ANALYSIS OF PESTICIDE RESIDUES IN HUMAN AND ENVIRONMENTAL SAMPLES

A COMPILATION OF METHODS SELECTED FOR USE IN PESTICIDE MONITORING PROGRAMS

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INTRODUCTION

The analytical methodology collected in this manual is intended for use by EPA Laboratories conducting analyses for pesticides in various segments of the environment and by the laboratories under contract with EPA to conduct community studies and the monitoring of pesticides in people.

One of the primary objectives of the Community Studies and Monitoring Laboratory program is to establish and maintain, in collaboration with other Federal Agencies, a broad surveillance and evaluation program concerned with the extent and significance of the contamination of man and his environment by pesticides and their metabolites. To accomplish this goal, data are continuously obtained on the levels of pesticides and their metabolites in the human population and various elements of the environment. It is important that uniform chemical methodology of utmost reproducibility and accuracy be used by participating laboratories so that analytical results can be correlated and directly compared between laboratories.

A prime responsibility of the Perrine Primate Research Laboratories is to make new and improved analytical procedures available to EPA and related laboratories and to those of State and local agencies working to assess pesticide residues in people and/or environmental media. Thus, the Division serves as the primary reference source for analytical quality control, and a research facility for sampling and analytical procedures, in addition to a major center for training chemists in pesticide analytical methodology.

The recommended methods collected in this volume include those which were presented at the Second Community Studies Chemists' Meeting, Tucson, Arizona, April, 1968. Revisions and changes have been made in the details of most of the procedures based on refinements and evaluations substantially contributed by the several Community Studies Laboratories as well as Perrine Laboratory research, development, and quality control. The present state of development notwithstanding, pesticide residue methodology is sufficiently dynamic to assure the need for additional revisions and additions as present procedures are improved or new ones are validated.

The analytical methodology compiled herein consists of both "multi-residue" methods and "specific residue" methods. Included also, are miscellaneous topics covering a number of important activities such as the cleaning of laboratory glassware, preparation of analytical standards, etc.

A numbering system has been initiated in this manual. Each page bears a date and numbers and/or letters designating the identity of the section and subsection. Additions, deletions and revisions will be distributed to manual holders as they are made available with each such issuance bearing appropriate section identification and revision date.

The cooperation of chemists in laboratories using this manual is solicited in helping to improve and up-date the manual. Suggestions for improvement in existing methods or for entirely new procedures based on your experience will be welcomed. Such suggestions or proposed new methods should be sent through your customary channels to :

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These suggestions and proposals will be reviewed and tested in the laboratory if indicated, and the suggester will be notified regarding their disposal.

In addition to acknowledging the contributions made to this manual by the scientists at Perrine and in the Community Pesticide Studies projects, a special note of thanks is due Mrs. Barbara Elwert, secretary for the Perrine Chemistry Branch who so graciously endured the many copy revisions.

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The mention of specific supplier names and/or brands does not constitute an endorsement of products or companies by the United States Government, but is included only for purposes of illustration and equivalency.

MISCELLANEOUS INFORMATION

A. CLEANING OF LABORATORY GLASSWARE

In the pesticide laboratory involved in the analysis of samples containing residues in the parts per billion range, the preparation of scrupulously clean glassware is mandatory. Failure to do so can lead to a myriad of problems in the interpretation of the final chromatograms due to the presence of extraneous peaks resulting from contamination. Particular care must be taken with glassware such as Kuderna-Danish flasks or evaporative concentrator tubes - or any other glassware coming in contact with an extract which will be evaporated to a lesser volume. The process of concentrating the pesticide in this operation may similarly concentrate the contaminating substance, resulting in extraneous chromatographic peaks which, in extreme cases, may completely overlap and mask out the pesticide peak pattern.

Chemists do not all agree on procedural details in the cleaning of glassware. However, the basic cleaning steps do find the majority in agreement. These are:

1. Removal of surface residuals immediately after use.
2. Hot soak to loosen and flotate most of soil.
3. Hot tap water rinse to flush away flotated soil.
4. Soak with deep penetrant or oxidizing agent to destroy traces of organic soil.
5. Hot tap water rinse to flush away materials loosened by deep penetrant soak.
6. Distilled water rinse to remove metallic deposits from the tap water.
7. Acetone rinse to flush off any final traces of organic material.
8. A preliminary flush of the glassware just before using with the same solvent to be used in the analysis.

Each of these eight fundamental steps will be discussed in the order in which they appear above.

1. As soon as possible after use of glassware coming in contact with known pesticides, i.e., beakers, pipets, flasks or bottles used for standards, the glassware should be acetone flushed before placing in the hot detergent soak. If this is not done, the soak bath may serve to contaminate all other glassware placed therein. Many instances of widespread laboratory contamination with a given pesticide are traceable to the glassware washing sink.

2. The hot soak consists of a bath of a suitable detergent in water of 50°C or higher. The detergent, powder or liquid, should be entirely synthetic and not a fatty acid base. There are very few areas of the country where the water hardness is sufficiently low to avoid the formation of some hard water scum resulting from the reaction between calcium and magnesium salts with a fatty acid soap. This hard water scum or curd would have an affinity, particularly for the chlorinated pesticides, and being almost wholly water insoluble, would deposit on all glassware in the bath in a thin film.

   There are many suitable detergents on the wholesale and retail market. Most of the common liquid dishwashing detergents sold at retail are satisfactory but are more expensive than other comparable products sold industrially. Alconox, in powder or tablet form is manufactured by Alconox, Inc., New York and is marketed by a number of laboratory supply firms. Sparkleen, another powdered product, is distributed by Fisher Scientific Company.

   NOTE: Certain detergents, even in trace quantities, may contain organics which will contribute significant background contamination by E. C. detection. For this reason any detergent selected should be carefully checked to insure freedom from such contamination. The following procedure is recommended:

   Add 25 ml dist. water previously checked for background contaminants to a 250 ml sep funnel. Add 1 drop of the liquid detergent or 50 mg if in powder form, followed by 100 ml hexane. Stopper funnel and shake vigorously for 2 minutes. Allow layer separation, draw off and discard aqueous layer. Add a pinch of anhydrous Na_2SO_4 to the hexant extract and shake 1 minute. Transfer extract to a Kuderna-Danish assembly fitted with a 10 ml evaporative concentrator tube containing one 3 mm glass bead. Reduce extract volume to ca. 3 ml in a hot water bath. Cool, rinse down T joint and sides of tube with hexane, diluting extract to exactly 5 ml. Stopper tube and shake on Vortex mixer 1 minute. Chromatograph by GLC, E.C. and evaluate chromatogram for contaminant peaks.

3. No comments required.

4. The most common and highly effective oxidizing agent for removal of traces of organic soils is the traditional chromic acid solution made up of H_2SO_4 and potassium or sodium dichromate. For maximum efficiency, the soak solution should be hot (40-50°C). Safety precautions must be rigidly observed in the handling of this solution. Prescribed safety gear should include safety goggles, and rubber gloves and apron. The bench area where this operation is conducted should be covered with lead sheeting as spattering will inevitably disintegrate the bench surface.

   The potential hazards of using chromic sulfuric acid mixture are great and have been well publicized. There are now commercially available substitutes that posses the advantage of safety in handling. These are biodegradable concentrates that claim cleaning strength equal to the chromic acid solution. They are alkaline, equivalent to ca. 0.1 N NaOH upon dilution and are claimed to remove dried blood, silicone greases, distillation residues, insoluble organic residues, etc. They further claim to remove radioactive traces and will not attack glass nor exert a corrosive effect on skin or clothing. One such product is Chem. Solv. 2157, manufactured by Mallinckrodt and available through laboratory supply firms. Another comparable product is "Detex" a product of Borer-Chemie, Solothurn, Switzerland.

5, 6, and 7 - No comments required.

8. There is always a possibility that between the time of washing and the next use, the glassware may pick up some contamination, airborne or from direct contact. To ensure against this, it is good practice to flush the item immediately before use with some of the same solvent that will be used in the analysis.

The drying and storage of the cleaned glassware is of great importance or the beneficial effects of the scrupulous cleaning may be nullified. Pegboard drying is not recommended as contaminants may be introduced to the interior of the cleaned vessels. Neoprene coated metal racks are suitable for such items as beakers, flasks, chromatographic tubes, etc., - any glassware then can be inverted and suspended to dry. Small articles like stirring rods, glass stoppers, bottle caps, etc., can be wrapped in aluminum foil and dried a short time if oven space is available. Under no circumstance should such small items be left in the open without protective covering. The dust cloud raised by the daily sweeping of the laboratory floor can most effectively recontaminate the clean glassware.

Pipet Washing

The efficient and effective washing of pipets offers some special problems. Self-contained equipment for the entire operation, while available commercially, is quite expensive.

The basic cleaning steps are the same as those listed earlier for miscellaneous glassware with the occasional exception of the chromic sulfuric acid soak. If pipets are properly cleaned this step may be safely eliminated for pipets except those which are to be used in a method involving concentration of the extract by evaporation. In this case, the additional insurance factor of the oxidizing action is recommended.

Hand washing performed by soaking in a pan or sink and then rinsing by holding under running water is highly unsatisfactory, particularly as applied to transfer or volumetric pipets. One simply cannot be sure of obtaining a complete rinse of all surfaces inside the bulb. Therefore, an automatic or semi-automatic washing system is strongly recommended. A complete unit is offered by Scientific Glass Apparatus Co. under catalog number P-6920 for $495 (1969 price). A modular system can be assembled to operate semi-automatically for about half the price of the complete commercial unit. Equipment required for the assembly is as follows:

1. Electric timer w/automatic cut-off.
   Fisher #6-656.

2. Electric heater, immersion, 66" length, 500 watts,
   Fisher #11-463-5B.

3. Pipet washer, stainless steel,
   Fisher #15-350-5.

4. Jars, cylindrical, 6" dia. x 18" height, Corning #6942,
   1-1/2 gal. (2 required).

5. Pipet baskets (2) stainless steel, fabricated by local tin shop per sketch in Figure 1. These are not available commercially.

One of the bell jars (Item 4) is filled with distilled water in which is dissolved a very small amount of synthetic detergent to function primarily as a wetting agent. After acetone rinsing the interior of the used pipets they are placed in a stainless steel basket and immersed in the weak detergent solution. They will remain here until enough are collected to run through the complete wash. The second jar is filled with a detergent solution. The same detergent used in regular glassware washing is not recommended in this application. Instead, a powdered detergent intended for use in automatic dishwashers is recommended. Many of these are available at wholesale or retail.

MISCELLANEOUS INFORMATION

D. EVALUATION OF QUALITY OF FLORISIL

I. INTRODUCTION:

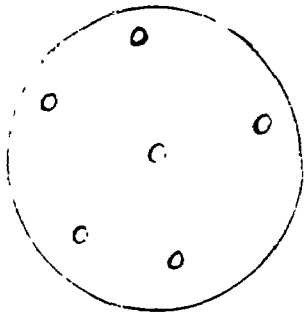
Florisil is purchased in 200 or 400-lb. lots from the Floridin Division of the Pennsylvania Glass Sand Corp. To insure the procurement of high quality material, direct contact is made with the plant engineer at the Hancock, W. Virginia plant. He is advised of our purchase requirement and is requested to be on the lookout for an especially high quality lot as indicated by the company's quality control checks. When such a batch is produced, 400 lbs., in 50-lb. fiber drums, is set aside, a representative sample of ca 1 lb. is taken and mailed to Perrine as an advance sample of the lot.

The advance sample is evaluated as described in the following subsection. If the quality indication is favorable, the company is so advised and the entire lot is shipped to Perrine. Upon receipt, a representative sample is taken and the quality evaluation is repeated as final assurance of the quality of the lot.

Immediately upon completion of the evaluation, the material is transferred from the polyethylene-lined fiber drums to specially cleaned wide-mouth jars, each jar holding 2 lbs. of Florisil.

II. SAMPLING:

Eight fiber drums containing 50 lbs. each of Florisil comprise an entire 400-lb. lot. Each drum is sampled by taking six plugs from top to bottom with a 36" x 1" grain trier. The plug pattern is approximately as shown in the following sketch:



The trier plugs from all drums are placed in a single container, mixed thoroughly, and a quantity transferred to a 500-ml Erlenmeyer flask. At the same time, three elution columns are prepared as described in Section 5,A,(1), page 6. The flask and the prepared columns are placed in a 130°C oven and held overnight or longer.

NOTE: If the laboratory's normal procedure is to pack the columns immediately before use, the prepacking of columns for overnight activation may be voided, but the flask of Florisil should be held in the 130° oven at least 24 hours before use.

III. STANDARD MIXTURES:

Prepare the two following standard mixtures with the concentration shown as picograms per microliter:

Florisil Check #1		Florisil Check #2
α-BHC----------20	o,p'-DDT----------50	β-BHC----------40
γ-BHC----------20	p,p'-DDT----------80	p,p'-DDE----------60
Heptachlor------20	Diazinon---------300	Hept. Epox.-------40
Aldrin----------20	M. Parathion-----250	Ronnel----------100
Dieldrin--------40	Malathion--------400	E. Parathion-----250
Endrin---------100	Trithion---------240	

IV. FLORISIL ELUTION:

1. Remove the prepacked columns from the oven so they may cool down before use.

2. Place a beaker or flask under each column and prewet the packing with 50 ml of pet. ether.

   NOTE: From this point and throughout the following elution process, the solvent level should not be allowed to go below the top of the Na_2SO_4 layer.

3. Read and record the % relative humidity in the room.

4. With 5-ml volumetric pipets transfer 5 ml each of mixtures 1 and 2 onto separate columns and 5 ml of hexane onto the third column as a control.

5. Place 150-ml beakers under each column and commence elution with 100 ml of 6% diethyl ether/pet. ether, the elution rate to be 5 ml per minute. The 100 ml portion of elution solvent is measured in a graduate and applied to the column when the prewetting liquid level just reaches the top of the Na_2SO_4 layer. At the instant the liquid level of the first 100 ml of eluting solvent just reaches the top of the Na_2SO_4 layer, place a second beaker under the column and quickly add another 100 ml of eluting solvent. Allow this second 100 ml of solvent to pass through the column.

6. Continue elution with 200 ml of 15% diethyl ether/pet. ether into a succession of two more beakers identified as 200-300 and 300-400, adding the eluting solvent in 100-ml portions as previously described.

7. Then continue the elution with 50% diethyl ether/pet. ether, following the same procedure of collecting the two 100-ml increments which are designated as 400-500 and 500-600 ml.

8. Place the 18 beakers containing the 100-ml eluate increments on a 35-40° water bath and evaporate under a nitrogen stream to ca 2-5 ml.

9. Add a pinch of anhydrous Na_2SO_4 to each beaker and transfer extracts to 10 ml grad. concentration tubes, rinsing beakers with ca 5 ml of hexane delivered by a syringe or 5-ml Mohr pipet. Evaporate under nitrogen stream to ca 3 ml, remove from bath, allow to cool and dilute with hexane to exactly 5 ml.

10. Stopper evap. conc. tubes and mix on Vortex mixer 1 minute.

V. GAS CHROMATOGRAPHY:

1. With a column of 1.5% OV-17/1.95% QF-1 installed in the instrument, prime column as described in Section 4,A, and equilibrate instrument.

   NOTE: Use only the column designated as the compounds in the respective mixtures will produce minimal peak overlaps.

2. Make 5-µl injections of the two original standard mixtures to obtain peak height data for calculations of recoveries.

3. Make a 5-µl injection of each of the concentrated eluate increments. In case of off-scale peaks or peaks of less than 10% f.s.d., make appropriate attenuation adjestment for both standard and eluates. However, the entire series should preferably be run at one attenuation.

VI. CALCULATIONS AND RECORDATION:

1. Measure all peak heights from original standards and eluate increments.

2. For each compound, and based on peak height measurement, calculate the percentage of the compound appearing in each 100-ml increment and in the original standard.

Example: Lindane, 0-100 eluate, peak ht. 30 mm.
100-200 eluate, peak ht. 60 mm.
Original standard 98 mm.

Percentages --- In 0-100 = $\frac{30}{30+60}$ x 100 = 33%

In 100-200 = $\frac{60}{30+60}$ x 100 = 67%

3. Compute the elution recovery by dividing the sum of the combined eluate peak heights by the peak height of the original standard.

   Working from the same data given above, we would have:

   $\frac{30 + 60}{98}$ x 100 = 92% Recovery

   With the possible exception of aldrin, the recoveries of the chlorinated compounds should fall in the range of 90-105%. Aldrin may not exceed 80%. Some of the OGP compounds may not yield high recoveries. For example, trithion may yield no higher than 40% recovery under certain conditions as outlined in the MISC. NOTES of Section 5,A,1.

4. Recordation of results is made on a standard form and should appear comparable to Table 1. The decision of acceptance or rejection of each lot is based on a consideration of the elution pattern and on the recovery efficiency of the pesticides of interest in the program.

VII. STORAGE OF FLORISIL:

It is imperative that the Florisil be transferred from the shipping drums into glass jars as soon as possible after the lot is evaluated and judged acceptable. The drums are lined with polyethylene which may contribute unwanted contamination over a period of time.

Glass jars which have been found suitable for storage are available from certain glass container distributors. The specific jar used bears Owens-Illinois mold #C-3122, with 100-400 finish, packed in cartons of 6 jars. Metal screw caps with coated paper liners are used.

The jars are washed by mechanical dishwashers, then rinsed with distilled water and acetone. After thorough drying of the jars, the Florisil is transferred with a 2-lb. aluminum sugar scoop previously washed and acetone rinsed. The net contents of each jar when filled to 1/2-inch from the rim with Florisil is ca 2 lbs. A square of aluminum foil is crimped over the rim of the jar and the cap is screwed on tight. Each jar is labelled with the lot number and now ready for distribution. A copy of the compound elution pattern (Figure 1) is enclosed with each shipment to

the field laboratories.

VIII. NOTES:

1. Factors influencing the recovery efficiency, particularly of certain organophosphorous compounds, include (1) presence of impurities in the pet. ether, and (2) presence of peroxides in the ethyl ether. This is discussed in more detail in the MISC. NOTES of Section 5,A,1.

2. Polarity of the elution solvents exerts a profound effect on the selective elution of a number of compounds. The ethyl ether must contain 2% v/v of ethanol to obtain compound elution patterns comparable to those shown in Table 1.

TABLE 1. ELUTION PATTERNS AND RECOVERY DATA FOR FLORISIL, LOT # 2854,
BY METHOD SECTION 5,A,(1)(MANUAL OF ANALYTICAL METHODS)
FLORISIL-COLUMN PREPACKED AND HELD IN 130°C OVEN AT LEAST 24 HRS BEFORE USE
RELATIVE HUMIDITY IN LABORATORY 65 %

ELUTION INCREMENTS (ml)

Compound	6% Fraction		15% Fraction		50% Fraction		Recovery, %
	0 - 100	100 - 200	200 - 300	300 - 400	400 - 500	500 - 600	
α-BHC	100						97
β-BHC	100						95
Lindane	100						96
Heptachlor	100						91
Aldrin	100						100
Hept. Epox.	78	22					105
Dieldrin			85	15			96
Endrin			89	11			99.6
p,p'-DDE	100						97
o,p'-DDT	100						99.6
p,p'-DDT	100						90
Ronnel	100						93
Methyl Parathion			47	53			103
Malathion					100		99
Ethyl Parathion			78	22			96
Diazinon			100				83
Trithion	100						43

Numerical values represent the percentage of each compound eluting in the given cut.

GAS CHROMATOGRAPHY-ELECTRON CAPTURE

INSTRUMENT

All references given presuppose that the gas chromatograph will be the Micro-Tek 220 manufactured by Tracor, Inc., Austin, Texas. This unit may be equipped with a detector with a radioactive source of Nickel 63 or 130 mc tritium. The electrometer may be the single channel model E2 or the solid state, dual channel unit, model SS.

In this section the intent is to provide the operator with an outline of the essentials for readying the instrument for routine use. Appendix I of this manual should be consulted for troubleshooting instructions.

I. FLOW SYSTEM:

This consists of the entire system through which nitrogen gas will flow, from the common point of entry at the exit of the filter drier branching to (1) the purge line running through the purge rotameter and flow controller thence through the detector, and (2) the carrier flow line running through the rotameters, the flow controllers and through the column, thence through the transfer line into the detector.

It is essential that no leaks exist anywhere in the flow system. Even a minute leak will result in erratic baselines with the ^{3}H detector. The ^{63}Ni detector will be even more seriously affected. Leaks can be detected by the application of "Snoop" at all connections or by spraying the connection with Freon MS-180 with the instrument operating and observing for recorder response. Spray short squirts close to the connection. Do not spray around the detector or injection port.

II. DETECTOR:

This subject is covered in detail later in Section 4,A,(3).

III. ELECTROMETER:

To insure proper daily operation of the unit, set the attenuators to OFF position and zero the recorder. Set the output attenuator at x1 and observe the baseline. A steady baseline with less than 1% noise is considered good. A check should occasionally be made of the electrometer electronic zero. Instructions for doing this may be obtained from the Electronics Shop in Perrine.

Zero and bucking controls should operate "smoothly" and should not cause erratic recorder response.

Check the "maximum" polarizing voltage available. If at least -130 vdc is not available on the rear panel, it is quite possible that the power printed circuit board (PCB) is not functioning properly and damage or noisy operation will result from continued use.

IV. TEMPERATURE PROGRAMMER:

The operator should be certain this unit is functioning properly. When the unit is operating properly, the column oven temperature should not show appreciable deviation. If the temperature fluctuation is excessive, baselines will cycle and, in all probability, retention measurements will be erratic.

In an emergency situation, a 10 amp variable transformer (Variac or Powerstat) may be used as a temporary measure. Constant use of this device is not advised as it does not operate on temperature demand, but simply supplies a fixed voltage to the heating elements. Therefore, oven temperature will vary with any changes in line voltage and room temperature.

V. PYROMETER:

The batteries of this unit should be checked monthly to be sure they are delivering full voltage under load. This can be done easily with a voltmeter set on the 3-volt range and shunting a 1 megohm resister across the voltmeter leads to constitute a load. If the voltage under this test situation falls below the rated voltage for the battery, replace battery. The battery contacts should also be cleaned by spraying with Freon MS-180 and wiping with dry cloth. To prevent shorts, it is recommended that electrical glass cloth tape be wound around each end of the battery at positions where the clamps hold the battery in place.

A hint of inaccurate pyrometer operation may be obtained by switching to one of the unused sensors and observing the readout. If the reading is more than 5°C from room temperature, faulty operation is suggested. This is suggested as a daily check to prevent straying gradually into grossly inaccurate temperature readings. Before final readings are made, gently finger tap the pyrometer frame in the area around the set screw.

VI. MISCELLANEOUS:

A. Septums - there are a number of different types available, ranging from the inexpensive plain black (or gray) silicone rubber to the sophisticated "sandwich" type selling at a significantly higher price.

Excellent results have been reported on the blue silicone rubber material marketed by Applied Science Laboratories as their "W" series.

The 13 mm precut septums are available in lots of 100 for $27 under catalog number W-13. The same material listed at "Type W" is available in sheets of 12" x 12" selling for $55. About 400 13-ml septums can be cut from this sheet with a No. 9 cork borer making the price per hundred septums about half that of the precut septums. The above prices are based on the 1972 catalog.

B. Column "O" Rings:

Very satisfactory results have been obtained with Viton "O" rings. One source of supply is:

John G. Shelly Co., Inc.
16 Mica Lane
Wellesley Hills, Mass.

C. Prepurified nitrogen gas shall be used for the D.C. mode of operation. This is piped to the instrument through a filter drier of molecular sieve, 1/16" pellets, Linde type 13X. When the filter drier is charged with fresh molecular sieve, the interior of the drier should be acetone rinsed and the drier unit placed in a 130°C oven for at least an hour. The bronze frit should be acetone rinsed and flamed. After filling, the unit should be heated at 350°C for 4 hours with a nitrogen flow of ca 90 ml/minute passing through the unit. If the activated unit is to be stored for a period of time before use, the ends should be tightly capped.

GAS CHROMATOGRAPHY-ELECTRON CAPTURE

COLUMNS

I. SPECIFICATIONS:

Column material shall be of borosilicate glass, 6 feet long, 1/4 inch o.d., 5/32 inch i.d. As off-column injection will be used, one side of the column shall be 1 inch longer than the other. The Swagelok nut, ferrule and silicone "O" ring are assembled as in Figure 4. Complete column specifications are given in Figure 11.

II. COLUMN SELECTION:

Of the three columns listed below, A and B, made up from precoated packings from Perrine, will be the working columns in laboratories conducting analyses on miscellaneous sample substrates. If blood is the predominant sample substrate, a preferable combination of working columns might be A and C.

A. 1.5% OV-17/1.95% QF-1 - liquid phases premixed and coated on silanized support, 80/100 mesh.

B. 4% SE-30/6% OV-210 - liquid phases premixed and coated on silanized support, 80/100 mesh.

C. 10% OV-210 - coated on silanized support, 100/120 mesh.

The three precoated packings are purchased in bulk from a commercial supplier under rigid performance specifications, and each batch is carefully evaluated upon receipt at Perrine. Each packing is available to the Community Studies Laboratories from Perrine in 25 gram units. A copy of the evaluation chromatograms will be included with each shipment to the field laboratories.

III. PACKING THE COLUMN:

Make certain the column is actually 6 feet long. A paper template tacked to the wall is a convenient and quick means of checking this. For off-column injection in the Micro-Tek Model 220, one column leg should be 1 inch shorter than the other.

With a china marking pencil, place a mark on the long colum leg 2 inches from the end. Place a similar mark 1-1/8 inch from the end of the short leg.

Add the packing to the column through a small funnel, ca 6 inches at

a time, and bounce the column repeatedly on a semihard surface. Rapid tapping up and down the column with a wooden pencil will promote settling of the packing. The packing is added until it reaches the mark on each leg and it is found that additional tapping will not produce any further settling.

NOTE: This operation should be done with great care, tapping the column a sufficient length of time to be certain that no further settling is possible by manual vibration. The use of mechanical vibrators is not advised as the packing can be packed too densely, thus introducing the possibility of an excessive pressure drop when carrier gas is applied.

Pack silanized glass wool into both ends of the column just tightly enough to prevent dislodging when carrier flow is applied. The glass wool should completely fill the space from the top of the packing to the end of the column.

NOTE: If the glass wool is manipulated by hand, the hands should be carefully prewashed with soap or detergent, rinsed and dried. This minimizes the possibility of skin oil contamination of the glass wool.

IV. COLUMN CONDITIONING:

The column is conditioned, or made ready for use, in two operations, (1) by heat curing, and (2) by silylation treatment.

1. Heat Curing

A Swagelok fitting is attached to the inlet port at the top of the oven. This is comprised of a 1/4-inch Swagelok to AN adapter, part number 400-A-4ANF, connected to a 1/4-inch male union, part number 400-6.

Before assembling, the bore of the union must be drilled out with a 1/4-inch drill and burnished with a rat-tailed file so that it will accept the 1/4-inch o.d. column glass.

The short column leg is attached to the above fitting, with the end of the long leg venting inside the oven. The nut, ferrule and "O" ring is assembled as shown in Figure 4. Make sure the nut is tight, as the "O" ring will shrink during the curing period, thus allowing carrier gas to escape.

NOTE: The outlet ports leading to the transfer line should be sealed off during the conditioning period to prevent traces of column effluent from seeping through to the detector. This is easily done by assembling a 1/4-inch Swagelok nut on a short piece of 6-mm glass rod with ferrule and "O" ring.

2. Silylating Treatment

Treatment with a silylating compound such as Silyl 8 serves to block active adsorption sites, particularly prevalent in a new column, thereby somewhat improving efficiency and resolution characteristics. The most drastic effect is in the improvement of endrin response and the near elimination of on-column breakdown of endrin. Silyl 8 is available in 1 and 25-ml septum capped bottles from the Pierce Chemical Company, P.O. Box 117, Rockford, Illinois 61105.

At the end of the prescribed heat curing period, adjust the oven tempset and carrier gas flow controllers to the appropriate settings to give the approximate recommended operating parameters for the given column. While the temperature is dropping, open the oven door and, wearing heavy gloves, retighten the Swagelok nut which will invariably loosen during heat curing. Close door and allow oven temperature to equilibrate. Make four consecutive injections of 25 µl each of Silyl 8, spacing the injections ca 1/2 hour apart. Allow an hour for the final injection to elute off the column before proceeding.

NOTE: Syringe used for Silyl 8 injections should be used for no other purpose, and should be rinsed immediately after use to avoid damage.

V. EVALUATION OF COLUMN:

Shut down oven and carrier gas flow, remove column from special fitting, remove fitting from inlet port, and connect column to detector, making sure that nuts are securely tightened. Replace Vykor glass injection insert with a clean one and install a fresh septum. Make certain that the stainless steel retainer for the insert is reinstalled with the slotted end up. Upside down installation will permit the escape of carrier gas. After septum nut is screwed down by hand, a little further tightening with pliers helps insure gas-tight septum installation. Raise oven temperature and carrier gas flow to the exact values given in Table 1 for the appropriate column. The oven temperature must be monitored by some means other than the built-in pyrometer, either a precalibrated dial face thermometer with the stem inserted through the oven door, or a

mercury thermometer down through an unused injection port. DO NOT RELY WHOLLY ON THE INSTRUMENT PYROMETER.

Check the carrier gas flow rate using the sidearm buret device sketched in Figure 4 (a) attached to the purge exit of the detector. DO NOT RELY ON THE INSTRUMENT ROTAMETER in adjusting the carrier flow. Allow an overnight period for complete equilibration of the column-detector system at normal operating parameters of temperature and carrier flow.

NOTE: If two columns are connected to the same detector, the carrier flow to the column not in use should be shut off while the flow rate through the column in use is being measured. Likewise, the purge line flow controller should be closed. The unused column flow should also be kept at zero while determining the background current.

After overnight equilibration, recheck the oven temperature and carrier gas flow rate. You are now ready to assess the performance characteristics of the column, and this should definitely be done before attempting to use the column for routine work.

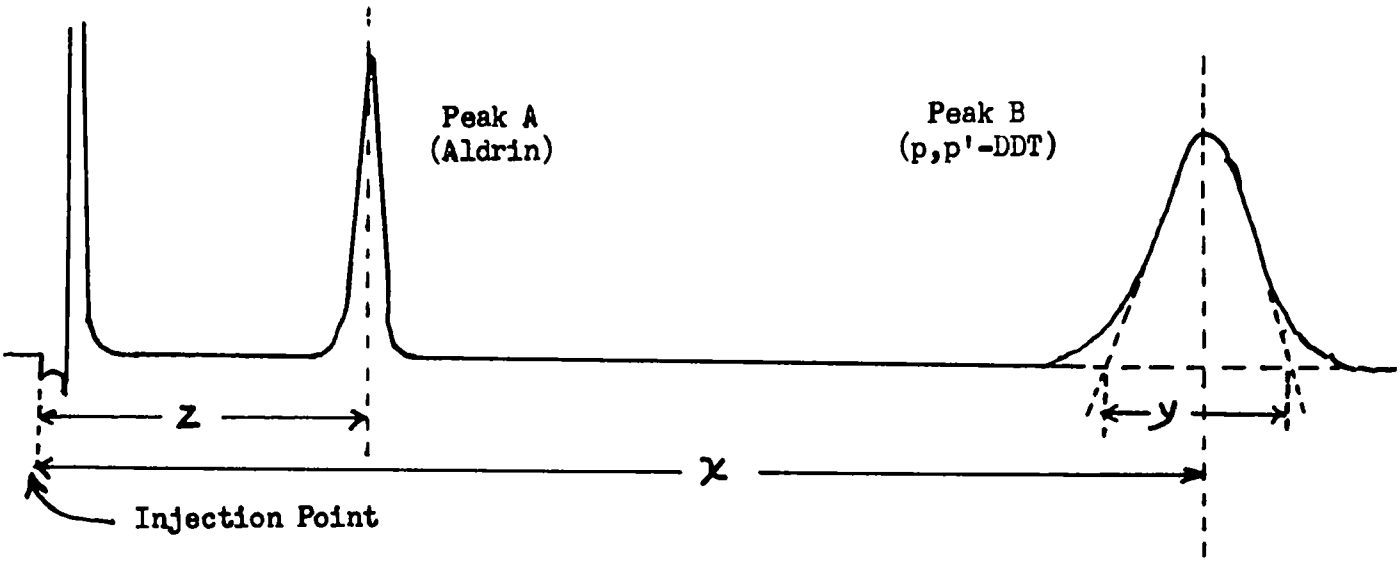
Run a background current profile at the normal operating parameters for the given column, with the purge line flow controller set at 4. Detailed instructions are given under Subsection 4,A,(3) DETECTOR. The BGC profile is particularly important in providing an assessment of detector behavior as affected by the column. It is presumed that a BGC profile was run on the same detector within a few days from the time of the present profile, so that the expected level of background current may be compared to the level obtained in the present test. If the present level falls far short of that expected, either the detector itself is faulty or the column is exerting an adverse effect on the detector. The column influence may be roughly determined by allowing several hours more for equilibration and repeating the BGC profile. If an increase in BG current is obtained, additional checks are made until no further increase is noted. A typical BGC profile is shown in Figure 5.

If the detector foil is new and the BG current is at a high level, it is acceptable practice to set the polarizing voltage at 85% of the full BGC profile. However, this practice is not reliable with an older, partially fouled detector. A more reliable method is to run a polarizing voltage/response curve as described in Subsection 4,A,(3) OPTIMUM RESPONSE VOLTAGE. A polarizing voltage/response curve is shown in Figure 6.

minutes from the Perrine value, it is indicated that (1) one or both operating parameters are off, or (2) column is not 6 feet long, or (3) the density of the packing is not comparable.

a. If the absolute retention is less than the Perrine value by more than 2 minutes, the oven may be running too hot or the carrier flow may be running too high, or both; the column may be packed too loosely, offering less surface area of coated support; the column may not be a full six feet in length.

b. Conversely, if the absolute retention value is high, the indications may be: a low column temperature, a low carrier flow, a column packed too densely, or some combination of two or more of these factors.



$$N = 16\ (\frac{x}{y})$$

$$R_a = \frac{8x}{5I} \text{ (at 1/4-inch chart speed)}$$

$$R_a = \frac{4x}{5I} \text{ (at 1/2-inch chart speed)}$$

$$R_r = \frac{x}{z}$$

where

N = column efficiency in total theoretical plates.

R_a = absolute retention, in minutes, for peak B.

R_r = retention ratio, relative to aldrin, for peak B.

x,y,z = measured in millimeters.

Based on the chromatograms of the evaluation mixture, a decision generally can be made as to the potential quality of the column. If, after making slight adjustments in the carrier gas flow rate, characteristics of efficiency, absolute retention and peak resolution do not compare reasonably well with the chromatograms and data furnished by Perrine, it is inadvisable to proceed further with the column. For example, if an efficiency value of over 2,700 theor. plates cannot be obtained on a new column, it is unlikely that the column would ever improve to much over 3,000 T.P. On the other hand, if the new column yielded 3,000 T.P., it is probable that it would improve to 3,300 or 3,500 T.P. after becoming "seasoned".

Assuming that a favorable indication is obtained from the mixture chromatograms, the next evaluation step is to determine the compound breakdown characteristics of the column. This may be done by injections of pure p,p'-DDT in sufficient concentration and appropriate attenuation to produce peak heights of 50 to 60% f.s.d. The DDT breakdown should not exceed 3%. The endrin response and breakdown characteristics may be determined similarly. This breakdown should not exceed 10%.

NOTE: This breakdown percentage is calculated by adding up the peak areas of main peak and breakdown peak(s). This value divided into the peak area value for the breakdown peak(s) x 100 is the breakdown percentage.

VI. MAINTENANCE AND USE OF COLUMN:

A column that is used and maintained properly should provide service for many months. It is difficult to make precise time estimates because of the variable in different laboratories. Data from a column performance survey showed one laboratory using the two working columns 3-1/2 months for an estimated number of 1,300 injections of predominantly fat extract. Another laboratory indicated their two columns to be at least a year old, and each had been subjected to an estimated 3,000 injections of blood extract. Neither laboratory's columns gave any indication of deterioration. In fact, the laboratory injecting fat predominantly was included in the group showing superior overall column performance.

The Vykor glass injection insert used in off-column injections serves as a trap to prevent a high percentage of dirty material from

befouling the front end of the column. However, if this insert is not changed frequently, column performance characteristics can be significantly altered. When a sufficient amount of residue collects in this insert, lowered efficiency, compound breakdown, peak tailing and depressed peak height response become evident. The changing of this insert should be on a daily basis if sample extracts of any kind are being injected.

The effects of Silyl 8 conditioning do not persist indefinitely. Any laboratory with an interest in endrin detection may find that resilylation may be necessary at intervals to be determined by weekly monitoring for breakdown.

A certain amount of extraneous matter is eluted through the glass insert and lodges in the glass wool plug at the column inlet. Indications from the survey mentioned above were that those laboratories changing the glass insert daily could go for long periods of time without changing the column plug. Daily compound conversion monitoring provides a constant check on the need for changing the glass wool plug.

When the column is idle overnight or over weekends, a low carrier flow of ca 25 ml per minute through the column is advised. Simultaneously, a purge flow of ca 25-30 ml through the detector is also advised. If a column is out of the instrument longer than two or three days, reconditioning is advised wherein the column is not connected to the detector, but is allowed to vent into the oven under a carrier flow of ca 60 ml per minute at a temperature ca 25° above the prescribed operating temperature.

An erratic and noisy baseline can indicate leaks in the column connections, or at some other point in the flow system, starting at the injection septum and on to the detector inlet. If the baseline has been stable and first became erratic upon installation of a new column, the probability is indicated of loose column connections.

If any laboratory has trouble obtaining performance characteristics equal to those indicated by the chromatograms and data furnished with each bottle of precoated packing, every effort should be made to pinpoint the trouble and correct it. If the foregoing instructions are followed with no deviations, trouble should not be experienced. If you are unable to overcome the difficulty, write or call the Perrine Quality Control group outlining your problem, including with your letter completely identified chromatograms of (1) the special evaluation mixture and (2) a chromatogram of pure p,p'-DDT at a concentration and attenuation producing a peak of 50-60% f.s.d. Give all instrumental and column operating parameters.

