usually adequate to provide a detection limit of 1 µg/l for industrial effluents.

1 µg/l for industrial effluents.

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[2]	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

2,4,5-T	1.72	1.51
2,4-D (minutes absolute)	2.00	1.62

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

[2]OV-210 may be substituted

8.2 Quantitatively transfer the proper aliquot of sample from the sample container into a two-liter separatory funnel. If less than 800 ml is analyzed, dilute to one liter with interference free distilled water.

9. Extraction

9.1 Add 150 ml of ether to the sample in the separatory funnel and shake vigorously for one minute.

9.2 Allow the contents to separate for at least ten minutes. After the layers have separated, drain the water phase into a 1-liter Erlenmeyer flask. Then collect the extract in a 250-ml ground-glass Erlenmeyer flask containing 2 ml of 37 percent aqueous potassium hydroxide.

9.3 Extract the sample two more times using 50 ml of ether each time, and combine the extracts in the Erlenmeyer flask. (Rinse the 1-liter flask with each additional aliquot of extracting solvent.)

10. Hydrolysis

10.1 Add 15 ml of distilled water and a small boiling stone to the

10. Hydrolysis

10.1 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.

10.2 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.

10.3 Acidify the contents of the separatory funnel by adding 2 ml of cold (4°C) 25 percent sulfuric acid (5.9). Extract the herbicides once with 20 ml of ether and twice with 10 ml of ether. Collect the extracts in a 125-ml Erlenmeyer flask containing about 0.5 g of acidified anhydrous sodium sulfate (5.8). Allow the extract to remain in contact the the sodium sulfate for approximately two hours.

11. Esterification (3,4)

11.1 Transfer the ether extract, through a funnel plugged with glass wool, 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 transfer.

11.1.1 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 11.2. Prepare diazomethane as directed in Appendix IV.

11.1.2 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 11.3.

11.2 Diazomethane Esterification

11.2.1 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.

11.2.2 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.

11.2.3 Dissolve residue in 5 ml of hexane. Analyze by gas chromatography.

11.2.4 If further clean-up of the sample is required, proceed as in 11.3.4 substituting hexane for benzene.

11.3 Boron Trifluoride Esterification

11.3.1 After the benzene solution in the ampul has cooled, add 0.5 ml of borontrifluoride-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.

11.3.2 Cool and add about 4.5 ml of a neutral 5 percent aqueous sodium sulfate solution so that the benzene-water interface is in the neck of the Kuderna-Danish ampul. Seal the flask with a ground glass stopper and shake vigorously for about one minute. Allow to stand for three minutes for phase separation.

11.3.4 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.

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 Appendix III.

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

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

B = Sample aliquot area

V_i = Volume of extract injected (µl)

V_t = Volume of total extract (µl)

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. "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.

3. Metcalf, L. D. and Schmitz, A. A., "The Rapid Preparation of Fatty Acid Esters for Gas Chromatographic Analysis", Analytical Chemistry, 33, 363 (1961).

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METHOD FOR VOLATILE CHLORINATED ORGANIC COMPOUNDS IN WATER AND WASTEWATERS

1. Scope and Application

1.1 This method covers the determination of various chlorinated organic compounds in water and wastewater.

1.2 The following chlorinated organic compounds may be determined individually by this method:

Parameter	Storet No.
Benzylchloride	---
Carbon tetrachloride	32102
Chlorobenzene	34301
Chloroform	32106
Epichlorohydrin	---
Methylene Chloride	34423
1,1,2,2-Tetrachloroethane	---
Tetrachloroethylene	34475
1,2,4-Trichlorobenzene	---
1,1,2-Trichloroethane	---

2. Summary

2.1 If the sample is turbid, it is initially centrifuged or filtered through a fiber glass filter in order to remove suspended matter

2.1 If the sample is turbid, it is initially centrifuged or filtered through a fiber glass filter in order to remove suspended matter. A three to ten microliter aliquot of the sample is injected into the gas chromatograph equipped with a halogen specific detector. The resulting chromatogram is used to identify and quantitate specific components in the sample. Results are reported in micrograms per liter. Confirmation of qualitative identifications are made using two or more dissimilar columns.

3. Interferences

3.1 The use of a halogen specific detector minimizes the possibility of interference from compounds not containing chlorine, bromine, or iodine. Compounds containing bromine or iodine will interfere with the determination of organochlorine compounds. The use of two dissimilar chromatographic columns helps to eliminate this interference and, in addition, this procedure helps to verify all qualitative identifications. When concentrations are sufficiently high, unequivocal identifications can be made using infrared or mass spectroscopy. Though non-specific, the flame ionization detector may be used for known systems where interferences are not a problem.

