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METHODS FOR ORGANIC PESTICIDES IN WATER AND WASTEWATER

National Environmental Research Center
Cincinnati, Ohio

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METHODS

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ORGANIC PESTICIDES

IN WATER AND WASTEWATER

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ENVIRONMENTAL PROTECTION AGENCY

NATIONAL ENVIRONMENTAL RESEARCH CENTER

CINCINNATI, OHIO

45268

PREFACE

The use of pesticides has become a routine practice in modern agriculture. While these compounds have great advantages in the control of predatory insects, they represent a possible danger to the aquatic environment when present in even trace concentrations.

The National Technical Advisory Committee on Water Quality Criteria has recommended "that environmental levels . . . not be permitted to rise above 50 nanograms/liter". Many of the states have incorporated pesticide criteria in their water quality standards. Therefore, the monitoring of surface waters for pesticides is an essential part of our measurement of water quality.

The Analytical Quality Control Laboratory, assisted by Environmental Protection Agency scientists experienced in the determination of pesticides, has prepared the following method for organochlorine pesticides. In the opinion of the AQC Laboratory and its advisors, this method is the best available procedure at this time.

Because methods development and selection is a dynamic process, requiring continual efforts toward improvement, comments on the application of the method and suggestions for improvements are solicited from the analysts in the field. These comments should be addressed to:

Director, Analytical Quality Control Laboratory
Environmental Protection Agency
National Environmental Research Center
Cincinnati, Ohio 45268

With the concurrence of the Office of Pesticides, the method has been adopted as the EPA Method for Organochlorine Pesticides and is recommended for use by all laboratories in acquiring data on the concentration of these materials in waters and wastewaters sampled by EPA.

Dwight G. Ballinger

Dwight G. Ballinger, Director
Analytical Quality Control Laboratory

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The critical review of this manual by several members of the Office of Pesticides, the Fish and Wildlife Service, and the U.S. Geological Survey is gratefully acknowledged.

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ORGANIC PESTICIDES IN WATER AND WASTEWATER

INTRODUCTION

Advances in the science of analytical chemistry in recent years are typified by constant new developments of methods which yield greater efficiency, selectivity, and sensitivity. As a result, the analytical chemist now has the means to measure minute quantities of pesticides, either singly or in combination. Laboratories capable of this degree of measurement are now commonplace; making possible pesticide pollution data for every major stream in the nation.

The increasing sophistication of data storage and retrieval systems and the every expanding toxicological and ecological information on the impact of pesticides, permits data obtained by several laboratories to be used in the combined assessment of pollution in a given river system or in the nation's major streams, no matter where located.

This growing use of data dictates development of effective means to minimize procedural error within each laboratory and optimize analytical agreement between laboratories through use of a standardized method. The Environmental Protection Agency's "Methods for Organic Pesticides in Water and Wastewater", presented herein, are designed to provide the means of obtaining such agreement and validity.

Part I of this manual, entitled "Recommended Practice for the Determination of Organic Pesticides in Water" presents a general discussion, helpful hints and suggestions, and precautionary measures required for pesticide analyses. Succeeding chapters present stepwise procedures for various types of pesticides and types of samples. This format was chosen to provide short, concise, and easy-to-follow methods, to facilitate the

inclusion of additional methods and revisions of existing methods as they are developed and found to be acceptable, and to emphasize the analytical quality control aspects of pesticide analysis.

The Environmental Protection Agency methods offer several analytical alternatives, depending on the analyst's assessment of the nature and extent of interferences and the complexity of the pesticide mixtures found. They are recommended for use only by experienced residue analysts or under the close supervision of such qualified persons.

PART I - RECOMMENDED PRACTICE FOR DETERMINATION OF ORGANIC PESTICIDES IN WATER AND WASTEWATER

1. General Information

1.1 Introduction - Part I of this manual is intended to provide general information, helpful hints and suggestions, and precautionary measures which experience has shown to be important in producing consistently reliable results. Part I is by no means complete and other references, such as the Food and Drug Administration's "Pesticide Analytical Manual" (1), The Canadian Department of Agriculture's "Guide to the Chemicals Used in Crop Protection" (2), and "Official Methods of Analysis of the Association of Official Analytical Chemists" (3), should also be consulted by pesticide residue analysts. Other helpful references for general practice and analytical quality control are ASTM Part 30 "Tentative Recommended Practice for General Gas Chromatography Procedures" (4), and the Environmental Protection Agency manual "Control of Chemical Analyses in Water Pollution Laboratories" (5). Additional recommended references giving fundamentals of chromatography in general, and gas chromatography in particular, are listed in the bibliography.

1.2 Sample Collection - Wide mouth glass bottles equipped with Teflon-lined screw caps should be used for sample collection. Plastic bottles must not be used since they are known to introduce interference and absorb pesticides. The size of the sample is dictated by the sensitivity required for a particular purpose and the detection system to be employed. The normal sample volume requirements are given in the individual methods of Part II. If analysis by more than one method is to be performed, sufficient sample must be collected to supply the need of each analysis. In addition,

sufficient sample should be collected to permit running of duplicate and spiked analyses. Breakage of glass sample bottles is overcome by shipping them in expanded polystyrene containers molded to fit the bottles. Refer to ASTM Standards, Part 23, D510 for further sampling recommendations (6).

1.3 Sample Handling - The sample collector should provide the following information in writing: date, time, location (coordinates or river mile, city, etc.), depth, suspected contaminants, type of sample (surface water, waste discharge, etc.), name of sample collector, as well as any other information that may be helpful in selecting the analytical approach as well as in interpreting results. Upon receipt in the laboratory, samples should be logged in immediately. Due to the instability of many of the pesticides in water (7) (8) (9), samples should be extracted and analyzed as soon as possible after collection. If samples must be stored, they should be placed in a cool dark place, preferably in a refrigerator. Holding time and conditions of storage should be reported along with results.

1.4 Glassware

1.4.1 Cleaning Procedure - It is particularly important that glassware used in pesticide residue 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 soap water, rinsing with tap water, distilled water, redistilled acetone and finally with pesticide quality hexane. Heavily contaminated glassware may require muffling at 400C for 15 to 30 minutes. High boiling materials,

such as some of the polychlorinated biphenyls (PCB's) 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 accumulation of dust or other contaminants. Store inverted or cover mouth with foil.

1.4.2 Calibration - Individual Kuderna-Danish concentrator tubes and/or centrifuge tubes used for final concentration of extracts must be 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. Class A volumetric ware should be used for preparing all standard solutions.

1.5 Standards, Reagents and Solvents

1.5.1 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.

1.5.1.1 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.

1.5.1.2 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 solution 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.

1.5.1.3 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.

1.5.1.4 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.

1.5.1.5 Cotton used at the top of the sodium sulfate column must be pre-extracted for about 40 hours in soxhlet using the appropriate solvent. A cheap grade of cotton is recommended. Red Cross cotton is not recommended.

1.5.2 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 concen-

trated 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.

1.5.2.1 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.

The great sensitivity of the electron capture detector 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.

1.6 Records

1.6.1 The progress of the sample through the analysis should be recorded in permanently bound notebooks or on laboratory data cards. Dates of extraction, clean-up and separation and G.C. analysis and date of reporting results should be recorded.

1.6.2 All evidence accumulated during the analysis: gas chromatograms, photographs of thin-layers, infrared spectra, etc. should be

retained for as long as may be required to fulfill the purpose for the analysis.

1.6.3 All results should be recorded on laboratory data cards or bound notebook so as to provide a permanent laboratory record. Where appropriate, the data should be entered into STORET.

2. Common Analytical Operations

2.1 Method Blank - A method blank must be determined whenever a sample or group of samples is analyzed. This is done by following the procedure step by step including all reagents, solvents, and other materials in the quantity required by the method concurrently and under conditions identical to those for the samples. Additional blanks are required whenever a new supply of any of the reagents, solvents, etc. is introduced.

2.2 Sample Transfer - The utmost in care and technique must be exercised in order to assure quantitative transfer of extract solutions from one vessel to another throughout the analysis. Careless technique will introduce determinate errors and produce inaccurate results. The internal wall of the vessel must be carefully rinsed several times (usually three) with a volume of the particular solvent appropriate for the analysis involved. Final flushing while pouring into the receiving vessel is recommended.

2.3 Concentration of Extracts

2.3.1 Kuderna-Danish (K-D) Evaporation - A Snyder column, evaporative flask and calibrated receiver ampul are employed. The evaporative flask should be filled to no more than 60% capacity. Set the K-D assembly over a vigorously boiling water bath or a live steam bath. The evaporation

must be carefully attended to avoid loss of pesticides. The water level should be maintained just below the lower joint, and the apparatus mounted so that the lower rounded surface of the flask is bathed in steam. Carry out the evaporation in a hood so that solvent vapors are exhausted. When the solvent no longer actively distills, the K-D apparatus is removed from the bath and allowed to cool. The condensed solvent is allowed to drain into the ampul before dismantling.

2.3.2 Final Concentration - Concentration below 5 ml is usually required when analyzing surface water samples. Final evaporation to a minimum of 0.2 ml may be accomplished in the ampul with the aid of a gentle stream of clean dry nitrogen or air in a warm water bath, adjusted to the temperature prescribed by the method. Final evaporation may also be accomplished in the ampul using a micro Snyder column to give a final volume of 0.2-0.4 ml. In the latter case, a small sand-size boiling chip is added to the ampul prior to evaporation. The extract volume is reduced to 0.1 ml below the volume sought so that the internal wall of the ampul may be rinsed. This step is carried out at least three times. Great care must be exercised to prevent the extract from going to dryness.