It should be borne in mind that before the precoated packing material of any batch received in Perrine is mailed to the field laboratories, a very thorough evaluation is conducted on a composite sample of all bottle units received. It is therefore a near impossibility for a laboratory to receive poor column packing. Any trouble experienced will almost certainly lie in some adverse contribution made by the operator in preparing and/or conditioning and/or operating the column.

VII. MISCELLANEOUS NOTES:

1. The carrier flow through the unused column should not be carried any higher than is required for positive pressure. Detector response is seriously affected by running both columns simultaneously at normal operating velocity. For instance, in a series of observations with a pair of nearly identical low-load columns in the oven, the peak height response for aldrin is reduced ca 25% when the off-column is carried at 70 ml/min., the same flow at which the on-column is being operated.

2. An obvious, but sometimes overlooked, point arises when only one column is installed in the oven. The transfer line commonly used is the dual type that conveys column effluent from the two-column outlet ports to the single detector. When one column is removed, its outlet port must be plugged or else a massive leak will be created. One easy means of doing this is to slip swagelok fittings and an "O" ring on the end of a short piece of 1/4" o.d. glass rod and install in the unused outlet port.

GAS CHROMATOGRAPHY-ELECTRON CAPTURE DETECTOR

Straight D.C. polarizing voltage shall be supplied to the detector from either an outboard power supply unit or from a strip on the back of the electrometer. Provided the column and all electronic circuits in the various modules of the instrument are functioning properly, the degree of sensitivity in the electron capture mode relate most probably to the condition of the interior of the detector. As radioactivity in the foil decreases, so does sensitivity of the system. Measurement of the background current gives an indication of the condition of the detector and should be run on a new or overhauled detector. Subsequent periodic measurements should be made to provide up-to-date information on the performance of the detector as influenced by the condition of the foil or by any other effects such as column bleed or contaminated carrier gas.

I. BACKGROUND SIGNAL PROFILE:

1. Zero recorder and electrometer in the normal manner.

2. With a well seasoned column such as OV-17/QF-1 in the instrument, set input attenuator on 10 and output attenuator on 256.

   NOTE: The given attenuation values apply to electrometer model E2. If the dual channel, solid state unit is used an equivalent setting would be 10^2 x 128.

3. Set column and detector temp. and carrier flow rate to the levels prescribed for the column in use. Apply ca. 70 ml/min. of purge gas.

4. Set OUTPUT POLARITY switch to the polarity opposite of that used in normal operation.

5. Reduce polarizing voltage to zero by control on power supply unit or in front of electrometer and adjust bucking control of electrometer to permit zeroing of pen on chart paper.

6. Set chart speed on 1/4 inch per minute, start chart drive, and allow about 1/2 inch horizontal trace.

7. Advance polarizing voltage control to 5 volts and allow sufficient time for trace to level off, then repeat for 10, 15, 20, 25 volts and so on, until a voltage value is reached which produced no further recorder deflection.

Generally, a new detector or one with a new tritium foil should be expected to produce a response of 60-80% full scale deflection. With aging, as the response level approaches about 30% f.s. deflection, a replacement of the foil

is indicated. Figure 5 shows a background signal profile on a detector in constant use for 2 months. At the time of original installation, the background signal profile produced 68% f.s. deflection.

II. OPTIMUM RESPONSE VOLTAGE:

It is important that a correct polarizing voltage be supplied to the detector to achieve maximum peak response with minimal overshoot. An incorrect voltage can result in (1) full potential sensitivity of the detector not being utilized, or (2) a strong overshoot in the peak downstroke which makes for difficult quantitation of peaks. The optimum response voltage is determined as follows:

1. Upon completion of the background signal profile, reset OUTPUT POLARITY switch back to normal operating position and set polarizing voltage control to the voltage that produced ca. 60% of the total BGC profile.

   NOTE: If you are fairly certain that the optimum polarizing voltage will fall in some fairly high range, i.e., 20-25 volts, time can be saved by starting about 7 volts under the expected optimum polarizing voltage.

2. Set oven and detector temperatures and carrier flow rate to the prescribed operating levels for the column in use, and allow system to equilibrate.

3. Set attenuators on the values appropriate for the condition of the detector.

4. Adjust bucking control to zero recorder pen.

5. Inject an aldrin standard in quantity known from current operation to produce a peak about 1/2 full scale at the attenuation being used.

   NOTE: The volume injected must be carefully measured and should not be less than 5 µl.

6. Repeat injection to obtain peaks from increments of 2.5 volts, i.e., 15-17.5-20, etc., until two peaks show less height than that obtained on the highest peak.

   NOTE: Occasionally a new detector will require only around 7 volts, and it may be found that the 2.5 volt intervals result in too much change in response. In this case, it may be advisable to use 1 volt intervals to set up the response curve.

7. Taking the exact peak height values, measured in millimeters, plot a peak height vs voltage curve on linear graph paper (see Figure 6). Usually the optimum polarizing voltage is that which produces the next to greatest peak height. However, if appreciable peak overshoot is evident at this voltage, it may prove desirable to set polarizing voltage slightly higher to minimize overshoot at some expense in response. Arrow in Figure 6 indicates the voltage selected in this particular case.

III. DETECTOR LINEARITY:

In making chromatographic runs for quantitation, it is mandatory that compound concentration be within the linearity range of the detector. As this characteristic may change with the age and use of the detector, standard curves for pesticides of interest should be run periodically to provide up-to-date linearity information. In most cases, operation at an output attenuation setting of 10 x 8 (or 16) on the E2 electrometer of 10^2 x 8 (or 16) on the SS will preclude the possibility of violating the linear range of the detector. If samples are diluted so that quantifiable peaks are produced at these settings, the large errors resulting from calculations based on non-linear response can be avoided.

GAS CHROMATOGRAPHY-ELECTRON CAPTURE

CHROMATOGRAPHY OF SAMPLE

When the chromatographic system has been idle for a number of hours, such as overnight or over the weekend, it is generally necessary to "prime" the column before quantitation may be attempted. The first early morning standard injection will frequently show relatively poor response. The second and third injections will usually improve the response to a constant level. This "priming" may be done by successive injections of a dilute working standard mixture or it may be accomplished by one injection of a highly concentrated mixture. One laboratory has reported good success with the latter system, and if other laboratories obtain comparable results, considerable daily "priming" time may be saved. The suggested priming mixture is given below and the concentration values in nanograms per microliter.

Lindane--------0.5	Dieldrin---------1.0
β-BHC----------1.5	o,p'-DDT---------1.5
Aldrin---------0.5	p,p'-DDD---------1.5
Hept. Epox.----1.0	p,p'-DDT---------1.5
p,p'-DDE-------1.0	

Forty microliters of this mixture is injected. If this one-shot system is used, a special syringe should be set aside and used solely for this purpose. Under no circumstances should the same syringe be used for routine injections.

In the early morning the priming may be conducted while the other daily instrument checks are being made. If more than one column in the instrument is to be used, the priming may be done simultaneously. After the priming mixture has eluted off the columns, the carrier flow should be carefully adjusted for the working column using the bubble device shown in Figure 4 (a). The chromatograph should now be ready for the first working standard injection.

A sample extract concentration of 10 ml from a 5 gram sample contains the tissue equivalent of 0.5 milligrams per microliter. A 5 µl injection of this extract (2.5 milligrams of sample) into an E. C. detector of average sensitivity should easily produce quantifiable peaks at pesticide concentrations of at least 0.1 ppm provided that instrumental attenuation is appropriately adjusted.

1. With the working column in the instrument, adjust column and detector parameters as prescribed in Table 1. If another column is in the oven, set a positive carrier gas pressure of ca. 20 ml/min., on this alternate column. Set attenuation at an estimated appropriate sensitivity.

NOTE: The specified GLC instrument has a high sensitivity potential provided that all modules are functioning properly. It is important to take full advantage of this potential by avoiding high output attenuation. For instance, some workers attempt to operate the electrometer at attenuations of 10 x 32 and 10 x 64, adjusting their sample concentration and standards to fit this attenuation range. With a new detector foil this high attenuation may be necessary, but as the BGC decreases, this practice, while resulting in a stable looking baseline, requires injections of relatively high sample concentration to produce quantifiable peaks. This tends to promote faster fouling of column and detector than would result from injections containing less sample material. This is particularly important when injecting eluate from the 15% ethyl-ether/pet. ether extract from fat. If all instrumental modules are functioning properly it should be possible to obtain a noise level not exceeding 2% of full scale at an attenuator setting of 10 x 8. If 10 x 8 should not meet this specification, then 10 x 16 should definitely produce an acceptable noise level. In the event that the electrometer noise level at 10 x 16 should exceed 2% full scale, the electronics shop at Perrine should be contacted.

2. Inject 5 µl of sample extract for an exploratory run, followed by one or more standard mixture runs. Evaluate the chromatogram using the appropriate relative retention/column temp. table to make tentative peak identifications.

   NOTE: By using the table, the operator can determine at this point whether the actual column temperature agrees with that shown on the instrument pyrometer readout. Comparison of the calculated RRR for p,p'-DDT with the values given in the table will advise the operator if temperature adjustment is needed.

3. If the data obtained from step 2 show one or more probable pesticide peaks, it is desirable at this point to run a chromatogram of a quantitating standard mix known to contain compounds of proper concentration to fall within the linearity range of the detector and also produce peaks comparable in size to those obtained from the sample chromatogram. This chromatogram should provide information concerning the degree of additional dilution of the sample extract to produce peaks of the higher concentration pesticides approximating those of the standard mix. It may also be possible at this point to quantitate some of the peaks obtained in the exploratory run.

NOTES: 1. It is desirable to use standard mixtures with the component pesticides at three concentration levels. This will enable the operator to select a mix whose concentrations will fall within the linear range of the detector and have a peak size comparable to the unknown peaks.

2. The height of sample and standard peaks should preferably vary by not more than 25%. It is sometimes alleged that this point is of no consequence provided both standard and sample are within the linear range of the detector. In theory this is true, but like many theoretical postulations the fact does not necessarily follow the theory. For example, the theory does not take into consideration minor response variations arising from injection error and/or instrumental sources. It can be easily demonstrated that a response variation of as little as 3 mm in peak height can result in a final error of 20 to 25% when a 13 mm sample peak is calculated against a 130 mm standard peak.

3. Electrometer attenuation should be adjusted to obtain a minimum sensitivity level of a peak of 50% f.s.d. resulting from the injection of 100 picograms of aldrin.

4. Prepare an estimated sample extract dilution and run another chromatogram on a 5 µl injection.

NOTE: It may be necessary to run several sample extract chromatograms of various concentrations and/or injection volumes to achieve reasonable approximation of peak sizes with those of the standard mixture.

5. At this point, detailed evaluations are made of all chromatograms. If there is reason to suspect any peak identification or quantitation, instrumental controls should be switched over to the alternate column for further scrutiny. The isomers of BHC, o,p'-DDE, and o,p'-DDT frequently pose identification problems. If such identification problems are present and cannot be confidently resolved by any of the three prescribed columns, further confirmatory work is required by electrolytic conductivity detection, and/or by T.L.C.

GAS CHROMATOGRAPHY-ELECTRON CAPTURE

QUANTITATION AND INTERPRETATION

There are several methods for quantitating chromatographic peaks. While we are not partial to any particular method, it is desirable in a system of laboratories providing data to a central point that some degree of uniformity be specified.

The preferred method of calculation is somewhat dependent on peak shape. The major categories of peak shapes are: (1) Tall, narrow and symmetrical, generally illustrated by a p,p'-DDT peak from a clean extract, (3) Overlapping peaks where the overlap is estimated not to obscure the peak height, (4) Unsymmetrical peaks such as are commonly encountered in an uncleaned extract.

Broadly speaking, quantitation methods recommended for the various types of peaks are:

I. PEAK HEIGHT:

A. Early eluting peaks, tall and narrow.

B. All peaks on the trace where there are no obscuring overlaps and where peaks are tall, symmetrical and fairly narrow (See Figure 7).

II. PEAK HEIGHT X WIDTH AT HALF HEIGHT:

A. Separated, symmetrical and fairly wide peaks (See Figure 8).

III. TRIANGULATION OR INTEGRATION:

A. Separated unsymmetrical peaks, or peaks on sloping baseline (Figure 9). Triangulation should not be attempted on very narrow peaks. Extreme care must be taken in the construction of the inflectional tangents and in measurements.

IV. INTERPRETATION:

Although this subject is listed last in this section devoted to E.C., GLC, it is far from being the least important. An excellent performance in all other areas may be nullified if the chromatograms are not properly interpreted.

The electron capture detector, being non-specific, responds to any electron capturing materials in addition to pesticides in the final extract being chromatographed. For this reason, the task of interpretation is one requiring careful study of the data and the application of sound judgement. The presence of chromatographic peaks which precisely match the absolute and relative retention values of those of

certain pesticides does not necessarily indicate the indisputable presence of those pesticides. For example, it is not uncommon to observe peaks from human tissue extract with retention characteristics precisely the same as α-BHC and/or o,p'-DDE. However, confirmation by ancillary techniques has never supported the E. C. indications. In one instance methyl parathion was reported in a blood sample. Had the individual conducting the interpretation exercised sound judgement, it should have been immediately apparent that the presence of parathion, per se, in body fluids other than gastro-intestinal would be a near impossibility.

The chromatographer must recognize that quite often peaks are obtained from a given sample substrate on one GLC column by E.C. detection, the retentions of which strongly suggest certain pesticidal compounds. If, based on experience, these particular compounds are not likely to be present in the sample material, some further confirmation is required. This may be done by (1) using an alternate column via E. C. detection, (2) applying electrolytic conductivity detection (3) thin-layer chromatography or (4) chemical derivatization.

1.5% OV-17/1.95% QF-1

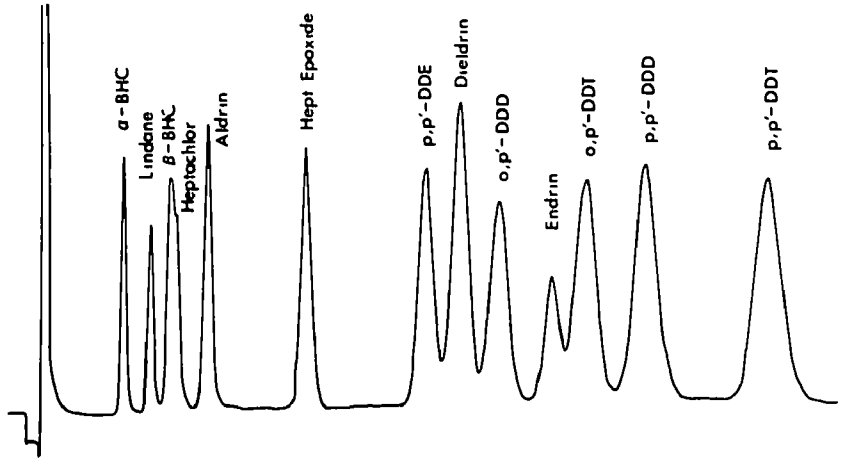


Figure 1

4% SE-30/6% OV-210

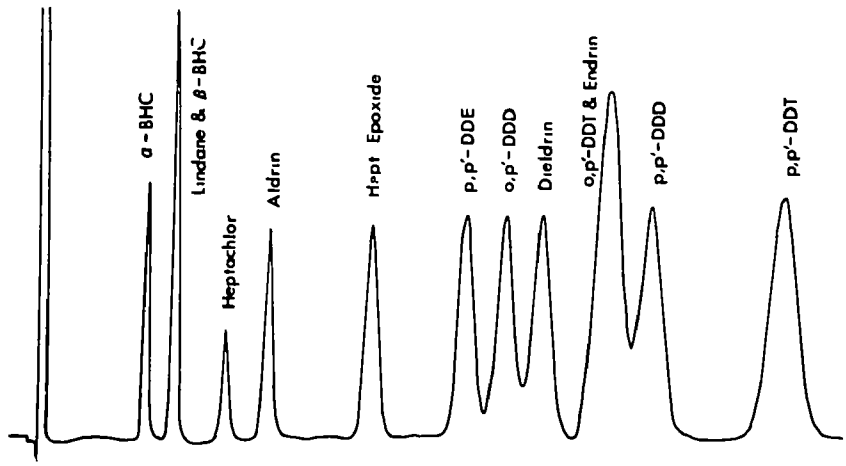


Figure 2

10% OV-210

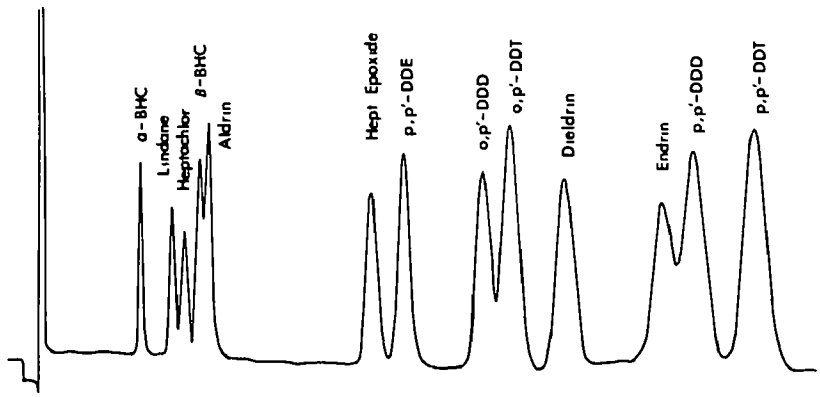


Figure 3

Fig. 4 — Column to Port Assembly – Exploded View

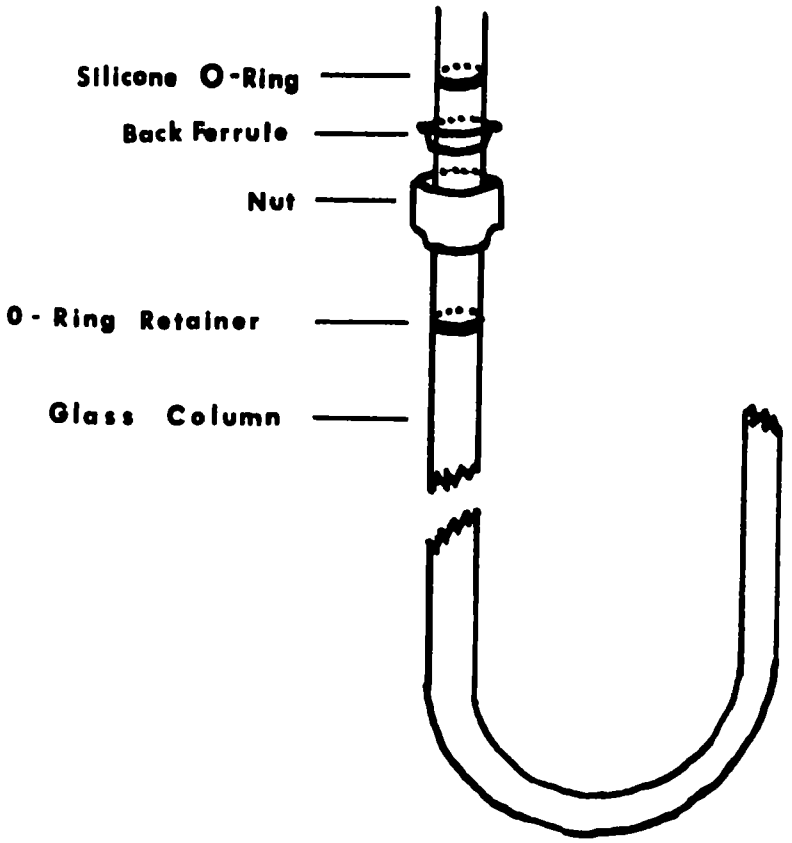


Fig. 4(a) — Bubble Flowmeter

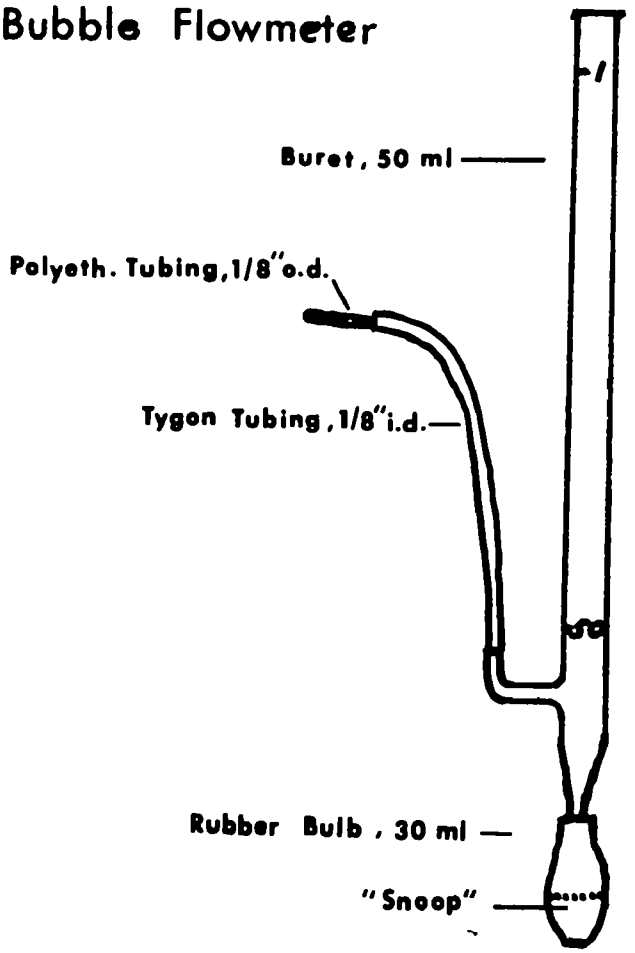
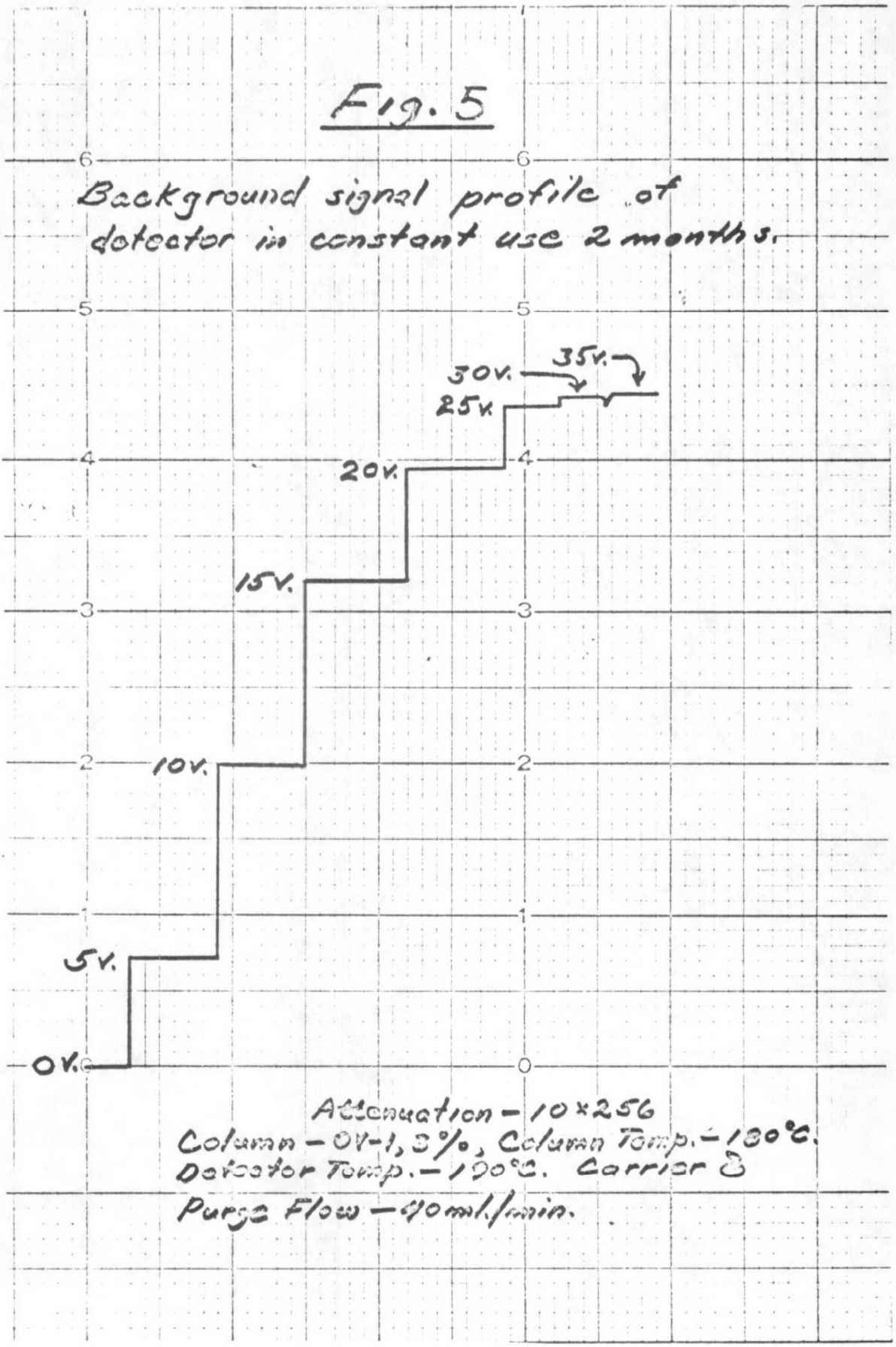
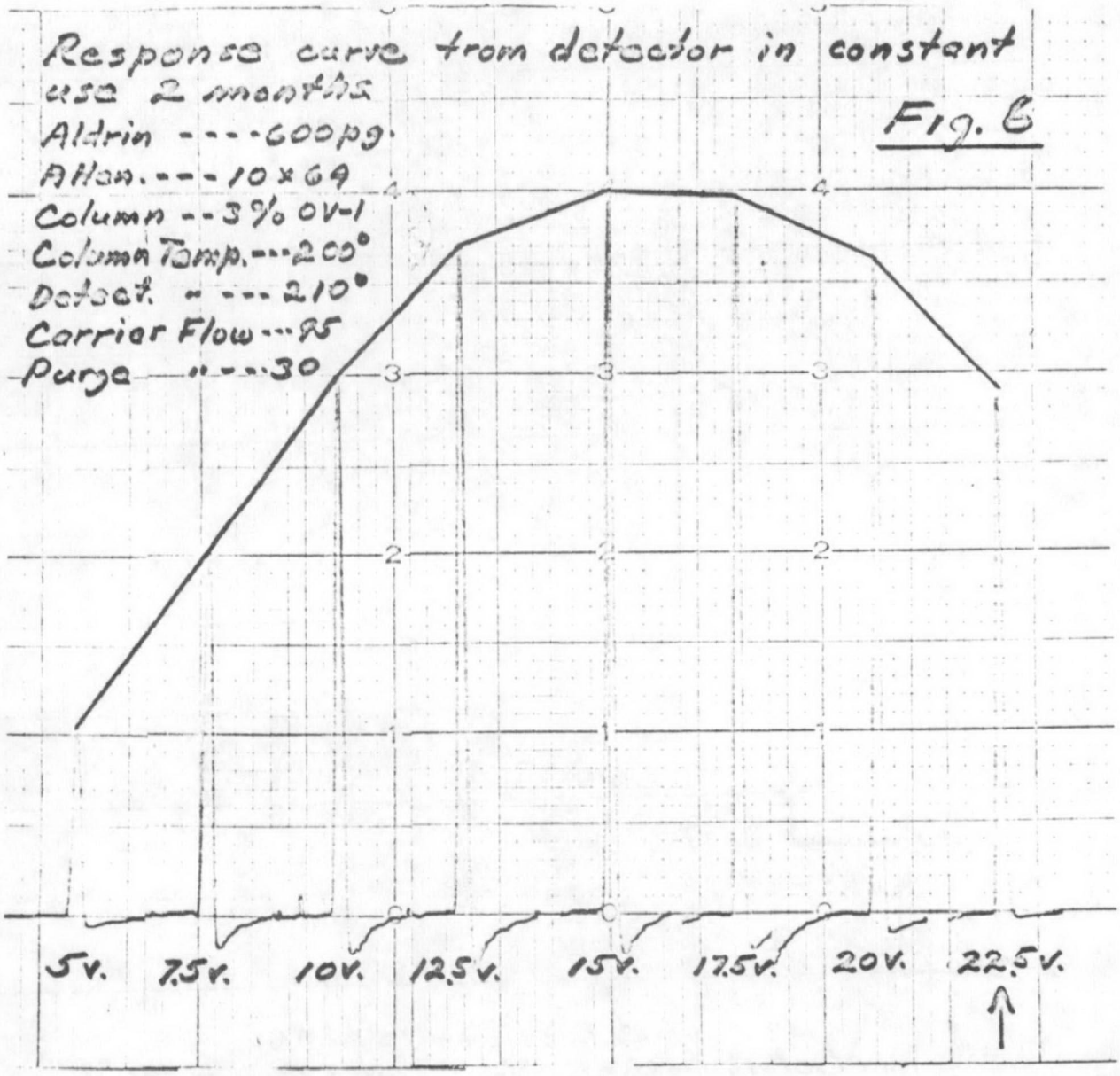


Fig. 5

Background signal profile of detector in constant use 2 months.



Attenuation – 10×256
Column – OV-1, 3%, Column Temp. – 180°C.
Detector Temp. – 190°C. Carrier 8
Purge Flow – 40 ml./min.


Response curve from detector in constant use 2 months
Fig. 6
Aldrin ----600pg.
Atten.---10 x 64
Column --3% OV-1
Column Temp.---200°
Detect. ----210°
Carrier Flow---95
Purge ----30
4
3
2
1
0
5v.
7.5v.
10v.
12.5v.
15v.
17.5v.
20v.
22.5v.

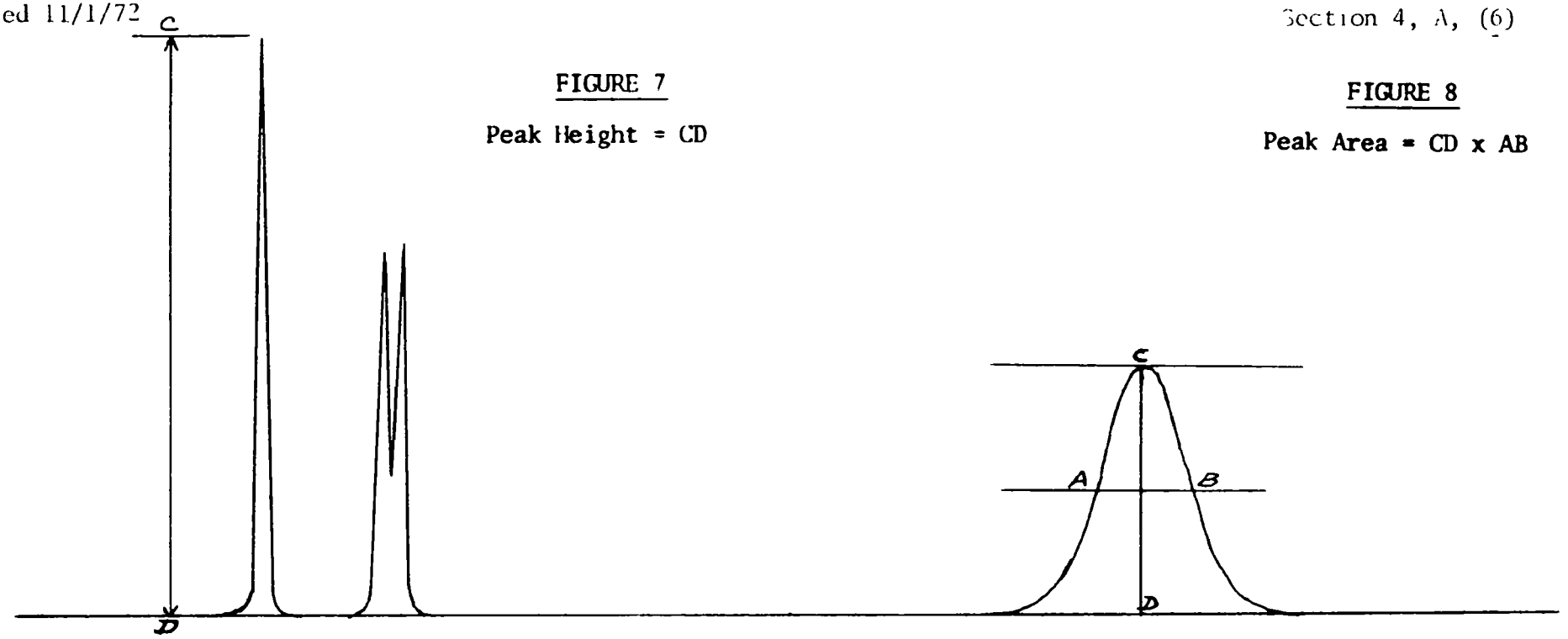


FIGURE 7

Peak Height = CD

FIGURE 8

Peak Area = CD x AB

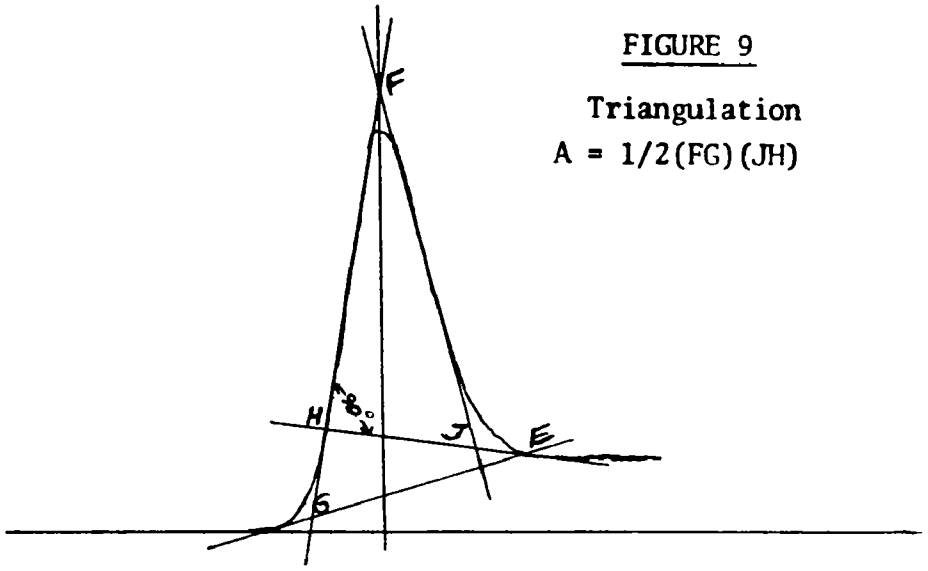
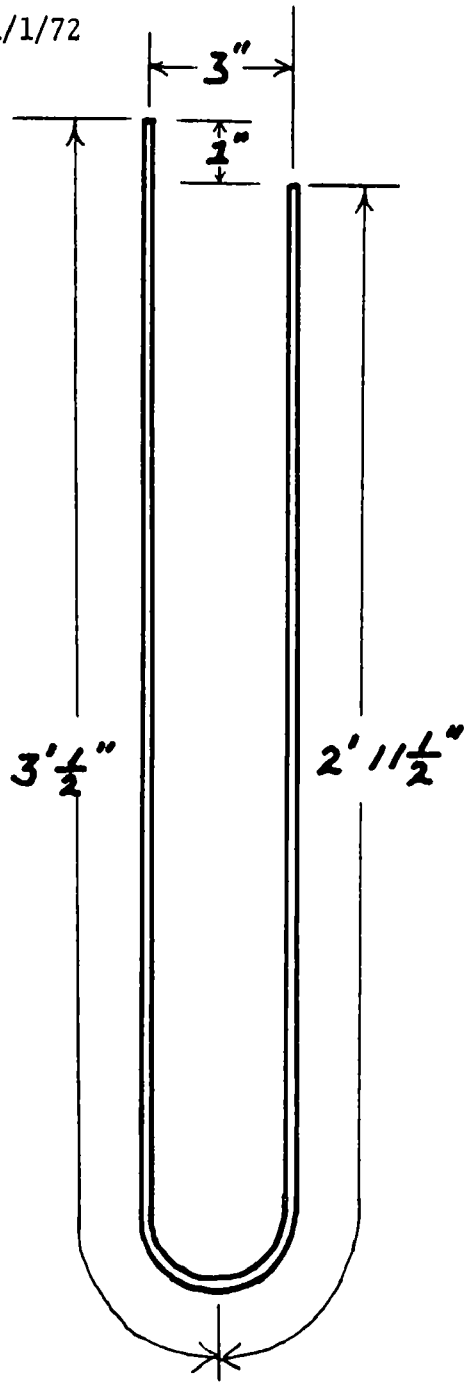


FIGURE 9

Triangulation

A = 1/2(FG)(JH)



Sketch of GLC Column for Off-Column Injection

To be of borosilicate glass, 1/4" o.d., 5/32" i.d., Corning No. 237300 or exact equivalent.

Ball joint to be on one side, not at U-bend.

Tubing to be sized to accomodate 1/4" Swagelok Nut.

Figure 11

Perrine Primate Research Laboratories, Environmental Protection Agency, Perrine, Florida 33157

Table 1. Conditioning, Operation Parameters and Performance Expectations for 6-ft. x 1/4-inch o.d. columns of Precoated Packings. 3% DEGS Included Solely as a Confirmatory Column, not for Routine Use.