3.2 Ghosting is usually attributed to the history of the chromatographic system. Each time a sample is injected, small amounts of various compounds are adsorbed on active sites in the inlet and at the head of the column. Subsequent injections of water tend to steam clean these sites resulting in non-representative peaks or displacement of the baseline. This phenomenon normally occurs when an analysis of a series of highly concentrated samples is followed by a low level analysis. The system should be checked for ghost peaks prior to each quantitative analysis by injecting distilled water in a manner identical to the sample analysis (1). If excessive ghosting occurs, the following corrective measures should be applied, as required, in the order listed:

1) Multiple flushes with distilled water

2) Clean or replace the glass injector liner

3) Replace the chromatographic column.

4. Apparatus and Materials

4.1 Gas Chromatograph - Equipped with programmed oven temperature controls and glass-lined injection port. The oven should be equipped with a column exit port and heated transfer line for convenient attachment to the halogen specific detector.

4.2 Detector Options:

4.2.1 Microcoulometric Titration

4.2.2 Electrolytic Conductivity

4.2.3 Flame Ionization

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

4.4 Syringes - 1 µl, 10 µl, and 50 µl.

4.5 BOD type bottle or 40 ml screw cap vials sealed with Teflon faced silicone septa.

4.6 Volumetric Flasks - 500 ml, 1000 ml.

4.7 Syringe - Hypodermic Lur-lock type (30 ml).

4.8 Filter glass fiber filter - Type A (13 mm).

4.9 Filter holder - Swinny-type hypodermic adapter (13 mm).

4.10 Glass stoppered ampuls - 10 ml

4.11 Chromatographic columns

4.11.1 Moderately-Polar Column - 23 ft x 0.1 in ID x 0.125 in OD stainless steel column #304 packed with 5% Carbowax 20 M on Chromosorb-W (60-80 mesh).

4.11.2 Highly-Polar Column - 23 ft x 0.1 in ID x 0.125 in OD stainless steel #304 packed with 5% 1,2,3-Tris-(2-cyano-ethoxy) propane on Chromosorb-W (60-80 mesh).

4.11.3 Porous Polymer Column - 6 ft x 0.1 in ID x 0.125 in OD stainless steel #304 packed with Chromosorb-101 (60-80 mesh).

4.11.4 Carbopack Column - 8 ft x 0.1 in ID x 0.125 in OD stainless steel #304 packed with Carbopack-C (80-100 mesh) + 0.2% Carbowax 1500.

5. Reagents

5.1 Chlorinated hydrocarbon reference standards

5.1.1 Prepare standard mixtures in volumetric flasks using contaminant-free distilled water as solvent. Add a known amount of the chlorinated compounds with a microliter syringe. Calculate the concentration of each component as follows:

$$mg/l = (\text{Density of Compound})(\mu l \text{ injected})\left(\frac{1000}{(\text{Dilution Volume (ml)})}\right)$$

6. Quality Control

6.1 Duplicate quantitative analysis on dissimilar columns should be performed. The duplicate quantitative data should agree within experimental error (±6 percent). If not, analysis on a third dissimilar column should be performed. Spiked sample analyses should be routinely performed to insure the integrity of the method.

should be routinely performed to insure the integrity of the method.

7. Selection Gas Chromatographic Column

7.1 No single column can efficiently resolve all chlorinated hydrocarbons. Therefore, a specific column must be selected to perform a given analysis. Columns providing only partially or non-resolved peaks are useful only for confirmatory identifications. If the qualitative nature of the sample is known, an efficient column selection can be made by reviewing the literature (2). In doing this, one must remember that injection of large volumes of water can cause two serious problems not normally noted using common gas chromatographic techniques:

1) Water can cause early column failure due to liquid phase displacement.

2) Water passing through the column causes retention times and orders to change when compared to common sample solvent media, i.e., hexane or air.

For these reasons, column life and the separations obtained by direct aqueous injection may not be identical to those suggested in literature.

8. Sample Collection and Handling

8.1 The sample containers should have a total volume in excess of 25 to 40 ml, although larger narrow-mouth bottles may be used.

8.1.1 Narrow mouth screw cap bottles with the TFE fluorocarbon

8.1.1 Narrow mouth screw cap bottles with the TFE fluorocarbon face silicone septa cap liners are strongly recommended. Crimp-seal serum vials with TFE fluorocarbon faced septa or

ground glass stoppered bottles are acceptable if the seal is properly made and maintained during shipment.