3. Gas-Liquid Chromatography

3.1 Gas Chromatographic System - The gas chromatographic system employed must be demonstrated to be suitable for the determination of pesticides with a minimum of decomposition and loss of compounds of interest. The analyst must evaluate the individual system to demonstrate such capability. Detectors, column packings, column conditioning as well as suggested operating conditions are given below.

3.1.1 Injection Systems - Many organic compounds, pesticides in particular, decompose at elevated temperatures when they come in contact with stainless steel. To prevent this decomposition, the inlet port of the gas chromatograph must be capable of accepting a quartz or pyrex glass insert. To avoid bleed off that may cause high background or discrete interferences, the septa should be preconditioned prior to use. The septa can be treated by heating in a vacuum oven at 250 C for two hours; changing the septum at the end of each work day to allow overnight purging of the system is also good practice.

3.1.2 Detectors - The analyst must thoroughly acquaint himself with the descriptive and theoretical information on the detectors employed. Review of technical papers and manufacturer's instructions on a specific detector is necessary to become familiar with its use and limitations.

3.1.2.1 Electron Capture Detector (EC) - The electron capture detector is extremely sensitive to electronegative functional groups, such as halides, conjugated carbonyls, nitriles, nitrates, and organometallics. It is virtually insensitive to hydrocarbons, amines, alcohols and ketones (10) (11). The selective sensitivity of halides makes this detector particularly valuable for the determination or organochlorine pesticides. It is capable of detecting picogram (10^{-12} gram) quantities of many organochlorine pesticides. Organophosphorus pesticides containing nitro- groups are also detected, although with much less sensitivity.

Electron capture detectors may be of parallel plate and concentric tube or concentral design and employ one of two ionization sources: tritium (H^3) or radioactive nickel (Ni^{63}). The tritium detector has a temperature limit of 225 C (It should not be operated above 210 C.)

which makes it susceptible to a buildup of high boiling contaminants which reduce its sensitivity and require frequent clean-up. The nickel detector, on the other hand, can be operated or baked out up to 400 C, to reduce contamination and cleaning problems.

3.1.2.2 Microcoulometric Titration Detector (MC) - The microcoulometric detector (12) is selective for halogen containing compounds, except fluorides, when used with the halogen cell. Under optimum conditions, this detector is capable of detecting 5-20 ng of organochlorine pesticides. Although the sensitivity of this detector is not as great as that of electron capture, the high degree of specificity makes it a very valuable instrument for qualitative identification as well as for minimizing sample clean-up. Under the proper oxidative-reductive conditions, the system can be made specific for sulfur, phosphorus, and nitrogen compounds.

3.1.2.3 Electrolytic Conductivity Detector (ECD) - The electrolytic conductivity detector has a sensitivity 2 to 3 times greater than the microcoulometric system. Although perhaps slightly less selective than the MC, it is, nonetheless, effective for qualitative identification, and cleanup appears to be less of a problem (13). Use of the electrolytic conductivity detector in the reductive mode with a platinum catalyst is recommended when determining halogen compounds. If the oxidative mode is used, a scrubber must be employed to remove SO_2, which also responds to the detector.

3.1.2.4 Flame Photometric Detector (FPD) - The flame photometric detector is selective for sulphur and phosphorus (14). With the use of a dual head, the detector is capable of simultaneously measuring both sulfur and phosphorus as well as a normal flame ionization response. Using a single head, either sulfur or phosphorus and normal flame ionization

response is measured. The characteristic optical emissions of sulfur and phosphorus are measured using filters with transmission at 394 mμ (sulfur) and 526 mμ (phosphorus). The FPD is capable of detecting sub-nanogram quantities of both sulfur (4×10^{-11} gram) and phosphorus (10^{-11} gram).

3.1.3 Columns - A well-prepared column is essential to an acceptable gas chromatographic analysis. The most advanced gas chromatographic instrumentation available is no better than the column used with it. A well-conditioned efficient column is a must. Column packings may be prepared by the analyst or purchased already prepared from a supply house that specializes in this service. The packing materials and column dimensions selected by EPA are specified under the individual methods in Part II.

3.1.3.1 Preparation of Column Packing - The analyst who prepares his own packing must develop a highly refined technique to produce consistently good efficient columns. Particular care should be taken to accurately measure loadings, uniformly distribute the liquid phase, and to preserve the structure of the fragile solid support. Improved column efficiency is obtained when the solid support is dried at 100 C overnight prior to coating. Several methods may be employed to coat the packing material: slurry (15), filtration (16), and frontal analysis (17).

The slurry technique consists of dissolving a weighed amount of the liquid phase in an appropriate solvent in a beaker and slowly pouring the weighed solid support into it with constant stirring. The beaker is immersed to the level of the solvent in a hot water bath and gently stirred until the bulk of the solvent has evaporated. Extra care must be exercised to minimize crushing of the solid support. The filtration

technique consists of mixing the solid support with the solution of liquid phase as above then carefully pouring into a Buchner funnel fitted with a filter flask. The excess solution is removed by vacuum. The frontal analysis technique consists of passing the solution through a column of the solid support until the effluent from the column is of the same composition as the original solution. The analyst should select the method that provides the best results for him. Drying of the coated support may be accomplished by spreading on a tray in a convection or vacuum oven at 100-120 C, with a rotary evaporator (18) or with a fluidized bed drier (19). The latter is recommended.

3.1.3.2 Column Material and Dimensions - The column should be constructed of borosilicate glass. The most useful length of column is about 6 ft. with a diameter of 1/4 in. O.D. to 1/8 in. O.D., depending on the detector employed and the volume of sample injected. Electron capture detectors of parallel plate design and the Tracor concentral design perform well with either 1/4 in. or 1/8 in. columns, while the Varian-Aerograph concentric tube design operates best with a 1/8 in. column. A 1/4 in. column for the microcoulometric detection system is recommended.

3.1.3.3 Packing the Column - It is important that the column be packed to a uniform density not so compact as to cause unnecessary back pressure and not so loose as to create voids during use. Care should be exercised so as not to crush the packing. Column tubing should be rinsed with solvent, eg. chloroform, and dried prior to packing. Columns are filled through a funnel connected by flexible tubing to one end. The other end of straight or coiled tubing is plugged with about 1/2 in. of silanized glass wool and filled with the aid of gentle vibra-

tion or tapping. A mild vacuum may also be applied to the plugged end. When filled the open end is also plugged with silanized glass wool. In a similar manner, one-half of a "U" shaped column is filled and then the other and the ends are plugged with silanized glass wool.

3.1.3.4 Column Conditioning - Proper thermal conditioning is essential to eliminate column bleed and to provide acceptable gas chromatographic analyses. A number of procedures may be used for this purpose. The procedure described below is used by the Analytical Quality Control Laboratory with excellent results:
Install the packed column in the oven. Do not connect the column to the detector. However, gas flow through the detector should be maintained. This can be done using the diluent gas line or, in dual column ovens, by connecting an unpacked column to the detector. Heat the oven to near the maximum recommended temperature for the liquid phase without gas flow for 2 hours. Reduce the oven temperature to approximately 40 C below the maximum recommended temperature and allow temperature to equilibrate for a minimum of 30 minutes still without flow. Then adjust the carrier gas flow to about 50 ml per minute for a 1/4 inch column and about 25 ml per minute for a 1/8 inch column. (Caution--bleed off of liquid phase will occur if not fully temperature equilibrated.) After one hour, increase the temperature to about 20 C above normal operating temperature with gas flow for 24-48 hours. (Do not exceed maximum recommended operating temperature.) Cool down and connect column to the detector system, then raise to normal operating temperature. Columns prepared and conditioned in this manner should yield good chromatograms with no further treatment.

3.1.3.5 Optimizing Operating Conditions - The analyst must determine the optimum conditions for obtaining the best results for the compounds

under study. Standard mixtures of interest should be chromatographed to determine retention times and maximum resolution that can be achieved. Parameters such as gas flow, temperature, column length and diameter, as well as the electronics and detector performance, must be evaluated and adjusted as required to achieve the desired results. Other important requirements for attaining optimum operating conditions include a clean injection block and a leak-free pneumatic system. The instrument must be operated within the linear range of the detector. The recorder gain and damping adjustments must be optimized. Regulated electric line current may be required for the electronic system. Detailed instructions for carrying out these operations are given in the manufacturer's instrument manuals.

Optimum detector sensitivity for each method is defined as the minimum acceptable response to a designated amount of a selected compound. The detector response for other compounds that may be determined by the method relative to the selected compound are listed in each method.

Continued optimum performance is maintained by following the routine maintenance and check program given in the instrument manuals. Frequent checks on the injection block, oven, and detector temperatures should be made. To avoid the risk of system or detector contamination from impurities in gas cylinders, replace the cylinders when the pressure reaches 200 psi. Use of molecular sieve gas-filter driers on all gases is recommended.

Column performance is monitored by observing daily response to a selected standard mixture and comparing it to the response obtained under previously established conditions. Changes in elution pattern, relative proportions of peaks, and peak geometry are signs of a deteriorating column, when all other parts of the system are properly maintained. Columns should be replaced when deterioration is observed.

3.2 Injection into the Gas Chromatograph

3.2.1 Loading Syringe and Measuring Volume Injected - The analyst must develop the ability to make accurate and reproducible injections into the gas chromatograph. Several techniques may be used. The analyst should select the one that provides the best results for him. One technique, preferred by some analysts is as follows:
Wet syringe needle and barrel with solvent solution of the standard or sample to be injected and expel all air bubbles. Draw the entire quantity of solution into the calibrated barrel and note volume. Inject into the chromatograph rapidly and withdraw syringe immediately. Then partially withdraw plunger and note volume remaining. Determine volume injected by subtracting this volume from the original volume. It is important that the syringe be thoroughly cleaned after each injection. Usually, several solvent rinses are adequate.