Parameters	1.5% OV-17 / 1.95% QF-1	4% SE-30 / 6% OV-210	10% OV-210	3% DEGS
Liquid phase(s)	Silicone OV-17 Silicone DC QF-1 (FS1265)	Silicone SE-30 Silicone DC QF-1 (FS1265)	OV-210 Trifluoropropyl Silicone	DEGS Stabilized Diethylene Glycol Succinate (Analabs G4)
Solid Support	Chromosorb W, H.P. 100/120 mesh	Chromosorb W, H.P., 80/100 mesh	Chromosorb W, H.P. 100/120 mesh	Gas-Chrom P 80/100 mesh
Heat Curing Temp °C Time Hours	245 48 (minimum)	245 72 (minimum)	245 48 (minimum)	235 20 (exact)
Operating Temp., °C	200	200	200	195
Detector Temp °C (tritium)	205	205	205	205
Carrier Flow ml/min.	50-70	70-90	45-60	70-90
Elution Time for p,p'-DDT Approx (min)	16-20	16-20	16-20	16-20
Expected Minimum Efficiency (Total theor. plates in 6-ft. column basis p,p'-DDT)	3000	3000	3000	2800

1.5%OV-17/1.95% QF-1
Column Temperature, °C.

170	172	174	176	178	180	182	184	186	188	190	192	194	196	198	200	202	204	
0 32	0 32	0 32	0 32	0 32	0 32	0 32	0 32	0 32	0 32	0 33	0 33	0 33	0 33	0 33	0 33	0 33	0 33	Phosdrin
0 44	0 45	0 45	0 45	0 45	0 45	0 45	0 46	0 46	0 46	0 46	0 46	0 46	0 47	0 47	0 47	0 47	0 47	2,4 D(ME)
0 48	0 48	0 48	0 49	0 49	0 49	0 50	0 50	0 50	0 50	0 51	0 51	0 51	0 51	0 52	0 52	0 52	0 52	Thimet
0 48	0 48	0 49	0 49	0 50	0 50	0 50	0 51	0 51	0 52	0 52	0 52	0 53	0 53	0 54	0 54	0 54	0 55	α-BHC
0 54	0 54	0 54	0 54	0 55	0 55	0 55	0 55	0 55	0 55	0 55	0 55	0 56	0 56	0 56	0 56	0 56	0 56	CDEC
0 56	0 56	0 56	0 56	0 56	0 56	0 56	0 56	0 56	0 56	0 56	0 56	0 56	0 56	0 56	0 56	0 56	0 56	2,4-D(IPE)
0 70	0 70	0 70	0 70	0 69	0 69	0 69	0 69	0 69	0 68	0 68	0 68	0 68	0 67	0 67	0 67	0 67	0 67	Simazine
0 69	0 69	0 69	0 68	0 68	0 68	0 68	0 68	0 68	0 68	0 67	0 67	0 67	0 67	0 67	0 67	0 67	0 66	Atrazine
0 67	0 67	0 66	0 66	0 66	0 66	0 66	0 65	0 65	0 65	0 65	0 65	0 65	0 64	0 64	0 64	0 64	0 64	Diazinon
0 68	0 67	0 67	0 67	0 67	0 67	0 67	0 68	0 68	0 68	0 68	0 68	0 68	0 69	0 69	0 69	0 69	0 69	Lindane
0 76	0 76	0 76	0 76	0 75	0 75	0 75	0 75	0 75	0 74	0 74	0 74	0 74	0 73	0 73	0 73	0 73	0 73	2 4,5-T(ME)
0 82	0 82	0 82	0 82	0 81	0 81	0 81	0 81	0 81	0 81	0 81	0 80	0 80	0 80	0 80	0 80	0 80	0 80	β BHC
0 86	0 85	0 85	0 84	0 84	0 84	0 83	0 83	0 82	0 82	0 81	0 81	0 80	0 80	0 79	0 79	0 78	0 78	2,4-D(BE)I
0 97	0 97	0 97	0 96	0 96	0 96	0 95	0 95	0 94	0 94	0 94	0 93	0 93	0 93	0 92	0 92	0 92	0 91	δ BHC
0 82	0 82	0 82	0 82	0 82	0 82	0 82	0 82	0 82	0 82	0 82	0 82	0 82	0 82	0 82	0 82	0 82	0 82	Heptachlor
0 94	0 94	0 93	0 92	0 92	0 91	0 90	0 90	0 89	0 88	0 88	0 87	0 87	0 86	0 85	0 85	0 84	0 83	2,4,5-T(IPE)
1 03	1 02	1 01	1 01	1 00	0 99	0 99	0 98	0 97	0 96	0 96	0 95	0 94	0 93	0 93	0 92	0 91	0 90	2,4-D(BE)II
1 02	1 02	1 01	1 01	1 01	1 01	1 01	1 00	1 00	1 00	1 00	1 00	0 99	0 99	0 99	0 99	0 99	0 98	Dichlone
1 17	1 16	1 15	1 14	1 13	1 11	1 10	1 09	1 08	1 07	1 06	1 05	1 03	1 02	1 01	1 00	0 99	0 98	Dimethoate
1 00	1 00	1 00	1 00	1 00	1 00	1 00	1 00	1 00	1 00	1 00	1 00	1 00	1 00	1 00	1 00	1 00	1 00	Aldrin (REFERENCE)
1 17	1 16	1 16	1 15	1 14	1 14	1 13	1 12	1 12	1 11	1 10	1 09	1 09	1 08	1 07	1 07	1 06	1 05	Ronnel
1 41	1 40	1 39	1 38	1 36	1 35	1 34	1 33	1 32	1 31	1 30	1 29	1 28	1 27	1 26	1 25	1 24	1 23	1 Hydroxychlordene
1 71	1 69	1 67	1 66	1 64	1 62	1 60	1 59	1 57	1 55	1 53	1 52	1 50	1 48	1 47	1 45	1 43	1 41	M Parathion
1 70	1 69	1 68	1 67	1 66	1 65	1 64	1 63	1 62	1 61	1 59	1 58	1 57	1 56	1 55	1 54	1 53	1 52	Heptachlor Epoxide
2 07	2 04	2 01	1 98	1 95	1 92	1 89	1 87	1 84	1 81	1 78	1 75	1 72	1 69	1 66	1 63	1 60	1 57	Malathion
1 82	1 80	1 78	1 76	1 74	1 72	1 70	1 68	1 66	1 64	1 62	1 60	1 58	1 56	1 54	1 52	1 50	1 48	Dacthal
2 15	2 13	2 11	2 09	2 07	2 05	2 02	2 00	1 98	1 96	1 94	1 91	1 89	1 87	1 85	1 83	1 81	1 79	Dyrene
2 14	2 12	2 09	2 07	2 05	2 03	2 01	1 98	1 96	1 94	1 92	1 90	1 88	1 86	1 84	1 82	1 79	1 77	o,p'-DDE
2 20	2 18	2 16	2 14	2 12	2 10	2 08	2 06	2 05	2 03	2 01	1 99	1 97	1 95	1 93	1 91	1 89	1 87	Chlorobenside
2 32	2 28	2 25	2 22	2 19	2 16	2 13	2 09	2 06	2 03	2 00	1 97	1 93	1 90	1 87	1 84	1 81	1 78	E Parathion
2 20	2 18	2 16	2 15	2 13	2 11	2 10	2 08	2 06	2 05	2 03	2 01	2 00	1 98	1 97	1 95	1 93	1 91	Thiodan I
2 75	2 72	2 68	2 64	2 61	2 58	2 54	2 51	2 47	2 43	2 40	2 37	2 33	2 30	2 27	2 23	2 20	2 17	p,p'-DDE
2 97	2 93	2 88	2 84	2 79	2 75	2 71	2 66	2 62	2 57	2 53	2 49	2 44	2 40	2 35	2 31	2 27	2 22	DDA(ME)
3 27	3 22	3 18	3 13	3 07	3 04	3 00	2 95	2 91	2 86	2 81	2 77	2 73	2 68	2 63	2 59	2 54	2 50	Captan
3 32	3 27	3 23	3 18	3 13	3 08	3 04	2 99	2 95	2 91	2 87	2 82	2 77	2 73	2 68	2 64	2 59	2 55	Folpet
2 80	2 77	2 75	2 72	2 69	2 67	2 64	2 61	2 59	2 56	2 53	2 51	2 48	2 45	2 43	2 40	2 37	2 35	Dieldrin
3 52	3 46	3 41	3 35	3 30	3 24	3 19	3 14	3 08	3 03	2 98	2 92	2 87	2 82	2 76	2 71	2 66	2 60	Perthane
3 34	3 29	3 25	3 20	3 15	3 11	3 06	3 01	2 97	2 92	2 88	2 83	2 77	2 74	2 69	2 65	2 60	2 56	o,p' DDD
3 98	3 94	3 88	3 83	3 77	3 71	3 66	3 60	3 54	3 48	3 43	3 38	3 32	3 27	3 21	3 16	3 10	3 04	o,p'-DDT
3 47	3 43	3 40	3 36	3 33	3 29	3 26	3 22	3 18	3 15	3 12	3 08	3 04	3 01	2 97	2 93	2 90	2 87	Endrin
3 26	3 23	3 19	3 16	3 13	3 09	3 06	3 03	3 00	2 96	2 93	2 90	2 87	2 83	2 80	2 77	2 74	2 70	Kepone
4 65	4 57	4 49	4 41	4 33	4 26	4 18	4 10	4 02	3 94	3 87	3 79	3 71	3 64	3 61	3 48	3 40	3 32	p,p' DDD
4 46	4 39	4 34	4 28	4 23	4 17	4 11	4 05	3 99	3 94	3 88	3 82	3 76	3 71	3 65	3 59	3 54	3 48	Thiodan II
6 1	5 97	5 85	5 73	5 61	5 49	5 36	5 24	5 12	5 00	4 88	4 76	4 64	4 52	4 40	4 28	4 16	4 04	Ethion
5 57	5 48	5 39	5 29	5 20	5 11	5 01	4 92	4 83	4 74	4 64	4 55	4 46	4 36	4 27	4 18	4 09	4 00	p,p' DDT
6 4	6 2	6 1	5 99	5 88	5 76	5 64	5 52	5 40	5 28	5 16	5 04	4 92	4 80	4 68	4 56	4 44	4 32	Trithion
8 0	7 9	7 7	7 5	7 4	7 2	7 1	6 9	6 7	6 6	6 4	6 3	6 1	5 95	5 80	5 62	5 48	5 34	Dilan I
7 7	7 6	7 5	7 3	7 3	7 1	7 0	6 9	6 8	6 7	6 6	6 5	6 4	6 3	6 2	6 1	6 0	5 85	Mirex
12 4	12 1	11 8	11 6	11 3	11 0	10 7	10 4	10 1	9 8	9 5	9 3	9 0	8 7	8 4	8 1	7 8	7 5	Methoxychlor
9 4	9 2	9 0	8 8	8 6	8 4	8 2	8 0	7 8	7 6	7 3	7 1	6 9	6 7	6 5	6 3	6 1	5 88	Dilan II
16 9	16 5	16 1	15 7	15 3	14 9	14 5	14 1	13 7	13 3	12 9	12 5	12 1	11 7	11 3	10 9	10 5	10 2	Tedion
24 0	23 4	22 8	22 2	21 6	21 0	20 4	19 8	19 2	18 6	18 0	17 4	16 8	16 2	15 6	15 0	14 4	13 8	Guthion
170	172	174	176	178	180	182	184	186	188	190	192	194	196	198	200	202	204	

Retention ratios relative to aldrin, of 48 pesticides on a column of 1 5%OV-17/1 95%QF-1 at temperatures from 170 to 204°C, support of Chromosorb W,H P , 100/120 mesh, electron capture detector, tritium source, parallel plate, all absolute retentions measured from injection point Arrow indicates optimum column operating temperature with carrier flow at 60 ml per minute

4%SE-30/6%OV-210
Column Temperature, °C.

170	174	178	182	186	190	194	198	202	204	
0 27	0 28	0 28	0 28	0 29	0 29	0 29	0 30	0 30	0 30	Phosdrin
0 39	0 39	0 39	0 40	0 40	0 40	0 41	0 41	0 42	0 42	2,4 D(ME)
0 43	0 43	0 43	0 44	0 44	0 44	0 45	0 45	0 45	0 45	Thimet
0 42	0 43	0 43	0 44	0 44	0 44	0 45	0 45	0 46	0 46	α BHC
0 44	0 44	0 45	0 45	0 46	0 46	0 46	0 47	0 47	0 48	CDEC
0 54	0 54	0 54	0 54	0 54	0 55	0 55	0 55	0 55	0 55	2,4 D(IPE)
0 52	0 52	0 52	0 53	0 53	0 53	0 53	0 53	0 53	0 53	Simazine
0 55	0 55	0 55	0 55	0 55	0 56	0 56	0 56	0 56	0 56	Atrazine
0 60	0 60	0 60	0 60	0 59	0 59	0 59	0 59	0 59	0 59	Diazinon
0 54	0 54	0 55	0 55	0 55	0 56	0 56	0 57	0 57	0 57	Lindane
0 66	0 66	0 66	0 66	0 65	0 65	0 65	0 65	0 65	0 65	2,4,5-T(ME)
0 57	0 57	0 57	0 58	0 58	0 58	0 59	0 59	0 59	0 59	β BHC
0 85	0 84	0 84	0 83	0 83	0 82	0 82	0 82	0 81	0 81	2,4-D(BE)I
0 65	0 65	0 66	0 66	0 66	0 66	0 67	0 67	0 67	0 67	δ-BHC
0 80	0 80	0 81	0 81	0 81	0 81	0 81	0 82	0 82	0 82	Heptachlor
0 89	0 89	0 88	0 88	0 87	0 87	0 87	0 86	0 86	0 85	2,4,5-T(IPE)
0 98	0 97	0 96	0 95	0 95	0 94	0 93	0 93	0 92	0 91	2,4-D(BE)II
0 82	0 82	0 82	0 82	0 82	0 82	0 82	0 82	0 82	0 82	Dichlone
0 96	0 95	0 94	0 94	0 93	0 93	0 92	0 91	0 91	0 90	Dimethoate
1 00	1 00	1 00	1 00	1 00	1 00	1 00	1 00	1 00	1 00	Aldrin (REFERENCE)
1 01	1 00	1 00	0 99	0 98	0 98	0 97	0 96	0 96	0 95	Ronnel
1 04	1 04	1 04	1 03	1 03	1 03	1 03	1 03	1 03	1 03	1 Hydroxychlordene
1 49	1 48	1 47	1 46	1 45	1 44	1 43	1 42	1 41	1 40	M Parathion
1 53	1 53	1 52	1 51	1 50	1 50	1 49	1 48	1 48	1 47	Heptachlor Epoxide
1 70	1 68	1 66	1 64	1 63	1 61	1 59	1 57	1 55	1 53	Malathion
1 64	1 63	1 61	1 60	1 59	1 57	1 56	1 54	1 53	1 52	Dacthal
1 53	1 52	1 51	1 50	1 49	1 48	1 46	1 45	1 44	1 43	Dyrene
1 67	1 66	1 65	1 63	1 62	1 60	1 59	1 57	1 56	1 55	o,p' DDE
1 62	1 61	1 59	1 58	1 57	1 56	1 55	1 54	1 53	1 52	Chlorobenside
2 09	2 07	2 05	2 02	2 00	1 98	1 96	1 94	1 91	1 89	E Parathion
1 99	1 98	1 97	1 95	1 94	1 93	1 91	1 90	1 89	1 87	Thiodan I
2 16	2 14	2 11	2 09	2 07	2 05	2 02	2 00	1 98	1 95	p,p' DDE
2 27	2 25	2 22	2 19	2 16	2 13	2 10	2 07	2 04	2 01	DDA(ME)
2 27	2 25	2 23	2 21	2 19	2 16	2 14	2 12	2 10	2 07	Captan
2 22	2 20	2 18	2 16	2 14	2 12	2 10	2 08	2 06	2 04	Folpet
2 43	2 41	2 39	2 37	2 35	2 33	2 31	2 29	2 27	2 25	Dieldrin
2 43	2 40	2 37	2 33	2 30	2 27	2 24	2 20	2 17	2 14	Perthane
2 34	2 31	2 29	2 27	2 24	2 22	2 19	2 17	2 15	2 12	o,p'-DDD
3 02	2 97	2 93	2 88	2 84	2 80	2 76	2 73	2 68	2 64	o p'-DDT
2 76	2 73	2 71	2 69	2 67	2 64	2 62	2 60	2 58	2 55	Endrin
2 97	2 94	2 91	2 89	2 86	2 83	2 80	2 78	2 75	2 72	Kepone
3 22	3 17	3 13	3 08	3 04	2 98	2 94	2 90	2 86	2 82	p,p' DDD
3 19	3 16	3 13	3 10	3 07	3 04	3 00	2 97	2 94	2 91	Thiodan II
4 08	4 02	3 96	3 89	3 81	3 76	3 68	3 60	3 53	3 47	Ethion
4 04	3 98	3 92	3 86	3 80	3 73	3 67	3 61	3 54	3 48	p,p'-DDT
4 08	4 02	3 96	3 90	3 83	3 78	3 72	3 66	3 59	3 52	Trithion
6 2	6 1	5 98	5 83	5 72	5 60	5 49	5 38	5 27	5 17	Dilan I
6 1	6 0	5 96	5 87	5 78	5 68	5 60	5 52	5 43	5 33	Mirex
6 7	6 5	6 4	6 3	6 1	5 98	5 84	5 70	5 57	5 42	Methoxychlor
7 4	7 2	7 1	6 9	6 8	6 6	6 5	6 3	6 2	6 0	Dilan II
11 6	11 3	11 1	10 8	10 6	10 3	10 1	9 8	9 6	9 3	Tedion
12 5	12 2	11 9	11 7	11 4	11 1	10 8	10 6	10 3	10 0	Guthion

170	174	178	182	186	190	194	198	202	204

194	198	202	204	
0 31	0 31	0 32	0 32	Phosdrin
0 43	0 43	0 44	0 44	2,4 D(ME)
0 46	0 46	0 47	0 47	Thimet
0 47	0 48	0 48	0 49	α BHC
0 48	0 49	0 49	0 50	CDEC
0 55	0 55	0 55	0 55	2,4 D(IPE)
0 53	0 54	0 54	0 54	Simazine
0 56	0 57	0 57	0 57	Atrazine
0 58	0 58	0 58	0 58	Diazinon
0 57	0 59	0 59	0 60	Lindane
0 65	0 64	0 64	0 64	2,4,5-T(ME)
0 59	0 60	0 61	0 61	β BHC
0 80	0 79	0 79	0 78	2,4-D(BE)I
0 67	0 68	0 69	0 69	δ-BHC
0 82	0 82	0 83	0 83	Heptachlor
0 86	0 85	0 84	0 83	2,4,5-T(IPE)
0 93	0 92	0 89	0 88	2,4-D(BE)II
0 82	0 83	0 83	0 83	Dichlone
0 91	0 89	0 88	0 87	Dimethoate
1 00	1 00	1 00	1 00	Aldrin (REFERENCE)
0 96	0 94	0 93	0 92	Ronnel
1 03	1 03	1 02	1 02	1 Hydroxychlordene
1 37	1 36	1 35	1 34	M Parathion
1 45	1 44	1 44	1 43	Heptachlor Epoxide
1 48	1 46	1 44	1 42	Malathion
1 52	1 51	1 49	1 48	Dacthal
1 41	1 39	1 38	1 37	Dyrene
1 52	1 50	1 49	1 47	o,p' DDE
1 49	1 48	1 47	1 46	Chlorobenside
1 85	1 83	1 80	1 78	E Parathion
1 85	1 83	1 82	1 80	Thiodan I
1 91	1 89	1 86	1 84	p,p' DDE
1 96	1 93	1 90	1 87	DDA(ME)
2 03	2 01	1 99	1 96	Captan
2 08	2 06	2 04	2 02	Folpet
2 29	2 27	2 25	2 22	Dieldrin
2 20	2 17	2 14	2 11	Perthane
2 17	2 15	2 12	2 10	o,p'-DDD
2 73	2 68	2 64	2 60	o p'-DDT
2 60	2 58	2 55	2 53	Endrin
2 78	2 75	2 72	2 69	Kepone
2 90	2 86	2 82	2 77	p,p' DDD
2 97	2 94	2 91	2 88	Thiodan II
3 60	3 53	3 47	3 40	Ethion
3 61	3 54	3 48	3 43	p,p'-DDT
3 66	3 59	3 52	3 47	Trithion
5 38	5 27	5 17	5 02	Dilan I
5 52	5 43	5 33	5 24	Mirex
5 70	5 57	5 42	5 29	Methoxychlor
6 3	6 2	6 0	5 87	Dilan II
9 8	9 6	9 3	9 0	Tedion
10 3	10 0	9 7	9 4	Guthion

Retention ratios, relative to aldrin, of 48 pesticides on a column of 4%SE 30/6%QF 1 at temperatures from 170 to 204°C, support of Chromosorb W,H P , 80/100 mesh, electron capture detector, tritium source, parallel plate all absolute retentions measured from injection point Arrow indicates optimum column operating temperature with carrier flow at 70 ml per minute

5%OV-210
Column Temperature, °C.

170	174	178	182	186	190	194	198	202	204	
0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	Phosdrin
0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	2,4-D(ME)
0.63	0.64	0.64	0.64	0.64	0.64	0.64	0.65	0.65	0.66	Thimet
0.62	0.62	0.62	0.63	0.63	0.64	0.64	0.65	0.65	0.65	α-BHC
0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.70	CDEC
0.88	0.87	0.87	0.87	0.86	0.86	0.86	0.85	0.85	0.85	2,4-D(IPE)
0.87	0.86	0.86	0.86	0.85	0.85	0.85	0.84	0.84	0.84	Simazine
0.88	0.88	0.87	0.87	0.86	0.86	0.86	0.85	0.85	0.84	Atrazine
0.76	0.75	0.75	0.75	0.75	0.75	0.75	0.74	0.74	0.74	Diazinon
0.80	0.80	0.80	0.80	0.80	0.81	0.81	0.81	0.81	0.82	Lindane
1.08	1.08	1.07	1.07	1.06	1.05	1.05	1.04	1.03	1.03	2,4,5-T(ME)
0.97	0.97	0.96	0.96	0.96	0.96	0.96	0.95	0.95	0.95	β BHC
1.30	1.29	1.28	1.27	1.26	1.25	1.23	1.22	1.21	1.20	2,4-D(BE)I
1.07	1.07	1.06	1.06	1.05	1.05	1.05	1.04	1.04	1.03	δ-BHC
0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	Heptachlor
1.36	1.34	1.33	1.32	1.30	1.29	1.27	1.26	1.25	1.23	2,4,5-T(IPE)
1.47	1.46	1.44	1.43	1.41	1.40	1.38	1.38	1.35	1.33	2,4-D(BE)II
1.51	1.51	1.50	1.49	1.48	1.48	1.47	1.46	1.45	1.45	Dichlone
2.18	2.15	2.12	2.09	2.06	2.04	2.01	1.98	1.95	1.92	Dimethoate
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Aldrin (REFERENCE)
1.41	1.40	1.39	1.38	1.37	1.36	1.35	1.34	1.33	1.32	Ronnel
1.43	1.42	1.41	1.40	1.39	1.38	1.38	1.37	1.36	1.35	1-Hydroxychlordene
3.17	3.12	3.07	3.02	2.97	2.92	2.88	2.83	2.78	2.73	M Parathion
2.02	2.00	1.98	1.97	1.95	1.93	1.91	1.89	1.87	1.85	Heptachlor Epoxide
3.20	3.14	3.08	3.02	2.96	2.91	2.85	2.79	2.73	2.67	Malathion
2.84	2.80	2.75	2.71	2.67	2.63	2.59	2.54	2.50	2.46	Dacthal
2.06	2.04	2.02	1.99	1.97	1.94	1.91	1.89	1.87	1.84	Dyrene
1.67	1.65	1.63	1.62	1.60	1.59	1.57	1.55	1.53	1.52	o,p'-DDE
1.97	1.95	1.93	1.90	1.88	1.86	1.84	1.82	1.80	1.78	Chlorobenside
4.24	4.17	4.09	4.02	3.94	3.87	3.78	3.71	3.63	3.56	E Parathion
2.63	2.60	2.57	2.54	2.51	2.48	2.45	2.42	2.39	2.36	Thiodan I
2.24	2.21	2.18	2.15	2.12	2.10	2.07	2.04	2.01	1.98	p,p'-DDE
3.10	3.05	3.00	2.94	2.89	2.84	2.79	2.74	2.69	2.64	DDA(ME)
4.47	4.40	4.32	4.24	4.17	4.09	4.01	3.93	3.85	3.77	Captan
4.04	3.98	3.92	3.85	3.78	3.72	3.65	3.59	3.53	3.46	Folpet
3.21	3.17	3.13	3.09	3.04	3.00	2.96	2.92	2.88	2.83	Dieldrin
2.07	2.04	2.01	1.98	1.95	1.92	1.89	1.86	1.83	1.80	Perthane
2.74	2.70	2.67	2.63	2.59	2.55	2.51	2.47	2.43	2.39	o,p'-DDD
2.92	2.87	2.83	2.79	2.74	2.70	2.65	2.61	2.57	2.52	o,p'-DDT
3.82	3.77	3.72	3.67	3.61	3.56	3.50	3.45	3.39	3.34	Endrin
2.76	2.73	2.70	2.67	2.64	2.61	2.58	2.55	2.52	2.49	Kepone
4.10	4.03	3.96	3.89	3.82	3.75	3.68	3.61	3.53	3.46	p,p'-DDD
4.98	4.91	4.83	4.75	4.67	4.59	4.51	4.43	4.35	4.27	Thiodan II
6.0	5.89	5.76	5.64	5.51	5.37	5.25	5.12	4.99	4.86	Ethion
4.47	4.39	4.31	4.23	4.15	4.07	3.98	3.90	3.82	3.74	p,p'-DDT
5.29	5.19	5.09	4.99	4.89	4.78	4.68	4.58	4.48	4.37	Trithion
12.3	12.0	11.7	11.4	11.1	10.8	10.4	10.1	9.8	9.5	Dilan I
4.06	4.01	3.95	3.89	3.84	3.78	3.73	3.68	3.62	3.57	Mirex
7.4	7.2	7.0	6.8	6.7	6.5	6.3	6.1	5.94	5.76	Methoxychlor
14.2	13.8	13.4	13.0	12.6	12.3	11.9	11.5	11.1	10.7	Dilan II
21.1	20.6	20.0	19.5	18.9	18.4	17.9	17.3	16.7	16.2	Tedion
24.7	24.0	23.3	22.6	21.9	21.2	20.6	19.9	19.2	18.5	Guthion
170	174	178	182	186	190	194	198	202	204	

Retention ratios, relative to aldrin, of 48 pesticides on a column of 5%OV-210 at temperatures from 170 to 204°C, support of Chromosorb W,H P , 100/120 mesh, electron capture detector, tritium source, parallel plate, all absolute retentions measured from injection point Arrow indicates optimum column operating temperature with carrier flow at 60 ml per minute

GAS CHROMATOGRAPHY-FLAME PHOTOMETRIC

INSTRUMENT

See Section 4,A,(1). This unit may be equipped with a Melpar Flame Photometric detector (F.P.D.).

I. FLOW SYSTEM:

See Section 4,A,(1), I. Flow systems should be tight but the F.P.D. is not as sensitive to leaks as electron capture detection.

In addition to the carrier gas, the F.P.D. requires Hydrogen, Oxygen and possibly air to operate. Leaks in these systems can be hazardous from the explosion standpoint.

II. DETECTOR:

This subject is covered in detail later in Section 4,B,(3).

III. ELECTROMETER:

See Section 4,A,(1), III. The electrometer for the F.P.D., must deliver at least 750 VDC. The electrometer should also be capable of delivering at least 1×10^{-6} amperes bucking current.

IV. TEMPERATURE PROGRAMMER:

See Section 4,A,(1), IV.

V. PYROMETER:

See Section 4,A,(1), V.

VI. MISCELLANEOUS:

See Section 4,A,(1), VI.

GAS CHROMATOGRAPHY-FLAME PHOTOMETRIC

COLUMNS

I. SPECIFICATIONS:

See Section 4,A,(2), I.

II. COLUMN SELECTION:

A. 4% SE-30/6% QF-1 - liquid phases premixed and coated on silanized support, 80/100 mesh.

B. 5% OV-210 - coated on silanized support, 100/120 mesh. See Section 4,A,(2), II.

III. PACKING THE COLUMN:

See Section 4,A,(2), III.

IV. COLUMN CONDITIONING:

1. Heat condition a 6-foot column of 4% SE-30/6% OV-210 (QF-1) according to Section 4,A,(2), IV, 1.

2. Carbowax treatment - Place a small wad (ca 1/2") silanized glass wool in one end of a 3" length of 1/4" o.d. glass tubing. Pack loosely with 2 inches of 10% Carbowax and place another wad of glass wool in the other end of the tube (See Fig. 1).

   Place Swagelok nut, ferrule and "O" ring on each end of the packed tube. Pass one end of the tube half way through the male union and attach Swagelok nut to union as tight as possible.

   Attach other end of tube to the column inlet port in the oven, tightening Swagelok nut as much as possible. Place 1/4" nut on inlet end of the 6-ft. GLC column (previously heat conditioned) and insert into the male union until it touches bottom end of 3" tube, then slack off very slightly to prevent glass ends from grinding together when nut is tightened. (See Fig. 2).

   Thoroughly tighten Swagelok nut to attach GLC column to male union and place some object on the floor of the oven to function as a retainer in case GLC column should slip out of the union during conditioning period. A littlejack works nicely. Bring oven heat up to 230-235°C and apply a carrier gas flow of 20 ml/min. Hold for a 17-hour period.

NOTES: 1. The combined parameters of temperature, time and carrier flow are critical in the assurance of uniformity of vapor phase deposition as affecting ultimate retention characteristics.

2. Special materials needed include:

(a) A 3-inch length of borosilicate glass tubing, 1/4" o.d. x 5/32" i.d. (or 6 mm x 4 mm).
(b) Silanized glass wool.
(c) 10% Carbowax 20M on a silanized support, 80/100 mesh.
(d) Swagelok male union #400-6, 1/4". This must be drilled out to accommodate the 1/4" o.d. tubing.

3. DO NOT USE A SILYLATED COLUMN WITH F.P.D. The Silyl-8 will bleed into the F.P.D. and fog the heat shield excessively.

V. EVALUATION OF COLUMN:

See Section 4,A,(2), V.

A mixture containing the following should give approximately equal peak heights.

Compound	ng/5µl injection
Ethyl Parathion	0.50
Methyl Parathion	0.38
Diazinon	0.17
Ronnel	0.25
Malathion	0.51
Trithion	1.22
Ethion	0.58

NOTE: A drastic reduction in the peak height of malathion can be an indication of a poor column, provided that the rest of the system is known to be operating properly.

VI. MAINTENANCE AND USE OF COLUMN:

See Section 4,A,(2), VI.

The effects of the vapor phase deposition from Carbowax appear to persist at least three months with a slow decrease in response becoming evident, depending on the particular column and the amount and type of use.

Each operator should monitor the response characteristics in relationship to the column just after treatment.

NOTES: 1. Response will sometimes drop rapidly for several days after treatment, then stabilize.

2. Retreatment of the same columns appears to rejuvenate the response, but will shift the RRR-p-values. Retreatment of the column is not recommended because the table of RRR-p-values would lose its usefulness.

GAS CHROMATOGRAPHY-FLAME PHOTOMETRIC DETECTOR

D.C. voltage shall be supplied to the detector from either an outboard power supply or from a strip on the back of the electrometer. Provided the column and all electronic circuits in the various modules of the instrument are functioning properly, the degree of sensitivity in the flame photometric mode relate to four factors: (1) condition of the photomultiplier (P.M.) tube; (2) voltage applied to P.M. tube; (3) flow rates of hydrogen, oxygen and air; and (4) condition of interior of detector.

I. OPTIMUM FLOW RATES:

A. Set hydrogen flow rate at 150-200 ml/min.

B. Maximum response by varying the oxygen flow with air flow at zero.

C. Maximize response by varying the air flow with oxygen set at optimum flow rate.

NOTES: (1) Some detectors respond best with no air flow.

(2) An increase in flow rates, while increasing response, may increase the baseline noise. Signal to noise ratio is a more accurate definition of sensitivity than response alone.

Suggested operating parameters for the phosphorus mode are as follows:

Temperatures		Flow Rates (ml/min.)	
Column	200°C	(2) Purge	70-80
Injection Block	225°C	Carrier	70-80
(1) Detector	165-200°C	Hydrogen	150-200
Transfer Line	235°C	Oxygen	10-30
(2) Switching Valve	235°C	Air	0-100

(1) Do not operate above 170°C unless fitted with heat shield.

(2) Appropriate only if using a Valco vent valve.

II. OPTIMUM RESPONSE VOLTAGE:

In order to determine the optimum response voltage for the P.M. tube, a variable power supply is necessary which allows the voltage to be increased with little increase in electronic noise. Increasing the voltage from the electrometer will increase the electronic noise inordinately.

A. With flows at optimum, set power supply at 750 V.D.C. Inject enough Ethyl Parathion to give 35-60 f.s.d.

B. Reset to 850 V. Inject the same amount of Ethyl Parathion as before.

C. Repeat in 100 V increments until the signal to noise ratio reaches maximum and starts to decrease. (See Fig. 4).

NOTES: (1) It should be necessary to attenuate to keep on scale. It is therefore mandatory to check the linearity of the electrometer at different attenuations.

(2) Comparison with a P.M. tube of known sensitivity will give indication of condition of P.M. tube.

III. DETECTOR LINEARITY:

The F.P.D. has a broad range of linearity. Excluding any effect from the instrument electronics, the effective range is from 1 to 50 times the minimum acceptable level of Ethyl Parathion (0.5 ng to 25 ng). The appropriate attenuation will be dependent upon the sensitivity of the particular system used. However, it is best to operate at the minimum detection level and dilute the sample when necessary.

IV. PHOSPHORUS MODE:

When the detector is fitted with a 526 mμ filter, it becomes selective for phosphorus. Large amounts of sulfur will give a response in this mode.

V. SULFUR MODE:

When the detector is fitted with a 394 mμ filter, it becomes selective for sulfur. Sensitivity for sulfur is usually an order of magnitude less than for phosphorus since sulfur response increases approximately as the square of the concentration.

GAS CHROMATOGRAPHY-FLAME PHOTOMETRIC

SAMPLE QUANTITATION AND INTERPRETATION

I. See Section 4,A,(4).

The priming mixture below is given in nanograms per microliter.

Ethyl Parathion	1.0	Malathion	1.0
Methyl Parathion	1.0	Ethion	1.0
Ronnel	0.5	Trithion	2.0
Diazinon	0.5		

Forty microliters of this mixture is injected. Do not inject with the same syringe used for routine work.

II. Peak Height:

See Section 4,A,(5),I.

III. Peak Height x width at half height:

See Section 4,A,(5),II.

NOTE: Both I. and II. presuppose that the absolute retention of standard and sample are the same.

IV. Triangulation or Integration:

See Section 4,A,(5),III.

V. Interpretation:

Because of the selectivity of the filters, interpretation is greatly simplified.

Identification of a thiophosphate can be accomplished in the following manner:

1. Retention ratio on a given column matched with a standard.
2. Suspect compound in the correct Florisil elution.
3. Response on a selective detector.
4. Sulfur to phosphorus ratio matched against a standard.