8.2 Sample Bottle Preparation

8.2.1 Wash all sample bottles and TFE seals in detergent. Rinse with tap water and finally with distilled water.

8.2.2 Allow the bottles and seals to air dry at room temperature.

8.2.3 Place the bottle in a 200°C oven for one hour, then allow to cool in an area known to be free of organics.

8.2.4 When cool, seal the bottles using the TFE seals that will be used for sealing the samples.

8.3 The sample is best preserved by protecting it from phase separation. Since the majority of the chlorinated solvents are volatile and relatively insoluble in water, it is important that the sample bottle be filled completely to minimize air space over the sample. Acidification will minimize the formation of nonvolatile salts formed from chloroorganic acids and certain chlorophenols. However, it may interfere with the detection of acid degradable compounds such as chloroesters. Therefore, the sample history must be known before any chemical or physical

sample history must be known before any chemical or physical preservation steps can be applied. To insure sample integrity, it is best to analyze the sample within 1 hour of collection.

8.4 Collect all samples in duplicate.

8.5 Fill the sample bottles in such a manner that no air bubbles pass through the sample as the bottle is filled.

8.6 Seal the bottles so that no air bubbles are entrapped in it.

8.7 Maintain the hermetic seal on the sample bottle until analysis.

8.8 Sampling from a water tap.

8.8.1 Turn on water and allow the system to flush. When the temperature of the water has stabilized, adjust the flow to about 500-ml/minute and collect duplicate samples from the flowing stream.

8.9 Sampling from an open body of water.

8.9.1 Fill a 1-quart wide-mouth bottle with sample from a representative area. Carefully fill duplicate 25 to 40 ml-sample bottles from the 1-quart bottle.

9. Sample Preparation

9.1 If the sample is turbid, it should be filtered or centrifuged to prevent syringe plugging or excessive ghosting problems. Filtering the sample is accomplished by filling a 30-ml hypodermic syringe with sample and attaching the Swinny-type hypodermic filter adaptor with a glass fiber filter "Type A" installed. Discard the first 5 ml of sample then collect the filtered sample in a glass stoppered sample filled to the top. (One should occasionally analyze the non-filtered sample to insure that the filtering technique does not

non-filtered sample to insure that the filtering technique does not adversely affect the sample).

10. Method of Analysis

10.1 Daily, analyze a standard containing 10.0 mg/l of each compound to be analyzed as a quality check sample before any samples are analyzed. Instrument status checks and lower limit of detection estimations based upon response factor calculations at two times the signal to noise ratio are obtained from these data. In

addition, response factor data obtained from this standard can be used to estimate the concentration of the unknowns.

10.2 Analyze the filtered sample of unknown composition by injecting 3 to 10 µl into the gas chromatograph. Record the injection volume and detector sensitivity.

10.3 Prepare a standard mixture consisting of the same compounds in concentrations approximately equal to those detected in the sample. Chromatograph the standard mixture under conditions identical to the unknown.

11. Calculation or Results

11.1 Measure the area of each unknown peak and each reference standard peak as follows:

Area = (Peak Height)(Width of Peak at 1/2 Height)

11.2 Calculate the concentration of each unknown as follows:

$$\text{mg/l} = \frac{(\text{Area of Sample peak})(\mu\text{l of Standard Injected})(\text{Conc'n of Standard})}{(\mu\text{l of Sample injected})(\text{Area of Standard Peak})}$$

12. Reporting Results

12.1 Report results in mg/l. If a result is negative, report the minimum detectable limit (see 10.1). When duplicate and spiked samples are analyzed, all data obtained should be reported.