3.2.2 Injection of Standard Solutions - The concentration of standard solutions should be such that the injection volume of the standard is approximately the same as that of the sample.

3.3 Qualitative Analysis - Qualitative identification of an unknown component is made by matching the retention time (R_t) of the unknown with that of a standard obtained under identical conditions. The R_t is the time lapsed from injection (time zero) to the peak maximum. The absolute and relative retention time (RR_t) are commonly recorded. The RR_t is defined as the R_t (component) ÷ R_t (reference compound). When solvent response is observed, as with an electron capture, the leading edge of the solvent peak is considered time zero. When no solvent response is observed, as with a microcoulometric detector, time zero is electrically or manually marked immediately after injection.

3.3.1 Confirmatory Identification - The analyst must be aware that a single gas chromatographic determination does not provide unequivocal identification of an unknown component. The retention time and peak geometry must be matched on two or more unlike columns. Co-injection of the sample with a standard of the suspected compound will assist in confirming the qualitative identification. Clean-up and separation techniques such as thin-layer and column chromatography also help to make the qualitative assignment. Identification of multicomponent pesticides requires not only matching of all retention times and overall peak geometry, but also the correct number and relative proportion of each peak - a so called "fingerprint" of the pesticide. Further corroboration using infrared spectroscopy and/or mass spectrometry should be obtained whenever possible.

3.4 Quantitative Analysis - The quantity of compound present is proportional to the area of the peak and can be used to determine the concentration of the components in a sample. The area measurement is usually preferred; however, peak height measurement may be more accurate when sharp narrow peaks occur. Peak area may be measured by electronic integrator, disc integrator, or by planimeter or may be calculated by taking the peak height x peak width at half height. The planimeter, although less precise than the other techniques, is recommended for measuring the area of unsymmetrical peaks that do not originate at the original baseline. To improve precision, measure area several times and take the average value.

3.4.1 Unresolved Peaks - To quantitate peaks that are not completely resolved, inject standards of the suspected compounds mixed in the same ratio as they occur in the sample and giving response equivalent to that

of the sample. An alternative method is to draw a line perpendicular from the baseline to the low point of the valley between the peaks. Shoulders on larger peaks may be measured, although not accurately, by using as the baseline a line drawn to conform to the shape of the major peak. Resolution may sometimes be accomplished using different G.C. columns and/or by preliminary separation using thin-layer and column chromatography.

3.4.2 Standard Calibration

3.4.2.1 Absolute Calibration (20) - Using the absolute method, pesticide concentrations are determined by direct comparison to a single standard when the injection volume and response are very close to that of the sample. The concentration of pesticide in the sample is calculated as follows:

$$\text{micrograms/liter} = \frac{(A)\quad(B)\quad(Vt)}{(Vi)\quad(Vs)}$$

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

B = sample aliquot area

Vi = volume of extract injected (µl)

Vt = volume of total extract (µl)

Vs = volume of water extracted (ml)

3.4.2.2 Relative Calibration (Internal Standardization) (20) - 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 constituent 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 and 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.

3.4.3 Linear range - Accurate quantitative analysis depends upon a linear relationship between concentration and detector response. The closer the linear relation the more accurate the analysis. The analysis range of a detector is defined as the ratio of the largest to the smallest concentration within which the detector is linear.

4. Column Chromatography

4.1 Adsorbents - A variety of adsorbents are used in the various pesticide methods to remove interferences and to separate individual

pesticides. These are usually purchased preactivated from the manufacturer. The adsorptivity of the adsorbent is checked by determining the elution pattern of specified dyes and/or of given pesticides. Recoveries of the pesticides must be determined prior to using the adsorbent for the analysis of samples.

4.1.1 Florisil - Florisil preactivated by the manufacturer at 1200 F is used. Prior to use, the Florisil is heated for at least 5 hours (overnight is convenient) at 130 C. Although the adsorbent tends to yellow when stored in this manner for several days, it remains satisfactory for use.

4.2 Packing the Column - To pack the column, slowly pour the adsorbent into the column while vigorously tapping it. This will assist in providing a uniform packing and minimize channeling during elution.

4.3 Eluting the Column - Liquids should be poured slowly down the inside wall of the column to avoid disturbing the surface of the adsorbent. Mixing of solvents above the adsorbent is minimized by adding succeeding solvents just as the last of the previous solvent reaches the adsorbent surface. However, the surface of the adsorbent must not be allowed to run dry, since introduction of air may cause channeling and reduce the efficiency of separation.

4.3.1 Pre-elution - Prior to addition of the sample, the column is pre-eluted with the solvent prescribed by the procedure. This is done to remove trapped air and trace contaminants that may interfere with the analysis. It may be necessary to tap the column to free all of the trapped air.

4.3.2 Introduction and Elution of Sample - The sample is introduced just as the last of the pre-eluting solvent reaches the surface of the adsorbent. The sample container is then rinsed with a few ml of the solvent and the rinse added to the column. Just as the last of this solvent reaches the surface a small volume of eluting solvent is used to rinse down the internal wall of the column. Then the remaining eluting solvent is added. Successive eluting solvents are added in a similar manner.

4.3.3 Eluate Composition - Since variations in elution pattern may occur from time to time, it is necessary to demonstrate that the eluate composition is proper for a given analyses. This can be done by eluting standard pesticides from the column and/or by using the activity test given in the "Official Methods of Analysis of the AOAC" (3).

5. Thin-Layer Chromatography

5.1 Equipment - Special equipment required for preparing layers and carrying out thin-layer chromatography is listed in the appendix. Layers prepared in the laboratory or purchased precoated layers may be used. The adsorbent is usually less tightly bound to the plate when prepared in the laboratory and is thus somewhat easier to scrape for subsequent elution and recovery of the pesticides.

5.2 Layer Preparation - Layers of desired thickness are prepared by making a homogeneous slurry of adsorbent in water. The slurry is poured into the applicator and the gate is quickly opened and the applicator is smoothly and rapidly passed over an aligning tray holding the glass plates. The layers are allowed to stand at room temperature for a time, then activated

in an oven, and stored in a desiccator for future use. Layers stored longer than one week should be reactivated before use. Prior to activation, marks should be made on the layer to define the spotting line and the upper limit of solvent development in order to minimize exposure to the atmosphere during the spotting operation.

5.3 Preparation of Developing Chamber - The developing solvent is added to the chamber, and two chromatography paper wicks, one on each of the long sides of the chamber, are placed so that the entire side is covered and the bottom edge contacts the solvent. The chamber is closed, shaken, and allowed to equilibrate. It is important that the chamber be protected from drafts and large temperature changes.

5.4 Spotting the Layer - Standards are spotted in the center and at least at one edge of the layer. The standards and samples should be dissolved in the same solvent and spotted in the same volume. Utmost care must be exercised to keep the spot small (less than 10 mm diameter). A gentle stream of clean dry air or nitrogen may be applied over the spot to facilitate close boundary evaporation, but this gas flow exposure should be kept to a minimum.

5.5 Developing the Layer - The spotted layer is placed in the pre-equilibrated chamber so that the bottom edge is in contact with the developing solvent and the lid is replaced. When the developing solvent reaches the upper reference mark, the layer is removed from the chamber and allowed to air-dry at room temperature.

5.6 Visualizing and Sectioning the Layer - After development, the portion of the layer containing the standards is sprayed evenly with a

chromogenic agent. The sprayed area is allowed to thoroughly dry and, where required, is further treated by exposure to short wave UV light or some other means. The location of each standard pesticide is marked.

5.6.1 The distance of travel for pesticides present in the unknown samples and recovery test standards will be, respectively, the same as those of the sprayed standards. Using this information, the vertical zone for each sample is divided into horizontal sections depending on the pesticides being determined.

5.7 Pesticide Removal from the TLC Plates - With the aid of a sharp pointed object, the silica gel sections of interest are individually ruled off. With the aid of a mild vacuum, the silica gel, first from the periphery of the section and then from the center of the section is collected. It is convenient to use a medicine dropper plugged at the tip with filtering grade glass wool (Figure 1). The pesticides are eluted quantitatively into a K-D ampul with a selected solvent to an appropriate volume for gas chromatographic analysis.

PART II - METHODS OF ANALYSIS

A. METHOD FOR ORGANOCHLORINE PESTICIDES

1. Scope and Application

1.1 This method covers the determination of various organochlorine pesticides, including some pesticidal degradation products and related compounds. Such compounds are composed of carbon, hydrogen, and chlorine, but may also contain oxygen, sulfur, phosphorus or nitrogen.

1.2 The following compounds may be determined individually by this method: BHC, lindane, heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin, Perthane, DDE, DDD, DDT, methoxychlor, endosulfan, γ-chlordane and sulphenone. Under favorable circumstances, Strobane, toxaphene, kelthane, chlordane (tech.) and others may also be determined.

1.3 When organochlorine pesticides exist as complex mixtures, the individual compounds may be difficult to distinguish. High, low, or otherwise unreliable results may be obtained through misidentification and/or one compound obscuring another of lesser concentration. Provisions incorporated in this method are intended to minimize the occurrence of such interferences.

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. This method is recommended for use only by experienced residue analysts or under the close supervision

of such qualified persons. Specifically, the procedure describes the use of an effective co-solvent for efficient sample extraction; provides, through use of thin-layer, column chromatography, and liquid-liquid partition methods for the elimation of non-pesticide interferences, and the pre-separation of pesticide mixtures. Identification is made by selective gas chromatographic separations through the use of two or more unlike columns. Detection and measurement is accomplished by electron capture, microcoulometric or electrolytic conductivity gas chromatography. Techniques for confirming qualitative identifications are suggested. Results are reported in micrograms per liter without correction for recovery data; but, such data is to be included in the report.