FIGURE 1

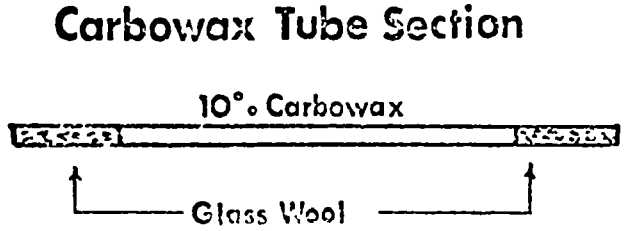


FIGURE 2

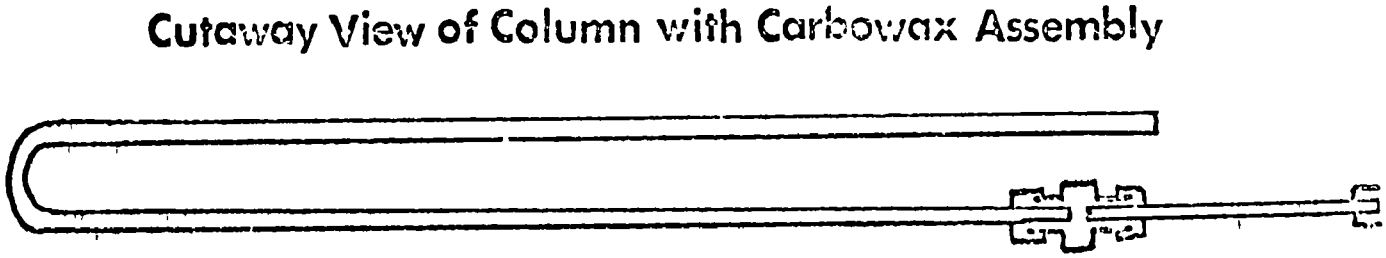


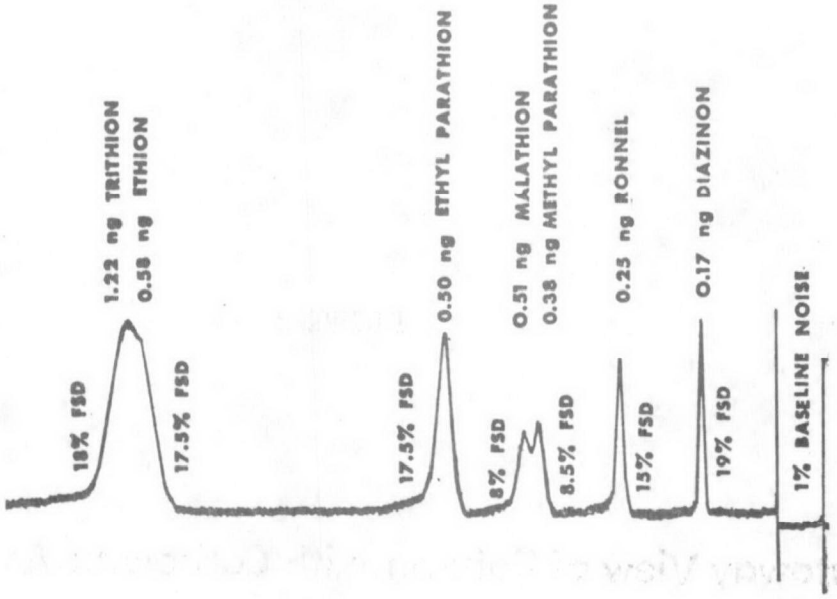
FIGURE 3

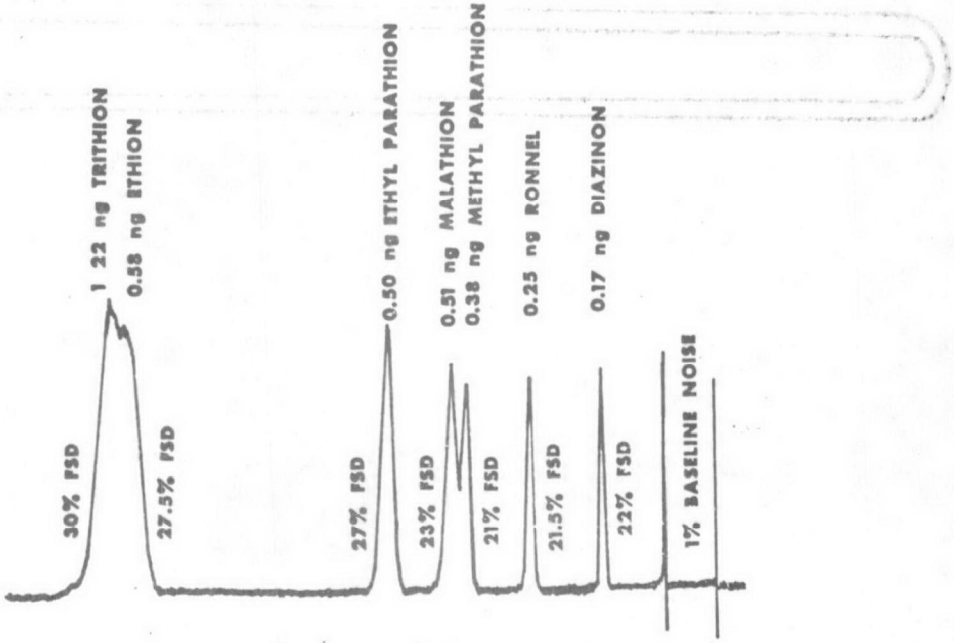
Chromatograms of a mixture of 7 organophosphorous pesticides on an untreated column of 4% SE-30/6% QF-1 (Fig.1),and on the same column treated with Carbowax(Fig.2)

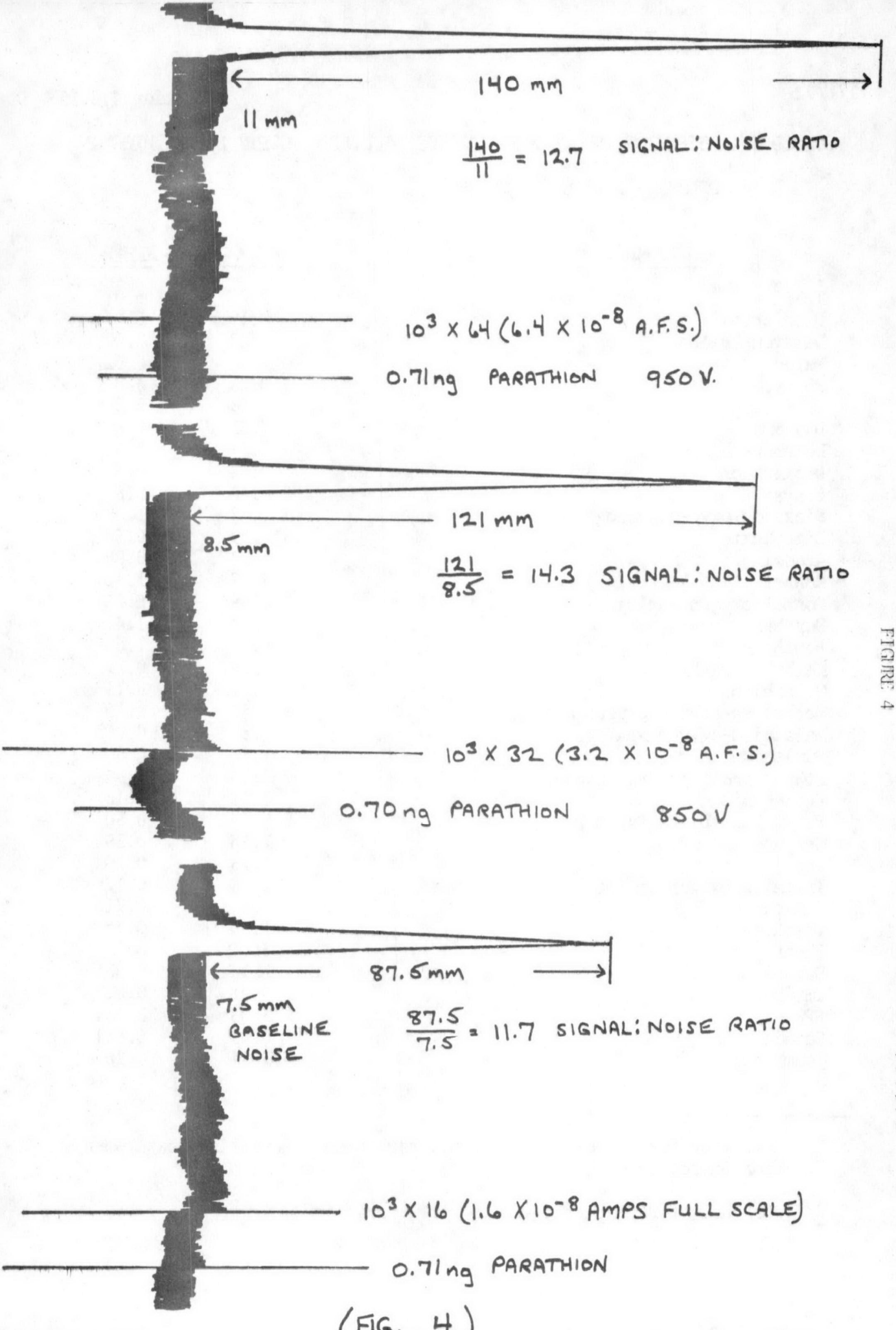
Column:4% SE-30/6% QF-1;amps,full scale 0.8 x10 -8 ;voltage 850 v.

OPERATING PARAMETERS

TEMP., C.		FLOW RATES,ml/min.	
Column	200	Carrier	60
Inlet	225	Vent	60
Detector	195	Oxygen	30
Transf.line	235	Hydrogen	180
Vent	235	Air	40







(FIG. 4)

FIGURE 4

TABLE I. RETENTION AND RESPONSE RATIOS, RELATIVE TO ETHYL PARATHION ON COLUMN, OF 4% SE-30/6% QF-1

COMPOUND	RRR-P[1/]	RPH-P[2/]
TEPP	0.08	5.0
Dichlorvos	.10	5.0
Demeton-Thiono	.22	2.0
Naled	.28	0.02
Phorate	.28	4.0
Sulfotepp	.28	5.2
Diazinon	.35	2.5
Demeton-thiolo	.37	2.0
Dioxathion	.38	0.5
Disyston	.40	3.8
Diazinon-oxygen analog	.42	1.0
Dimethoate	.49	0.50
Azodrin	.54	0.08
Ronnel	.57	1.42
Ronnel-oxygen analog	.58	0.25
Dursban	.68	1.4
Fenthion	.72	1.6
Methyl Parathion	.75	0.71
Malathion	.81	0.71
Methyl Parathion-oxygen analog	.83	0.10
Malathion-oxygen analog	.85	0.063
Sumithion	.85	0.80
Ethyl Parathion (reference)	*1.00*	*1.00*
Phosphamidon	1.02	0.16
Ethyl Parathion-oxygen analog	1.10	0.50
Merphos	1.23	0.35
DEF	1.25	0.80
Trithion-oxygen analog	1.78	0.12
Ethion	1.83	0.71
Trithion	1.90	0.36
Phenkapton	3.04	0.20
Dasanit	3.16	0.03
Imidan	3.91	0.02
EPN	3.95	0.134
Guthion	6.03	0.044
Coumaphos	11.84	0.20

1/ Retention ratio, relative to ethyl parathion. Retention measurements from injection point.

2/ Peak height ratio, relative to ethyl parathion.

★ANALYSIS OF HUMAN OR ANIMAL ADIPOSE TISSUE*

(MODIFIED MILLS, ONLEY, GAITHER PROCEDURE)

I. EQUIPMENT:

1. Aluminum foil, household type.
2. Beakers, 250 ml, stainless steel or heavy duty glass.
3. Beakers, 250 ml, Griffin low form.
4. Stirring rods, glass 10 mm diameter.
5. Water bath with temperature adjustment of 90-100°C.
6. Filter paper - Whatman No. 1, 15 cm diameter.
7. Funnels, glass, ca 60 ml diameter.
8. Separatory funnels - 125 ml and 1 liter, Kimble 29048-F, or equiv.
9. Chromatographic columns - 25 mm o.d. x 300 mm long, with Teflon stopcocks, without fritted glass plates, Kontes 420530, Size 241.
10. Filter tubes, 150 x 24 mm, such as Corning #9480.
11. Erlenmeyer flasks - 500 ml capacity.
12. Kuderna-Danish concentrator fitted with grad. evaporative concentrator tube. Available from the Kontes Glass Company, each component bearing the following stock numbers:
    a. Flask, 500 ml, stock #K-570001
    b. Snyder column, 3-ball, stock #K-503000
    c. Steel springs, 1/2", stock #K-662750
    d. Concentrator tubes, 10 ml, size 1025, stock #K-570050
13. Modified micro-Snyder columns, 19/22, Kontes K-569251.
14. Glass beads, 3 mm plain, Fisher #11 - 312 or equivalent.

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* This method, with appropriate modifications, may be used for the analysis of other tissues if original sample size is adequate.

15. Glass wool - Corning #3950 or equivalent.

II. REAGENTS:

1. Petroleum ether - Pesticide Quality, redistilled in glass, b.p. 30° - 60°C. (See Note 7, p. 10).

2. Diethyl ether - AR grade, peroxide free, Mallinckrodt #0850 or the equivalent. The ether must contain 2% (v/v) absolute ethanol. Some of the AR grade ethers contain 2% ethanol, added as a stabilizer, and it is therefore unnecessary to add ethanol unless peroxides are found and removed.

   NOTE: To determine the absence of peroxides in the ether, add 1 ml of freshly prepared 10% KI solution to 10 ml of ether in a clean 25-ml cylinder previously rinsed with the ether. Shake and let stand 1 minute. A yellow color in ether layer indicates the presence of peroxides which must be removed before using. See Misc. Note 4 at end of procedure. The peroxide test should be repeated at weekly intervals on any single bottle or can as it is possible for peroxides to form from repeated opening of the container.

3. Eluting mixture, 6% (6+94) - purified diethyl ether 60 ml is diluted to 1000 ml with redistilled petroleum ether and anhydrous sodium sulfate (10-25 g) is added to remove moisture.

4. Eluting mixture, 15% (15+85) - purified diethyl ether 150 ml is diluted to 1000 ml with redistilled petroleum ether and dried as described above.

   NOTE: Neither of the eluting mixtures should be held longer than 24 hours after mixing.

5. Florisil, 60/100 mesh, PR grade, to be stored at 130°C until used.

   NOTE: (1) In a high humidity room, the column may pick up enough moisture during packing to influence the elution pattern. To insure uniformity of the Florisil fractionation, it is recommended to those laboratories with sufficiently large drying ovens that the columns be packed ahead of time and held (at least overnight) at 130°C until used.

   (2) Florisil furnished to the contract laboratories by the Perrine laboratory on order, has been activated

by the manufacturer, and elution pattern data is included with each shipment. However, each laboratory should determine their own pesticide recovery and elution pattern on each new lot received, as environmental conditions in the various laboratories may differ somewhat from that in Perrine. Each new batch should be tested by the procedure described in Section 3,D for assurance that the operator can obtain recoveries and compound elution patterns comparable to the data given on the accompanying table.

6. Acetonitrile, reagent grade, saturated with pet. ether.

   NOTE: Occasional lots of CH_3CN are impure and require redistillation. Generally, vapors from impure acetonitrile will turn litmus paper blue when the moistened paper is held over the mouth of the bottle.

7. Anhydrous sodium sulfate, reagent grade granular, Mallinkrodt stock #8024 or the equivalent.

   NOTE: When each new bottle is opened, it should be tested for contaminants that will produce peaks by Electron Capture Gas Liquid Chromatography. This may be done by transferring ca 10 grams to a 125 ml Erlenmeyer flask, adding 50 ml pet. ether, stoppering and shaking vigorously for 1 minute. Decant extract into a 100-ml beaker and evaporate down to ca 5 ml. Inject 5 µl into the Gas Liquid Chromatograph and observe chromatogram for contaminants. When impurities are found, it is necessary to remove them by extraction. This may be done by using hexane in a continuously cycling Soxhlet extraction apparatus or by several successive rinses with hexane in a beaker. The material is then dried in an oven and kept in a glass-stoppered container.

8. Sodium Chloride solution, 2%, from reagent grade NaCl.

   NOTE: See NOTE for sodium sulfate, Step 7, above.

9. Sand, quartz, which has been acid washed and extracted with hexane to produce a zero background in the determinative step.

10. MgO-Celite mixture (1:1) weigh equal parts of reagent grade MgO and Celite 545 and mix thoroughly.

11. Hexane, redistilled, pesticide quality.

III. SAMPLE PREPARATION & EXTRACTION:

1. On a cupped sheet of lightweight aluminum foil, weigh 5 grams of the previously minced fat. Transfer entire cup to a 250 ml stainless steel or heavy duty glass beaker.

2. Add ca 10 grams of clean, sharp sand, ca 10 grams of anhydrous Na_2SO_4 and 1.0 ml of hexane solution containing 200 nanograms of aldrin.

   NOTE: The aldrin is added here for the dual purpose of (1) providing a built-in retention marker for direct peak identification on all chromatograms of the first fraction extract, and (2) as a quantitative recovery check for the procedure. This inoculation should, of course, not be made if aldrin is suspected to be in the substrate.

3. Grind the mixture with a heavy glass rod and continue adding portions of Na_2SO_4 to give a uniform, dry granular mass.

4. Add 50 ml of pet. ether and warm carefully on a water bath with continuous stirring until solvent boils gently.

5. Place Whatman No. 1 filter paper in glass funnel and rinse several times with pet. ether. Place funnel over previously tared 250-ml beaker and transfer extract to funnel by decantation.

6. Extract the contents of the first beaker with two more 50-ml portions of pet. ether as described in steps 4 and 5.

7. Transfer insoluble material to the filter paper and rinse beaker and paper with a final 10 ml of pet. ether.

8. Place beaker on a 40°C water bath and evaporate just to dryness under a stream of nitrogen. Check odor to be sure all solvent is removed and allow to cool to room temperature in a dessicator.

9. Weigh beaker and record for calculation of percent fat in the sample.

10. Accurately weigh between 2.8 and 3.0 grams of the fat obtained in Step 9 into a 125-ml separator. Add 12 ml of pet. ether previously saturated with acetonitrile.

NOTE: In the case of highly saturated animal fat, it will be necessary to add 17 ml of pet. ether to the separator. In such a case the amount of acetonitrile used in the partitioning step should be increased to 40 ml.

IV. LIQUID-LIQUID PARTITIONING:

1. Add 30 ml of acetonitrile, previously saturated with pet. ether. Stopper funnel and shake vigorously for 2 minutes.

2. Allow phases to separate and draw off the acetonitrile layer into a 1-liter separator containing 700 ml of a 2% solution of NaCl and 100 ml of pet. ether.

3. Similarly extract the pet. ether layer in the 125-ml separator three more times with 30-ml portions of acetonitrile, combining all acetonitrile extracts in the 1-liter separator.

4. Stopper, invert 1-liter separator, vent off pressure and mix by shaking for two minutes, releasing pressure as required.

5. Allow the layers to separate and drain aqueous layer into a second 1-liter separator.

6. Add 100 ml pet. ether to second separator, and after a 30-second vigorous shaking, discard aqueous phase and transfer pet. ether phase into first 1-liter separator.

7. Wash pet. ether with two 100-ml portions 2% NaCl and discard the aqueous washings.

8. Prepare a 2-inch column of anhydrous, granular Na_2SO_4 in a 150 x 24 mm filter tube and position over a 500-ml K-D evaporator fitted with a 10-ml grad. concentrator tube containing one glass bead. Dry the pet. ether by filtering through this column. Rinse the separator twice with 10-ml portions of pet. ether and finally rinse down sides of the filter tube with 10 ml pet. ether.

9. Attach a 3-ball Snyder column to the top of the K-D evaporator and place in a 90-100°C water bath. Approximately 1-1/2 inches of the concentrator tube should be below the surface of the water.

10. Concentrate the extract to ca 5 ml, rinse down the sides of K-D evaporator and the ground glass joint with a total of 3 ml pet. ether. Reconcentrate extract to ca 5 ml under a gentle stream of nitrogen at room temperature.

V. FLORISIL FRACTIONATION:

1. Prepare a chromatographic column containing 4 inches (after settling) of activated Florisil topped with 1/2 inch of anhydrous, granular Na_2SO_4. A small wad of glass wool, preextracted with pet. ether, is placed at the bottom of the column to retain the Florisil.

   NOTES: (1) If the oven is of sufficient size, the columns may be prepacked and stored in the over, withdrawing columns a few minutes before use.

   (2) The amount of Florisil needed for proper elution should be determined for each lot of Florisil.

2. Place a 500-ml Erlenmeyer flask under the column and prewet the packing with pet. ether (40-50 ml, or a sufficient volume to completely cover the Na_2SO_4 layer).

   NOTE: From this point and through the elution process, the solvent level should never be allowed to go below the top of the Na_2SO_4 layer. If air is introduced, channeling may occur, making for an inefficient column.

3. Using a 5-ml Mohr or a long disposable pipet, immediately transfer the tissue extract (ca 5 ml) from the evaporator tube onto the column and permit it to percolate through.

4. Rinse tube with two successive 5-ml portions of pet. ether, carefully transferring each portion to the column with the pipet.

   NOTE: Use of the Mohr or disposable pipet to deliver the extract directly onto the column precludes the need to rinse down the sides of the column.

5. Prepare two Kuderna-Danish evaporative assemblies complete with 10 ml graduated evaporative concentrator tubes. Place one glass bead in each concentrator tube.

6. Replace the 500-ml Erlenmeyer flask under each column with a 500-ml Kuderna-Danish assembly and commence elution with 200 ml of 6% diethyl ether in pet. ether (Fraction I). The elution rate should be 5 ml per minute. When the last of the eluting solvent reaches the top of the Na_2SO_4 layer, place a second 500-ml Kuderna-Danish assembly under the column and continue elution with 200 ml of 15% diethyl ether in pet. ether (Fraction II).

7. To the second fraction only, add 1.0 ml of hexane containing 200 nanograms of aldrin, place both Kuderna-Danish evaporator assemblies in a water bath and concentrate extract until ca 5 ml remain in the tube.

8. Remove assemblies from bath, and cool to ambient temperature.

9. Disconnect collection tube from Kuderna-Danish flask and carefully rinse joint with a little hexane.

10. Attach modified micro-Snyder column to collection tubes, place tubes back in water bath and concentrate extracts to 1 ml. If preferred, this may be done at room temperature under a stream of nitrogen.

11. Remove from bath, and cool to ambient temperature. Disconnect tubes and rinse joints with a little hexane.

    NOTE: The extent of dilution or concentration of the extract at this point is dependent on the pesticide concentration in the substrate being analyzed and the sensitivity and linear range of the Electron Capture Detector being used in the analysis. See Section 4,A in this manual.

12. Should it prove necessary to conduct further cleanup on the 15% fraction, transfer 10 grams MgO-Celite mixture to a chromatographic column using vacuum to pack. Prewash with ca 40 ml pet. ether, discard prewash and place a Kuderna-Danish receiver under column. Transfer concentrated Florisil eluate to column using small portions of pet. ether. Force sample and washings into the MgO-Celite mixture by slight air pressure and elute column with 100 ml pet. ether. Concentrate to a suitable volume and proceed with Gas Liquid Chromatography.

    NOTE: Standard Recoveries should be made through column to insure quantitative recoveries.

VI. ASSESSMENT OF EXTRACT CONCENTRATION:

1. Inject 5 µl of each fraction into the gas chromatograph for the purpose of determining the final dilution. If all peaks are on-scale and quantifiable, it will not be necessary to proceed with any further adjustment in concentration. With human fat, however, it is probable that there will be several sizable on-scale peaks and one or more off-scale peaks in the 6% fraction.

2. If off-scale peaks are obtained in either fraction it will be necessary to dilute volumetrically with hexane to obtain a con-

centration that will permit quantitation of those peaks from a 5 µl injection.

NOTE: A 5-ml dilution of a 3.0 gram sample containing .01 ppm of a given pesticide will yield 30 picograms of the pesticide per 5-microliter injection. Provided the detector is operating at average sensitivity, it should be possible to obtain quantifiable peaks of most compounds likely to be present at this concentration.

VII. MISCELLANEOUS NOTES:

1. The two fractions from the Florisil column should never be combined for examination by Gas Liquid Chromatography. By so doing, a valuable identification tool is voided.

2. Meticulous cleaning of glassware is absolutely essential for success with this procedure. All reagents and solvents must be pretested to insure that they are free of contamination by electron capturing materials at the highest extract concentration levels. Reagent blanks should be run with each set of samples.

3. The method, as described, is known to be capable of producing recoveries of most of the chlorinated pesticides of from 85-100%. Each laboratory should conduct their own recovery studies to make certain of their capability to achieve this recovery range. A clue may be obtained from the recovery of the aldrin spike. The recovery of this compound should not be less than 70%.

4. For the removal of peroxides from the ethyl ether, place an appropriate volume in a separatory funnel and wash it twice with portions of water equal to about 1/2 the volume of ether. The washed ether is shaken with 50-100 ml of saturated NaCl solution and all of the aqueous layer is discarded. The ether is then transferred to a Ƒ flask containing a large excess of anhydrous sodium sulphate and shaken vigorously on a mechanical shaker for 15 minutes. This treatment should not be attempted on ether containing ethanol, as the amount of ethanol that would remain is indeterminate.

5. If the presence of malathion is suspected, it is necessary to pass 200 ml of 50% diethyl ether in pet. ether through the Florisil column into a third K-D evaporator assembly, concentrating the eluate as described for the 6% and 15% eluates.

6. Table 1 gives the elution pattern for a number of common pesticides. On occasion it may be observed that a portion of a given compound

may elute into a different fraction than the one given. For example, some operators have difficulty eluting all the dieldrin in the 15% fraction. This is generally due to either moisture in the system or the use of solvents of different polarity than those specified in the reagent list. For example, it is essential that the diethyl ether contain 2% (v/v) ethanol. Ether without the ethanol or with too much would expectedly result in an altered elution pattern.

7. If this method is used for the detection and quantitation of organophosphorous compounds, some special factors must be considered. The presence of any peroxides in the ethyl ether and/or impurities in the pet. ether can result in extremely low recoveries. Recovery efficiency should be predetermined on standard mixtures containing the specific compounds of interest. If low recoveries are obtained, it may be necessary to try an alternate manufacturer's pet. ether.

TABLE I. ELUTION PATTERN OF SOME COMMON PESTICIDES FROM FLORISIL PARTITIONING

	6% Eluate	15% Eluate	50% Eluate
α-BHC	X		
β-BHC	X		
γ-BHC	X		
Heptachlor	X		
Hept. Epoxide	X		
Aldrin	X		
o,p'-DDE	X		
p,p'-DDE	X		
Dieldrin		X	
Endrin		X	
o,p'-DDD	X		
p,p'-DDD	X		
o,p'-DDT	X		
p,p'-DDT	X		
Perthane	X		
Methoxychlor	X		
Chlordane	X		
Chlorobenside	X		
Chlorobenzilate		X(80%)	X
2,4,5-T, isooctyl ester		X	

TABLE I. ELUTION PATTERN OF SOME COMMON PESTICIDES FROM FLORISIL PARTITIONING (CONTINUED)

	6% Eluate	15% Eluate	50% Eluate
2,4,5-T,isopropyl ester		X	
2,4,5-T, n-butyl ester		X	X
2,4-D, isobut. ester		X	
2,4-D, isooctyl ester		X(80%)	
2,4-D, isopropyl ester		X	
Tedion		X	
Endosulfan I		X	
Endosulfan II			X
Dilan			X
Ronnel	X		
Diazinon		X	
Methyl Parathion		X	
Ethyl Parathion		X	
Malathion			X
Ethion	X		
Trithion	X		

MICRO METHOD FOR THE DETERMINATION OF CHLORINATED PESTICIDES IN HUMAN TISSUE

I. MATERIALS AND REAGENTS:

1. MICROCOLUMN:

Place a small loose plug of glass wool in the tip of a size "B" Chromaflex column. (Kontes Cat. No. 42100 Size 22-7 mm.). Pack the column with 1.6 gms of 60-100 mesh Florisil which has been activated by the manufacturer at 1200°F. (PR grade Florisil should be used for this method). The column packing is added in increments followed by a gentle tapping. Add 1.6 gms of sodium sulfate, granular, to the top of the column. Wash the column with 50 ml of Nanograde hexane followed by 50 ml of Nanograde methanol. Dry and store columns in a 130°C oven. The columns should be conditioned at 130°C at least over night before using. For routine work it is convenient to prepare a large number of columns at one time.

2. SODIUM SULFATE, ANHYDROUS, GRANULAR:

Store in glass stoppered bottles in an oven at 130°C. Extract a portion of the sodium sulfate, equivalent to the amount used in the Florisil column, with hexane. Concentrate the extract to 50 microliters and inject 5 microliters into the gas chromatograph. The results will indicate whether it is necessary to extract the batch of sodium sulfate with hexane and methanol prior to storing in the oven.

3. NANOGRADE SOLVENTS:

Hexane, acetonitrile, methanol.

4. DISTILLED WATER:

Extract 8.0 ml. with hexane. Concentrate the extract to 300 µl, and inject 5 µl into the gas chromatograph. If extraneous peaks result then the distilled water must be extracted with hexane prior to use.

5. TISSUE GRINDER:

Dual tissue grinder Size 22 or 23 (Kontes Cat. No. K-885450).

6. MIXER:

Vortex Junior or equivalent.

7. CENTRIFUGE:

8. EVAPORATIVE CONCENTRATOR:

   Complete with modified micro Snyder column, ℉ joint 19/22, Kontes #K-569250.

9. CONCENTRATOR TUBE:

   Size 1025 Kontes Cat. No. K-570050.

10. CONCENTRATOR TUBE:

    Size 2525 Special Order - Kontes Cat. No. K-570050.

11. TEST TUBE:

    25 ml cap. with ℉ 19/22, joint with hooks Special Order Kontes Cat. No. K-897900.

II. SAMPLE PREPARATION - LIVER, KIDNEY, BONE MARROW, ADRENAL, GONADS:

1. Extract a 500-mg sample of tissue in a size 22 or 23 dual tissue grinder with 2.5 ml of acetonitrile. Add 20 nanograms of aldrin, in 0.1 ml of hexane, to the tissue grinder. This will serve as a recovery check as well as a marker for relative retention time.

   NOTE: Run a complete reagent blank with each set of samples.

2. Centrifuge and pour supernatant into a 50-ml round bottom test tube. Repeat extraction twice more, collecting supernatants in the test tube.

3. Add 25 ml of 2% aqueous sodium sulfate to the test tube and mix the contents with the aid of a Vortex mixer.

4. Extract the aqueous acetonitrile mixture with one 5-ml and two 2-ml portions of hexane. Combine the extracts in a 10-ml evaporative concentrator.

5. Concentrate the extract to 300 microliters with the aid of a modified micro Snyder column* and a 3-mm glass bead in the tube.

6. Proceed to Subsection III.

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* J. Burke et al., J.A.O.A.C. 49 (5), 999-1003, 1966.

III. FLORISIL FRACTIONATION:

1. Remove a Florisil column from the oven and allow it to cool to room temperature.

2. Pre-wet the column with 10 ml of hexane and discard the eluate.

3. Transfer the 0.3 ml of extract remaining after step (5), to the top of the Florisil column with the aid of a disposable pipet fitted with a rubber bulb. Begin immediate collection of eluate in a 25 ml capacity concentrator tube.

4. Rinse the 10 ml concentrator tube with 0.25 ml of hexane transferring this to the top of the column. Repeat this step a second time.

5. Proceed with the elution and collection using a total of 12 ml of hexane followed by 12 ml of 1% methanol in hexane. This 24 ml represents fraction one, and will contain heptachlor, aldrin, p,p'-DDE, o,p'-DDT and p,p'-DDT.

6. Collect a second fraction by eluting with a second 12 ml portion of 1% methanol in hexane. This fraction will contain dieldrin, heptachlor epoxide, endrin, β-BHC*, Lindane*, and p,p'-DDD.* See Table 1.

   *A small amount of β-BHC, Lindane, and/or p,p'-DDD may appear in the first fraction.

7. Add 20 nanograms of aldrin in 0.1 ml of hexane to fraction two, evaporate both fractions using a modified micro Snyder column and a 3 mm glass bead in the tube.

8. Adjust the volumes in fractions (1) and (2) to 500 and 300 microliters, respectively, and proceed with the GLC portion as outlined under ELECTRON CAPTURE GAS CHROMATOGRAPHY, diluting basic concentrates as required to obtain quantifiable peaks with minimal tissue concentration.

IV. ANALYSIS OF BRAIN:

Proceed with steps (1) thru (4) as described under II. Sample Preparation.

5. Concentrate the combined hexane extracts to 500 µl in a 25 ml test tube fitted with a modified micro Snyder column and using a 3 mm glass bead in the tube.

6. Add 0.3 ml Acetic Anhydride and 0.3 ml pyridine and incubate in a water bath at 60-65°C for 1/2 hour.

7. Add 9 ml of 2% Na_2SO_4 and extract with 2-3 ml portions of hexane.

8. Concentrate the combined extracts to 300 ml in a 10 ml evaporative concentrator fitted with a modified micro Snyder column using a glass bead for a boiling chip.

9. Proceed as described under III. FLORISIL FRACTIONATION.

Table 1. Elution pattern for microflorisil column.

Fraction 1 12 ml hexane followed by 12 ml 1% methanol in hexane
- aldrin
- heptachlor
- o,p'-DDE
- p,p'-DDE
- o,p'-DDT
- p,p'-DDT
- heptachlor
- polychlorinated biphenyls

Split between Fraction 1 and Fraction 2
- α-BHC
- lindane
- diazinon
- o,p'-DDD
- p,p'-DDD
- TOK
- toxaphene (mostly in fraction 1)

Fraction 2 12 ml 1% methanol in hexane
- DDA (methyl ester)
- dieldrin
- endrin
- ethion (recovery variable with Florisil)
- ethyl parathion
- β-BHC
- δ-BHC
- heptachlor epoxide
- 1-hydroxychlordene (80-90%)
- malathion
- methoxychlor
- methyl parathion
- ronnell
- thiodan I and II
- paradichlorobenzophenone

Neither Fraction 1 nor Fraction 2
- trithion

ANALYSIS OF HUMAN BLOOD OR SERUM

I. Because of its availability and probable diagnostic value with regard to extent of both chronic and acute exposure to chlorinated hydrocarbon and other classes of pesticides, blood specimens present a convenient tissue for study, providing meaningful data pertinent to the Community Study and Monitoring laboratory program. Of several methods available in the literature, the Dale, et al., (1966) method provided some desirable features in rapidity, simplicity and sensitivity for the determination of chlorinated insecticides and related materials in blood. A method including these features is essential in the monitoring situation involving analyses of large numbers of samples. The following procedure utilizes the direct solvent extraction principle of the Dale, et al. method. It is to be considered a general survey method for the determination of chlorinated hydrocarbon pesticide levels in blood, particularly DDT and its metabolites. For an in-depth study of total pesticide residue levels in this tissue, it is recommended that a cleanup method for the determination of chlorinated pesticides in human tissue, [i.e., Section 5,A,(1) in this manual] be applied, together with confirmatory determination such as TLC and chemical derivatization techniques.

REFERENCE: Dale, W. E., A.Curley, and C. Cueto, (1966), Hexane Extractable Chlorinated Insecticides in Human Blood, Life Sciences 5: 47.

II. PRINCIPLE:

A 2-ml aliquot of serum is extracted with 6 millilliters of hexane in a round-bottom tube. The extraction is conducted for 2 hours on a slow-speed rotating mixer. The formation of emulsion is unlikely, but if it should occur, centrifugation may be used to effect separation of the layers. A 5-ml aliquot of the hexane layer is quantitatively transferred to an evaporative concentrator tube to which is affixed a modified micro-Snyder column. The extract is concentrated in a water or steam bath and the final volume is adjusted to correspond to the expected concentration of the pesticide residue. A suitable aliquot is analyzed by electron capture gas chromatography.

APPARATUS AND REAGENTS

1. Rotary mixing device, "Roto-Rack™", Fisher Scientific Company, Catalog #14-057.

2. Tubes, Culture, 16 x 125 mm., fitted with screw caps, size 15-415 with Teflon-faced rubber liners, Corning #9826.

3. Micro-Snyder column, modified, with 19/22 ℉ joint, Kontes #K-569251.

4. Concentrator tube, 10 ml, grad. 0 to 1 x 0.1 and 2 to 10 x 1, 19/22 ℉ joint, size 1025, Kontes #K-570050.

5. Syringe, 100 µl, Hamilton #710 or equivalent.

6. Vortex Genie mixer.

7. Pipet, Mohr type, 1 ml in 0.01 ml grad., Corning #7063 or equivalent.

8. Pipets, transfer, 2, 5 and 6 ml Corning #7100 or the equivalent.

9. Beads, solid, glass, 3 mm., Corning #7268 or the equivalent.

10. Six-place tube carrier, stnls. steel. May be fabricated at local tin shop per attached sketch.

11. Water bath capable of holding temp. of 95 to 100°C.

12. Centrifuge with head to accomodate the Corning #9826 tube, capable of speed of 2,000 rmp.

13. Hexane, distilled in glass, pesticide grade.

III. PROCEDURE:

1. Mix blood serum sample thoroughly and, with a volumetric pipet, transfer 2 ml to a 15 ml round bottom culture tube.

2. Add 6 ml hexane from a volumetric pipet. Tightly stopper the culture tube with a Teflon-lined screw cap. Place tube on rotator.

3. Set rotator speed at 50 rpm and rotate for 2 hours.

NOTES: (1) This speed may vary from 50 to 55 rpm but should be confined to this range.

Figure 1. ROTO-RACK^{R} Mixer, variable speed

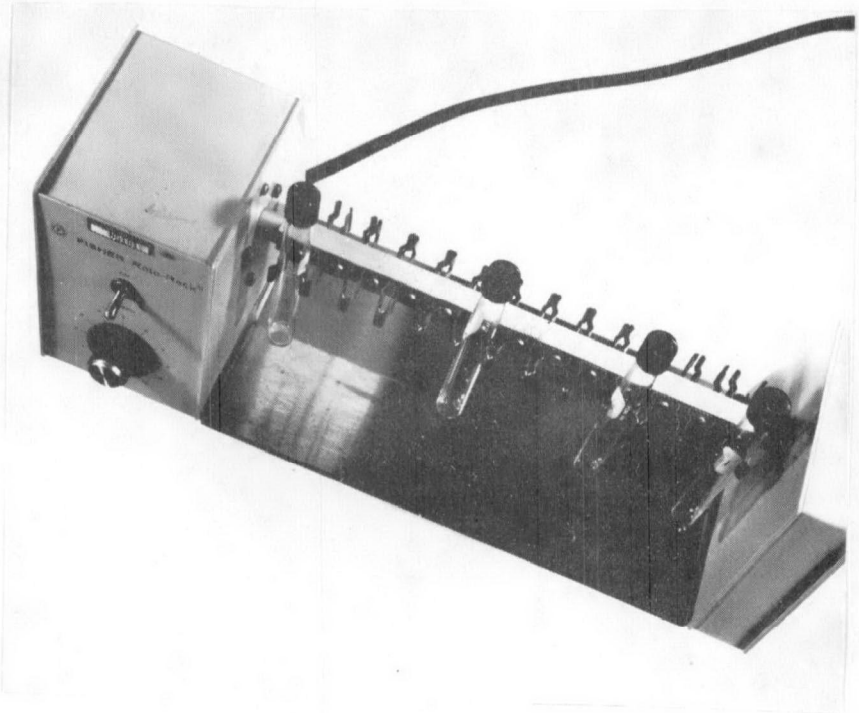
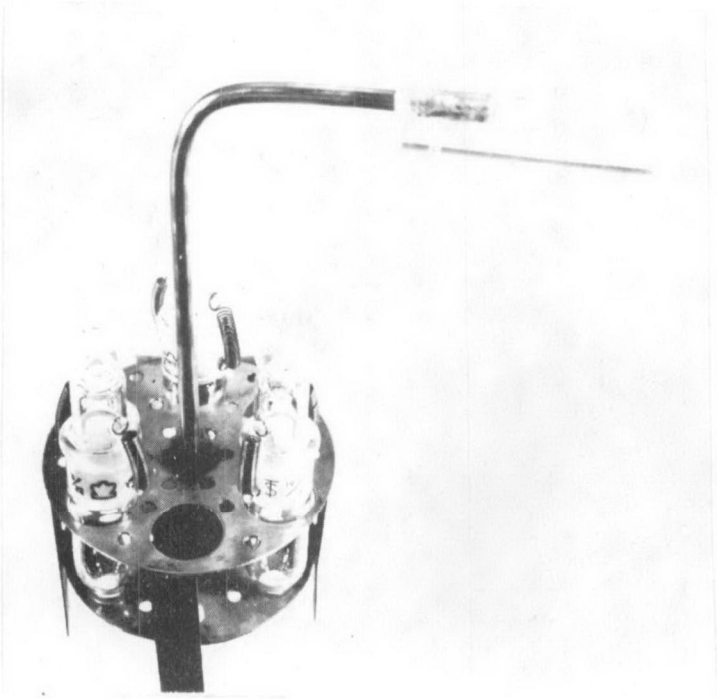
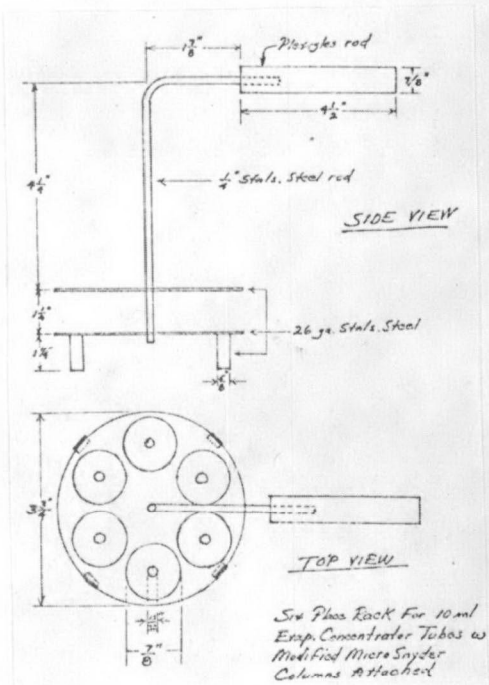


Figure 2. Evaporative concentrator tube holder, 6-place, stainless steel





This should pose no problem on the other two columns used in the program as the RR values at 200°C are:

	SE-30/QF-1	OV-210
2,4-D(ME)	0.44	0.09
PCP(ME)	0.63	0.56

4. All reagents including the distilled water used in the method must be extracted with hexane before use as they may be contaminated with PCP or other materials which may cause interferences. Glassware should be washed with dilute NaOH solution followed by deionized water and acetone rinsed. Care should be taken not to permit contact between wooden or paper materials and glassware as peg boards and some brands of absorbent paper products have been found to contain PCP.