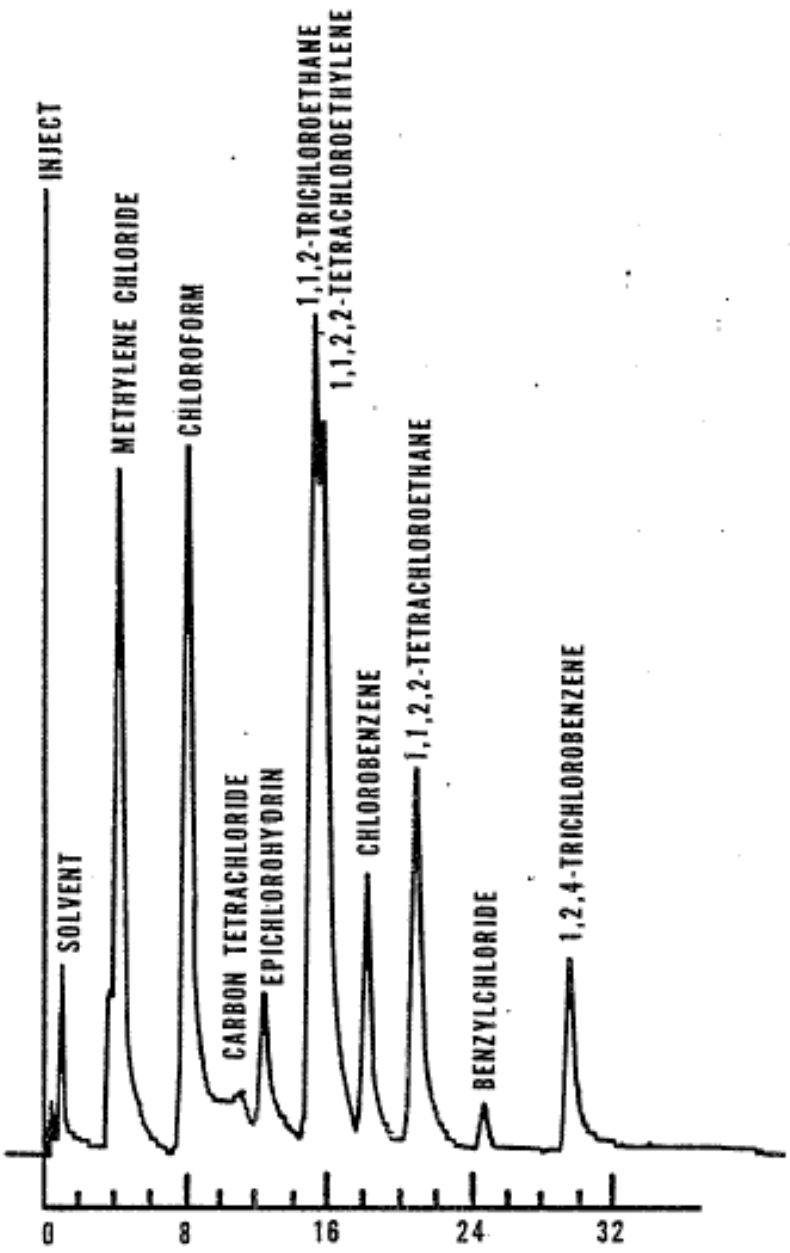

Figure 1. Column: Chromosorb-101, Temperature Program: 125 C for 4 min then 4 C/min up to 280 C., Carrier Gas: Nitrogen at 36 ml/min, Detector: Microcoulometric.

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2. "Gas Chromatography Abstracts", Knapman, C. E. H., Editor, Institute of Petroleum, 61 New Cavendish Street, London W1M8AR, Annually 1958 to date, since 1970, also includes Liquid Chromatography Abstracts.

METHOD FOR PENTACHLOROPHENOL IN WATER AND WASTEWATER

1. Scope and Application

1.1 This method covers the determination of pentachlorophenol (PCP) in water and wastewater.

2. Summary

2.1 Pentachlorophenol is extracted from the acidified water sample (pH 3) with toluene, methylated with diazomethane, and analyzed by electron-capture gas chromatography, using the columns listed in the organochlorine pesticide method. (Page 7, this manual)

2.2 Further identification of pentachlorophenol is made with a mass spectrometer.

3. Interferences

3.1 Chlorinated pesticides and other high boiling chlorinated organic compounds may interfere with the analysis of PCP.

3.2 Injections of samples not treated with diazonmethane indicate, to a certain degree, whether interfering substances are present.

4. Precision and Accuracy

4.1 Single laboratory accuracy and precision reported for this method when analyzing five replicates of tap water spiked with 0.05 to 0.07 µg/l of PCP is as follows:

0.07 µg/l of PCP is as follows:

Recovery - mean 95.9%, range 88.1 to 100.2%

Standard Deviation - 6.0%

REFERENCE:

1. "Analysis of Pentachlorophenol Residues in Soil, Water and Fish," Stark, A., Agricultural and Food Chemistry, 17, 871 (July/August 1969).