3. Significance

3.1 The extensive and widespread use of persistent organochlorine pesticides has resulted in their presence in all parts of our environment. Their occurrence in surface waters throughout the country is common. Such common occurrence coupled with the toxic nature of these materials is cause for concern. The known lethal effects of these substances to fish and wildlife and the unknown long term consequences to humans make it imperative that we identify and quantitate the pesticides present in the environment. Effective evaluation and control programs require such information.

3.2 Because of the concept of biological concentration, we need to detect minute quantities (low nanogram amounts) of pesticides in water. The method presented here is capable of detecting these small quantities.

4. Interference

4.1 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 is required. (Refer to Part 1, Sections 1.4 and 1.5)

4.2 Sample treatment required to remove non-pesticide materials which cause interference may result in the loss of certain organochlorine pesticides. Methods for eliminating or minimizing interferences are described below in the section on Clean-up and Separation Procedures (5.2). It is beyond the scope of this method to describe procedures for overcoming all of the possible interferences that may be encountered, particularly, in highly contaminated water and wastewater.

4.3 Polychlorinated Biphenyls - Special attention is called to industrial plasticizers and hydraulic fluids such as the chlorinated biphenyls (PCB's, Aroclors[1]) which are a potential source of interference in pesticide analysis. Chlorinated biphenyls containing 4 to 8 chlorine atoms per molecule have been reported in extracts of birds, fish, mussels and water. Possible interferences from these compounds are indicated by unresolved peaks (shoulders and nongaussian peaks), slight discrepancies in retention times, and peaks which elute later than p,p'-DDT. With some dependence upon the relative concentrations, a number of chlorinated biphenyl isomers may interfere with the determination of DDE, DDD and DDT isomers. The authenticity of the DDT identification may be determined by treating the extract with alcoholic KOH which converts DDT to DDE (21).

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[1]Tradename of the Monsanto Company, St. Louis, Missouri

Particularly severe PCB interference will require special separation procedures, such as those of Reynolds (22), Armour and Burke (23), and Mulhern, et al (24).

4.4 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, since these materials are not detected by the latter three detectors.

4.5 Organophosphorus Pesticides - A number of organophosphorus pesticides, notably those containing a nitro group, eg, parathion, also respond to the electron capture detector and may interfere with the determination of the organochlorine pesticides. The presence of such compounds is indicated in samples which respond to both the electron capture and flame photometric detectors but not to the microcoulometric halogen detector.

5. Method for Analysis Using Electron Capture Gas Chromatography

5.1 Extraction of Sample

5.1.1 The size of sample taken for extraction 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 help to determine 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 electron capture analysis. The extract should not be concentrated further than required to meet the sensitivity dictated by the purpose for the analysis. Each time a set of samples is extracted, an aliquot of solvent equivalent to that used for extraction is carried through the entire procedure to provide a method blank. To assist in interpretation of results, the pH of the sample is taken prior to extraction. When the volume of the sample permits, one set of duplicates and one doped sample should also be analyzed as a quality control check.

5.1.2 A measured volume (1-liter) of sample is drained into a 2-liter separatory funnel equipped with a Teflon stopcock, and extracted with 60 ml of 15% ethyl ether in hexane by shaking vigorously for two minutes. The sample container is rinsed with each aliquot of extracting solvent prior to extraction of the sample.

5.1.3 The mixed solvent is allowed to separate from the water; the water is then drawn into the original sample container or into a second 2-liter separatory funnel. The organic layer is passed through a small column of anhydrous sodium sulfate topped with a pledget of cotton (previously rinsed with hexane) and collected in a 500 ml Kuderna Danish flask equipped with a 10 ml ampul. The extraction is repeated and the solvent treated as above. Approximately 35 ml of sodium sulfate saturated water is then added to the sample and a third extraction is completed with 60 ml of hexane (not hexane-ethyl ether). This solvent, too, is passed through the sulfate column and collected in the flask. The column is rinsed with several small portions of hexane and this solvent is recovered in the collection flask containing the combined extracts. The extract is concentrated in the Kuderna-Danish evaporator as described in Part I, Section 2.3.1.

5.1.4 Concentration of Extract - The final concentration volume for samples of high pesticide content (eg. pesticide plant wastewater samples)

is adjusted as necessary. Samples containing small quantities of pesticides (low nanogram amounts, eg. most surface water samples) are concentrated to 1 ml. The volume of the initial K-D concentrate is 5 to 6 ml. This is reduced to 1 ml in a warm water bath (70 C) with a gentle draft of clean dry air or nitrogen. The internal wall of the ampul is rinsed several times during this operation. Initial gas chromatographic analysis is made on this volume. Up to 10 µl of extract are injected. If insufficient pesticide is present for detection at this volume and greater sensitivity is required, the extract is concentrated further to a minimum volume of 0.2 ml in the manner described above. The extract volume is reduced below the volume sought so that the internal wall of the ampul may be rinsed. (See Part I, Section 2.3.2) (The volume should never be reduced below 0.1 ml). Repeat this operation three times, exercising great care to prevent the extract from going to dryness.

5.2 Clean-up and Separation Procedures

5.2.1 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) will indicate whether clean-up is required. When these interfere with measurement of the pesticides, proceed as directed below. Whether required for quantitative analysis or not, all extracts should be subjected to these procedures, subsequent to the initial analysis and rechromatographed for qualitative corroboration of the results. Another clean-up technique, acetonitrile partition, although not ordinarily required for surface water extracts, is sometimes useful for cleaning up high organic wastewater samples. Refer to

the FDA methods manual (1) for this procedure.

5.2.2 Florisil Column Adsorption Chromatography - The sample extract previously concentrated to 1 ml or less is diluted to 10 ml. A 15 g charge of activated Florisil is placed in a column over a small layer (one-half inch) of anhydrous granular sodium sulfate. After tapping the Florisil into the column, about a three-fourths inch layer of granular sodium sulfate is added to the top. The column, after cooling, is pre-eluted with about 75 ml of hexane. The pre-eluate is discarded, and just prior to exposure of the sulfate layer to air, the sample extract is quantitatively transferred into the column by decantation and subsequent hexane washings. The elution rate is adjusted to about 5 ml per minute with two eluates collected separately in 500 ml K-D apparatus equipped with 10 ml ampuls. The first elution is performed with 200 ml of 6% ethyl ether in hexane, and the second elution with 200 ml of 15% ethyl ether in hexane. The K-D apparatus containing the eluates are connected to three ball Snyder columns and the solvents are evaporated as described in Part I, Section 2.3. The concentrated extract may be analyzed directly by injecting suitable aliquots from the K-D ampuls into the gas chromatograph. If the residues are high in total organics, they may be further cleaned up and separated by thin-layer chromatography prior to gas chromatographic analyses.

5.2.2.1 Eluate Composition - If the Florisil has been properly activated and stored, and if the reagents are carefully prepared, the following eluate compositions will be obtained when the pesticides are present. The first eluate (6% ethyl ether in hexane) will contain:

lindane	DDE	methoxychlor
BHC	DDD	toxaphene
kelthane	DDT	Strobane
aldrin	Perthane	chlordane (γ & tech)
heptachlor	heptachlor epoxide	endosulfan I

The second eluate (15% ethyl ether in hexane) will contain:

dieldrin	endosulfan II
endrin	lindane (possible trace of total)
	kelthane (possible trace of total)

Polychlorinated biphenyls are recovered in the first eluate and phthalate esters in the second eluate.

5.2.3 Thin-Layer Chromatography - The sample extract (5.1.4) or the eluates from the Florisil clean-up (5.2.2) may be subjected to thin-layer chromatography according to the procedure described below. (Refer to Part I, Section 4.3 for general discussion and conditions for preparation of thin-layers). Silica gel G layers, 250 μ thick, are employed.

5.2.3.1 Spotting the Extract - The extract volume should be adjusted so that no more than 100 μl must be spotted in order to retain adequate GC sensitivity in the TLC eluates when they are reduced to the minimum volume of 0.2 ml. Up to 100 μl of the eluate is then spotted on the thin-layer.

5.2.3.2 Spotting of Standards - A mixture of reference standard pesticides is spotted at the center and at one edge of the layer, in 10-20 μg amounts of each pesticide, to confirm the separation of the individual pesticides by visual observation. It is convenient to use a 100 ng/μl mixture

of endrin, lindane, DDD and DDT. Standards for recovery and instrumental measurement are spotted in the 20 to 100 nanogram range and handled just as a sample would be treated in the subsequent steps of the procedure.

5.2.3.3 Developing the Layer - The layer is developed with carbon tetrachloride. When the solvent front reaches the upper reference mark (10 cm), the layer is removed from the chamber and allowed to air dry at room temperature. The developing solvent should be checked frequently for contamination and changed as necessary.

5.2.3.4 Visualizing and Sectioning the Layer - The portions of the layer containing the samples are covered with a glass plate or cardboard. The portion of the layer containing the standards is sprayed evenly with a fairly heavy coat of Rhodamine B (0.1 mg/ml in ethanol). The sprayed area is allowed to thoroughly dry (about 5 minutes) and then is exposed to and viewed under short wave UV light. The pesticides show up as quenched areas (dark) on a fluorescent background. Mark the location of each pesticide. From this information, the vertical zone for each sample is divided into five horizontal sections. The sections are identified with Roman numerals as shown in Figure 2. Examples of respective Rf and Rr values for various pesticides are listed in Table 1.

5.2.3.5 Removal of Pesticide From the TLC Layer - Using the spotting template as a ruler, and with the aid of a sharp pointed object, the silica gel sections of interest are individually ruled off. With the aid of a mild vacuum, the silica gel, first from the periphery of the section and then from the center of the section, is drawn into a medicine dropper which is plugged at the tip with filtering grade glass wool (Figure 1). The pesticides adsorbed on this silica gel are eluted quantitatively into a 10 ml

K-D ampul with successive small washes of ethyl ether-petroleum ether (1+1) to a total volume of 5 to 10 ml. The ampul is glass stoppered and the contents are retained for gas chromatographic analysis. Prior to gas chromatographic analysis, the extracts are concentrated as required. Refer to 5.1.4.