5. If the recommended volumes are used calcualtions are simplified and are as follows:

   PCP (in ppb) in serum = pg/µl injected X 10.

Table 1. Percent recovery of PCP from samples fortified before extraction

Sample	PCP Found ppm	PCP Added, ppm	PCP Recovered, ppm	Recovery %
Blood Plasma	0.19	0.50	0.67	96
			0.65	92
			0.68	98
		5.00	4.58	88
			4.70	90
			4.70	90
			4.70	90
		50.0	46.9	93
			48.5	97
			42.0	84
				Mean 92[b]
Urine	0.01[a]	0.50	0.46	90
			0.50	98
			0.48	94
		5.00	4.86	97
			4.70	94
			4.89	98
		50.0	49.0	98
			49.8	100
			49.8	100
				Mean 96[c]

[a]Limit of detectability for blood and urine.
[b]Standard deviation, blood plasma --- ± 4.5%
[c]Standard deviation, urine --- ± 3.5%

DETERMINATION OF 2,4-D AND 2,4,5-T IN URINE

I. INTRODUCTION:

A number of derivatives of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) are applied extensively as selective herbicides in the control of terrestrial and aquatic broad-leaf plants. Because of their widespread use and relatively lengthy persistence, particularly in treated lakes and streams, potential human exposure to these materials may occur via several routes. These include consumption of contaminated edible plants, livestock, and water, as well as direct exposure by agricultural spraymen and herbicide formulators. Thus, rapid, sensitive procedures for the detection of the free acids and chlorinated phenol degradation products in human and animal urine assumes an important role in the toxicological and environmental monitoring of these herbicidal compounds.

REFERENCE: A Method for Determination of Low Levels of Exposure to 2,4-D and 2,4,5-T, Shafik, M. T., Sullivan, H. C. and Enos, H. F., Journal of Environmental Analytical Chemistry, 1971, Vol. 1 pp 23-33.

II. PRINCIPLE:

The phenolic conjugates are subjected to acid hydrolysis, the free phenols and acids are extracted and ethylated with diazoethane. Cleanup of the derivatized products is carried out on a silica gel column, the resulting eluate is concentrated to an appropriate extent and subjected to analysis by electron capture GLC, chromatographing on a column of 4% SE-30/6% QF-1.

III. EQUIPMENT:

1. Gas chromatograph with E.C. detector fitted with a glass column 6' x 1/4" o.d. packed with 4% SE-30/6% QF-1. Column operating parameters are those prescribed in Section 4 of this manual. Injection port, transfer line and detector as maintained in normal operation.

2. Chromatographic columns, Size 22, Kontes #420100.

3. Boiling water or steam bath.

4. Distilling column (condenser), 200 mm jacket, fitted with tight glass stopper at top, Kontes #286810.

5. Circulating water pump.

6. Vortex mini-mixer.

7. Evaporative concentrator tubes, grad., 25 ml ℨ 19/22, Kontes #570050.

8. Conical centrifuge tubes, conical, grad., 15 ml with ℨ stoppers, Corning #8084 or the equivalent.

9. Disposable pipets, Pasteur, 9 inch.

10. Dry nitrogen. Tank fitted with 2-stage pressure regulator.

11. Volumetric flasks, 50 and 100 ml.

12. Mohr pipets, 0.2, 0.5 and 5 ml.

13. Transfer (vol) pipets, 1 through 5 ml.

14. An exhaust hood with a minimum draft of 150 linear feet per minute.

IV. REAGENTS:

1. Benzene, pesticide quality.

2. Hexane, pesticide quality.

3. Hydrochloric Acid, conc., A.R. Grade.

4. Silica gel, Woelm, Activity Grade I.

   NOTE: Dry adsorbent for 48 hours at 170°C and store in a desiccator. On day of use deactivate the silica gel by adding 15 microliters of water and 1 gram of silica gel to a 125 ml Erlenmeyer flask. Stopper and rotate until the water is evenly distributed throughout the adsorbent. Allow to equilibrate for 2 to 3 hours with periodic shaking. Prepare the chromatographic columns just prior to use.

5. N-ethyl-N'-nitro-N-nitrosoguanidine, Aldrich Chemical Co.

6. Distilled water. All distilled water used throughout procedure must be benzene extracted.

7. Ethylating Reagent, Preparation:

   a. In a 125 ml Erlenmeyer flask dissolve 2.3 grams of KOH, A.R. grade in 2.3 ml of distilled water. When solution is complete allow to cool to room temperature.

   b. Add 25 ml hexane and cool flask in a -18°C freezer for 15 min.

   c. In a VERY HIGH DRAFT hood, add 1.6 grams of N-ethyl-N'-nitro-N-nitrosoguanidine in small portions at a time, mixing contents of flask after each addition.

   d. Decant the hexane layer into a bottle with a Teflon-lined screw cap. This may be stored for periods up to a week at -18°C.

      NOTES: (1) Because of demonstrated carcinogenicity and toxicity, do not allow the nitrosoguanidine or the diazoethane to come in contact with the skin. Disposable gloves and safety goggles should always be worn when handling.

      (2) Do not use ground glass stoppered bottles or bottles with visible interior etching.

8. Analytical grade standards for 2,4-D and 2,4,5-T. Available from the Perrine Repository.

9. Preparation of ethylated standard mixtures;

   a. Weigh 20 mg of each of the two analytical standards into separate 100-ml vol. flasks, dissolve, and make to volume with benzene. These concentrated stock solutions will contain 200 ng/µl of the respective compounds.

   b. Transfer aliquots from each of the concentrated stock solutions into a single 50-ml vol. flask in the following volumes:

      2,4-D----------1.0 ml 2,4,5-T--------0.5 ml

   c. Add diazoethane dropwise with a disposable pipet until a definite yellow color persists.

d. Allow solution to stand 15 minutes, then bubble nitrogen through the solution until yellow color disappears (ca 5-10 minutes). THIS OPERATION MUST BE DONE IN A HIGH DRAFT HOOD. Dilute to volume with benzene. This is the alkylated stock standard mixture of the following concentrations in nanograms per microliter:

2,4-D----------4 2,4,5-T----------2

e. Prepare an ethylated working standard mixture of highest usable concentration by pipetting 5 ml of the alkylated stock mixture (d. above) into a 50-ml vol. flask and make to volume with benzene. This will yield a dilute mixture of the following concentrations:

Alkylated 2,4-D----------400 pg/µl
Alkylated 2,4,5-T----------200 pg/µl

Injection of 5 µl of this mix into the gas chromatograph will provide information on the final concentration range needed for further diluted standards.

NOTE: These alkylated standards should be stored at -18°C when not in use and discarded after one month.

V. PROCEDURE:

Extraction and Alkylation

A control sample of urine from an unexposed donor should be carried through the entire procedure parallel with the sample(s) being tested.

1. Pipet 1 to 5 ml of urine into a 25-ml evap. conc. tube.

NOTE: The precise volume is predicated on the expected residue level.

2. Add dropwise a volume of conc. HCl equal to 1/5 the volume of urine and mix well.

3. Fit a stoppered reflux condenser to the tube and heat in boiling water bath for one hour, cooling the condenser with circulating ice water.

4. Remove from bath, cool and rinse inside walls and condenser tip with 3 ml benzene.

5. Mix contents of tube for two minutes on a Vortex mixer set at high speed and centrifuge.

6. By means of a disposable pipet, carefully transfer the benzene (upper) layer to a 15-ml centrifuge tube taking special care not to transfer any water.

7. Repeat the extraction with another 3-ml portion of benzene, adding the second benzene to the centrifuge tube.

8. Add diazoethane reagent dropwise with a disposable pipet until the yellow color persists (ca 2 ml).

9. Allow tube to stand 15 minutes, then bubble nitrogen through the solution to remove excess reagent.

10. Concentrate the ethylated extract to ca 0.3 ml at room temperature or on a 40°C water bath under a gentle stream of nitrogen.

Silica Gel Elution Pattern

The elution pattern of the ethylated compounds must be determined before using the silica gel column for cleanup of the ethylated urine extracts. The column preparation and elution pattern evaluation is outlined in the following steps:

a. Place a small wad of glass wool at the bottom of a Chromaflex column and add 1 gram of the partially deactivated silica gel. Top this with 1/2 inch of anhydrous, granular Na_2SO_4.

b. Prewash the column with 10 ml of hexane and discard the eluate.

c. When the surface level of the hexane reaches a point on the column ca 2 cm from the top of the Na_2SO_4, add 0.3 ml of the alkylated stock standard mixture (subsection IV,9,c) to the column. Elute successively with 10 ml of each of the solvent systems listed in the following table, collecting each fraction separately. Inject from 5 to 10 µl from each fraction into the gas chromatograph and calculate the percent of each compound present in the fraction.

A typical elution pattern is shown in the table:

Eluting Solvents	2,4-D 5 µg	2,4,5-T 2 µg
20% Benzene-Hexane	0	0
40% Benzene-Hexane	0	0
60% Benzene-Hexane	0-2%	20-25%
80% Benzene-Hexane	98-100%	75-80%
100% Benzene	0	0

Silica Gel Sample Cleanup

1. Prepare a chromatographic column of silica gel as described on the previous page and prewash column with 10 ml of hexane exactly as described, discarding the eluate.

2. Transfer the concentrated extract to the column, rinsing centrifuge tube with two successive portions of 5 ml each of 20% benzene/hexane, collecting the eluate.

   NOTE: If chlorinated phenols are present they should eluate in this fraction.

3. Finally, add 10 ml of 60% benzene/hexane followed by 10 ml of 80% benzene/hexane, collecting both these fractions in a single tube. The ethylated 2,4-D and 2,4,5-T are contained in these fractions.

   NOTE: If the individual analyst has determined that his elution pattern differs from that given in the author's table and he is able to obtain a consistent altered pattern, some appropriate revision in the eluate collection instructions may be indicated.

Gas Chromatography

Inject into the gas chromatograph 5-10 µl of the 20% fraction for the determination of the phenols and 5-10 µl of the combined 60-80% fraction for the determination of the chlorophenoxyacetic acids. Injections of 5-10 µl

can also be made from fractions which have been concentrated to 5 ml, if necessary, in case of lower levels of exposure. The elution pattern of the 2 compounds extracted from a fortified urine sample must be established as described below. The limits of detectability for 2,4-D and 2,4,5-T are 0.05 and 0.01 ppm, respectively. Quantitation is conducted by mathematical comparison of sample peaks against peaks resulting from the injection of working standard (subsection IV,9,e).

The retention values, relative to aldrin, of the two ethylated compounds on the SE-30/QF-1 column at 200°C are:

2,4-D----------0.51

2,4,5-T----------0.78

Recovery Determination

Recovery runs are essential for the operator to determine the efficiency of alkylation and cleanup. From the concentrated stock standard of IV,9,a, transfer the aliquots specified in Step 9,b to a single 50-ml vol. flask. Dilute to volume with benzene without ethylating. Transfer a 2-ml aliquot to a 15-ml grad. centrifuge tube and add an equal volume of 1 N NaOH. Mix well and allow to stand for 10 minutes, agitating from time to time. Centrifuge 5 minutes at 2,000 rpm and discard benzene (upper) layer. Fortify 5 ml of control urine with aliquots of 0.1 to 1 ml of the aqueous extract and proceed as described in subsection V starting at Step 2.

Partitioning

NOTE: Because of differences in ambient temperature and relative humidity from one laboratory to another, it is imperative that each laboratory establishes silica gel elution patterns under local conditions. Should the compounds of interest elute in a later fraction (i.e., in 100% benzene instead of 60% or 80% benzene-hexane) the percent water added to the silica gel must be increased by 1% increments until desired elution pattern is established. If the compounds of interest elute in an earlier fraction (i.e., in 20% B-H instead of 60% or 80% B-H), the amount of water initially added to silica gel must be decreased (use spiked control urine, not standard compounds to determine pattern).

METHOD FOR DETERMINATION OF METABOLITES OR HYDROLYSIS PRODUCTS OF ORGANOPHOSPHORUS PESTICIDES IN HUMAN URINE

I. INTRODUCTION:

The metabolism and urinary hydrolysis of organophosphorus pesticides in mammals results in the excretion of a variety of alkyl phosphates. These include the salts of dimethyl or diethyl phosphate, phosphorothioate and phosphorodithioate. The gas chromatographic separation and quantitation of such products in urine may be of value in estimating the extent of exposure to the parent organophosphorus pesticide. The procedure permits the determination of six major metabolites and hydrolysis products of organophosphorus pesticides. In addition, the method possesses high sensitivity and allows for elimination of interferences from inorganic phosphates.

REFERENCES:

1. Bowman, M. C., and Beroza, M., 1968, Gas Chromatographic Detector for Simultaneous Sensing of Phosphorus and Sulfur-Containing Compounds for Flame Photometry, Anal. Chem. 40; 1448.

2. Shafik, M. T. and Enos, H. F., 1969. Determination of Metabolites and Hydrolysis Products of Organophosphorus Pesticides in Human Blood or Urine. J. Ag.& Food Chem. 17 (6); 1186.

3. Shafik, M. T., Bradway, D., Biros, F. J. and Enos, H. F., 1970. Characterization of Alkylation Products of Diethyl Phosphorothioate. J. Ag. & Food Chem., 18 (6); 1174.

II. PRINCIPLES

Organophosphate metabolites or hydrolysis products in urine are extracted with a 1:1 (v/v) mixture of acetonitrile and diethyl ether after acidification with 6 N HCl. An aliquot of the extraction solvent containing the dialkyl phosphates is treated with diazopentane to convert the dialkyl phosphates to the more volatile and gas chromatographable trialkyl phosphates. It is necessary to clean up the alkyl phosphate derivatives using silica gel chromatography to eliminate interferences arising from inorganic phosphate and other materials and to obtain separations of compounds producing closely adjacent peaks. Determination of trialkyl phosphates is accomplished by gas chromatography using flame photometric detection with a phosphorus filter (526 mμ). The presence of sulfur-containing trialkyl phosphates may

be confirmed by use of the flame photometric detector with a sulfur filter (394 mμ).

III. APPARATUS:

1. Dual flame photometric detector, Tracor, Inc., Austin, Texas, equipped with 526 mμ filters for the determination of sulfur and phosphorus compounds, respectively. This detection system was fitted to a Micro-Tek MT-220 gas chromatograph for the initial development of this method, but can be adapted for use with any gas chromatograph.

2. Gas chromatograph modifications. The following modifications are suggested for operation of the gas chromatograph with flame photometric detection and are generally applicable to any gas chromatograph.

   (a) Buckout control on electrometer type E-2 equipped with a 20 megohm Victoreen resistor to reduce detector signal to a level acceptable to electrometer circuitry.

   (b) Valco switching valve #CV 4 HT interfaced between gas chromatographic column and flame photometric detector. The valve is mounted in a modified block and is coupled with R 100 dead volume Swagelok unions. The heater in the block heats both the transfer lines and the valve. The valve permits interchange of column effluent and nitrogen purge. The purge flow rate is adjusted to equal the flow from the GLC column so that when an interchange of flows is made for the purpose of venting solvent, no change is observed in the recorder baseline. This arrangement prevents the flame from being extinguished when injections are made.

3. Gas chromatographic column - Borosilicate glass, 6' x 1/4" o.d. packed with 5% OV-210 on Chromosorb W, H.P., 80/100 mesh.

   Prepare and condition column as follows:

   Fill each column leg to a point 1-3/4" from the end and insert small plugs of silane-treated glass wool. Heat condition column overnight at 240°C with an applied carrier gas flow of ca 30 ml/min. Cool column and add at inlet end 1-3/4" of 10% Carbowax 20M coated on Chromosorb W, H.P. Insert another small plug of glass wool to retain Carbowax. Place column back in oven for another heat conditioning overnight at 225°C with an applied carrier flow of ca 10 ml/min. Upon completion of this period, cool column and dump out Carbowax by carefully tapping inverted

column. Fill the void left by the Carbowax removal with silane-treated glass wool and install column in the operating mode.

NOTE: An alternate column, which may be used for confirmation of identity of peaks, is a 6' x 1/4" o.d. borosilicate glass column packed with 4% SE-30/6% OV-210 on 80/100 Gaschrom Q. Condition as described above.

4. Centrifuge tubes - 15 ml capacity, grad., conical, with ℨ ground glass stoppers.

5. Pipets - Disposable glass, Pasteur type, (9" length) fitted with rubber bulbs (Fisher Scientific Co.).

6. Column, chromatographic, Size 23, (Kontes #420100).

7. Vortex-Genie Mixer - Model K-550-G or equiv. (Scientific Industries, Inc., Springfield, Mass.).

8. Evaporative concentrator tube, 25 ml, (Kontes #570050).

9. Bottles - Reagent, narrow mouth, capacity 1 oz., complete with polyseal screw type caps. (A. H. Thomas Co., Phila., Pa., Cat. No. 2203-C, bottles; and 2849-E, polyseal caps.)

10. Exhaust hood with a minimum draft of 150 linear feet per minute.

IV. REAGENTS:

1. Acetonitrile - "Pesticide Grade" solvent, distilled from all-glass apparatus (Mallinckrodt Nanograde solvent or equiv.).

2. Diethyl ether - AR, or USP grade with 2% ethanol, Mallinckrodt, Cat. No. 0850 or equivalent.

NOTE: Under no conditions should anhydrous ether be used for the preparation of diazopentane because of the danger of explosion.

3. Methylene chloride, dist. from all-glass apparatus.

4. Silica gel, Woelm, activity grade I (Waters Associates, Inc.), activated at 130°C for 48 hours and stored in desiccator.

5. Potassium hydroxide, pellets, AR grade.

6. Sodium chloride, AR grade.

7. N-amyl-N'-nitro-N-nitrosoguanidine - Aldrich Chemical Co., Inc., Milwaukee, Wis.

8. Extracting solvent - 1:1 (v/v) mixture of acetonitrile and anhydrous diethyl ether.

9. Formic acid reagent - make up a 1% solution of formic acid in benzene.

10. Diazopentane reagent - Preparation:

    a. Dissolve 2.3 grams of KOH in 2.3 ml of dist. H_2O in a 125-ml Erlenmeyer flask. When solution is complete, cool in a freezer for 30 minutes.

    b. Add 25 ml of cold diethyl ether (Mallinckrodt #0850), cover flask mouth with foil, and cool in a -18°C freezer 15 minutes.

    c. In a VERY HIGH DRAFT hood, add 2.1 grams of N-amyl-N'-nitro-N-nitrosoguanidine to the flask in small portions over a period of a few minutes, swirling the flask vigorously after each addition.

    d. Decant the ether layer into a 1-oz. reagent bottle fitted with a Teflon-lined screw cap. This may be stored at -20°C for periods up to a week.

    NOTES: (1) Because of the demonstrated carcinogenicity and skin irritating characteristics, DO NOT ALLOW THE NITROSOGUANIDINE OR THE DIAZOALKANE TO COME IN CONTACT WITH THE SKIN. Wear disposable vinyl gloves and safety goggles while handling. Avoid breathing vapors.

    (2) Do not use ground glass stoppered bottles or bottles with visible interior etching. Avoid strong light.

13. Standards

For brevity's sake, the names of various phosphate compounds will be abbreviated from this point on. The identities of the

abbreviations are given in Table 3. The standard solutions are prepared as described in the following:

a. In 15-ml grad., glass stoppered centr. tubes, accurately weigh ca 10 milligrams of each of the following dialkyl phosphate standards:

(1) Sodium* dimethyl phosphate
(2) Potassium dimethyl phosphorothionate
(3) Potassium dimethyl phosphorodithioate
(4) Diethyl* phosphate
(5) Potassium diethyl phosphorothionate
(6) Potassium diethyl phosphorodithioate

*For consistency, convert the weights of these compounds to the weight of an equivalent amount of the potassium salt For sodium dimethyl phosphate the conversion factor is 1.109; for diethyl phosphate it is 1.247.

b. The alkylation of these standards is carried out as follows:

(1) To each tube add 2 drops of 6 N HCl.
(2) Add sufficient diazopentane to produce a persistent yellow color (2 to 5 ml usually suffices), mix and allow to stand for 20 minutes.
(3) Remove excess reagent by adding the formic acid/ benzene reagent dropwise until the yellow color just disappears. Avoid an excess of this reagent.

NOTE: The procedure, up to this point, must be carried through without interruption. Solutions of dialkyl phosphates are unstable, especially in an acidic medium. Therefore, the length of time in contact with HCl must be kept to a minimum. After alkylation, the trialkyl derivatives are stable indefinitely.

(4) Dilute the solution(s) exactly to 10 ml with benzene, stopper and mix thoroughly. Store in the freezer in glass stoppered containers when not in use.

c. These alkylated concentrated stock standards may be diluted individually or more generally prepared as mixtures at an intermediary concentration range. For the mixtures, pipet aliquots of appropriate volumes into three volumetric flasks

to obtain the concentrations given in the following, in nanograms per microliter:

Mixture 1		Mixture 2		Mixture 3	
DMTP	10	DMDTP	5	DMP	10
DETP	10	DEDTP	5	DEP	10

NOTE: Because of the scarcity of the potassium salts of the dialkyl phosphates, the Ferrine Laboratory will supply the Community Studies Laboratories with sealed ampoules of the intermediary concentration mixtures listed above. All that will be required is a dilution with benzene in a 1:50 ratio.

d. Working Standard Mixtures:

(1) Dilute each of the intermediary stock mixtures in a 1:50 ratio with benzene.

(2) To establish that the working standard mixtures are in a proper concentration range, observe the recorder response resulting from the injection of 5 µl of each into the gas chromatograph.

Photometric tubes vary somewhat in sensitivity and it may prove necessary to either further dilute or to prepare higher concentrations of the working standards. Injection volumes may be varied from 5 to 25 µl.

V. SAMPLE PREPARATION, EXTRACTION AND ALKYLATION:

1. Store urine samples in a freezer until ready for analysis. Do not store acidified samples. When urine sample is thawed, centrifuge and discard solids. Pipet a 2-ml aliquot into 15-ml centr. tube.

NOTE: At this point, a sample of control urine from an unexposed donor should be started and carried through the entire procedure. The donor should be an individual known to have no contact with organophosphorus pesticides for at least a week.

2. If necessary, pre-extract urine samples with two 5-ml portions of diethyl ether. Centrifuge for one minute to obtain separation of phases and discard extracts.

NOTE: Pre-extraction of urine is usually necessary only when the urine has been contaminated with feces as would be the case with urine collected from experimental animals housed in cages. The pre-extraction step does not remove any of the metabolites determined by the method.

3. Add 2 grams of NaCl and pipette 4.0 ml of extracting solvent (IV,8) to the centrifuge tubes.

4. Add 1 ml of 6 N HCl, stopper tubes and mix on a Vortex mixer for one minute. It is advisable to do samples in pairs from this point until diazopentane is added.

5. Centrifuge at 2,000 rpm for one minute.

NOTE: No interruptions nor delays are permissible until Step 7 has been completed.

6. With a disposable pipet, transfer **2.0 ml of the organic solvent** layer to a grad., 15 ml, glass-stoppered centr. tube.

7. Working in a high draft hood, add ca 2 ml of diazopentane. A pronounced orange color should persist after mixing. If necessary, add slightly more of the alkylating reagent until the color persists. Allow to stand for 20 minutes.

8. Concentrate solution to 0.2 to 0.3 ml under a gentle stream of nitrogen, either at room temperature or on a 40°C water bath. Dilute to 5 ml with dist. water, add ca 5 gm of NaCl and 3 ml of hexane. Stopper and mix vigorously on Vortex mixer for 1 minute. Allow layers to separate and centrifuge in case of interfacial emulsion.

9. Prepare silica gel as follows: Partially deactivate 10 gm of silica gel by shaking 2 hours with 2.0 ml dist. water. Transfer 2.4 gm to a size 23 chromatographic column with a small wad of glass wool at the bottom. Top the column with ca 2 gm of anhydrous Na_2SO_4 and prewash with 10 ml of hexane.

NOTE: Elution patterns may vary from one laboratory to another depending on the temperature and relative humidity. This dictates the need for each laboratory to establish an elution pattern of standards and spiked control urine samples under local conditions before attempting to analyze samples.

10. Transfer hexane extract (Step 8 above) to the silica gel column. Re-extract aqueous layer with 2 ml of hexane and add to column. Extract with another 2 ml of hexane and add to column. All eluate to this point is discarded.

11. Place a 25-ml concentrator tube under column and add 15 ml of methylene chloride to the column. This fraction contains DMTP, DETP, DMDTP and DEDTP.

12. Add 15 ml of 1% acetone in methylene chloride and discard this eluate.

    NOTE: This eluate contains most of the triamyl phosphate and other interfering substances.

13. Place another 25-ml concentrator tube under column and add 20 ml of 3% acetone in methylene chloride. This fraction contains DMP and DEP.

VI. GAS CHROMATOGRAPHIC DETERMINATION:

1. Inject 5-25 µl aliquots of the silica gel column fractions and working standard mixtures into the gas chromatograph. Dilution or concentration of the extract will be governed by chromatographic response from the initial injections.

2. Suggested operating conditions:

Column temperature	165°-175°C
Injection block temperature	200°C
Detector temperature	200°C
Transfer line temperature	200°C
Switching valve temperature	200°C
Nitrogen (carrier) flow rate	30-40 ml/min.
Nitrogen (purge) flow rate	30-40 ml/min.
Hydrogen flow rate	180 ml/min.
Air flow rate	80 ml/min.
Oxygen flow rate	10 ml/min.

    NOTE: It may be necessary to adjust air and oxygen flow rates to obtain optimum response.

3. Examine the two eluates by gas chromatography. Quantitate sample alkyl phosphate peaks by mathematical comparison with peaks obtained from working standards using the phosphorus response.

NOTE: DMTP and DETP isomerize upon alkylation producing thionates and thiolates. Quantitation is based on the DMATP and DEATP.

4. Confirm the presence of sulfur-containing alkyl phosphates by use of the flame photometric detector with sulfur filter (394 mμ).

See Figure 1 for chromatograms of the amyl derivatives of dialkyl phosphates. This figure was obtained using the phosphorus filter (526 mμ).

Table 2 lists the relative retention times, and detector sensitivity for the amyl derivatives of dialkyl phosphates using the phosphorus filter.

Do not inject Silyl 8 on a column connected to the FPD. If column needs reconditioning, disconnect exit end on column from the detector and condition as required.

NOTES: (1) Often after extended use of the gas chromatographic system, extraneous peaks may appear. They arise chiefly from the accumulation of underivatized compounds on the column. Since the appearance of the extraneous peaks is not obvious, it is recommended that the operator inject about 1 μl of diazopentane solution periodically (2 weeks). If underivatized compounds are present in the column, they show as peaks following the diazopentane injection. If this is the case, recondition the column.

(2) Quantitation is based on the amyl derivatives, DMAP, DEAP, DMATP, DEATP, DMADTP, and DEADTP. Some inorganic phosphate is extracted which is converted to TAP.

(3) Confirmation and Specificity.

a. The ability to interchange the sulfur and phosphorus filters in the single detector, or the use of the base assembly for dual phototube operation with both filters (Bowman and Beroza 1968), greatly enhances the specificity of this method. Suspected thiophosphate can be confirmed using the sulfur filter by simply increasing the concentration of the compound injected into the gas chromatograph by a factor of 5 to 10.

b. Confirmation of any particular compound can be accomplished by preparing the hexyl derivative. The method described for diazopentane is followed except that N-hexyl-N'-nitro-N-nitrosoguanidine is used as the diazoalkane precursor.

c. Further confirmation is achieved using the silica gel column when the sulfur-containing derivatives are eluted in the methylene chloride fraction and the non-sulfur derivatives eluted in the ethyl acetate fraction.

(4) Before attempting to analyze any samples, the chemist must run fortified urine for assurance of satisfactory recoveries. A spike of 0.1 ppm is suggested of both sodium dimethyl phosphate and diethyl phosphate. Proceed as follows:

a. To 5-ml aliquots of control urine in separate 15-ml centrifuge tubes add 0.5 microgram (in aqueous solutions) of NaDMP to one and like amount of DEP to the other, converting the weights to the potassium salt equivalents (see subsection IV,11,a).

b. Proceed with extraction and alkylation as described in subsection V,3-13 and the GLC as outlined in subsection VI.

Table 1. Amyl derivatives of dialkyl phosphates. Concentration ranges, in nanograms, of standards which produce linear detector response for normal GLC operating conditions.

	Phosphorus Filter (526 mμ)
DMAP	0.1-25 ng
DEAP	0.1-50 ng
DMATP	0.1-50 ng
DEATP	0.1-50 ng
DMADTP	0.1-50 ng
DEADTP	0.1-50 ng

Table 2 Data from isothermal gas chromatography of trialkyl phosphates with phosphorous flame photometric detection system. Column 5% OV-210, Temp. 173°C, carrier flow 40 ml/min. of N_2, noise ±1%.

Compound	Retention (Min)	Relative Ret. Value (Basis DMADTP)	Response % fsd/ng	Sensitivity (ng) 4:1 signal/noise
DMATP	1.46	0.59	37.2	0.11
DEATP	1.81	0.73	27.5	0.14
DMAP	2.36	0.95	57.0	0.07
DMADTP	2.48	1.00	67.8	0.06
DEAP	3.03	1.22	45.3	0.09
DEADTP	3.11	1.25	58.7	0.07
DMAPTh	3.46	1.40	-	-
DEAPTh	4.37	1.76	-	-
TAP	16.6	6.69	-	-

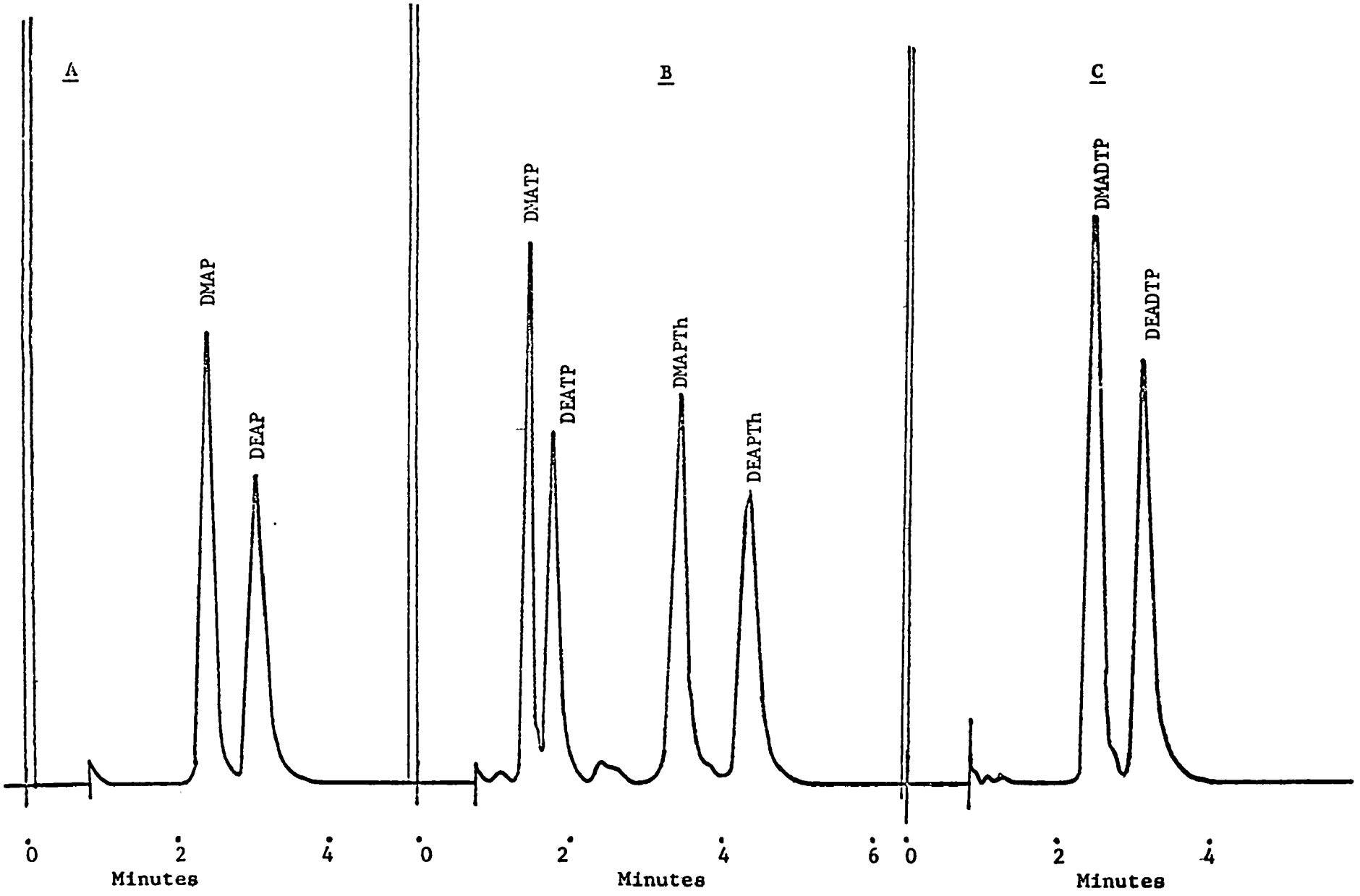
Table 3. Compound identifications for abbreviations used in text:

DMP	O,O-Dimethyl phosphate
DEP	O,O-Diethyl phosphate
DMTP	O,O-Dimethyl phosphorothionate
DETP	O,O-Diethyl phosphorothionate
DEDTP	O,O-Diethyl phosphorodithioate
DMDTP	O,O-Dimethyl phosphorodithioate
DMAP	O,O-Dimethyl-O-amyl phosphate

Table 3. Compound identifications for abbreviations used in text (Cont'd)

DEAP	O,O-Diethyl-O-amyl phosphate
DMATP	O,O-Dimethyl-O-amyl phosphorothionate
DEATP	O,O-Diethyl-O-amyl phosphorothionate
DMAPTh	O,O-Dimethyl-S-amyl phosphorothiolate
DEAPTh	O,O-Diethyl-S-amyl phosphorothiolate
DMADTP	O,O-Dimethyl-S-amyl phosphorodithioate
DEADTP	O,O-Diethyl-S-amyl phosphorodithioate
TAP	O,O,O-Triamyl phosphate

Figure 1. Chromatograms of standard solutions. A DMAP-0.59 ng, DEAP-0.53 ng, B DMATP-1.13 ng, DEATP-1.00 ng, C-DMADTP-0.63 ng, DEADTP-0.54 ng.



★CHOLINESTERASE ACTIVITY IN BLOOD

I. INTRODUCTION:

The cholinesterase enzyme in blood is inhibited in varying degrees by organophosphate pesticides and is loosely correleated with the decrease of acetylcholinesterase activity in the nervous system which in turn is accompanied by an increase in the concentration of acetylcholine. Therefore, a scheme for measuring the level of activity of the cholinesterase is one means of establishing possible exposure.

The technique of continuous titration of the acetic acid released from acetylcholine by the enzyme cholinesterase overcomes many of the undesirable features of other methods. This method does not utilize buffers, is temperature and atmospherically controlled, and has easily calculated units. In addition, the substrate and enzyme concentrations can be adjusted and maintained at levels which allow optimal enzyme activity.

REFERENCES:

1. Michel, H. O. An electrometric method for the determination of red blood cell and plasma cholinesterase activity. J. Lab Clin. Med. 34; 1964 (1949).

2. Nabb, D. P. and F. Whitfield. Determination of cholinesterase by an automated pH-stat method. Arch. Environmental Health 15:147 (1967).

3. Pearson, J. R. and G. F. Walker. Conversion of acetylcholinesterase activity values from the Michel to the pH-stat scales. Arch. Environmental Health, 16:809 (1968).

II. PRINCIPLES:

The whole blood sample is separated into the plasma and RBC (red blood cells). Each fraction is placed in the reaction vessel of a pH-stat and mixed with an excess of acetylcholine iodide. The cholinesterase present in the blood fraction reacts with the AChI releasing acetic acid as illustrated in the following:

Acethycholine (ACh)

Choline + Acetic Acid HA

A standardized solution of dilute NaOH is used as the titrant for the released HA. The automatic titrator records the amount of titrant delivered to the reaction in a given time period. A feedback signal from the pH measuring electrodes controls the titrant delivery rate. A high ChE activity produces a large hydrogen ion release in a fixed time period, calling for a faster titrant delivery rate than will a lower ChE activity.