APPENDIX I

CONSIDERATIONS FOR GLASSWARE AND REAGENTS USED IN ORGANIC ANALYSIS*

1. Glassware

1.1 Cleaning Procedure - It is particularly important that glassware used in trace organic analyses be scrupulously cleaned before initial use as well as after each analysis. The glassware should be cleaned as soon as possible after use, first rinsing with water or the solvent that was last used in it. This should be followed by washing with hot soap water, rinsing with tap water, distilled water, redistilled acetone and finally with pesticide quality hexane. Heavily contaminated glassware may require muffling at 400°C for 15-to 30-minutes. High boiling materials, such as some of the polychlorinated biphenyls (PCBs) may not be eliminated by such heat treatment. NOTE: Volumetric ware should not be muffled. The glassware should be stored immediately after drying to prevent

glassware should be stored immediately after drying to prevent accumulation of dust or other contaminants. Store inverted or cover mouth with foil.

1.2 Calibration - Individual Kuderna-Danish concentrator tubes and/or centrifuge tubes used for final concentration of extracts must be

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*Methods for Organic Pesticides in Water and Wastewater," 1971, Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio, 45268

accurately calibrated at the working volume. This is especially important at volumes below 1 ml. Calibration should be made using a precision microsyringe, recording the volume required to bring the liquid level to the individual graduation marks. Glass A volumetric ware should be used for preparing all standard solutions.

2. Standards, Reagents and Solvents

2.1 Analytical Standards and Other Chemicals - Analytical reference grade standards should be used whenever available. They should be stored according to the manufacturer's instructions. Standards and reagents sensitive to light should be stored in dark bottles and/or in a cool dark place. Those requiring refrigeration should be allowed to come to room temperature before opening. Storing of such standards under nitrogen is advisable.

2.1.1 Stock Standards - Pesticide stock standards solutions should be prepared in 1 µg/µl concentrations by dissolving 0.100-grams of the standard in pesticide-quality hexane or other appropriate solvent (Acetone should not be used since some pesticides degrade on standing in this solvent) and diluting to volume in a 100 ml ground glass stoppered volumetric flask. The stock solution is transferred to ground glass stoppered reagent bottles. These standards should be checked frequently for signs of degradation and concentration, especially just prior to preparing working standards from them.

2.1.2 Working Standards - Pesticide working standards are prepared from the stock solutions using a micro syringe preferably equipped with a Chaney adapter. The concentration of the working standards will vary depending on the detection system employed and the level of pesticide in the samples to be analyzed. A typical concentration (0.1 ng/µl) may be prepared by diluting 1 µl of the 1 µg/ul stock to volume in a 10-ml ground glass stoppered volumetric flask. The standard solutions should be transferred to ground glass stoppered reagent bottles. Preparation of a fresh working standard each day will minimize concentration through evaporation of solvent. These standards should be stored in the same manner as the stock solutions.

2.1.3 Identification of Reagents - All stock and working standards should be labeled as follows: name of compound, concentration, date prepared, solvent used, and name of person who prepared it.

2.1.4 Anhydrous sodium sulfate used as a drying agent for solvent extracts should be prewashed with the solvent or solvents that it comes in contact with in order to remove any interferences that may be present.

2.1.5 Glass wool used at the top of the sodium sulfate column must be pre-extracted for about 40-hours in soxhlet using the appropriate solvent.

2.2 Solvents - Organic solvents must be of pesticide quality and demonstrated to be free of interferences in a manner compatible with whatever analytical operation is to be performed. Solvents can be checked by analyzing a volume equivalent to that used in the analysis and concentrated to the minimum final volume. Interferences are noted in terms of gas chromatographic response - relative retention time, peak geometry, peak intensity and width of solvent response. Interferences noted under these conditions can be considered maximum. If necessary, a solvent must be redistilled in glass using a high efficiency distillation system. A 60-cm column packed with 1/8 inch glass helices is effective.

2.2.1 Ethyl Ether - Hexane - It is particularly important that these two solvents, used for extraction of organochlorine pesticides from water, be checked for interferences just prior to use. Ethyl ether, in particular, can produce troublesome interferences. (NOTE: The formation of peroxides in ethyl ether creates a potential explosion hazard. Therefore it must be checked for peroxides before use.) It is recommended that the solvents be mixed just prior to use and only in the amount required for immediate use since build-up of interferences often occurs on standing.

2.2.2 The great sensitivity of the electron capture detector requires that all solvents used for the analysis be of

requires that all solvents used for the analysis be of pesticide quality. Even these solvents sometimes require

redistillation in an all glass system prior to use. The quality of the solvents may vary from lot to lot and even within the same lot, so that each bottle of solvent must be checked before use.