5.3 Gas Liquid Chromatography

5.3.1 Reasonably positive identification of a pesticide is obtained by corroborating the results using, at least two different types of gas chromatographic columns. To achieve this, a relatively less polar packing [5% OV-17 on Gas Chrom Q (80-100 mesh)] and a more polar packing [5% QF-1 (FS-1265) plus 3% DC-200 on Gas Chrom Q (80-100 mesh)] are employed. Other packings recommended for this purpose are 3% OV-101 on Gas Chrom Q (80-100 mesh) and 3% OV-210 on Gas Chrom Q (80-100 mesh). Packings and packed columns can be obtained from commercial sources or may be prepared in the laboratory.

5.3.2 Preparation of Columns - To prepare the column packings, dissolve 5 g of OV-17 in 225 ml of methylene chloride - chloroform (1+1) in a 500 ml beaker and add 95 g of Gas Chrom Q. Similarly, dissolve 5 g of QF-1 plus 3 g of DC-200 in methylene chloride - chloroform (1+1) and add 92 g of Gas Chrom Q. Dissolve 3 g of OV-101 in chloroform and add to 97 g of Gas Chrom Q. Dissolve 3 g of OV-210 in acetone and add to 97 g of Gas Chrom Q. Proceed as described in Part I, Section 3.1.3.1.

5.3.2.1 Columns of borosilicate glass, 6 ft. long x 1/4 in. O.D. (5/32 in. I.D.) or 1/8 in. O.D. (1/16 in. I.D.) are packed and conditioned according to directions in Part I, Sections 3.1.3.3 through 3.1.3.5. The column oven operating temperature is approximately 210 C for the OV-17 column, 185 C for the QF-1/DC-200 column, 190 C for the OV-101 column, and 185 C for the OV-210 column. The operating conditions are optimized for

the individual instrument as described in Part I. Section 3.1.3.5. Operating conditions are considered acceptable for an electron capture system when the response to 0.3 ng of aldrin is at least 50% of full scale while operating within the linear range of the detector.

5.3.3 Sample Measurement - The volume of sample extract (5.14), the Florisil eluates, or the TLC eluates is noted and suitable aliquots (5-10 µl) are analyzed by gas chromatography, employing at least two columns of varying polarity for identification and quantitation. Standards are injected frequently, as a check on the stability of the operating conditions. Gas chromatograms of several standard pesticides are shown in Figures 3, 4, 5, and 6. The elution order, as well as elution ratios for various pesticides in Table 2, are provided only as a guide. It is the responsibility of the analyst to develop his own identification keys to fit the chosen operating conditions of the instrument.

5.4 Confirmatory Evidence - The qualitative identification of a pesticide should be confirmed using infrared spectroscopy or mass spectroscopy whenever the instrumentation is available and/or the quantity of the compound permits. If this is not possible, gas chromatographic analysis using additional unlike columns and other selective detectors is recommended. Lack of response to the flame photometric detector is negative evidence which supports the identification of organochlorine compounds. Determination of the p-values of an unknown pesticide in several solvent systems will assist in confirming the identification (25).

5.5 Calculation of Results - The pesticide concentrations are determined using the absolute or the relative calibration procedure described in Part I, Section 3.4.2.

5.6 Reporting Results - Report results in micrograms per liter without correction for recovery data. The percent recoveries of known pesticides added to samples or to distilled water as well as the step in the procedure where they were added must also be reported. The recoveries of several organochlorine pesticides from natural waters during collaborative testing are listed in Table 3. The precision of the method within the designated range varies with the concentration as shown in Table 4.

5.6.1 If a sample is reported negative for a given pesticide, the minimum detectable limit for that compound should also be reported. If favorable conditions prevail and ultimate sensitivity is required by the purpose for the analysis, sample response of less than two times the detector noise level (N) should be reported as negative. For sample response at two times the detector noise level, list the result as presumptive. Responses of greater than 2N should be quantified if possible. In cases of questionable identification, the analyst should qualify the reported result to insure the subsequent misinterpretation will not occur.

6. Method for Analysis Using Microcoulometric or Electrolytic Conductivity Gas Chromatography

6.1 Extraction of Sample

6.1.1 The size of sample taken for extraction 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 help to determine the sample size required as well as the final volume to which the extract needs to be concentrated. A 3-liter sample is usually taken for microcoulometric analysis. Since the conductivity

detector is 2 to 3 times more sensitive than the microcoulometric detector, less than 3 liters may be required when using the conductivity detector. If such is the case, the volume of extracting solvents and other reagents are correspondingly decreased.

6.1.2 A measured volume (3 liters) of sample is drained into a 4-liter separatory funnel equipped with a Teflon stopcock, and extracted with 150 ml of 15% ethyl ether in hexane by shaking vigorously for two minutes. The sample container is rinsed with each aliquot of extracting solvent prior to extraction of the sample.

6.1.3 The mixed solvent is allowed to separate from the water and this water is drawn off into the original container or into a second 4-liter separatory funnel. The organic layer is passed through a small column of anhydrous sodium sulfate topped with a pledget of cotton (previously rinsed with hexane) and collected in a 600 ml tall form beaker. The extraction is repeated and the solvent treated as above. Approximately 100 ml of sodium sulfate saturated water is then added to the sample and a third extraction is completed with 150 ml of hexane (not hexane-ethyl ether). This solvent too, after separation, is passed through the column of sodium sulfate. The column is then rinsed with several small portions of hexane and this solvent is recovered in the collection beaker containing the combined extracts. The contents of the beaker are partially evaporated to about 300 ml in a water bath at 70 C applying no air or vacuum and quantitatively transferred to a 500 ml K-D evaporator equipped with a 10 ml receiver ampul. The extract is concentrated in the K-D evaporator as described in Part I, Section 2.3.1.

6.1.4 Concentration of Extract - The final concentration volume of sample extract is adjusted as necessary according to 5.1.4. The use of

a "keeper" is recommended when concentrating below 0.3 ml. Two milligrams of "keeper" is placed in the concentrated extract through syringe addition of 100 µl of 20 µg/µl of Nujol in hexane. This "keeper" will not interfere with mocrocoulometric detection and will prevent major residue losses in this exhaustive evaporation step. Because of interference possibilities, it is not advisable to use a "keeper" in extracts to be analyzed by electron capture.

6.2 Clean-up and Separation Procedures

6.2.1 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) will indicate whether clean-up is required. When these interfere with measurement of the pesticides, proceed as directed below. Whether required for quantitative analysis or not, all extracts should be subjected to these procedures, subsequent to the initial analysis and rechromatographed for qualitative corroboration of the results. Another clean-up technique, acetonitrile partition, although not ordinarily required for surface water extracts, is sometimes useful for cleaning up high organic wastewater samples. Refer to the FDA methods manual (1) for this procedure.

6.2.2 Florisil Column Adsorption Chromatography - Refer to 5.2.2.

6.2.3 Thin-Layer Chromatography - Refer to 5.2.3.

6.3 Gas Liquid Chromatography

6.3.1 Reasonably positive identification of a pesticide is obtained by corroborating the results using, at least two different types of gas chromatographic columns. Refer to 5.3.1.

6.3.2 Preparation of Columns - Refer to 5.3.2. Columns of borosilicate glass, 6 ft. long x 1/4 in. O.D. are employed. The operating conditions are optimized for the individual instrument as described in Part I, Section 3.1.3.5. Conditions are considered optimum when the response to 15 ng and 30 ng of aldrin is at least 50% of full scale for the electrolytic conductivity and microcoulometric detectors, respectively, while operating within the linear range.

6.3.3 Sample Measurement - The volume of sample extract (6.1.4), the Florisil eluates, or the TLC eluates is noted and suitable aliquots (20-100 µl) are analyzed by gas chromatography, employing at least two columns of varying polarity for identification and quantitation. Standards are injected frequently, as a check on the stability of the operating conditions. Gas chromatograms of several standard pesticides are shown in Figures 3, 4, 5, and 6. The elution order as well as the elution ratios for various pesticides are provided in Table 2, only as a guide. It is the responsibility of the analyst to develop his own identification keys to fit the chosen operating conditions of the instrument.

6.4 Confirmatory Evidence - Refer to 5.4

6.5 Calculation of Results - The pesticide concentrations are determined using the absolute or the relative calibration procedure described in Part I, Section 3.4.2.

6.6 Reporting Results - Refer to 5.6.

TABLE 1

SOME Rf AND Rr VALUES OF PESTICIDES DEVELOPED WITH CCl_4 ON SILICA GEL G THIN LAYER

Pesticide	Rf Value	Rr Value	Section
Dieldrin	0.17	0.33	
Endrin	0.20	0.37	II
Heptachlor Epoxide	0.29	0.52	
Lindane	0.37	0.69	
DDD	0.54	1.00	III
γ-Chlordane	0.55	1.02	
Heptachlor	0.67	1.24	
DDT	0.68	1.26	IV
DDE	0.72	1.33	
Aldrin	0.73	1.35	

Rf = distance traveled by the compound divided by the distance traveled by the solvent front.

Rr = distance traveled by the compound divided by the distance traveled by standard p,p'-DDD.