III. EQUIPMENT:

1. *pH-stat, recording, complete with micro glass reference combination electrode, thermistor temperature sensing element, a buret assembly for delivery of 0.5 and 2.5 ml, reaction vessels.

2. Vortex mixer.

3. Centrifuge capable of spin velocity of 2000 r.p.m.

4. Aspirator for connection to suction pipet.

5. Pipet, volumetric, 2 ml.

6. Pipets, measuring, 0.2, 0.5 and 5.0 ml, Corning 7064 or the equivalent.

7. Centrifuge tubes, 5 ml, glass stoppered, Corning 8061 or the equivalent.

IV. REAGENTS:

1. Sodium chloride, reag. grade.

   Prepare 0.9% solution by dissolving 9.0 grams and diluting to a liter with dist. water.

2. Potassium acid phthalate (P.A.T.) - analytical primary standard, available from the National Bureau of Standards, Washington, D.C.

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*Manufacturers of equip. applicable for automatic pH titration are:

Burkland Scientific, 919 North Michigan Avenue, Chicago, Illinois.
Fisher Scientific, 711 Forbes Avenue, Pittsburgh, Pennsylvania.
Joseph Kaye, 737 Concord Avenue, Cambridge, Massachusetts.
Metrohm-Brinkmann, Cantiague Road, Westbury, New York.
Precision Scientific, 3737 West Cortland, Chicago, Illinois.
Radiometer-London, 811 Sharon Drive, Westlake, Ohio.
E. H. Sargent, 4647 West Foster Avenue, Chicago, Illinois.

Standard Solution - Dry standard P.A.T. in 105°C oven at least 4 hours before use. Store dried salt in desiccator. Weigh exactly 0.20423 grams and transfer to a 1-liter vol. flask, dissolving and making to volume in freshly boiled dist. water.

2. Titrant standard solutions prepared from sodium hydroxide, reag. grade, Fisher S-318 or the equivalent, 98.7% NaOH.

   a. Stock solution (1.0 N NaOH): Weigh 4.053 grams NaOH, dissolve in freshly boiled dist. water, cool and dilute to 100 ml. Store in Pyrex bottle with neoprene rubber stopper.

   b. Working solution (0.01 N NaOH): Pipet 1.0 ml of the 1.0 N solution into a 100-ml vol. flask and make to volume with freshly boiled dist. water. Standardize using potassium acid phthalate as the primary reference.

3. Standardization of titrant working solution.

   This solution should be restandardized each time that a series of samples is run. Triplicate standardizations are run to obtain an average, with the deviation between replicates no greater than 2 RU (recorder units). It is not mandatory that the titrant working solution be exactly 0.01 N as long as the exact normality is known, but the value should fall in the range of 0.0095 to 0.0105.

   The standard solution of P.A.T. contains 0.001 meq/ml. The neutralization of each ml of the P.A.T. solution will require 0.001 meq of NaOH. The NaOH working solution contains 0.01 meq/ml. Therefore, 0.1 ml of the working titrant ≙ 1.00 ml of the P.A.T. solution.

   a. With a vol. pipet, transfer 2 ml of the standard P.A.T. solution into a clean titration vessel and normalize instrument operating temperature to 37°C.

   b. Set titrant delivery to appropriate position for 2.5 ml delivered full scale, set pH to 7.0, position pen at zero and turn on function switch.

   c. The end point is reached when pen levels off at a certain RU level. Record this level and compute the titrant normality as follows:

$$N = \frac{2.0 \text{ ml} \times 0.001 \text{ meq/ml}}{\text{RU} \times 0.005 \text{ ml/RU}} = \frac{0.4}{\text{RU}} \text{ meq/ml}$$

NOTE: Total syringe delivery in the assumed system is 0.50 ml, indicated by 100 RU, or 100% of full syringe travel. It is not convenient to use the entire syringe volume for a titrant standardization or assay. For other syringe delivery capacities, such as 0.25 or 1.0 ml, adjust the RU value accordingly.

4. Acetylcholine iodide available from Calbiochem Company, P.O. Box 54282, Los Angeles, California 90054.

Substrate solution - Weigh 0.7510 grams of AChI into a 25-ml vol. flask. Dissolve and make to volume at room temperature. Store in an amber bottle in the refrigerator and hold no longer than 2 weeks.

NOTE: Weigh sample without delay since all salts of acetylcholine are hygroscopic and weight will change rapidly.

V. SAMPLE HANDLING AND PREPARATION:

The sample preparation and analysis of blood should be carried out as soon as possible after drawing sample. If a few hours delay is unavoidable, keep samples refrigerated. If the delay will be overnight or longer, blood should be centrifuged and plasma separated from RB cells and the latter taken through the following steps (a through f) before storage.

a. Place blood tube in centrifuge and spin for 20 minutes at 2000 r.p.m.

b. Pipet plasma into clean tubes for storage.

NOTE: If a part of this sample will eventually be analyzed by GLC, E.C., the tube cap should be ground glass or screw cap with Teflon or foil liner.

c. Using vacuum pipet, remove and discard fluffy layer.

d. Resuspend red cell mass in an equal volume of 0.9% NaCl solution. This is done by gently inverting tube.

e. Centrifuge again for 10 minutes and remove supernatant NaCl solution by vacuum pipet.

f. Repeat steps d. and e. The RBC mass is now ready for assay.

VI. PREPARATION OF RBC HEMOLYSATE:

1. Pipet 1.8 ml of dist. water into a 5-ml conical centrifuge tube and then pipet in 0.2 ml of packed red cells.

   NOTE: Care must be taken to wipe the tip of the pipet with tissue while still retaining the full 0.2 ml contents. This may require some practice.

2. With a rubber bulb attached to the pipet, draw the hemolysate solution up into the bore of the pipet repeatedly until all adhering cells are washed into the water.

3. Stopper tube and mix on Vortex mixer about 30 seconds or until all cells have hemolyzed. The hemolysate so prepared may remain up to 20 minutes at room temperature before assaying. For longer periods, hold in refrigerator.

VII. CHOLINESTERASE ASSAY:

1. Calibrate instrument with reference buffers, following manufacturer's instructions. This is best done by using two buffers, one below and one slightly above pH 8. Normalize the instrument to a 37°C operating temperature.

2. Into a clean titration vessel pipet 4.2 ml of 0.9% NaCl solution and 0.15 ml of plasma, (or 4.2 ml of 0.9% NaCl and 0.50 ml of RBC).

3. Place titration vessel on instrument and be sure that instrument end point is set at pH 8.0. As the initial pH of sample and NaCl solution will nearly always be a little low, it is necessary to adjust the pH to 8.0 with the 0.01 N NaOH titrant.

4. Check to be sure that recorder and titrant delivery systems are set at zero.

5. Add substrate to mixture in titration vessel:

   a. If plasma assay, add 0.6 ml of acetylcholine iodide solution.

   b. If RBC assay, add 0.10 ml of AChI solution.

6. Start recorder and titrant flow and allow titration to proceed until recorder describes a line with constant slope, then mark off the start and end of a 3-minute period of titration. See Figure 1.

NOTE: None of the line included should be nonlinear. It is good practice to allow titration to proceed for a minute or so before counting the RU.

VIII. SAMPLE CALCULATIONS:

Refer to Figure 1:

1. Standardization of titrant.

Average of three replicate titrations:

$$\begin{array}{r} 54.0 \text{ RU} \\ 54.0 \\ 53.9 \\ \hline 3\overline{)161.9} \end{array} = 53.961 \text{ RU}$$

From formula (1), $N = \frac{0.600}{53.961} = \frac{0.600}{53.961} = 0.011$ N

2. Calculation of ChE activity.

A. Factor, from Table 1, for plasma ChE, based on 0.011 N titrant, is 0.1222. The observed RU value is multiplied by this factor:

Plasma 1: 46.5 x 0.1222 = 5.682 µM/min/ml (first replicate)
48.5 x 0.1222 = 5.927 µM/min/ml (second replicate)

Plasma 2: 30.5 x 0.1222 = 3.727 µM/min/ml (only one replicate shown)

B. Alternative method, without using factors:

Plasma 1: $\frac{\text{RU x 0.005 ml/RU}}{\text{Minutes}} = \text{ml/minute} = \frac{\text{Substituted: } 46.5 \text{ x } 0.005}{3} = 0.0775$ ml/min.

ml/minute x Normality = meq/minute = 0.0775 x 0.011
= 0.000853 meq/minute

meq/minute x 1000 µM/meq = µM/minute = 0.000853 x 1000 µM/meq
= 0.853 µM/minute

$\frac{\mu\text{M/minute}}{\text{sample volume}} = \mu\text{M/min/ml} = \frac{0.853}{0.15} = 5.687$ µM/min/ml

IX. MISCELLANEOUS NOTES:

1. Because of the variety of instruments available, operating instructions are not given for pH-stat equipment. Detailed instructions furnished by the manufacturer will govern operating details. A step-by-step outline of sample preparation, reagent preparation and standardization, plus the scheme for performing calculations are outlined.

2. Heparin is preferable to other anticoagulants, because oxalate, citrate and EDTA will sequester calcium and magnesium, which are required co-factors for ChE. In an emergency, these anticoagulants can be used, but ChE assay results are likely to be lower than if heparin were used.

3. Considering the low pipetting volumes, meticulous care must be taken to obtain reproducible aliquots. This is particularly true with packed red blood cells.

4. Centrifugation speeds and times should be consistent from one sample lot to the next. Formation of packed red cell mass after final saline wash is critical. Each successive red cell pack should be the same, otherwise differences in density will yield different results from sample to sample.

5. Be Gentle. Hemolysis of red cells in contact with plasma will liberate acetylcholinesterase into plasma, thus altering the true enzyme activity of the latter. Mix red cells with plasma or saline solution by gentle inversion of tubes, or by gentle stirring with glass rod or wooden applicator stick.

6. The approximate lower limits of normal ChE activity for human blood assayed by the present method are:

   2.0 μM/min/ml - Plasma
   8.0 μM/min/ml - Red Cells

   An individual laboratory should, of course, establish its own normal ranges based on experience with the method.

7. Assay results obtained by the pH-stat method are expressed as:

   micromoles (acetic acid liberated)/minute/ml sample (either packed red cells or plasma). Abbreviated: μM/min/ml.

8. Standard sources of enzyme are available from Sigma and are useful in intralaboratory quality control.

Figure 1. Sample of strip chart record of pH-stat assay of cholinesterase activity.

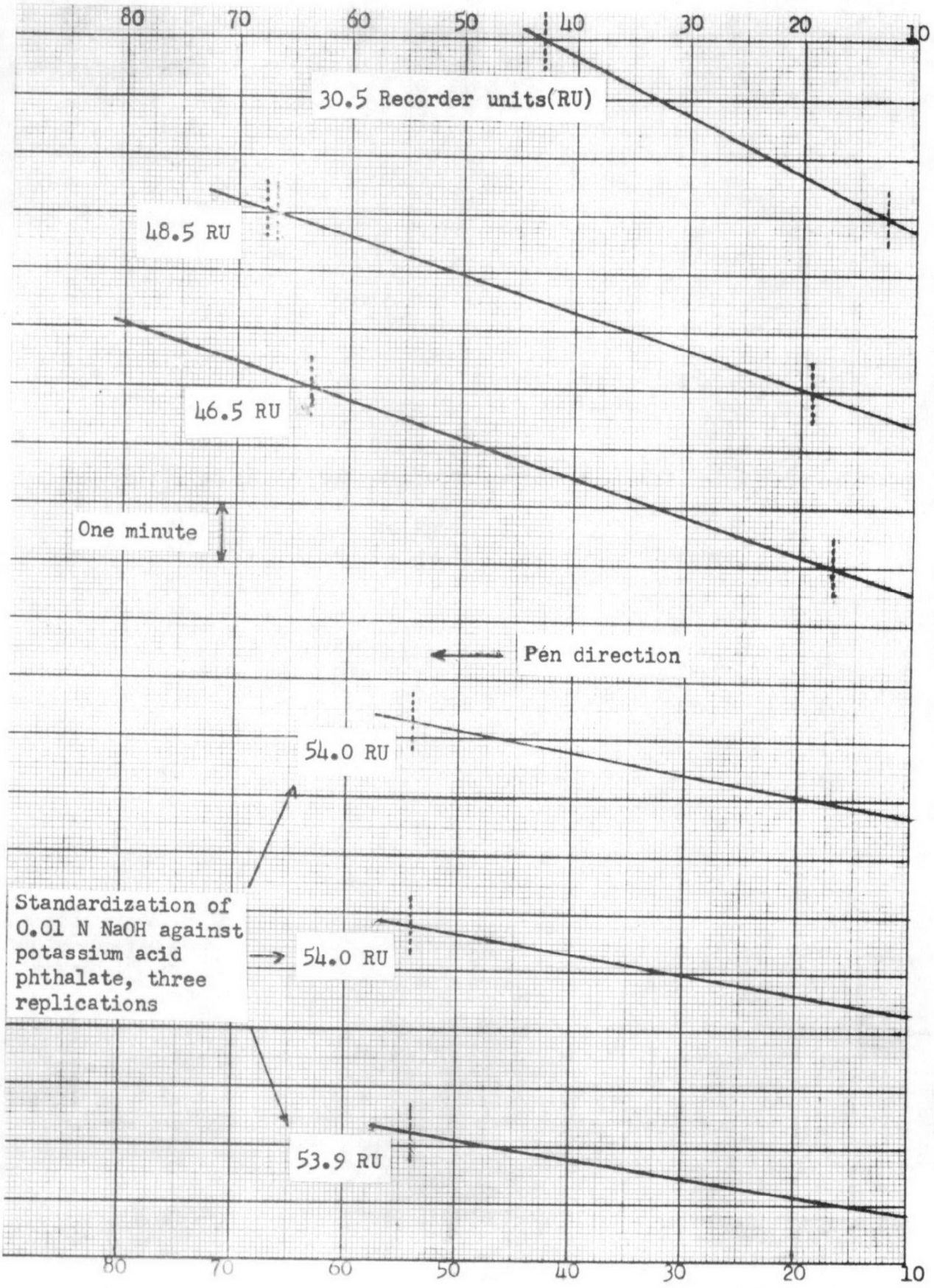

Table 1. Factors for Three Minute Titrations

Normality NaOH Solution	Factor	Plasma ChE Factor
0.0095	0.3167	0.1055
0096	3200	1067
0097	3233	1078
0098	3267	1089
0099	3300	1100
0100	3333	1111
0101	3367	1122
0102	3400	1133
0103	3433	1144
0104	3467	1156
0105	3500	1167

These factors are derived as follows:

$$\text{Red cell factor} = \frac{\text{ml/min x meq/ml x 1000 } \mu\text{M/meq}}{0.05}$$

$$\text{Plasma factor} = \frac{\text{ml/min x meq/ml x 1000 } \mu\text{M/meq}}{0.15}$$

These factors are valid only if a 0.5-ml syringe is used for titrant delivery, so that the ml/min factor in the equation becomes $\frac{0.005}{3}$, or 0.00167 ml/min.

DETERMINATION OF 1-NAPHTHOL IN URINE

I. INTRODUCTION:

Humans exposed industrially to the insecticide carbaryl (1-naphthyl-N-methyl carbamate) excrete relatively large quantities of 1-naphthol, conjugated either as the sulfate or glucuronide. Quantitative determination of 1-naphthol in human urine has been generally accomplished using a colorimetric procedure. This method lacks both the sensitivity and specificity necessary for determining the relatively small amounts of 1-naphthol excreted in the urine of agricultural workers exposed to low levels of this insecticide.

The 1-naphthol resulting from the hydrolysis of carbaryl has been used as an indirect measure of the residue level of the parent insecticide on a variety of agricultural crops. Argauer has described a procedure for chloroacetylating phenols and 1-naphthol for subsequent detection by electron capture gas chromatography. In a recent publication this procedure was utilized to determine a number of carbamate insecticides. However, it was indicated that further modification would be necessary if the method was to be extended to carbaryl.

The method described in this section utilizes the enhanced electron capture characteristics of the monochloroacetate derivative. This, coupled with a silica gel cleanup results in a method sensitivity down to 20 ppb of 1-naphthol.

REFERENCES:

1. Shafik, M. T., Sullivan, H. C., Enos, H. F., Bull. of Envir. Contamin. & Toxic., Vol. 6, No. 1, 1971 pp 34-39.

II. PRINCIPLES:

A small sample of urine is subjected to acid hydrolysis. The 1-naphthol present is extracted in benzene and derivatized with chloroacetate anhydride solution. After silica gel cleanup, the resulting 1-naphthyl chloroacetate is quantitatively determined by E.C., GLC, comparing sample peaks against peaks obtained from pure 1-naphthol standard, similarly derivatized.

III. APPARATUS:

1. Gas chromatograph equipped with E.C. detector and fitted with a 6' x 1/4" o.d. column of 1.5% OV-17/1.95% QF-1. Operating parameters for the column are those prescribed in Section 4,B of this manual.

III. APPARATUS (Continued)

2. Chromatographic column (Chromaflex), size 22, Kontes #420100.

3. Evap. concentrator tubes grad., glass stoppered, 25 ml, ℱ 19/22, Kontes #570050.

4. Distilling column (condensor), 200 mm jacket, Kontes #286810, fitted with tight glass stopper at top.

5. Centrifuge capable of 2000 r.p.m. that will accept metal shield, I.E. Company No. 367.

6. Volumetric flasks, 10 and 100 ml.

7. Pipets, Mohr, 0.2, 0.5 and 10 ml, Corning 7064 or the equivalent.

8. Pipets, transfer, 2, 3 and 5 ml, Corning 7100 or the equivalent.

9. Vortex-Genie mixer.

10. Disposable pipets, Pasteur, 9 inch.

11. Graduated conical centrifuge tubes, 15 ml, with ℱ glass stoppers, Corning 8084 or the equivalent.

12. Circulating water pump.

13. Boiling water or steam bath.

IV. SOLVENTS AND REAGENTS:

1. Benzene, pesticide quality.

2. Hexane, pesticide quality.

3. Pyridine, Spectro Grade, Eastman #13098.

4. Chloroacetic anhydride, Eastman #335 - prepare a 2% solution in benzene and hold no longer than one week.

   NOTE: The chloroacetic anhydride must be dry and therefore should be stored in a desiccator as it is extremely hygroscopic.

5. Hydrochloric Acid, reag., conc.

6. Sodium sulphate, anhydrous, granular - preextract on a Soxhlet with benzene for about 50 discharge cycles, remove excess solvent and store in 130°C oven.

7. Sodium sulphate, 3% solution in dist. water. Use preextracted Na_2SO_4.

   NOTE: Deionized or distilled water, preextracted with benzene, is used throughout the procedure.

8. Mixtures of benzene/hexane as follows: 20/80, 40/60 and 80/20.

9. 0.1 N and 1.0 N NaOH solutions.

10. Silica gel, Woelm, activity grade I, Waters Associates, Inc., DO NOT SUBSTITUTE.

11. Preparation of silica gel.

    Dry adsorbent for 48 hours at 170°C and store in the same oven. On day of use, cool the silica gel in a desiccator and deactivate with 1.5% water in the following manner: Add the necessary volume of water to a 125-ml glass stoppered Erlenmeyer flask, rotating the flask to coat the sides with water. Add the weighed amount of silica gel, stopper and mix until the water is evenly distributed throughout the adsorbent. Allow to equilibrate for 2 to 3 hours with periodic shaking. Chromatographic columns are prepared just prior to use.

12. 1-Naphthol, Eastman #170.

13. Preparation of standard solutions:

a. Stock Standard I. Weigh 20 mg of 1-naphthol into a 100-ml vol. flask, dissolve and dilute to volume with benzene. This is the conc. stock of 200 ng/µl and may be held several months at -18°C.

b. Stock Standard II. With a 0.5-ml Mohr pipet, transfer 0.25 ml of stock standard I to a 50-ml vol. flask and make to volume with benzene. This intermediary stock standard of 1 ng/µl is used for preparation of working standards and sodium naphthoxide for recovery studies (See Misc. Note, 1, a).

c. Prepare reagent blank and working standards by transferring aliquots of stock standard II of 0, 0.1 and 0.5 ml to separate 25 ml evap. concentrator tubes. Dilute each to 5 ml with benzene.

(1) Add 2 ml of 2% chloroacetic anhydride and 0.2 ml of pyridine to each tube. Stopper and mix vigorously on Vortex mixer for 2 minutes. Allow to stand 10 minutes.

(2) Add 5 ml of dist. water, stopper and reagitate on Vortex for one minute.

(3) Allow layers to separate and, with a disposable pipet, carefully remove and discard as much as possible of the lower (aqueous) layer.

(4) Repeat water wash (Steps (2) and (3) above) twice more.

(5) Place tubes in centrifuge, spin 5 minutes at 2,000 r.p.m. and remove any final traces of water from bottom of tubes. Dilute to exactly 10 ml with benzene and mix thoroughly. The three tubes will contain concentrations of 1-naphthyl chloroacetate of 0, 10 and 50 pg/µl.

d. Prepare another intermdeiary stock standard (III) of derivatized 1-naphthol (1-naphthyl chloroacetate) by transferring 0.5 ml of stock standard I to a 25-ml evap. concentrator tube and dilute to 5 ml with benzene. Proceed with derivatization as described above in c, (1), (2), (3), (4) and (5) but instead of diluting to 10 ml as described in step (5), transfer extract through a glass funnel into a 100-ml vol. flask, rinsing tube with several portions of benzene and finally making to volume with benzene. From this derivatized stock of 1 ng/µl concentration of 1-naphthyl chloroacetate, dilutions may be made and used to check the derivatized working standards finalized in step c, (5) above.

NOTE: The derivatized standards are relatively stable. Assuming no solvent losses from repeated opening of the storage bottles, it is probable that the standards could be held for 6 months, storing in the refrigerator when not in use.

V. PROCEDURE:

Hydrolysis, Extraction and Derivatization

When the following procedure is started there should be no interruption until the final derivatized extract is obtained in Step 10. It is highly desirable that a sample of control urine from an unexposed donor be carried along parallel with the sample(s) under test.

1. Pipet 5 ml of urine into a 25 ml evap. concentrator tube, add 1 ml of conc. HCl, stopper and mix on the Vortex mixer 2 minutes.

2. Fit concentrator tube with a glass stoppered condenser (distilling column) and reflux mixture in a hot water or steam bath 90 minutes, cooling the condenser with circulating ice water.

NOTE: The top of the condenser should be tightly stoppered.

3. After removal from the bath and cooling, wash down bore and condenser tip with 2 ml of 0.1 N NaOH followed by 3 ml of benzene.

4. Stopper and mix vigorously for 2 minutes on the Vortex mixer.

5. Place conc. tube in centrifuge and spin for 10 minutes at 2,000 r.p.m.

6. Carefully transfer as much of the benzene (upper) layer as possible to a clean 25 ml concentrator tube, using a disposable pipet fitted with a rubber bulb.

7. Add 3 ml more of benzene and repeat Steps 4, 5 and 6.

NOTE: Extreme care should be taken to prevent water from being transferred as this would seriously affect derivatization efficiency.

8. Wash benzene extract with two 3 ml portions of 5% Na_2SO_4 solution, centrifuging and discarding each successive aqueous layer.

9. To the combined benzene extract add 2 ml of 2% chloroacetic anhydride solution and 0.2 ml of pyridine. Stopper tube, mix on Vortex mixer for 2 minutes and allow to stand 10 minutes at room temperature.

10. Refer to and follow identically Steps (2),(3),(4), and (5) of subsection IV., 13, C but placing tubes of derivatized extract in a 40°C bath and evaporating to 0.5 ml under a dry nitrogen stream.

NOTE: Under no circumstances should final volume be permitted to go below 0.3 ml.

Silica Gel Cleanup

1. Place a small wad of glass wool at the bottom of a Chromaflex column and add 1 gram of the partially deactivated silica gel. Top this with ca 1/2-inch of anhydrous, granular Na_2SO_4.

2. Prewash the column with 10 ml of hexane, discarding the eluate.

3. When the surface level of the hexane reaches a point on the column ca 2 cm from the top of the Na_2SO_4 transfer the concentrated benzene extract to the column with a disposable pipet and rinse tube with two portions of 0.5 ml of 20/80 benzene/hexane solvent applied with another disposable pipet, directing stream so as to wash down walls of tube. Follow this with 8.5 ml of 20/80 benzene/hexane solvent, discarding all eluates up to this point.

4. Place a 15 ml grad. conical centrifuge tube under column and add 10 ml of 60/40 benzene/hexane solvent, collecting this fraction which contains the 1-naphthyl chloroacetate derivative. Finally, adjust volume of extract to exactly 10 ml with benzene.

NOTE: 1. The foregoing instructions are based on the premise that the operator obtains an elution pattern identical to that obtained by the authors. However, this should not be assumed until the operator has confirmed it by actual trial. This may be readily done by applying 0.5 ml of the derivatized 200 ng/ul stock standard to a silica gel column in the manner described in Steps 1, 2 and 3 but collecting the 10 ml of hexane prewash and the 20/80 benzene/hexane eluates instead of discarding them. In addition to this, 10 ml each of benzene/hexane mixtures of 40/60 60/40, 80/20 and 100% benzene should be eluted through the column, collecting each of the cuts for

examination by GLC to determine whether any of the derivatized compound is eluting out of place. If so, it may prove necessary for the individual operator to make appropriate revisions in the eluate collection instructions.

2. At no time during the elution should the liquid level in the column be allowed to go below the top of the Na_2SO_4 layer.

Gas Chromatography

After adjusting operating parameters of the gas chromatograph to the values prescribed in Section 4,B of this manual, commence injections of derivatized sample and standard extracts. Assuming an average background current, it should be possible to quantify as little as 50 picograms of the derivatized 1-naphthol Using the 1.5% OV-17/1.95% QF-1 column, the relative retention value for 1-naphthyl chloroacetate should be ca 0.92 with respect to aldrin.

VI. MISCELLANEOUS NOTES:

1. Elution patterns may vary from one laboratory to another depending on the temperature and relative humidity. This emphasizes the need for establishing an elution pattern of standards and spiked control urine samples under local conditions before attempting to analyze samples. The procedure for spiked control urine samples is as follows:

   a. In a 15-ml conical grad. centrifuge tube mix 2 ml of 1.0 N NaOH and 2 ml of the diluted, underivatized standard described in the NOTE in Step b, of subsection IV, 13.

   b. Stopper tube and mix 2 minutes on a Vortex mixer. Allow to stand 10 minutes and centrifuge 5 minutes at 2,000 r.p.m.

   c. Pipet aliquots of 0.1, 0.25 and 0.5 from the aqueous layer (sodium naphthoxide solution) into separate 25-ml grad. evap. concentrator tubes.

   d. From this point on, conduct the hydrolysis and derivatization as previously described in subsection V, starting with Step 1, under Hydrolysis, Extraction and Derivatization, ending at Step 4 under Silica Gel Cleanup. The final extracts contained in the three 15-ml centrif. tubes should have concentrations of 10, 25 and 50 pg/µl of derivatized 1-naphthyl chloroacetate. Recovery data is obtained by chromatographing these extracts against the derivatized working standards.

SAMPLING AND ANALYSIS OF PESTICIDES IN AIR

B. ANALYSIS

I. INTRODUCTION:

The analytical method described presupposes the collection of the air sample with the sampling device developed by Mid West Research Institute* utilizing a vacuum pump which draws air through ethylene glycol contained in glass impingers. A customary sample consists of 400 ml of ethylene glycol representing the contents of four impingers of 100 ml each, two of which are operated simultaneously for 12 hours, and then two more for another 12 hours. The total air volume sampled in the 24-hour period is generally ca 80 cubic meters.

II. PRINCIPLE:

The ethylene glycol contents of four 100-ml impingers are transferred to a large separatory funnel and diluted with water. Pesticides are extracted with hexane and transferred to a Kuderna-Danish evaporator. After concentration to a suitable volume, a preliminary gas chromatographic evaluation is made using electron capture detection for organochlorine compounds and flame photometric detection for the organophosphates.

The extract is then subjected to column cleanup with Florisil, partitioning into two or three eluate fractions, each of which is reconcentrated to a suitable volume and subjected to final gas chromatographic analysis.

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*Stanley et al., Envir. Sci. Technol., 5, (5) 430 (1971).

III. EQUIPMENT:

1. Chromatographic columns 22 mm i.d. x 300 mm length, Teflon stopcocks, without filter frits.

2. Separatory funnel - 1 and 2-liter capacity, Teflon stopcocks.

3. Hot water bath - 95-100°C.

4. Glass beads 3 mm plain, Fisher #11-312 or equivalent.

5. Glass wool - angel hair, fine.

6. Kuderna-Danish concentrator.

   Evaporative concentrator flask - Kontes Catalog No. K-570001, 500 and 1,000 ml, lower joint 19/22 ℸ, upper joint 24/40 ℸ.
   Snyder column - 3 ball, lower joint 19/22 ℸ, upper joint 24/40 ℸ, Kontes Cat. No. K-503000.
   Tube - 10 ml grad., 19/22 ℸ joint, Kontes No. K-570050, Size 1025.

7. Gas Chromatographic Columns.

   1.5% OV-17/1.95% QF-1, 4% SE-30/6% QF-1 and 10% OV-210, preparation, conditioning and operating parameters as outlined in Section 4,A.

IV. REAGENTS:

1. Ethylene Glycol, pesticide quality. Must meet the criteria described in Section 8,C.

2. Hexane, redistilled in glass. Must be evaluated for background interference by gas chromatography, electron capture, of a 1-ml concentrate from 180 ml of original solvent.

3. Petroleum ether - pesticide quality, redistilled in glass, E.P. 30-60°C.

4. Ethyl ether - AR grade, peroxide free.

   The above solvents should be free of materials that give a response to the electron capture detector. Preparation of peroxide-free ethyl ether is given in Section 5,A,(1).

IV. REAGENTS (CONTD.)

5. Distilled water - check for interferences. If necessary, extract with benzene and pet. ether as described in Section 8,C for glycol extraction.

6. Florisil, P. R. grade. See MISCELLANEOUS NOTE 4, Page 7.

7. Anhydrous sodium sulfate.*

8. Eluting mixture (6+94 ether in pet. ether). (Used for Fraction I.)*

9. Eluting mixture (15+85 ethyl ether in pet. ether). (Used for Fraction II.)*

10. Eluting mixture (50+50 ethyl ether in pet. ether). Prepare same as other ether mixtures. (Used for Fraction III.)*

V. PROCEDURE:

All glassware to be used must be scrupulously cleaned and given a final hexane rinse immediately before use.

1. Combine the impinger contents representing the collection of 81 m^3 of air (presumably 4-100 ml increments of glycol).

2. Transfer the 400 ml of E.G. to a 2-liter sep. funnel (No. 1). Wash the sample bottle with a measured amount of dist. water and add washings to sep. funnel. Dilute with a total of 1400 ml of water.

NOTE: All procedural instructions from here on assume the extraction of 400 ml of E.G. Should a smaller volume be extracted, the glassware sizes and reagent volumes should be scaled down proportionately.

3. Transfer the prefilter glass cloths to a 400-ml beaker with a clean pair of forceps. Add 240 ml of hexane, stopper and shake funnel vigorously for 2 minutes. If emulsions are formed at this point, add 10 ml of a saturated NaCl solution to the separatory funnel (NaCl and distilled H_2O should be free of interferences - pre-extract if necessary).

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* Instructions and comments re purity of these materials may be found in Section 5,A,(1).

V. PROCEDURE (CONTD.)

4. Transfer the aqueous layer to a second 2-L sep. funnel (No. 2), and the hexane layer to a 1-L sep. funnel (No. 3). Add another 240-ml portion of hexane to funnel No. 2, stopper and shake vigorously for 2 minutes. Allow a few minutes for layer separation and transfer aqueous layer back into funnel No. 1. Combine the hexane layer with that already in funnel No. 3. Add a final 240-ml portion of hexane to funnel No. 1 and repeat shaking and settling. Discard aqueous layer and transfer hexane layer to funnel No. 3.

5. Add 200 ml of dist. water to S.F. No. 3, stopper and shake vigorously 1 minute. Discard aqueous layer and repeat extraction with more of the 200-ml portion of dist. water, again discarding aqueous layer.

6. Place a glass wool retaining plug in the bottom of a 300-mm chromatographic column, add 2 inches of Na_2SO_4 and pass the combined hexane extract through the column, collecting eluate in a 1,000-ml K-D flask which has been fitted to a 10-ml evaporative concentrator tube containing one 3-mm glass bead.

7. When extract has eluted from the column, rinse the sep. funnel with three consecutive portions of 10 ml each of hexane, washing down the walls of the column. Allow each rinse to elute off the column before adding the next. Finally, rinse the column walls with two more 10-ml portions of hexane.

8. Place K-D assembly in a boiling water bath and concentrate extract to ca 5 ml. Remove from bath, cool, disconnect tube from K-D flask, rinsing joint with a little hexane. Place tube under a nitrogen stream at room temp., and further concentrate extract to ca 0.5 ml. Rinse down tube sidewalls with hexane delivered from a 100-ul syringe, diluting extract back up to exactly 1.0 ml. Stopper and mix on Vortex 30 seconds.

9. Make exploratory injections into the gas chromatograph via FPD detection. In the event that no peaks appear with relative retention values suggestive of organophosphorus compounds, it may be concluded that the sample contains none of these compounds, or that any compounds present are below the minimum detection levels. (See Section 4,B for sensitivity guidelines.) If peaks of greater than 10% f.s.d. appear by FPD, and their identities can be determined as organophosphorous pesticides, quantitations should be conducted at this point.

V. PROCEDURE (CONTD.)

NOTE: It is probable that at this extract concentration ratio (400:1), background interference would make electron capture appraisal difficult or impossible. However, if the detectors are so arranged in one instrument that an E.C. chromatogram can be obtained simultaneously with the FPD chromatogram, some useful, preliminary information may be obtained.

Florisil Partitioning

1. Dilute extract up to ca 5 ml with hexane and transfer to a Florisil column. [Prepare column as described in Section 5,A,(1).] Begin eluate collection immediately on adding hexane to column and collect the eluate in a 500-ml Kiderna-Danish concentrator fitted with a 10-ml grad. concentrator tube containing one glass bead. When hexane reaches the Na_2SO_4 layer, wash the tube with 3- to 5-ml portions of pet. ether, rinsing the column walls each time, then wash column walls with 1- to 10-ml portion of pet. ether. When final wash reaches Na_2SO_4 level, elute column with 200 ml of the 6% mixed ethers and collect until ethers reach Na_2SO_4 layer (Fraction I).

2. Change Kuderna-Danish concentrators and elute with 200 ml of the 15% mixed ethers in the same manner (Fraction II).

3. Change Kuderna-Danish concentrators and elute with 200 ml of the 50% mixed ethers (Fraction III).

4. Attach Snyder columns on the K-D flasks, immerse tubes in hot water bath and concentrate eluates to slightly less than 5 ml. Cool and detach tubes from K-D flasks, rinsing joints with a little hexane.

5. Adjust the volume of the Fraction I and II extracts to exactly 5.0 ml and analyze by electron capture detection.

NOTE: If the 1-ml concentrate of the unpartitioned extract was examined via electron capture, it may be possible to estimate the degree of dilution necessary to bring peaks of chlorinated pesticides into quantifiable range by electron capture or electrolytic conductivity detection.

V. PROCEDURE (CONTD.)

6. After completing GLC examination of Fractions I and II, continue extract concentration, along with Fraction III, down to ca 0.5 ml under a nitrogen stream at room temperature. Rinse down sides of tube with hexane delivered from a 100-µl syringe, diluting extract to exactly 1.0 ml. Stopper and shake on Vortex 30 seconds.

NOTES: (1) Do not use air for blow down and do not permit evaporation to dryness. Organophosphate compounds may be seriously affected by either of these practices.

(2) If predetermined Florisil elution patterns have indicated that any chlorinated pesticides are eluting in Fraction III (endrin or dieldrin are sometimes troublesome), the volume of this fraction should also be adjusted to exactly 5 ml, and examination by electron capture conducted.

7. Analyze the 1-ml extract concentrates of all three fractions by FPD, carefully observing sensitivity guidlines set forth in Section 4,B.

NOTE: The FPD analysis on the partitioned extract is run for assurance of identifications obtained on unpartitioned extract. There will probably be no need for requantitating these O.G.P. compounds except in cases of distorted or overlapped peaks in the original chromatograms.

VI. MISCELLANEOUS NOTES:

1. Use the Ov-17/QF-1 and SE-30/QF-1 columns for electron capture GLC, and the SE-30/QF-1, Carbowax-treated, for all FPD work. An alternate column that may prove useful is the OV-210. At this point in time, relative retention and response data given in Section 4,B, Table 1 is restricted to the SE-30/QF-1 Carbowax-treated column. Data will eventually be forthcoming for the columns of 1.5% OV-17/1.95% and 10% OV-210.

2. The pesticides of interest may be in any of the three fractions and can be identified on the electron capture or flame photometric detector systems as needed. (i.e., Fraction I contains organochlorine pesticides; Fraction II contains organochlorine, chlorophenoxy acid esters and organophosphate pesticides; and Fraction III

VI. MISCELLANEOUS NOTES (CONTD.)

contains malathion.) Information on any other organochlorine or organophosphate pesticide of particular interest may be characterized by monitoring the appropriate fraction with either the electron capture or flame photometric detector.

3. As a reiteration, the miscellaneous notes discussed in the Section 5,A,(1), under the "Analysis of Adipose Tissue", are very pertinent to this methodology and should be thoroughly understood.

4. The analyst must know from predetermined tests of recoveries and compound elution patterns which compounds will appear in each Florisil fraction eluate of the particular lot of Florisil being used. Those laboratories operating under contract with EPA are supplied with standardized Florisil of known adsorptive characteristics. It may prove necessary to use more or less than the 4 inches of Florisil specified to achieve the partitioning indicated in Table 1, this section or in Table 1 of Section 5,A,(1).