APPENDIX II

STANDARDIZATION OF FLORISIL COLUMN BY WEIGHT ADJUSTMENT BASED ON ADSORPTION OF LAURIC ACID

1. Scope

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

2. Apparatus

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

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

   2.3 Pipet -- 10 and 20 ml transfer.

2.3 Pipet -- 10 and 20 ml transfer.

2.4 Volumetric flasks -- 500 ml.

3. Reagents and Solvents

3.1 Alcohol, ethyl. -- USP or absolute, neutralized to phenolphthalein.

3.2 Hexane -- Distilled from all glass apparatus.

3.3 Lauric acid -- Purified, CP.

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).

3.5 Phenolphthalein Indicator - Dissolve 1 g in alcohol and dilute to 100 ml.

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 1250 ml Erlenmeyer flask. Add 50 ml neutralized ethyl alcohol and 3 drops phenolphthalein indicator; titrate to permanent end point. Calculate mg lauric acid/ml 0.05 N NaOH (about 10 mg/ml).

4. Procedure

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 min. Let adsorbent settle and pipet 10.0

lauric acid solution (400 mg), stopper, and shake occasionally for 15 min. Let adsorbent settle and pipet 10.0 ml of supernatant into 125 ml Erlenmeyer flask. Avoid inclusion of any Florisil.

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

5. Calculation of Lauric Acid Value and Adjustment of Column Weight

5.1 Calculate amount of lauric acid adsorbed on Florisil as follows:

Lauric Acid value = mg lauric acid/g Florisil = 200 - (ml required for titration X mg lauric acid/ml 0.05N NaOH).

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 6.

6. Test for Proper Elution Pattern and Recovery of Pesticides

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 others in the 6% eluate.

7. References

7.1 "Pesticide Analytical Manual," U.S. Department of Health, Education and Welfare, Food and Drug Administration, Washington, D.C.

7.2 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).

APPENDIX III

CHROMATOGRAPHIC CALIBRATION TECHNIQUE

Relative Calibration (Internal Standardization):

A relative calibration curve is prepared by simultaneously chromatographing mixtures of the previously identified sample constituent and a reference standard in known weight ratios and plotting the weight ratios against area ratios. An accurately known amount of the reference material is then added to the sample and the mixture chromatographed. The area ratios are calculated and the weight ratio is read from the curve. Since the amount of reference material added is known, the amount of the sample consitituent can be calculated as follows:

$$\text{micrograms/liter} = \frac{Rw \times Ws}{Vs}$$

Rw = Weight ratio of component to standard obtained from calibration curve

Ws = Weight of internal standard added to sample in nanograms

Vs = Volume of sample in milliliters

Using this method, injection volumes need not be accurately measured the detector response need not remain constant since changes in response will not alter the ratio. This method is preferred when the internal standard meets the following conditions:

a) well-resolved from other peaks

b) elutes close to peaks of interest

c) approximates concentration of unknown

d) structurally similar to unknown.

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

APPENDIX IV

PREPARATION OF DIAZOMETHANE IN ETHER

1. Scope

   1.1 Diazomethane is prepared by reaction of Carbitol and Diazald in the presence of KOH. Solutions of diazomethane decompose rapidly in the presence of solid material such as copper powder, calcium chloride, boiling stones, etc. These solid materials cause solid polymethylene and nitrogen gas to form.

2. Apparatus

   2.1 Distilling flask with condenser, 125 ml, long neck with dropping funnel.

   2.2 Erlenmeyer flasks - 500 ml and 125 ml.

   2.3 Water bath.

3. Reagents and Solvents

   3.1 Ether

   3.2 Potassium hydroxide pellets.

   3.3 Carbitol (diethylene glycol monoethyl ether).

   3.4 Diazald in ether. Dissolve 21.5 g of Diazald in 140 ml ether.

4. Procedure

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

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

4.2 Dissolve 6-g KOH in 10 ml water in the distilling flask (no heat). Ad 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.

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

5. Cautions

5.1 Diazomethane is very toxic. It can explode under certain conditions. The following precautions should be observed.

5.1.1 Use only in well-ventilated hood.

5.1.2 Use safety screen.

5.1.3 Do not pipette solution of diazomethane by mouth.

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

5.1.5 Do not heat solutions at 100°C (EXPLOSIONS).

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

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

5.1.8 Keep solutions away from alkali metals (EXPLOSIONS).

6. Reference

6.1 "Pesticide Analytical Manual," U.S. Department of Health, Education and Welfare, Food and Drug Administration, Washington, D.C.

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