4C

TABLE 2

RETENTION TIMES OF ORGANOCHLORINE PESTICIDES RELATIVE TO ALDRIN

Liquid Phase[1]	3% DC-200 + 5% QF-1	5% OV-17	3% OV-101	3% OV-210	Relative[2] Sensitivity to EC Detector
Column Temp.	200 C	200 C	175 C	160 C	
Pesticide	RRt[3]	RRt[3]	RRt[3]	RRt[3]	
α-BHC	0.40	0.45	0.33	0.54	1.0
Lindane	0.51	0.61	0.42	0.75	1.0
Heptachlor	0.80	0.79	0.76	0.82	1.0
Aldrin	1.00	1.00	1.00	1.00	1.0
Kelthane	1.19	1.52	1.12	2.46	0.1
Heptachlor Epoxide	1.38	1.58	1.30	2.16	0.5
γ-Chlordane	1.53	1.82	1.55	2.12	0.5
Endosulfan I	1.77	2.00	1.70	2.89	0.4
p,p'-DDE	1.93	2.67	2.18	2.91	0.5
Dieldrin	2.10	2.54	2.08	3.65	0.5
Endrin	2.43	3.21	2.33	4.46	0.3
o,p'-DDT	2.62	3.97	3.02	4.04	0.1
Endosulfan II	2.62	3.97	2.45	5.96	0.3
p,p'-DDD	2.68	4.13	2.94	5.61	0.1
p,p'-DDT	3.41	5.19	3.97	6.28	0.2
Methoxychlor	5.26	11.17	6.88	13.52	0.1
Aldrin (Minutes Absolute)	3.76	3.84	2.64	2.28	

[1]All columns glass, 6 ft. long x 4 mm ID, solid support Gas-Chrom Q (80/100 mesh), nitrogen carrier flow 80 ml/min.

[2]Sensitivity factors relative to aldrin.

[3]Retention times relative to aldrin.

TABLE 3

Recovery of Organochlorine Pesticides from Natural Waters

Pesticide	Added Level ng/liter	Recovery Without Cleanup, %	Added Level ng/liter	Recovery With Cleanup, % [a]
Aldrin	15	69	25	68
	110	72	100	65
Lindane	10	97	15	94
	100	73	85	70
Dieldrin	20	108	25	70
	125	85	130	65
DDT	40	101	30	118
	200	77	185	71

[a] Florisil column clean-up used.

TABLE 4

Precision of Method for Organochlorine Pesticides in Natural Waters

Pesticide	Pretreatment	Mean Recovery ng/liter	Precision, ng/liter[a] S_T	S_0
Aldrin	No	10.42	4.86	2.59
	Cleanup	79.00	32.05	20.19
	Cleanup[b]	17.00	9.13	3.48[c]
		64.54	27.16	8.02[c]
Lindane	No	9.67	5.28	3.47
	Cleanup	72.91	26.23	11.49[c]
	Cleanup[b]	14.04	8.73	5.20
		59.08	27.49	7.75[c]
Dieldrin	No	21.54	18.16	17.92
	Cleanup	105.83	30.41	21.84
	Cleanup[b]	17.52	10.44	5.10[c]
		84.29	34.45	16.79[c]
DDT	No	40.30	15.96	13.42
	Cleanup	154.87	38.80	24.02
	Cleanup[b]	35.54	22.62	22.50
		132.08	49.83	25.31

[a] S_T = Overall precision, and

S_0 = Single-Operator precision.

[b] Use of Florisil column cleanup prior to analysis

[c] $S_0 < S_{T/2}$

FIGURE 1

SILICA GEL COLLECTION ASSEMBLY

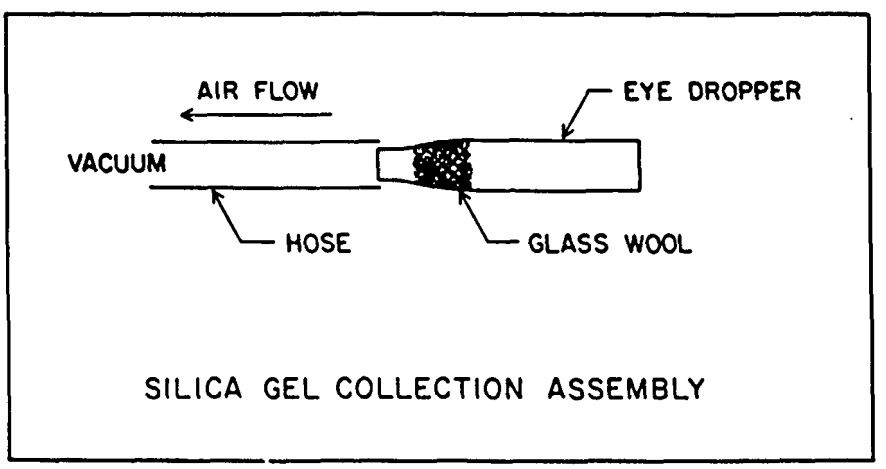


FIGURE 2

DIAGRAM OF DESIGNATION OF TLC SECTIONS IN THE CLEANUP AND SEPARATION ON SILICA GEL PLATES

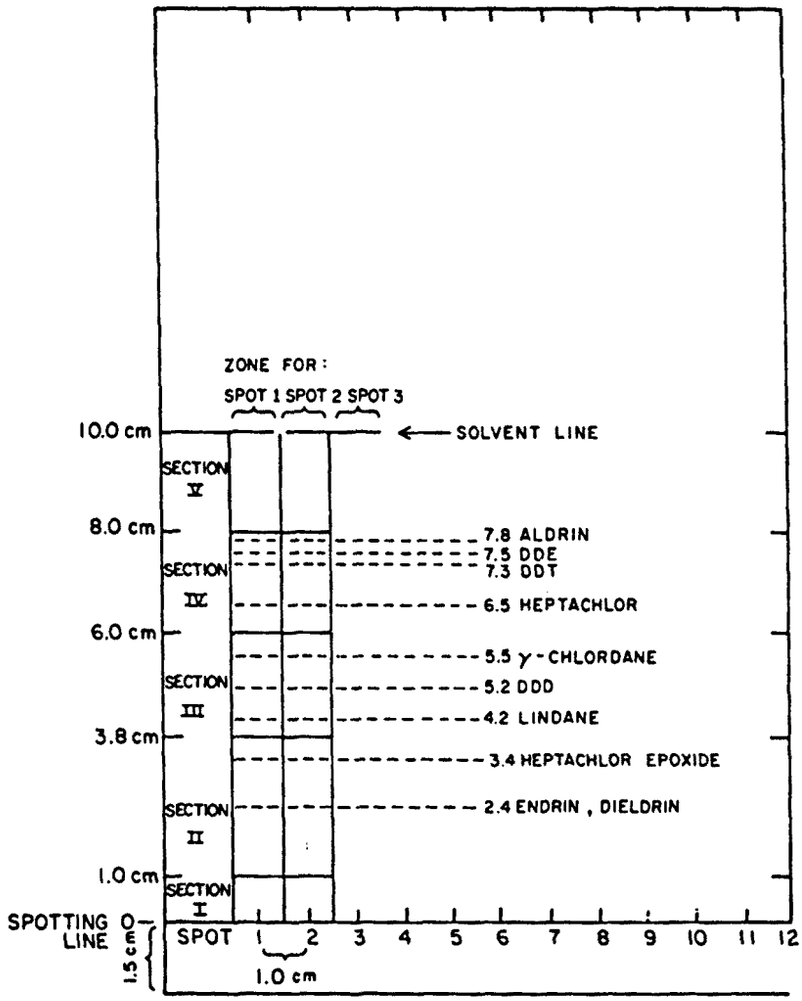
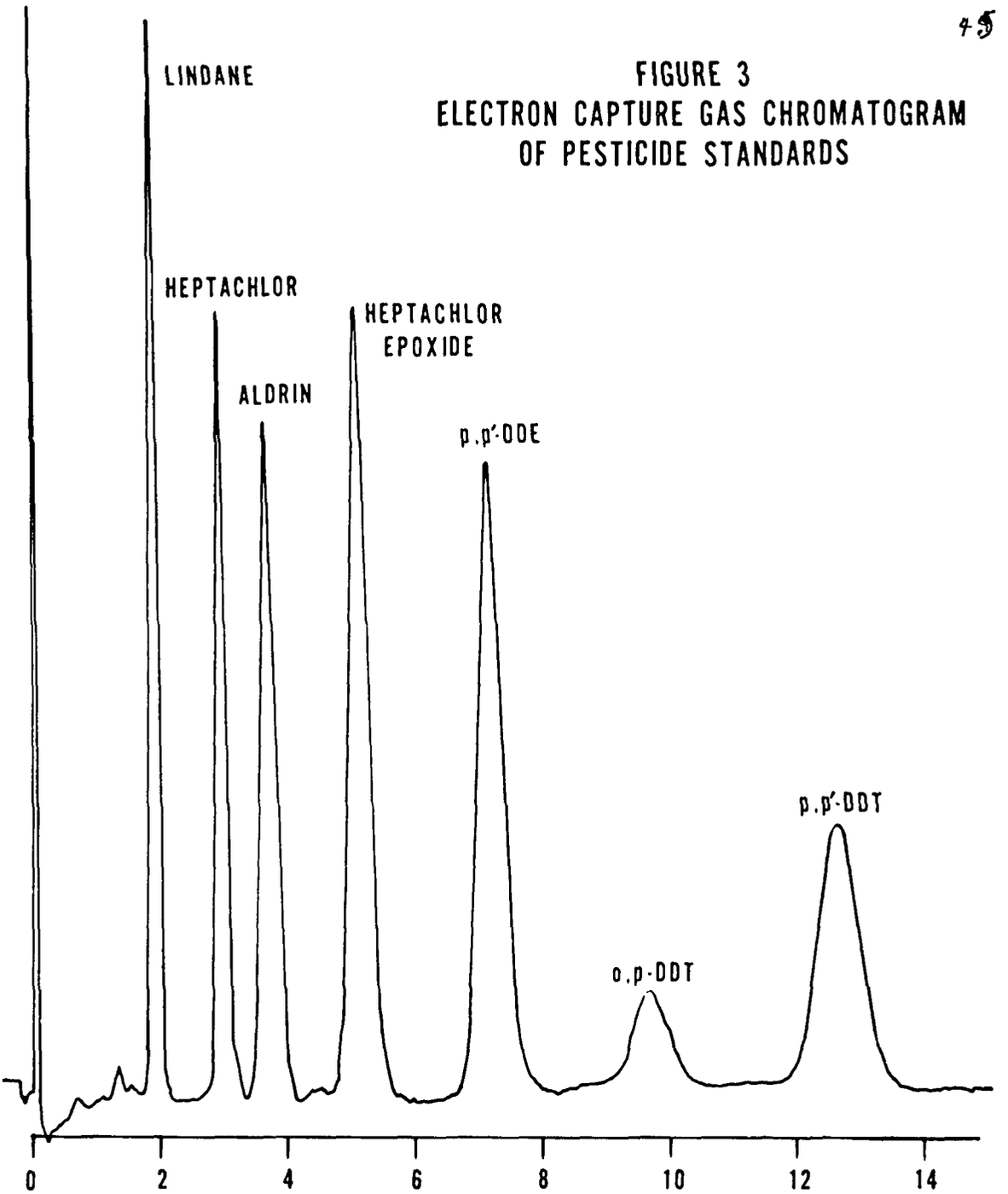