5. Occasions arise when positive compound identification by electron capture is not possible. This situation is not unusual with certain of the organochlorine compounds at low concentration levels. One confirmation technique involving minimum effort is TLC. This is described in Section 12,B, pp 7 and 8. Another relatively rapid technique is the p-value procedure described in Section 12,C. Those laboratories equipped with electrolytic conductivity or microcoulometric detectors can utilize this equipment for both qualitation and quantitation.

TABLE 1. FLORISIL ELUTION PATTERNS, RECOVERY EFFICIENCY, AND LOWER LIMITS OF DETECTION OF SOME COMMON PESTICIDES EXTRACTED FROM ETHYLENE GLYCOL BY PROCEDURE OUTLINED IN SUBSECTION III.

Compound	Elution Fraction	Lower Detection Limits (ng/m^3) 4% SE-30/6% QF-1	1.5% OV-17/1.95% QF-1
α-BHC	I	0.2	0.2
Diazinon	II	.4	-
β-BHC	I	.8	1.2
Lindane	I	.4	.4
δ-BHC	I	.4	.4
Heptachlor	I	.4	.4
Ronnel	I	.6	-
Aldrin	I	.6	.6
Heptachlor Epoxide	I	.8	.8
Dieldrin	II	1.2	1.8
Endrin	II	2.6	4.0
o,p'-DDE	I	1.4	2.0
Chlorobenside	I	1.6	1.2
p,p'-DDE	I	1.0	1.2
Thiodan I	II	-	-
o,p'-DDD	I	2.0	2.8
*Perthane	I	167.	167.
p,p'-DDD	I	1.6	2.0
o,p'-DDT	I	2.0	2.6
Thiodan II	III	-	-
p,p'-DDT	I	2.0	1.5
Tedion	II	25.	
Ethyl Parathion	II	1.3	
Methyl Parathion	II	.9	
Malathion	III	1.3	
Ethion	I	1.5	
Chlordane	I	-	
Carbophenothion	I	3.1	
Aroclor 1254	I	-	
2,4-D esters	II		
*ME			
*BE		13.6	10.2
*IOE		50.	44.
IPE		6.2	9.
*BOEE		43.	22.
*PGBEE		19.	15.

TABLE 1 (CONTD.)

Compound	Elution Fraction	Lower Detection Limits (ng/m^3) 4% SE-30/6% QF-1	1.5% OV-17/1.95% QF-1
2,4,5-T esters	II		
*IOE		18.	7.6
*IPE		1.4	1.6
*n-Butyl			

Lower detection limits of organophosphorous compounds determined by flame photometric detection; all other compounds by electron capture.

*Recovery efficiencies not established for these compounds. Recoveries of 85% or better determined on all other compounds listed.

TABLE 2. ELUTION AND RECOVERY OF ORGANOPHOSPHOROUS PESTICIDES BY MOG METHOD
Copied from F.D.A. Release

Pesticide Name	% Recovery	Elution from Florisil 6%	15%	50%
Abate[a]	-			
Azinphosethyl	50%	-	-	-
Azinphosmethyl	0	-	-	-
Azodrin	0	-	-	-
Bensulide[a]	-			
Bidrin	0	-	-	-
Bomyl	0	-	-	-
Bromophos ethyl	102%	+	-	-
Carbophenothion Oxygen Analog	0	-	-	-
Ciodrin	0	-	-	-
Compound 4072	0	-	-	-
Coumaphos	0	-	-	-
Def	91%	-	+	+
Demeton	0	-	-	-
Dicapthan	76%	-	+	+
Dichlorvos[a]	-	-	-	-
Dimethioate Oxygen Analog	0	-	-	-
Dimethioate	0	-	-	-
Dioxathion	0	-	-	-
Disulfoton	51%	+	-	-
Disulfoton Oxygen Analog	0	-	-	-
Disulfoton Sulfone	0	-	-	-
Dyfonate	91%	+	-	-
Falone[a]	-	-	-	-
Famphur	0	-	-	-
Fenthion	45%	+	+	-
Fensulfathion	0	-	-	-
Fensulfathion Oxygen Analog	0	-	-	-
Fensulfathion Sulfone	0	-	-	-
Gardona	0	-	-	-
Gophacide[a]	-	-	-	-
Hercules 14503	95%	-	+	-
Imidan	0	-	-	-
Malathion Oxygen Analog	0	-	-	-
Maretin[a]	-	-	-	-
Merphos	133%[b]	-	+	-
Methyl Parathion Oxygen Analog	0	-	-	-
Methyl Trithion	82%	+	-	-
Mevinphos	0	-	-	-
Mocap	55%	-	-	+

TABLE 2 (CONTD.)

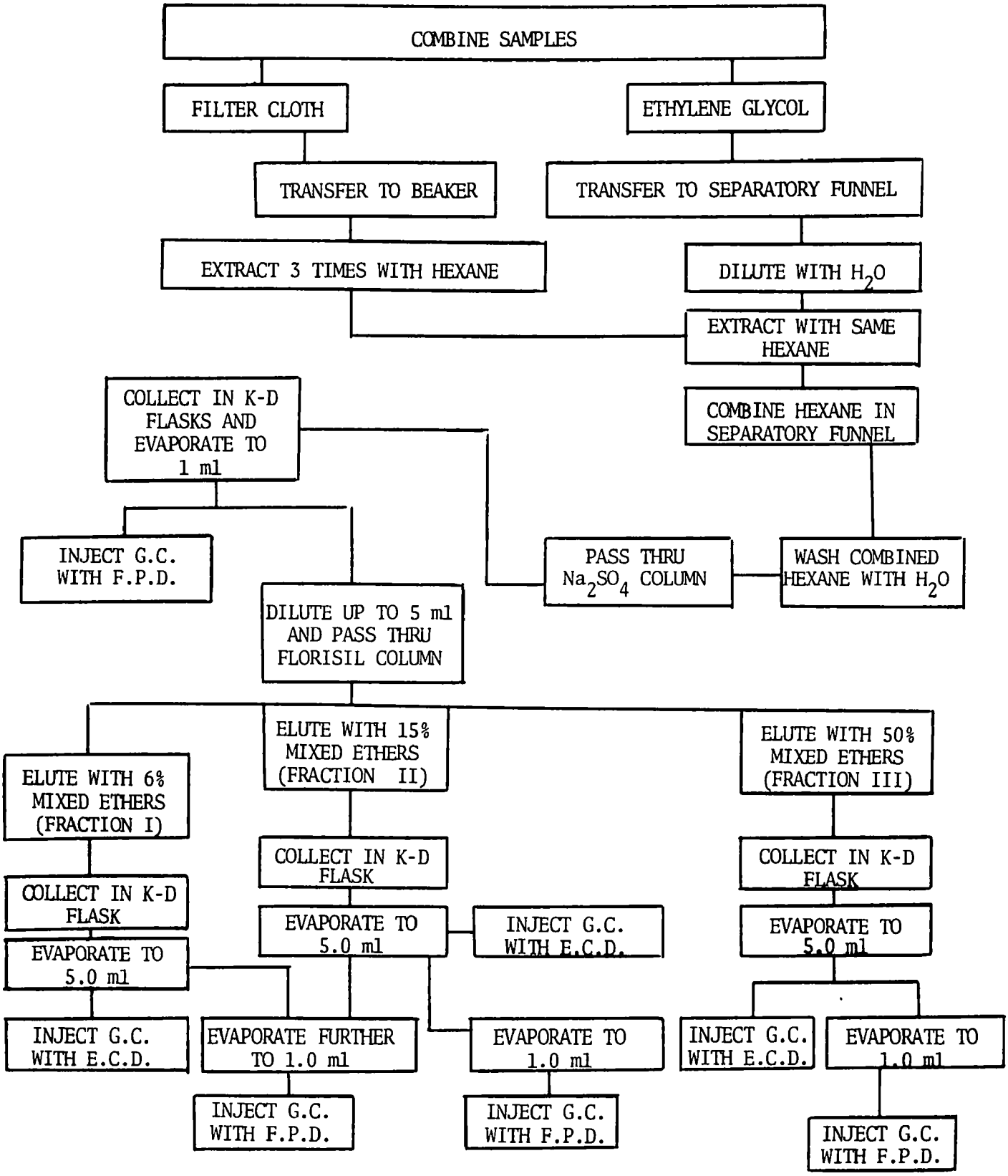
Pesticide Name	% Recovery	Elution from Florisil 6%	15%	50%
Naled	0	-	-	-
Nemacide	110%	+	-	-
OMPA	0	-	-	-
Parathion Oxygen Analog	0	-	-	-
Phenkaptan	95%	+	-	-
Phorate Sulfone Oxygen Analog	0	-	-	-
Phosalone	83%	-	-	+
Phosphamidon	0	-	-	-
Phostex	67%	+	-	-
Ronnel Oxygen Analog	0	-	-	-
Ruelene[c]	0	-	-	-
SD 7438[c]	98%	-	+	-
Sulfatepp	85%	+	-	-
Sumithion	81%	-	+	-
Supracide	33%	-	-	+
Trichlorfon[a]	0	-	-	-
Zinophos	59%	-	+	-
Zytron	100%	+	-	-

[a]Recovery of pesticide not determined because the compound was not satisfactorily chromatographed using the GLC conditions of this experiment. (6 ft. x 4 mm 10% DC 200 on 80/100 Gas Chrom Q; C.T. = 200°C, D.T. = 205°C, Inlet = 225°C; N_2 flow = 120 ml/min; KClTD detector.)

[b]% recovery determined by averaging results of 3 determinations. The majority of the pesticide was obtained in the 15% elution; however, a portion was obtained in the 50% fraction in one determination and in the 6% fraction in two of the determinations.

[c]Value listed for SD 7438 is the result of a single determination. Except for this pesticide and for Merphos, all recovery values which are listed are the average of two determinations.

Figure 1. Flow Sheet Diagram of Analysis of Air Sample



SAMPLING AND ANALYSIS OF PESTICIDES IN AIR

C. EVALUATION OF ETHYLENE GLYCOL

I. INTRODUCTION:

Ethylene Glycol (hereinafter referred to as E.G.) is purchased in 4-kg glass bottles. Each lot received must be sampled and subjected to analysis by GLC, E.C. and FPD, to insure that the material does not contain contaminants which will result in chromatographic peaks that interfere with peaks resulting from pesticides. The following procedure is designed to provide this information.

II. APPARATUS & REAGENTS:

1. Erlenmeyer flask, 500 ml with ℨ glass stopper.
2. Grad. cylinders, 50 and 500 ml.
3. Separatory funnels, with Teflon stopcocks, 250 and 500 ml.
4. Chromatographic columns, 300 mm column length x 22 mm i.d. (no frit). Kontes #420530, size 241, or the equivalent.
5. Evaporative concentrator tubes, 10 ml, Kontes #570050, size 1025, or the equivalent.
6. Kuderna-Danish flasks, 500 ml, Kontes #570001 or equivalent.
7. Snyder columns, size 121, Kontes #503000 or Kjeldahl bulbs, ℨ 24/40, Ace Glass #5230-1.
8. Hexane, pesticide grade, distilled in glass, prechecked for zero background by GLC, E.C. at a concentration of 180:1.
9. Distilled water, prechecked for contaminants by GLC, E.C. (See NOTE in III., 4, next page).
10. Sodium Sulphate, anhydrous, A.R. grade. Prechecked for contaminants and, if found, extracted as described in Section 5,A,(1).
11. Glass wool, pre-extracted with hexane.

III. PROCEDURE:

All glassware used must be scrupulously cleaned and given a final hexane rinse immediately before use.

1. The E.G. will be contained in 4-kg or 1-gal. amber glass bottles with Teflon-lined screw caps. One composite sample of each manufacturer's lot will be taken for evaluation. The number of bottles to be sampled depends on the size of the lot. If the lot consists of less than a dozen bottles, the composite should be comprised of some fluid from each individual bottle. If the lot is 12 to 24 bottles, half the bottles should be sampled. From 24 to 48, a quarter of the total bottles in the lot should be sampled.

2. Shake each bottle by inverting end for end about 15 times and pour 25 ml into a grad. cylinder. Transfer each 25 ml into a 500-ml Erl. flask. Stopper flask and shake ca 50 times to insure complete mixture of composite.

3. Carefully measure 80 ml of the E.G. composite into a 100-ml grad. cylinder and retransfer this to a 500-ml sep. funnel.

4. Measure 350 ml of dist. water into a 500-ml cylinder and transfer to the sep. funnel containing the E.G., rinsing the 100-ml cylinder used to measure the E.G. composite with several portions of the water.

   NOTE: At this point, prepare a 350-ml water blank and carry through all following steps.

5. Add 60 ml of hexane, stopper and shake vigorously two minutes.

6. Allow layers to separate, draw off lower aqueous layer into a second 500-ml sep. funnel (No. 2), and the upper hexane layer into a 250-ml sep. funnel (No. 3).

7. Add 60 ml of hexane to the aqueous solution in Funnel 2 and again shake vigorously two minutes.

8. Draw off lower aqueous layer into Funnel 1 and hexane layer into Funnel 3.

9. Repeat the extraction a third time, discarding the final aqueous layer and combining all three hexane extracts in Funnel 3.

III. PROCEDURE (CONTD.)

10. Add 50 ml of H_2O to Funnel 3, stopper and shake 1 minute, discard aqueous layer and repeat scrubbing with another 50-ml portion of water also discarding the final aqueous layer.

11. Place a glass wool retaining plug in the bottom of a 300-mm chromatographic column, add 2 inches of Na_2SO_4 and pass the combined hexane extract through the column, collecting eluate in a 500-ml K-D flask which has been fitted to a 10-ml evaporative concentrator tube containing one 3-mm glass bead.

12. When extract has eluted from the column, rinse the sep. funnel with three consecutive portions of 10 ml each of hexane, washing down the walls of the column. Allow each rinse to elute off the column before adding the next. Finally, rinse the column walls with two more 10-ml portions of hexane.

13. Place K-D assembly in a boiling water bath and evaporate to 5 ml. Remove the tube from K-D flask rinsing joint with a little hexane. Concentrate to exactly 1 ml under a nitrogen stream at room temp.

14. Conduct GLC, E.C. with the 1.5% OV-17/1.95% QF-1 column, following instructions given in Section 4,A of this manual.

15. At room temperature and under a nitrogen stream, evaporate extract to exactly 0.2 ml and conduct GLC, FPD using the 4% SE-30/6% QF-1, Carbowax-treated column (See Section 4,B).

IV. INTERPRETATION OF CHROMATOGRAPHIC DATA:

1. It is necessary that the contaminant peaks contributed by the water be subtracted from the peak structure obtained in the sample chromatogram. If the water blank yields troublesome contaminant peaks, it may be necessary to purify the water by multiple extractions with benzene and hexane.

2. Generally, if the contaminant peaks from the E.G. do not exceed 10% f.s.d. and whose R.R. ratios do not indicate an overlap with any pesticide peaks, the E.G. would be considered acceptable. If such peaks match the R.R. ratios of any of the organophosphate compounds via E.C. but are not detected by flame photometric, the E.G. would be considered acceptable.

   If contaminant peaks are produced of less than 10% f.s.d. which match the R.R. ratios of any of the common chlorinated pesticides,

IV. INTERPRETATION OF CHROMATOGRAPHIC DATA (CONTD.)

the SE-30/QF-1 column should be tried. Should this also produce overlaps, consideration may be given to rejection of the lot depending on the size and location of the contaminant peak(s).

3. If contaminant peaks greater than 10% f.s.d. are obtained by E.C. but do not produce R.R. ratios overlapping any of the pesticides, acceptance of the sample would probably be indicated. One possible exception to this would be an extremely late eluting peak which, although not interfering with any pesticide, might entail excessive delays between injections.

SEPARATION OF SOME POLYCHLORINATED BIPHENYLS FROM CERTAIN ORGANOCHLORINE PESTICIDES

I. INTRODUCTION:

Polychlorinated biphenyls (PCB) are a group of chemicals with industrial applications. They are stable (resistant to alkali and acid) and persistent; their residues have been found in wild life. Commercially, PCB are manufactured in this country by Monsanto and sold as a series of products tradenamed Aroclors. Most Aroclors actually consist of many different chlorobiphenyls, although some, partially or totally, consist of members of another group of compounds, chloroterphenyls.

The various components of PCB residues are partially or completely recovered through multiresidue methodology for organochlorine pesticides; they are eluted from the Florisil column by the 6% ethyl ether/petr ether eluant. PCB residues exhibit complex gas chromatographic patterns because of the various components represented. These peaks appear in and beyond the retention time region of the organochlorine pesticides. If present in high enough concentration, relative to pesticides present, PCB can interfere with the determination of some organochlorine pesticides. Likewise, the presence of certain organochlorine pesticides can interfere with the determination of PCB.

> NOTE: The polychlorinated terphenyls (PCT) are also recovered through the multiresidue methodology used for the analysis of organochlorine pesticides and PCB. However, the PCT elute from the GLC column much more slowly than either the pesticides or PCB and so do not interfere with the determination. In order to analyze for the PCT, it is necessary to use a GLC column and operating parameters which permit much more rapid elution and greater sensitivity for the chloroterphenyl components.

A number of procedures have been proposed for dealing with the various pesticide PCB combinations encountered. These include the silicic acid column chromatographic separation technique presented in detail here and several other published approaches, some of which are noted in Subsection VII. The residue analyst must make judicious use of the available techniques in order to obtain accurate results. The proper course of action in the determination of residues of PCB and pesticides found together depends on the suspected identity of each and on the estimated amounts of each. Some combinations will permit quantitation of both pesticides and PCB without their separation from one another on the silicic acid column. Other combinations of PCB and pesticides must

be separated before quantitation; still other combinations cannot be separated by silicic acid, yet cannot be determined in the presence of one another. The relative amounts of residues of pesticides and PCB may also influence the decision on whether or not to perform silicic acid separation prior to quantitation. Even when the residues will not be completely separated by this technique, its use may be the best means of achieving quantitative estimation of the residues when one chemical is present in much larger amounts than the other.

REFERENCES:

1. Armour, J., and Burke, J., JAOAC 53, 761-767 (1970).
2. Masumoto, H. T., JAOAC, in press.
3. Pesticide Analytical Manual, Vol. 1, Section 251, U. S. Food & Drug Admin.

II. PRINCIPLES:

The silicic acid column chromatographic procedure given here permits separation of DDT and its analogs from some of the PCB, including those with which they interfere. The silicic acid is standardized before use by addition of enough water to effect the best possible separation between p,p'-DDE and Aroclor 1254. The interfering PCB are eluted with petroleum ether from a column of the standardized silicic acid. The DDT compounds, most other organochlorine pesticides, and some other PCB are then eluted from the column with a mixture of hexane, methylene chloride, and acetonitrile. See Table I for list of chemicals eluting in each of the two eluates.

The method is applicable to the 6% Florisil eluate [Sect. 6,A,(1)] obtained in the analysis of fatty tissues or to extracts cleaned up by other methods for gas chromatography. Extracts in polar solvents must be transferred to nonpolar solvents prior to separation.

III. APPARATUS:

1. Chromatographic column 400 x 22 mm i.d., with 24/40 ℥ outer joint with coarse fritted plate and Teflon stopcock, Kontes No. K-420550, C-4, or the equivalent.

2. Grad. Cylinder, 250 ml.

3. Kuderna-Danish Assembly as follows:

   Evaporative concentrator flask - Kontes Catalog No. K-570000, 500-ml capacity, lower joint 19/22 ℱ, upper joint 24/40 ℱ; Snyder column - 3 ball, lower joint 19/22 ℱ, upper joint 24/40 ℱ; Tube - 10 or 15 ml capacity, 19/22 ℱ upper joint.

4. Air pressure regulator - for pressure reduction to deliver ca 1 lb. psig; air must be clean and dry.

5. Separatory funnel - Used for column eluant reservoir, 250 ml, with Teflon stopcock, 24/40 ℱ joint at top, Kontes No. K-633030, or the equivalent.

6. Hot water bath adjustable to temp. of 90-100°C.

IV. REAGENTS:

1. Petroleum ether, acetonitrile, hexane, and methylene chloride, all pesticide quality.

2. Celite 545, Johns-Manville.

3. Silicic acid, Mallinckrodt, 100 mesh powder; "specially prepared for chromatographic analysis by the method of Ramsey and Patterson," Analyt. Reagent No. 2847.

V. PREPARATION OF SPECIAL REAGENTS:

1. Celite 545 - must be dry and free of electron capturing substances. If electron capturing substances are extracted by petroleum ether, treat as follows: Slurry Celite with 1 + 1 hydrochloric acid - H_2O while heating on steambath; wash with H_2O until neutral; wash successively with several portions each of methanol and acetone (to remove H_2O); then ethyl acetate and petr ether. Remove solvents by suction and air drying. Hold a 1- to 2-inch layer of Celite in 130°C oven for at least seven hours to remove water and other volatile substances. After washing treatment and/or drying, store Celite in closed glass container.

2. Eluant for Pesticides - 1% acetonitrile, 19% hexane, 80% methylene chloride (v/v/v). Pipette 10 ml acetonitrile into 1-liter volumetric flask, add 190 ml hexane, and fill to volume with methylene chloride.

3. Silicic acid - Place silicic acid to depth of about 1 inch in open beaker and heat for a minimum of 7 hours, but preferably up to 24 hours, in 130°C oven to remove water. After heating, place beaker in desiccator and allow to cool to room temperature. Quickly weigh silicic acid into glass-stoppered bottle and add 3% H_2O by pipette (97 g silicic acid + 3 ml H_2O = 3% H_2O). Stopper bottle tightly and seal with tape to insure that container is air tight. Shake well until all H_2O is absorbed; make sure that no lumps remain. Place sealed container in desiccator and allow to equilibrate for 15 hours.

   To determine the separation achieved with the treated silicic acid, prepare a column as described in VI, below, and add to it a standard solution containing 40 µg Aroclor 1254 and 3 µg p,p'-DDE in hexane. Elute as described and determine recoveries in each eluate. Inadequate separation of PCB from p,p-DDE will require that further testing be done with other heated batches of silicic acid, treated with different amounts of H_2O as needed to achieve the desired separation. Increments of 0.25% or 0.5% more or less H_2O are recommended for the testing. More H_2O is required when the initial test results show PCB eluting in the polar solvent with the p,p'-DDE; less H_2O when p,p'-DDE elutes in the petr ether fraction.

   This testing and standardization is required for each new lot of silicic acid obtained from the manufacturer.

   Once a batch of standardized silicic acid is prepared, it should be stored in a desiccator between uses. Desired activity remains for about 5 days.

VI. PROCEDURE:

SEPARATION OF PCB FROM ORGANOCHLORINE PESTICIDES

1. Weigh 5 g Celite, then 20 g activated silicic acid and combine in 250-ml beaker. Immediately slurry with 80 ml petr ether, mixing well.

2. Pour slurry into a chromatographic column with coarse frit, keeping stopcock open. Complete transfer of silicic acid - Celite mixture by rinsing beaker with small portions of petr ether.

NOTE: Apply air pressure to top of column as much as necessary to force enough petr ether from column to allow space for all silicic acid - Celite.

3. Stir material in column with long glass rod to remove air bubbles, applying air pressure to settle adsorbent and to force petr ether through column. Continue application of air pressure until petr ether level is ca 3 mm above surface of gel.

NOTE: Do not allow column to go dry or to crack at any time during the procedure. Close stopcock when air pressure is not being applied. At this point, column of adsorbent should be firm and should not lose its shape if tipped.

4. Place 250-ml grad. cylinder under column for collection of eluate and take a suitable aliquot of 6% Florisil extract for addition to the column.

NOTE: Large amounts of PCB and pesticides placed on column may result in incomplete separation. Choose aliquot to contain amounts of PCB and pesticides required for determination. The weight of sample equivalent placed on the column may also affect separation by causing p,p'-DDE to appear in the petr ether eluate. Should this occur, an amount of extract equivalent to a smaller weight of sample should be used. In analysis of samples by this procedure, it is suggested that no more than 0.3-0.4 g fat equivalent be placed on the silicic acid column.

5. Add aliquot carefully to column being careful not to disturb top of adsorbent.

6. Apply slight air pressure until solvent level is ca 3 mm above adsorbent and then complete transfer of sample extract to column using small portions of petr ether and again applying slight air pressure until solvent level again reaches the 3 mm point above adsorbent.

7. Position a 250-ml sep. funnel containing 250 ml of petr ether on top of column. Open funnel stopcock and slowly apply air pressure to reservoir until an elution rate of ca 5 ml/min. is established. Continue elution until eluate volume in the graduate is exactly 250 ml.

8. Quantitatively transfer eluate to a 500-ml Kuderna-Danish evaporator fitted with a 5-ml evap. concentrator tube. Rinse graduate with small portions of pet. ether.

9. Place a second 500-ml K-D flask assembly under the column for collection of any remaining petr ether eluant and second eluate described in Step 10.

10. Apply air pressure until petr ether eluant level is ca 3 mm above adsorbent and add 200 ml of CH_3CN-hexane-CH_2Cl_2 (1:19:80) eluant to upper reservoir. Open stopcock and slowly reapply air pressure, continuing elution until all of eluant passes through column into the K-D concentrator.

11. Place Snyder columns on both K-D assemblies, place in a hot water bath and reduce eluate volumes to 5 ml in preparation for exploratory GLC analyses.

NOTE: The first (petr ether) eluate should contain the PCB's and the combined solvent eluate should contain the chlorinated pesticides.

DETERMINATION OF POLYCHLORINATED BIPHENYLS

Determine quantity of PCB in the sample by electron capture GLC or by halogen specific microcoulometric or electrolytic conductivity GLC. Compare the total area of response for the residue to the total area of response for a known weight of the Aroclor(s) reference with most similar GLC pattern(s). The pattern of GLC peaks for a sample containing PCB is often not exactly like that from any of the Aroclor standards. This is probably due to a combination of circumstances, e.g., weathering and/or metabolism of the residue; and perhaps slight variation in the recovery of the different PCB components through the methodology. Sometimes the GLC curve clearly indicates the presence of components of more than one Aroclor. In this case, quantitate the PCB residues separately if possible, using the appropriate Aroclor references for the respective portions of the GLC curve. Choosing the appropriate Aroclor reference(s) against which to measure a residue requires good judgment on the part of the analyst.

GLC with halogen specific microcoulometric or electrolytic conductivity detection is often preferable as means of quantitation. This type of detection system also provides confirmatory evidence for the identification of the residue as PCB.

VII. REFERENCES TO ADDITIONAL PROCEDURES:

The discussion under INTRODUCTION indicates the complexity involved in analysis of samples containing combinations of PCB and pesticides, including combinations of more than one Aroclor. The following procedures can be used to provide additional information on the identification and quantitation of residues found by the GLC determination. In certain cases, use of one or more of these techniques may be the only way to determine the residues. Knowledge of the behavior of compounds of interest in each of these procedures is necessary in making a decision on the best approach to a particular analysis.

The papers cited here do not always contain such complete information, so it may be necessary for the residue analyst to generate additional recovery data before using a method.

1. Alkaline hydrolysis. Young, S. J. V., and Burke, J. A., Micro Scale Alkali Treatment for Use in Pesticide Residue Confirmation and Sample Cleanup, Bull. Environ. Contam. Toxicol., in press. Vol. 7, No. 1 (1972); Krause, R. T., Quantitative Dehydrochlorination of Perthane Residues and Effect of Alcoholic Potassium Hydroxide on Other Pesticides, JAOAC, in press.

   The stability of PCB to alkali permits the use of alkaline hydrolysis as a test for confirming the identity of PCB residues. At the same time, the conversion of DDT to DDE by alkali treatment provides a means of removing some interferences from the quantitative measurement of PCB. Alkaline hydrolysis also provides an additional cleanup for many sample types. An appropriate sequence of determination step(s) and alkaline hydrolysis can be chosen to permit quantitation and confirmation of both PCB and the DDT compounds.

2. Oxidative treatment. (a) Mulhern, B. M., Cromartie, E., Reichel, W. L., and Belisle, A. A., Semiquantitative Determination of Polychlorinated Biphenyls in Tissue Samples by Thin Layer Chromatography, JAOAC, 54, 548-550 (1970).

   Cleaned up sample extracts are reacted with a chromic acid-acetic acid mixture to convert DDE to 4,4'-dichlorobenzophenone. The authors follow this reaction with TLC in which the PCB migrate as a single spot and are not separated from DDE, but are separated from the benzophenone. Sequential use of the alkaline hydrolysis and oxidation on a sample containing PCB, DDE, and DDT would result in the conversion of both o,p'- and p,p'-DDT and o,p'- and p,p'-DDE to the respective chlorobenzophenone while leaving the PCB unchanged.

(b) This manual, Section 9,D, Semi-Quantitative Estimation of Polychlorinated Biphenyls in Adipose Tissue.

3. Two-dimensional TLC. Fehringer, N. V., and Westall, J. E., Separation and Identification of DDT analogs in the Presence of Polychlorinated Biphenyl Compounds by Two-Dimensional Thin-Layer Chromatography, J. Chromatography 57, 397-405 (1971).

   Thin-layer chromatography is used in a manner designed to separate PCB from DDT and its analogs. After an initial development in n-heptane, the TLC place is rotated, spotted with additional standards and developed with 2% acetone in n-heptane in a direction perpendicular to that of the first development. The pattern of spots indicates the identity of residues in the sample.

4. Reversed-phase partition TLC. de Vos, R. H., and Peet, E. W., Thin-Layer Chromatography of Polychlorinated Biphenyls, Bulletin Environ. Contam. & Toxicol. 6, 164-170 (1971).

   A stationary phase of liquid paraffin on Kieselguhr G adsorbent is utilized in a TLC system which involves three successive developments of the spotted plate with a mixture of acetonitrile, acetone, methanol, and water. Visualization is by the usual silver nitrate spray and UV irradiation. These parameters permit separation by TLC or DDE, DDT and several other organochlorine pesticides from the components of Aroclor 1260, and cause the PCB to appear as a number of distinct spots rather than a long streak as sometimes occurs with other TLC systems.

   [Editor's Note: The ability of this TLC system to separate Aroclor 1260 into several spots suggests the possibility that further work would show the system capable of distinguishing among the different Aroclors.]

Table 1. Pesticides and Other Chemicals Recovered Through Silicic Acid Column Chromatographic Separation of Some Polychlorinated Biphenyls (PCB) from Certain Organochlorine Pesticides.[a]

Petroleum Ether Eluate	Acetonitrile, Methylene Chloride, Hexane Eluate
Aldrin	Aroclor 1221[b]
Aroclor 1221[b]	Aroclor 1242[b]
Aroclor 1242[b]	Aroclor 1248[b]
Aroclor 1248[b]	Aroclor 5442[d]
Aroclor 1254	Aroclor 5460[c,d]
Aroclor 1260	BHC (all isomers)
Aroclor 1262	chlordane (technical)
Aroclor 4465	p,p'-DDE
Aroclor 5460[c,d]	o,p'-DDT
hexachlorbenzene	p,p'-DDT
mirex[e]	dieldrin
octachloro-dibenzo-p-dioxin	endrin
polychlorinated naphthalenes[f]	heptachlor
2,3,7,8-tetrachloro-dibenzo-p-dioxins	heptachlor epoxide
	lindane
	Perthane[g]
	p,p'-TDE
	toxaphene

[a]Method tested only with chemicals listed.

[b]Divides between the two eluates. The earliest (GLC) eluting peaks in any of these Aroclors are the most likely to elute in the polar eluate.

[c]Divides between the two eluates.

[d]Aroclors 5442 and 5460 are composed of polychlorinated terphenyls and must be chromatographed on a GLC column that permits rapid elution; e.g., 1% OV-101 on 100/120 Gas Chrom Q at 240°C, 120 ml N_2/min. (Wieneke, W., private communication, Jan. 1972).

[e]Mirex may be separated from Aroclors 1260 and 1254 by collecting the first 100 ml petr ether separately. This fraction will contain the mirex. (Gaul, J., private communication, July 13, 1971).

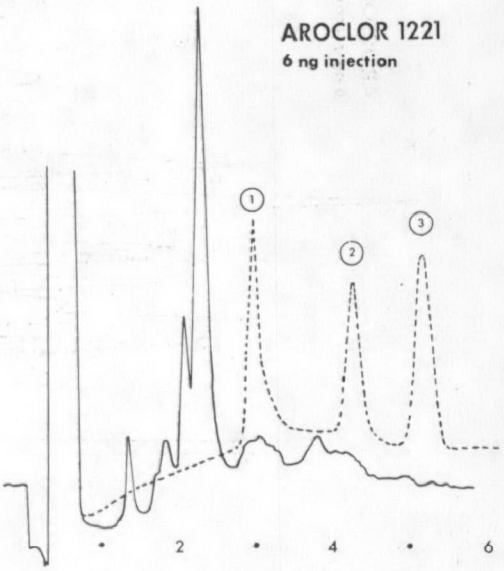
[f]Method tested with commercial polychlorinated naphthalenes: Halowaxes 1014, 1099 (Armour, J., and Burke, J., JAOAC 54, 175-177 (1971).

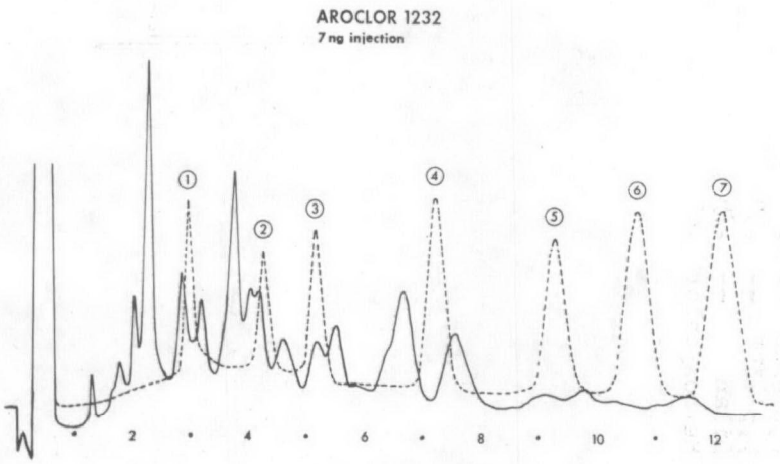
[g]Krause, R. T., in press. JAOAC.