FIGURE 3
ELECTRON CAPTURE GAS CHROMATOGRAM
OF PESTICIDE STANDARDS



RETENTION TIME IN MINUTES
(Chart speed one-half inch per minute)

Column Packing - 3%DC-200+5%QF-1 on Gas Chrom Q (80/100 Mesh)
Carrier Gas - Nitrogen at 80 ml/min.
Column Temperature - 200°C

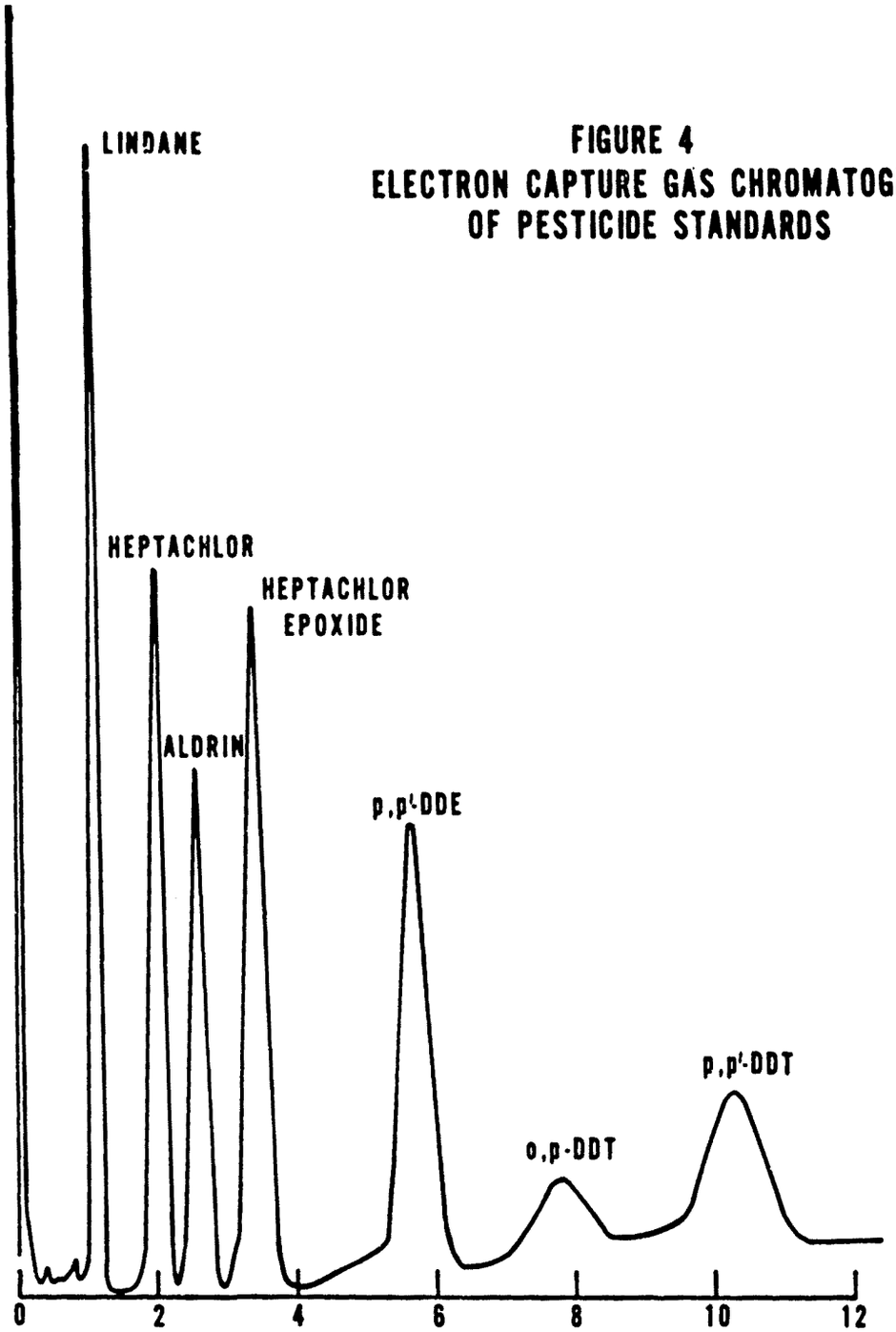
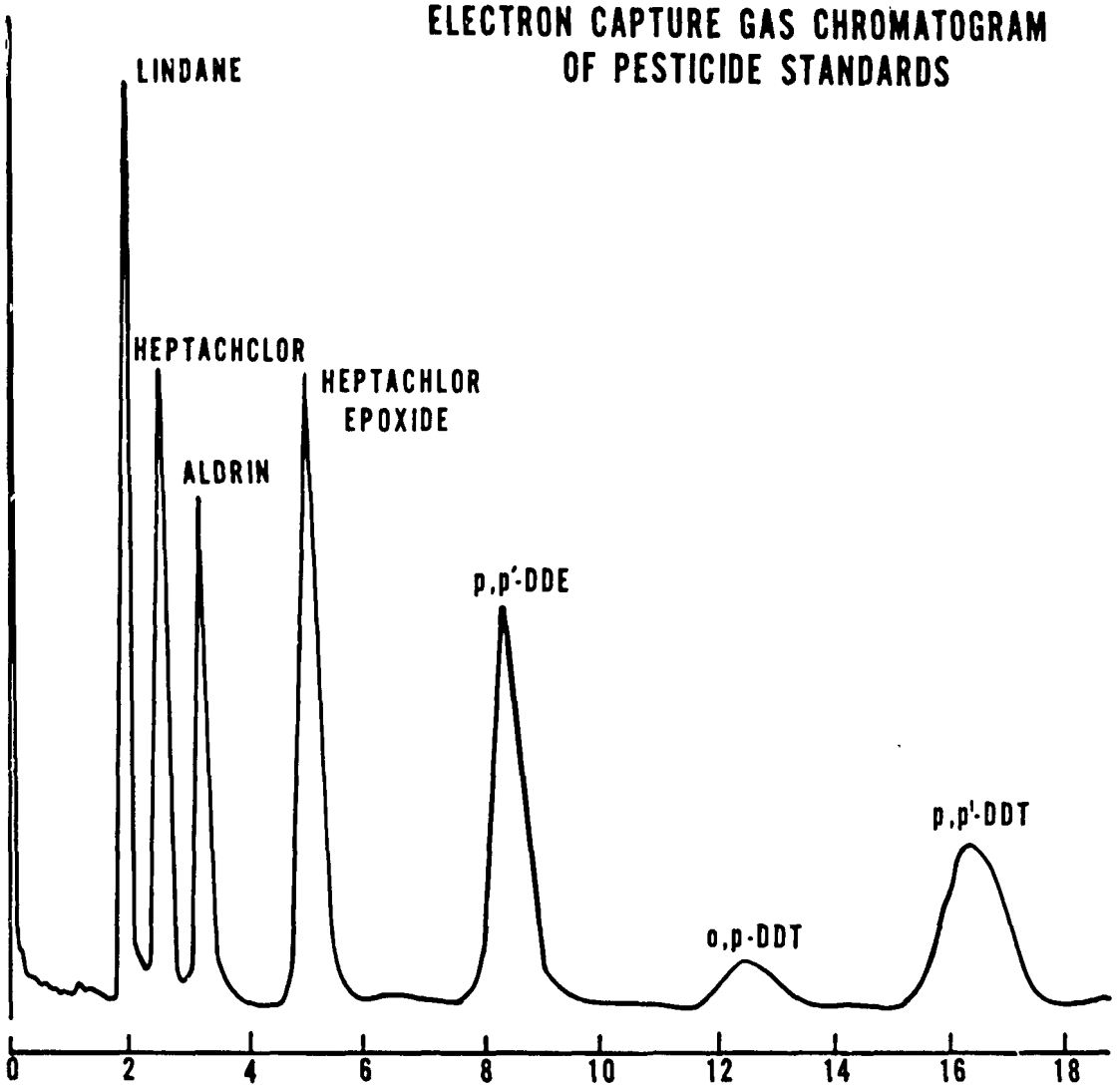


FIGURE 4
ELECTRON CAPTURE GAS CHROMATOGRAM OF PESTICIDE STANDARDS

(Chart speed one-half inch per minute)
Column Packing - 3% OV-101 on Gas Chrom Q (80/100 Mesh)
Carrier Gas - Nitrogen at 80ml/min.
Column Temperature - 175°C

FIGURE 5

ELECTRON CAPTURE GAS CHROMATOGRAM
OF PESTICIDE STANDARDS



(Chart speed one-half inch inch per minute)

Column Packing- 5%OV-17 on Gas Chrom Q (60/80 Mesh)

Carrier Gas - Nitrogen at 80ml/min.

Column Temperature - 200°C

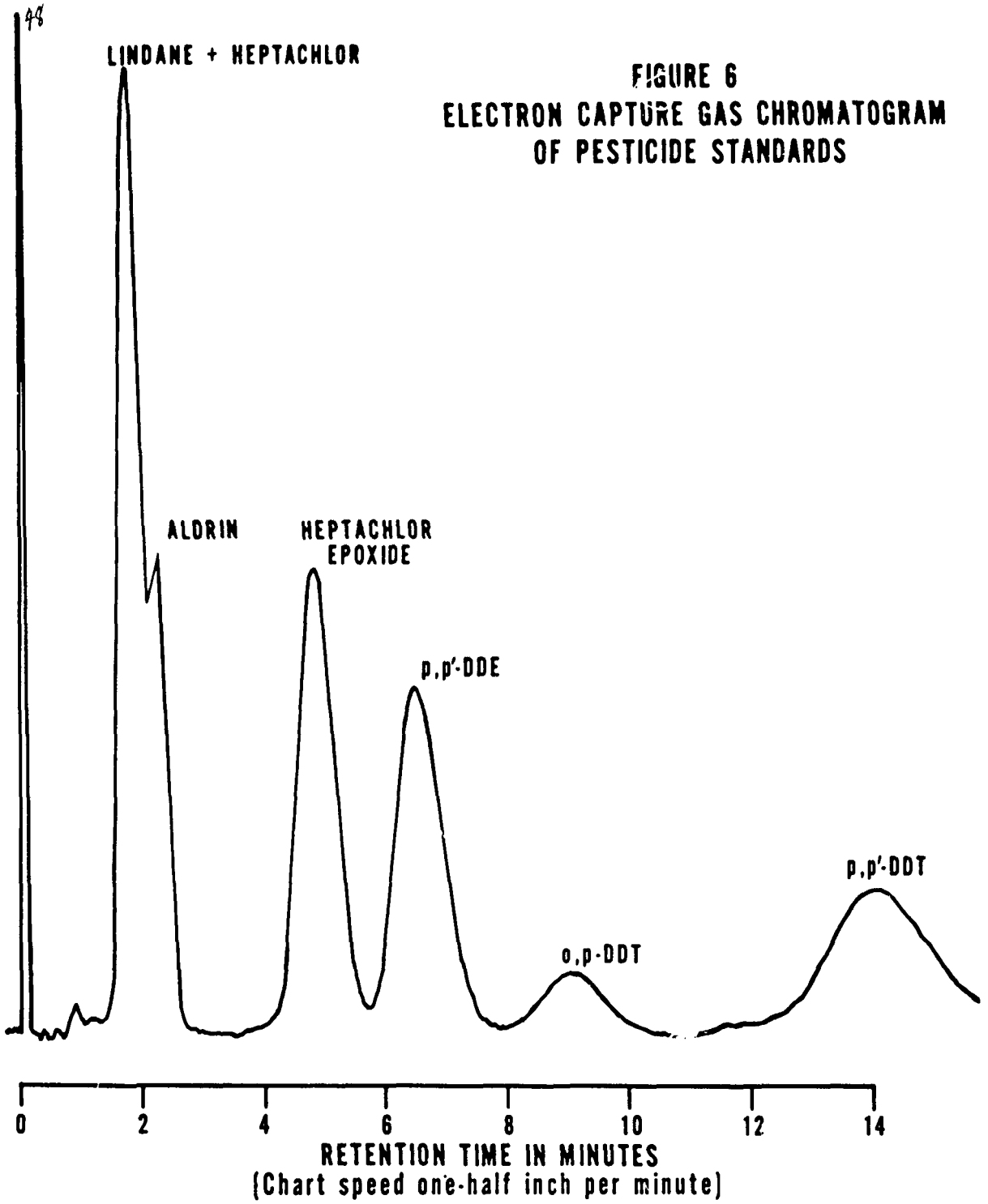


FIGURE 6
ELECTRON CAPTURE GAS CHROMATOGRAM
OF PESTICIDE STANDARDS

Column Packing - 3% OV-210 on Gas Chrom Q (80/100 Mesh)
Carrier Gas - Nitrogen at 80 ml/min.
Column Temperature - 160°C

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APPENDIX

7. SPECIAL EQUIPMENT, REAGENTS, AND SOLVENTS

7.1 Equipment

7.1.1 Gas Chromatograph - Suitable gas chromatographs are available from many manufacturers.

7.1.2 Detectors

7.1.2.1 Electron Capture - Radioactive Source (tritium or nickel-63)

a. Concentric tube design

Varian-Aerograph
2700 Mitchell Drive
Walnut Creek, California 94598

b. Unique concentral design

Tracor, Inc.
6500 Tracor Lane
Austin, Texas 78721

c. Parallel plate design

Perkin-Elmer Corporation
Norwalk, Connecticut 06852

Also supplied by many other manufacturers.

7.1.2.2 Microcoulometric (T-300-S)

Dohrmann Instruments Company
1062 Linda Vista Avenue
Mountain View, California 94040

7.1.2.3 Electrolytic Conductivity

Tracor, Inc.
6500 Tracor Lane
Austin, Texas 78721

7.1.2.4 Flame Photometric

Also from Tracor, Inc.

7.1.3 Recorder - 1 millivolt, 1 second full scale potentiometric strip chart. This type of recorder is supplied by many instrument manufacturers.

7.1.4 Kuderna-Danish Glassware

Snyder Column - three ball (macro) and one ball (micro)

Evaporative Flasks - 125 ml, 250 ml, and 500 ml

Receiver Ampuls - 10 ml

Ampul Caps

Kontes Glass Company
Vineland, New Jersey 08360

Dohrmann Instruments
1062 Linda Vista Avenue
Mountain View, California 94040

7.1.5 Column Chromatography - Pyrex column (I.D. 19 mm, length 400 mm) 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 Glass Company
Vineland, New Jersey 08360

7.1.6 Micro Syringes - (1, 5, 10, 25, 50, and 100 µl)

Hamilton Company
Post Office Box 307
Whittier, California 90608

7.1.7 Separatory Funnels - two liter and four liter funnels with Teflon stopcock.

Pyrex or Kimble supplied through many distributors.

7.1.8 Thin-Layer Chromatography - Applicator, aligning tray, spotting template, developing chamber, and UV light source.

Applied Science Laboratories, Incorporated
Post Office Box 440
State College, Pennsylvania 16501

Brinkmann Instruments, Incorporated
Cantiague Road
Westbury, New York 11590

also - from many other suppliers

7.2 Standards, Reagents and Solvents

7.2.1 Pesticide standards - highest available purity

City Chemical Company
132 West 22nd Street
New York, New York 10011

Applied Science Laboratories, Incorporated
Post Office Box 440
State College, Pennsylvania 16501

Environmental Protection Agency
Perrine Primate Research Branch
P.O. Box 490
Perrine, Florida 33157

also - from the manufacturer

7.2.2 Florisil (60/100 mesh) - purchased activated at 1200 F and stored at 130 C.

Floridin Company
2 Gateway Center
Pittsburgh, Pennsylvania 15222

7.2.3 Sodium sulfate (A.C.S.) - granular, anhydrous

7.2.4 Pyrex wool - filtering grade

7.2.5 Solvents - hexane, diethyl ether, acetone, benzene, xylene, carbon tetrachloride, acetonitrile, methylene chloride - high purity, distilled in glass for pesticide analyses--either Nanograde type or purified in lab.

Burdick and Jackson, Incorporated
1953 South Harvey Street
Muskegon, Michigan 49442

Mallinckrodt Chemical Works
2nd and Mallinckrodt Streets
St. Louis, Missouri 63160

Matheson Coleman and Bell
Post Office Box 85
East Rutherford, New Jersey 07073

7.2.6 Gas Chromatographic Column Materials

Gas-Chrom Q (80-100 mesh)

Glass Wool (silanized with dimethyldichlorosilane)

OV-17

OV-101

OV-210

DC-200 (12,500 centistokes)

QF-1 (FS-1265)

Tubing (Pyrex 1/8 in. and/or 1/4 in. O.D.)

Applied Science Laboratories, Incorporated
Post Office Box 440
State College, Pennsylvania 16501

Ohio Valley Specialty Chemical, Inc.
Marietta, Ohio 45750

7.2.7 Silica gel-G with gypsum binder (No. 8076)

Warner-Chilcott Laboratories
Instruments Division
200 South Garrard Boulevard
Richmond, California 94801

7.3 Sample Collection Bottles and Shipping Containers

7.3.1 One-Quart Jars - Standard 32 oz. 63-400 flint (C-5020),

FTK cap P/O (63-400)

Cincinnati Container Co.
2833 Spring Grove Avenue
Cincinnati, Ohio 45225

7.3.2 Teflon Insert for Bottle Cap - 2-7/16 in. diameter, 0.020 in.

thick

Cadillac Plastics
3818 Red Bank Road
Cincinnati, Ohio 45227

7.3.3 Shipping Containers - Expanded polystyrene packer for one-quart

jars

Preferred Plastics Corp.
Route 12
North Grosvenordale, Connecticut

Polystyrene packers are also available for half-gallon and

one-gallon bottles.

Mention of products and manufacturers
is for identification only and does not imply
endorsement by the Water Quality Office,
Environmental Protection Agency.

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