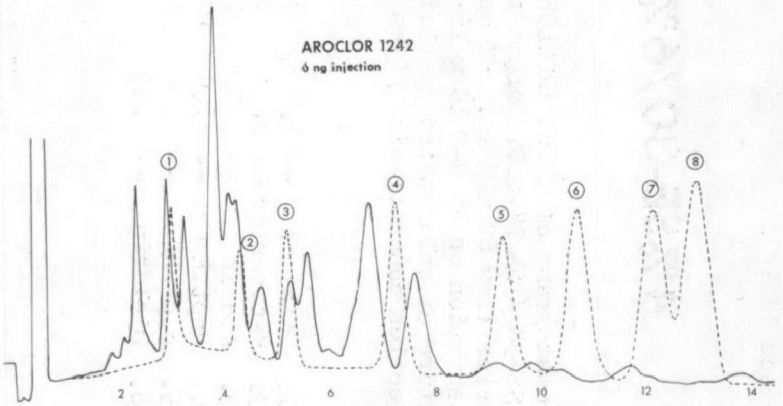
4%SE-30/6%OV-210

Chromatograms of three AROCLORS on column of 4% SE-30 / 6% OV-210. Column temp. 200°C., carrier flow 60 ml/min., ^{3}H detector, electrom. attenuation on an E-2 10 x 16; dotted line a mixture of chlorinated pesticides, identity and injection concentration given below:

1.	Diazinon	-- 1.5 ng	7.	o,p'-DDT	-- 0.24 ng
2.	Heptachlor	-- 0.03	8.	p,p'-DDD	-- .24
3.	Aldrin	-- .045	9.	p,p'-DDT	-- .30
4.	Hept.Epox.	-- .09	10.	Dilan	-- .75
5.	p,p'-DDE	-- .09	11.	Methoxychlor	.60
6.	Dieldrin	-- .12			



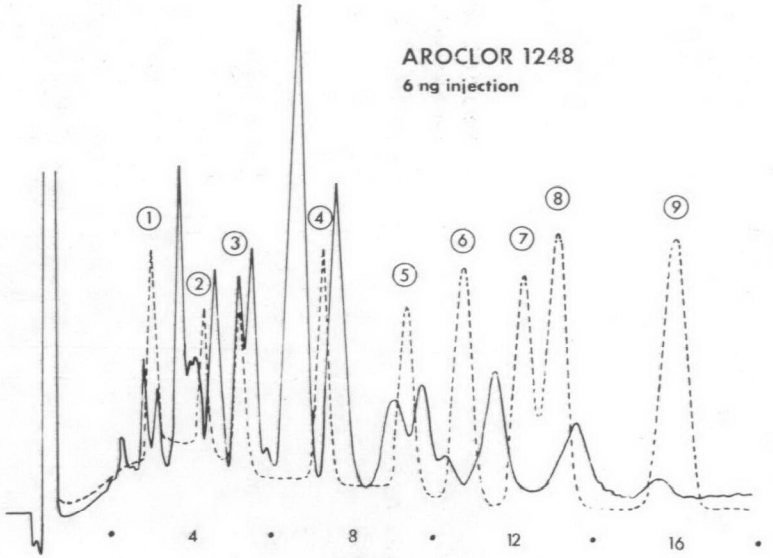


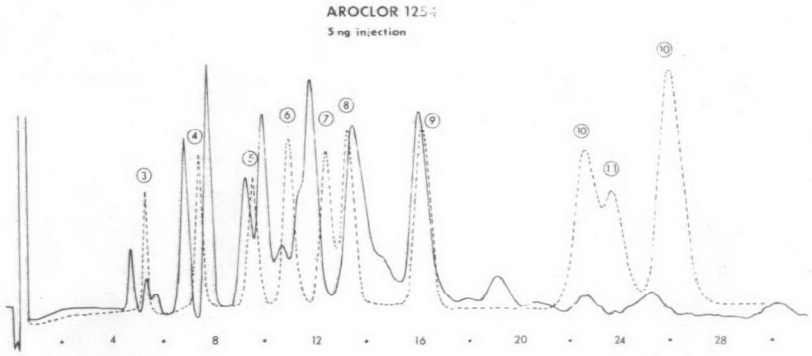


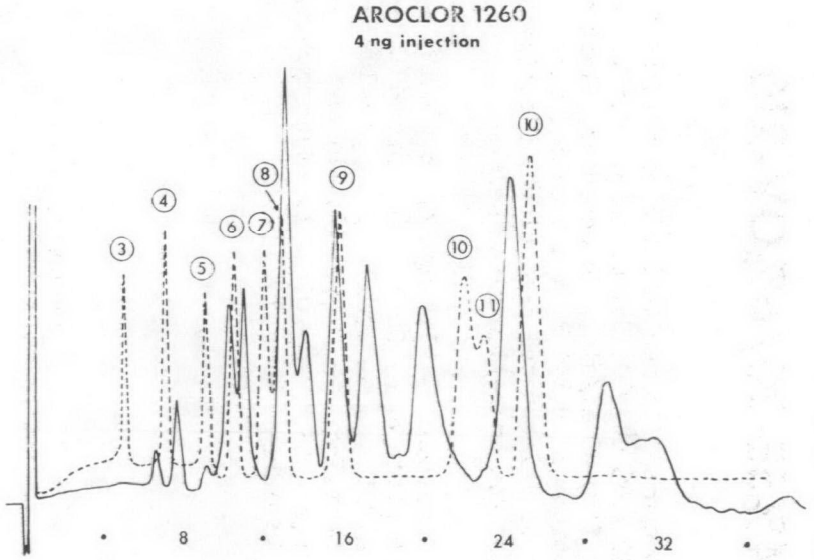
4%SE-30/6%OV-210

Chromatograms of three AROCLORS on column of 4% SE-30 / 6% OV-210. Column temp. 200°C., carrier flow 60 ml/min., ^{3}H detector, electrom. attenuation on an E-2 10 x 16; dotted line a mixture of chlorinated pesticides, identity and injection concentration given below:

1.	Diazinon	-- 1.5 ng	7.	o,p'-DDT	-- 0.24 ng
2.	Heptachlor	-- 0.03	8.	p,p'-DDD	-- .24
3.	Aldrin	-- .045	9.	p,p'-DDT	-- .30
4.	Hept.Epox.	-- .09	10.	Dilan	-- .75
5.	p,p'-DDE	-- .09	11.	Methoxychlor	.60
6.	Dieldrin	-- .12			





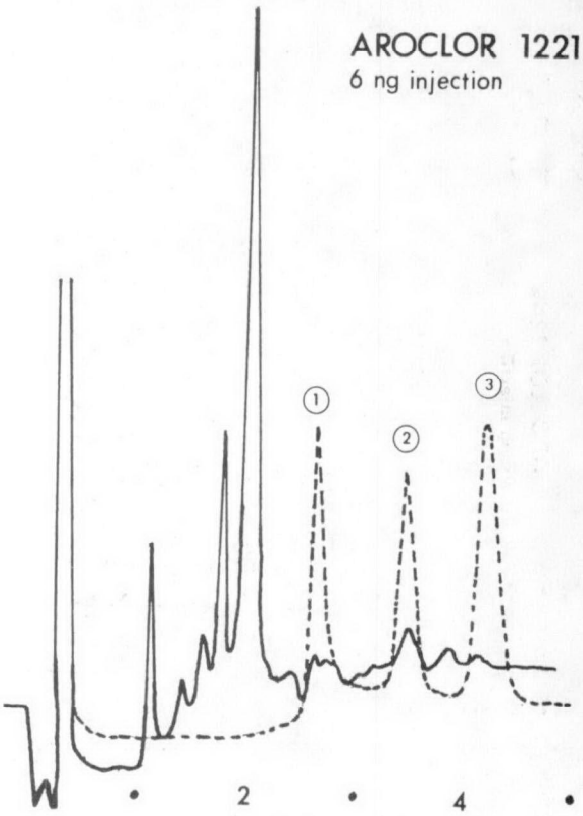


1.5% OV-17/1.95% QF-1

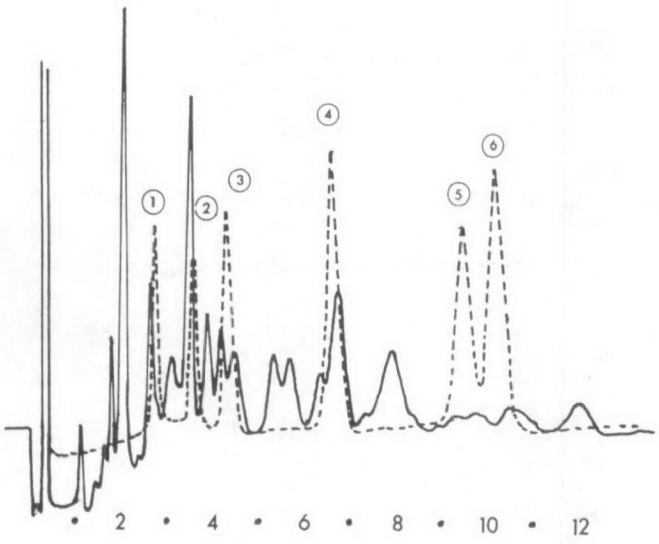
Chromatograms of three AROCLORS on column of 5% OV-17 / 1.95% QF-1. Column temp. 200°C., carrier flow 60 ml/min., ^{3}H detector, electrometer atten. 10 x 16 on an E-2; dotted line a mixture of chlorinated pesticide compounds, identity and injection concentration given below:

1.	Diazinon	-- 1.5 ng	7.	o,p'-DDT	-- 0.24 ng
2.	Heptachlor	-- 0.03	8.	p,p'-DDD	-- .24
3.	Aldrin	-- .045	9.	p,p'-DDT	-- .30
4.	Hept.Epox.	-- .09	10.	Dilan	-- .75
5.	p,p'-DDE	-- .09	11.	Methoxychlor	.60
6.	Dieldrin	-- .12			

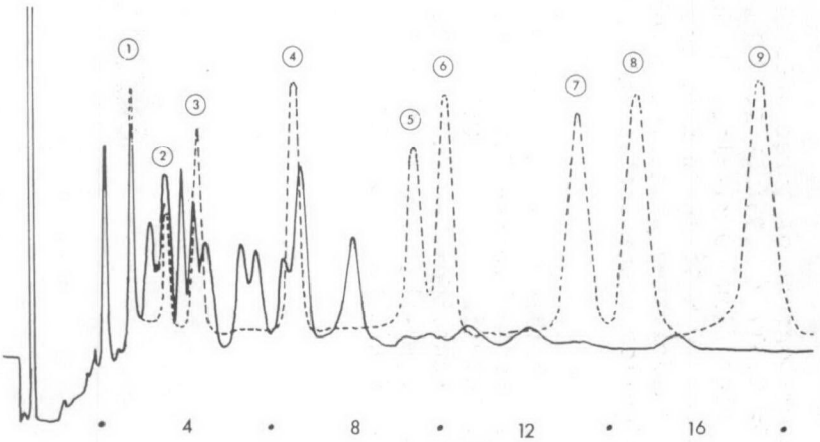
AROCLOR 1221
6 ng injection



AROCLOR 1232
7ng injection



AROCLOR 1242
5 ng injection

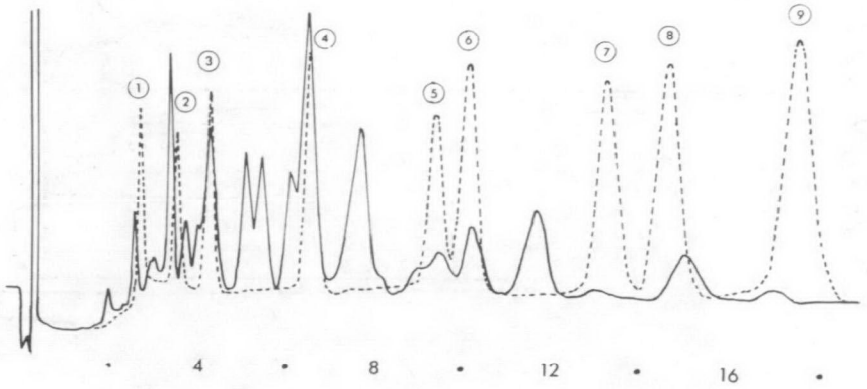


1.5% OV-17/1.95% QF-1

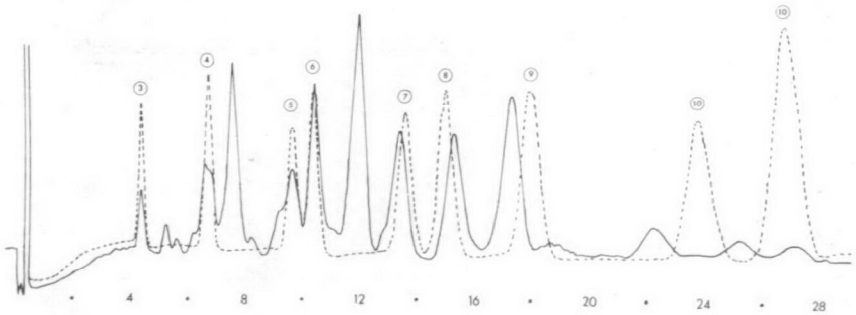
Chromatograms of three AROCLORS on column of 1.5% OV-17 / 1.95% QF-1. Column temp. 200°C., carrier flow 60 ml/min., ^{3}H detector, electrometer atten. 10 x 16 on an E-2; dotted line a mixture of chlorinated pesticide compounds, identity and injection concentration given below:

1.	Diazinon -- 1.5 ng	7.	o,p'-DDT	--	0.24 ng
2.	Heptachlor -- 0.03	8.	p,p'-DDD	--	.24
3.	Aldrin -- .045	9.	p,p'-DDT	--	.30
4.	Hept.Epox. -- .09	10.	Dilan	--	.75
5.	p,p'-DDE -- .09	11.	Methoxychlor		.60
6.	Dieldrin -- .12				

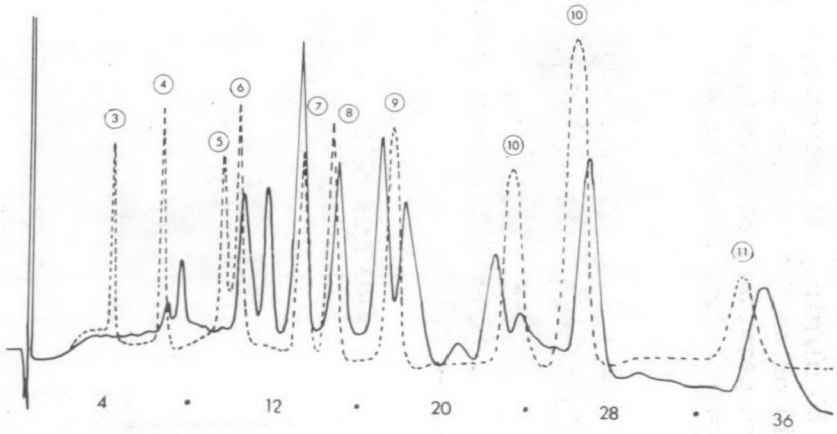
AROCLOR 1248
4 ng injection



AROCLOR 1254
5 ng injection



AROCLOR 1260
3 ng injection



Retention Values, Relative to Aldrin and Response Values, Relative to the Major Peak of Six of the Aroclor Compounds (poly-chlorinated biphenyls).

Column. Pyrex glass , 6-ft., 4 mm. i.d., 1.5% OV-17/1.95% QF-1, 200°C Column Temp. Carrier flow 60 ml/min.

Detector. Electron capture, ^{3}H, parallel plate, 210°C.

Misc.[1] Pesticides		#1221		#1232		#1242		#1248		#1254		#1260	
		RRR[2]	RPH[3]	RRR	RPH	RRR	RPH	RRR	RPH	RRR	RPH	RRR	RPH
	RRR[2]	0.27	0.19	0.27	0.17								
Phosdrin	0.32	.34	.03	.33	.03								
		.40	.05	.40	.07								
		.43	.27	.43	.27								
Thimet	.50	*.49*	*1.00*	*.47*	*1.00*	0.48	0.34	0.48	0.08				
Diazinon	.63	.62	.05	.61	.38	.63	.41	.63	.34				
	*			.73	.20	.74	.22	.74	.15				
Heptachlor	* .83	.82	.04	.80	.85	*.82*	*1.00*	*.83*	*1.00*				
δ-BHC	.92	.91	.02	.90	.33	.90	.38	.92	.31				
				.95	.26	.97	.32	.98	.24				
Aldrin	1.00			1.03	.13	1.05	.23	1.06	.68	1.04	0.23		
	*			1.22	.20	1.24	.23	1.26	.62	1.24	.14		
				1.30	.19	1.33	.21	1.34	.56	1.34	.08		
Methyl Parathion	1.43			1.44	.13	1.49	.15	1.50	.42	1.48	.07		
Hept. Epoxide	1.54			1.54	.27	1.57	.29	1 58	.96	1.56	.29		
Malathion	1.63									1.62	.33	1 61	0.09
Ethyl Parathion	* 1.81			1.83	.19	1.88	.21	1.88	.65	1.80	.58	1.78	.16
										1.97	.13		
p,p'-DDE	2.23			2.26	.03			2.21	.13	2.20	.19		
								2.32	.17	2.29	.36	-	
Dieldrin	2.40			2.43	.05	2.52	.03	2.50	.24	2.47	.68	2.53	.39
Endrin	2.93			2.78	.07	2.85	.04	2.85	.32	*2.82*	*1.00*	2.81	.42
o,p'-DDT	3.17			3.13	.02			3.18	.24	3.16	.53	*3.20*	*1 00*
p,p'-DDD	* 3.49			3.57	.04	3.67	.02	3.66	.16	3.60	.50	3.50	.52
p,p'-DDT	4.18			4.04	.03			4.12	.05	4.08	.66	4.10	.74
Ethion	4.44									4.42	.08	4.38	.58
												5.0	.07
										5.28	.11	5.4	.38
Dilan I	5.7											5.7	18
										5.95	.09		
Dilan II	6.4									6.4	.09	6.4	90
Methoxy-chlor	8.3									8.4	.05	8.4	43

[1]Relative retention ratios given for some common pesticides for comparison purposes.

[2]RRR - Means the retention relative to aldrin.

[3]RPH - Means the peak height response relative to that of the tallest peak shown in *italics*.

*Indicates chlordane peaks eluting in the appropriate area.

Retention Values, Relative to Aldrin and Response Values, Relative to the Major Peak of Six of the Aroclor Compounds (poly-chlorinated biphenyls)

Column: Pyrex glass, 6-ft., 4 mm. i.d., 4% SE-30/6% QF-1, 200°C. Column temp. Carrier flow 70 ml/min.

Detector: Electron capture, ^{3}H, parallel plate, 210°C.

Misc.[1] Pesticides		#1221		#1232		#1242		#1248		#1254		#1260	
		RRR[2]	RPH[3]	RRR	RPH	RRR	RPH	RRR	RPH	RRR	RPH	RRR	RPH
	RRR[2]	0.24	0.37	0.25	0.26								
Phosdrin	0.32	.32	.07	.32	.07								
	*	.34	.13	.35	.13	0.34	0.04						
		.39	.40	.39	.33	.38	.08						
2,4-D(ME)	.44	*.44*	*1.00*	*.43*	*1.00*	.43	.52	0.44	0.14				
Thimet	.47					.47	.03						
Simazine	.54	.55	.04	.55	.37	.53	.54	.55	.42				
Lindane	.60	.60	.05	.62	.28	.61	.38	.62	.25				
δ-BHC	.69	.73	.06	.72	.70	*.72*	*1.00*	.73	.99				
2,4-D(BE)	* .78			.79	.31	.77	.43	.80	.34				
Heptachlor	* .85			.82	.27	.81	.36	.83	.36				
Ronnell	.91			.90	.14	.89	.18	.90	.61	0.90	0.38		
Aldrin	1.00			1.02	.16	1.00	.22	1.03	.66	1.02	.25		
	*			1.09	.20	1.07	.27	1.10	.68	1.08	.14		
				1.16	.03	1.15	.02	1.18	.10				
M Parathion	1.34			1.31	.24	1.30	.30	*1.32*	*1.00*	1.29	.79	1.30	0.10
Hept. Epoxide	*1.43			1.48	.19	1.47	23	1.50	.78	1.48	.95	1.52	.17
p,p'-DDE	1.82			1.77	.03	1.77	.03	1.80	.19	1.75	.53	1.80	.07
Captan	1.94			1.96	.04	1.90	.03	1.92	.25	1.87	.83	1.93	.08
o,p'-DDD	1.98					2.02	.03			2.00	.09	2.02	.36
Dieldrin	2.12									2.14	.28	2.18	.40
								2.24	.07	*2.22*	*1.00*		
o,p'-DDT	2.39			2.27	.05	2 27	.02	2.30	.27				
Endrin	2.42									2.51	.78		
p,p'-DDD	*2.55									2.62	.38	*2.59*	*1.00*
Thiodan II	2.72					2.69	.03	2.73	.18				
												2.81	35
p,p'-DDT	3.12							3.12	.06	2.99	.91	3.10	.67
Trithion	3.20											3.39	.59
										3.55	.12	3.71	.06
												3.95	.42
Dilan I	4.40									4.20	.10		
Methoxy chlor	4.6									4.60	.12	4.85	.9
Dilan II	5.1									5.54	.05	5.77	.32
												6.2	.20

[1]Relative retention ratios given for some common pesticides for comparison purposes.

[2]RRR - Means the retention relative to aldrin.

[3]RPH - Means the peak height response relative to that of the tallest peak shown in *italics*.

*Indicates chlordane peaks eluting in the approximate area.

Retention values, relative to aldrin and response values, relative to the major peak, of six of the Aroclor compounds (polychlorinated biphenyls).

Column: Pyrex glass, 5 ft., 5/32" i.d., 5% OV-210, 200°C column temp., carrier flow 45 ml/min.

Detector: Electron capture, ^{3}H, parallel plate, 205°C.

Miscellaneous[1] Pesticides		#1221		#1232		#1242		#1248		#1254		#1260	
		[2]RRR	[3]RPH	RRR	RPH	RRR	RPH	RRR	RPH	RRR	RPH	RRR	RPH
	[2]RRR	0.33	0.27	0.33	0.19								
		.41	.10	.42	.07								
		.46	.31	.46	.24	0.47	0.01						
		.51	*1.00*	*.52*	*1.00*	.53	.43	0.52	0.08				
		.61	.02	.62	.30	.62	.50	.60	.23				
Diazinon	0.73	.72	.06	.70	.20	.71	.22	.69	.11				
		.79	.08	.80	.84	*.81*	*1.00*	.80	.58				
		.88	.03	.88	.13	.88	.16	.86	.05				
β-BHC	.94	.92	.03	.93	.59	.94	.66	.92	.68	0.91	0.25		
				1.12	.29	1.12	.29	1.08	.54	1.08	.13		
1-Hydroxychlordene	1.30			1.29	.27	1.29	.17	1.28	.49	1.27	.38	1.28	0.05
				1.41	.54	1.41	.52	*1.37*	*1.00*	1.39	.26		
								1.47	.06	*1.46*	*1.00*	1.49	.20
				1.62	.15	1.62	.17	1.59	.24				
Hept. Epoxide	1.73							1.77	.10	1.77	.41	1.72	.04
Dimethoate	1.75			1.83	.03							1.86	.44
p,p'-DDE	1.81			1.96	.07	1.97	.04	1.92	.22	1.92	.75		
o,p'-DDT	2.26											2.06	.48
Malathion	2.32			2.32	.13	2.32	.08	2.28	.32	2.28	.77		
M. Parathion	2.44									2.40	.57	2.44	.89
										2.63	.11	2.66	.22
				2.82	.05	2.86	.04	2.78	.19	2.79	.20		
p,p'-DDT	3.25			2.99	.07			2.96	.08	2.93	.80	*2.98*	*1.00*
								3.60	.02	3.63	.21	3.58	.43
								4.32	.02	4.29	.13		
Methoxychlor	4.53											4.41	.76
										5.32	.06	5.38	.34
												6.5	.05
Dilan II	8.4											8.0	.10
	[4]RRA		.014		.0097		.02		.02		.025		.027

[1]Relative retention ratios given for some common pesticides for comparison purposes.

[2]Indicates the retention ratio, relative to aldrin.

[3]Indicates the peak height response relative to that of the tallest peak shown in *italics*.

[4]Indicates the peak height response of the tallest peak relative to that obtained from an equivalent amount of aldrin.

SAMPLE PREPARATION AND ANALYSIS OF SOILS AND HOUSE DUST

I. INTRODUCTION:

The analytical method described below is similar in principle to the method presented in the Analytical Manual distributed at the annual Chemist's Meeting in Tucson in 1968. The main difference lies in the incorporation of the standard Mills, Onley, Gaither Florisil cleanup technique for which all laboratories have equipment and a degree of expertise in manipulation. This is preceded by percolation through an alumina column for further removal of contaminants.

II. PRINCIPLES:

Organochlorine pesticides, together with other lipid-soluble substances, are extracted from homogenized samples by continuous Soxhlet extraction with acetone-hexane. Bulk of solvent is removed by evaporation in Kuderna-Danish equipment. Interfering lipid-soluble materials are then partially removed from the extracts by successive cleanup on aluminum oxide and Florisil columns. Extracts are adjusted to appropriate concentration for determinative analysis by E.C. and F.P.D., confirming as needed by M.C. and/or T.L.C.

III. EQUIPMENT:

1. Soxhlet extraction apparatus, complete with 125-ml ℱ 24/40 flask, extraction tube with ℱ 24/40 lower and ℱ 34/45 upper joints and Friedrichs condenser with ℱ 34/45 joint. Kimble #24010 or the equivalent for the entire assembly.

2. Soxhlet extraction thimbles, paper, Whatman, 25 x 80 mm, Fisher #9-656-c or the equivalent.

3. Sieves, U. S. Standard, #10 mesh, #18 mesh and #60 mesh with top covers and bottom pans, 8" dia. x 2" depth, stainless steel.

4. Chromatographic columns, 22 x 300 mm with Teflon stopcock, without glass frit. Size #241, Kontes #420530 or the equivalent.

5. Kuderna-Danish concentrator fitted with grad. evaporative concentrator tube. Available from the Kontes Glass Company, each component bearing the following stock numbers:

   a. Flask, 250 and 500 ml, stock #K-570001.

   b. Snyder column, 3 ball, stock #K-503000.

c. Steel springs, 1/2", stock #K-662750.

d. Concentrator tubes, 10 ml grad., size 1025, stock #K-570050.

6. Modified micro-Snyder columns, 19/22, Kontes K-569251.

7. Glass beads, 3 mm plain, Fisher #11 - 312 or equivalent.

8. Evap. concentrator tubes, grad., 25 ml, ℉ 19/22, Kontes #570050.

9. Water or steam bath.

10. Glas Col heating mantles with variable autotransformers, size to match 125-ml Soxhlet flasks.

11. Filter paper, Whatman No. 1, 15 cm.

IV. REAGENTS AND SOLVENTS:

1. Acetone, pesticide quality.

2. Hexane, pesticide quality.

NOTE: Both solvents must be carefully checked for background contaminants as outlined in Section 3,C of this manual.

3. Extraction mixture - acetone/hexane, 1:1.

4. Aluminum oxide, Merck reagent grade, stock #71695 acid-washed. Prepare for use by shaking with 10% distilled water (w/w) for partial deactivation. ◦ Shelf life of 10 days if stoppered tight.

NOTE: The distilled water must be prechecked for contaminant background. If any interferences are detected, the water must be hexane extracted before use.

5. Diethyl ether - AR grade, peroxide free. The ether must contain 2% (v/v) absolute ethanol. Most of the AR grade ethyl ether contains 2% ethanol, added as a stabilizer, and it is therefore unnecessary to add ethanol unless peroxides are found and removed.

NOTE: To determine the absence of peroxides in the ether,

add 1 ml of ether in a clean 25-ml cylinder previously rinsed with ether. Shake and let stand 1 minute. A yellow color in either layer indicates the presence of peroxides which must be removed before using. See Misc. Note 4 at end of procedure. The peroxide test should be repeated at weekly intervals on any single bottle or can as it is possible for peroxides to form from repeated opening of the container.

6. Eluting mixture, 6% (6+94)-purified diethyl ether - 60 ml is diluted to 1000 ml with redistilled petroleum ether, and anhydrous sodium sulfate (10-25 g) is added to remove moisture.

7. Eluting mixture, 15% (15+85)-purified diethyl ether - 150 ml is diluted to 1000 ml with redistilled petroleum ether, and dried as described above.

   NOTE: Neither of the eluting mixtures should be held longer than 24 hours after mixing.

8. Florisil, 60/100 mesh, PR grade, to be stored at 130°C until used. Furnished by Perrine on order.

   NOTE: (1) In a high humidity room, the column may pick up enough moisture during packing to influence the elution pattern. To insure uniformity of the Florisil fractionation, it is recommended to those laboratories with sufficiently large drying ovens that the columns be packed ahead of time and held (at least overnight) at 130°C until used.

   (2) Florisil furnished by the Perrine Laboratory has been activated by the manufacturer, and elution pattern data is included with each shipment. However, each laboratory should determine their own pesticide recovery and elution pattern on each new lot received, as environmental conditions in the various laboratories may differ somewhat from that in Perrine. Each new batch should be tested with a mixture of β-BHC, aldrin, heptachlor epoxide, dieldrin, p,p'-DDE, p,p'-DDD, and p,p'-DDT, eluting the standard mixture as described in Section 5,A,(1) of this manual. Dieldrin should elute entirely in the 15% diethyl ether fraction, whereas all other compounds should be in the 6% fraction.

9. Anhydrous sodium sulfate, reagent grade granular, Mallinckrodt

Stock #8024 or the equivalent.

NOTE: When each new bottle is opened, it should be tested for contaminants that will produce peaks by Electron Capture Gas Liquid Chromatography. This may be done by transferring ca 10 grams to a 125-ml Erlenmeyer flask, adding 50 ml pet. ether, stoppering and shaking vigorously for 1 minute. Decant extract into a 100-ml beaker and evaporate down to ca 5 ml. Inject 5 µl into the Gas Liquid Chromatograph and observe chromatogram for contaminants. When impurities are found, it is necessary to remove them by extraction. This may be done using hexane in a continuously cycling Soxhlet extraction apparatus or by several successive rinses with hexane in a beaker. The material is then dried in an oven and kept in a glass-stoppered container.

V. PROCEDURE:

Sample Preparation

1. Soils and vacuum cleaner bag dusts are analyzed in the air-dry state. If a soil sample is obviously damp, it is allowed to equilibrate its moisture content with room air before handling. Trials have shown that house dust screenings generally contain approximately 0.1% moisture, possibly more in areas of high relative humidity.

2. Vacuum cleaner bag contents are sieved on U. S. Standard #10 and #60 sieves to remove hair, fibers and large particles. The resulting "fines" are separated into sealed glass jars until analyzed. Soils are sifted on a U. S. Standard #18 sieve to remove stones and other foreign material. Store the sieved soil in a sealed glass jar until analyzed.

3. The 15-cm filter paper and the Soxhlet extraction thimbles should be preextracted with the acetone/hexane extraction solvent prior to use. This may be conveniently done by folding several sheets of filter paper and placing in the Soxhlet extractor. Allow to cycle ca 2 hours, remove and dry. Wrap in aluminum foil and store in desiccator. The thimble is similarly preextracted and may be used repeatedly with no need for reextraction as long as it remains in good physical shape.

Sample Extraction

1. Weigh sample (2 grams of soil or 1 gram of dust) onto a sheet of 15-cm filter paper. Carefully fold paper to form a half-circle with the sample in the center (along the diameter line). Fold in the ends of the half-circle towards the center, the total resulting length to be ca 70 mm; then, starting at the diameter line, roll into an approximately cylindrical shape and insert into the extraction thimble.

2. As a recovery check, another portion of the same dust (or soil) should be spiked and carried through the entire procedure. This is done as follows:

   a. Weigh exactly 3.0 grams of the soil or 2.0 grams of dust into an evaporating dish. Add sufficient hexane to make a slurry.

   b. Prepare a standard mixture of the following compounds, the concentration expressed in micrograms per milliliter:*

Lindane----------5.0	Dieldrin------7.5
Hept. Epoxide-----5.0	p,p'-DDD-----10.0
Aldrin-----------5.0	o,p'-DDT-----10.0
p,p'-DDE----------7.5	p,p'-DDT-----10.0

   *In case your testing program indicates the presence of any other compounds or metabolites, standards of these should be included.

   c. Add 1.5 ml of this mixture to the soil sample or 1.0 ml to dust. Mix gently with a glass rod and evaporate the solvent at 40°C under a nitrogen stream, stirring from time to time.

   d. After removal of the solvent, allow the spiked sample to equilibrate to room temperature and humidity, and weigh the sample for extraction as outlined above in Step 1.

3. At this point, a reagent blank should be initiated, starting with the folded filter paper and carrying through the entire extraction, cleanup and determinative procedures.

4. Place the sample, reagent blank and spiked sample thimbles into separate Soxhlet extractors. Fill the boiling flasks, each containing six glass beads, about half full with the 1:1 acetone/hexane co-solvent, assemble the extraction apparatus, position in the heating mantles and start extraction.

NOTE: Each laboratory will need to determine the setting of their voltage controller. There should be sufficient heat to result in 1 discharge cycle about every 5 minutes, or ca 60 syphon discharges in a 5-hour period. This should be an adequate number of cycles to insure complete extraction.

5. At the completion of the extraction period disassemble the extraction apparatus, rinsing the joint between flask and extractor with a few ml of hexane.

6. Assemble a Kuderna-Danish evaporator with the 250-ml K-D flask attached to a 10-ml evap. concentrator tube containing one 3-mm glass bead.

7. Transfer the extract from the 125-ml Soxhlet flask to the K-D flask, rinsing the Soxhlet flask with 3 portions of 5 ml each of hexane. Attach the Snyder column and immerse evap. concentrator tube about 1-1/2 inches into the boiling water bath. Evaporate extract down to ca 3 ml, remove from bath and cool. Extract is now ready for cleanup.

Alumina and Florisil Cleanup

1. Prepare an alumina column as follows:

   a. Place a small wad of prerinsed glass wool at the bottom of a 22 x 300 mm chromatographic column.

   b. Add preextracted anhydrous Na_2SO_4 to a depth of 1/2 inch.

   c. Close stopcock and fill column with hexane.

   d. In a 50-ml grad. beaker, fill exactly to the 30 ml mark with alumina (this should be ca 30 grams). Add this slowly to the column, allowing all the alumina to settle to the bottom. Top this with a 1-inch layer of Na_2SO_4. When settling is complete, open stopcock and allow the hexane to elute through the column down to a point ca 1/8 inch above the top of the upper Na_2SO_4 layer, then close stopcock.

      NOTE: This column packing technique minimizes the density that may be obtained in dry packing. The volume of hexane specified provides sufficient column prerinse.

2. Position a second K-D flask fitted with 10-ml evap. concentrator tube under column.

3. Transfer the 3 ml of concentrated extract from the first K-D evaporation to the column. Rinse tube with three portions of 3 ml each of hexane transferring the rinsings to the column.

4. Open stopcock and add 85 ml of hexane to the column, open stopcock wide and elute into the K-D flask.

5. Concentration of the eluate from the alumina column is conducted exactly the same as outlined above in Step 7 under Sample Extraction, taking extract down to 3 ml. This extract is now ready for Florisil partitioning.

6. Florisil column: Prepare the column as described in Section 5,A,(1) of this manual under FLORISIL FRACTIONATION, Steps 1 and 2, substituting hexane for pet. ether.

7. Assemble two more K-D apparatus but with 500-ml flasks and position the flask of one assembly under the Florisil column. However, at this point use 25-ml grad. evap. concentrator tubes instead of the 10-ml size for previous concentrations.

8. Using a 5-ml Mohr or a long disposable pipet, immediately transfer the extract from the evaporator tube in Step 5, above, onto the column and permit it to percolate through. Rinse tube with two successive 5-ml portions of hexane, carefully transferring each portion to the column with the pipet.

   NOTE: Use of the Mohr or disposable pipet to deliver the extract directly onto the column precludes the need to rinse down sides of the column.

9. Commence elution with 200 ml of 6% diethyl ether in pet. ether (Fraction I). The elution rate should be ca 5 ml per minute. When the last of the eluting solvent reaches a point ca 1/8 inch from the top of the Na_2SO_4 layer, place the second 500-ml Kuderna-Danish assembly under the column and continue elution with 200 ml of 15% diethyl ether in pet. ether (Fraction II). Place both Kuderna-Danish evaporator assemblies in a water bath and concentrate extract to ca 20 ml.

   NOTE: If there is reason to suspect the presence of malathion in the sample, have a third 500-ml K-D assembly

ready. At the end of the 15% fraction elution, add 200 ml of 50% diethyl ether in pet. ether (Fraction III), evaporating the eluate in the same manner.

10. Remove K-D assemblies from bath, cool and rinse S joint between tube and flask with a little pet. ether. Finally, dilute both extracts to exactly 25 ml and proceed with the GLC determinative step.

NOTE: A relatively high dilution is suggested as it has been observed that residues are generally sufficiently high to warrant this. Furthermore, the concentration of contaminants remaining after cleanup is hereby reduced.

Determinative by GLC

1. Inject 5 µl of each fraction extract into the gas chromatograph (E.C. mode) primarily to determine whether the extracts will require further adjustment by dilution or concentration.

2. When appropriate dilution adjustments have been made in the extracts and column oven is set to a known temperature, the relative retention values of the peaks on the chromatograms should be calculated. When these values are compared with the values in the printed table for the appropriate column, the operator should be able to make tentative compound identifications. Microcoulometry and/or TLC may be required for positive confirmation of some of the suspect chlorinated compounds, whereas FPD may be utilized for the organophosphate suspects.

TYPICAL RECOVERY DATA - Soxhlet Method

-Pesticides-

	Lindane	Hep. Epox.	p,p'-DDE	Dieldrin	p,p'-TDE	p,p'-DDT
SOILS:						
Mean percent recovery:	85.25	87.83	83.08	88.25	91.17	94.17
S.D. :	5.446	9.446	6.345	6.210	6.886	8.922
n :	12	12	12	12	12	12
HOUSE DUSTS:						
Mean percent recovery:	87.33	80.58	82.92	86.27	90.00	87.78
S.D. :	6.997	12.85	6.345	12.89	9.715	20.20
n :	12	12	12	11	12	9

SAMPLE PREPARATION AND ANALYSIS OF BOTTOM SEDIMENT

I. INTRODUCTION:

The examination of sediment from the bottom of a stream or lake provides information concerning the degree of pollution resulting from pesticides, particularly the organochlorine compounds which are not readily biodegradable. This information combined with residue data obtained by analysis of the water and tissues from resident marine life contribute in the development of an overall profile of the pesticidal contamination of a given body of water.

REFERENCES:

1. Column Extraction of Pesticides from Fish, Fish Food and Mud, Hesselberg, R. J. and Johnson, J. L., in press.

2. Sediment Extraction Procedure, Southeast Water Laboratory, EPA, Athens, Georgia, Method Number SP-8/71.

II. PRINCIPLES:

The sediment sample is partially dried and extracted by column elution with a mixture of 1:1 acetone/hexane. The extract is washed with water to remove the acetone and then the pesticides are extracted from the water with 15% CH_2Cl_2 in hexane. The extract is dehydrated, concentrated to a suitable volume, subjected to Florisil partitioning, desulfurized if necessary, and analyzed by gas chromatography.

III. EQUIPMENT AND REAGENTS:

1. Pans, approximately 14" x 10" x 2-1/2".
2. Oven, drying.
3. Muffle furnace.
4. Desiccator.
5. Crucibles, porcelain, squat form, size 2.
6. Omni or Sorvall mixer with chamber of ca 400 ml.
7. Chromatographic columns, 300 mm x 22 mm with Teflon stopcock.

8. Separatory funnels, 500 ml and 250 ml with Teflon stopcocks.

9. Filter tube, 180 mm x 25 mm.

10. Kuderna-Danish concentrator fitted with grad. evaporative concentrator tube. Available from the Kontes Glass Company, each component bearing the following stock numbers:

    a. Flask, 250 ml, stock #K-570001.

    b. Snyder column, 3 ball, stock #K-503000.

    c. Steel springs, 1/2", stock #K-662750.

    d. Concentrator tubes, 10 ml, size 1025, stock #K-570050.

11. Pyrex glass wool - preextracted with methylene chloride in a Soxhlet extractor.

12. Hot water bath, temp. controllable at 80°C.

13. Sodium sulfate, anhydrous, Baker, prerinsed or Soxhlet extracted with methylene chloride.

14. n-Hexane, pesticide quality.

15. Acetone, pesticide quality.

16. Methylene chloride, pesticide quality.

17. Acetone-hexane, 1:1.

18. Diethyl ether, pesticide quality, free of peroxides.

19. Distilled water, suitable for pesticide residue analysis.

20. Sodium sulfate solution, saturated.

21. Methylene chloride-hexane, 15% v/v.

IV. PROCEDURE:

1. Decant and discard the water layer over the sediment. Mix the sediment to obtain as homogeneous a sample as possible and transfer

to a pan to partially air dry for about 3 days at ambient temperatures.

NOTE: Drying time varies considerably depending on soil type and drying conditions. Sandy soil will be sufficiently dry in one day, whereas muck requires at least three days. The silt and muck sediment is sufficiently dry when the surface starts to split, but there should be no dry spots. Moisture content will be 50-80% at this point.

2. Weigh 50 gm of the partially dried sample into a 400-ml Omni-Mixer chamber. Add 50 gm of anhydrous sodium sulfate and mix well with a large spatula. Allow to stand with occasional stirring for approximately one hour.

NOTE: As the final calculations will be made on a "bone dry" basis, it is necessary at this point to initiate the test for percent total solids in the sample being extracted for pesticide evaluation. Immediately after weighing the 50gm sample for extraction, weigh ca 5 gm of the partially dried sediment into a tared crucible. Determine the percent solids by drying overnight at 103°C. Allow to cool in a desiccator for half an hour before weighing. Determine the percent volatile solids by placing the oven-dried sample into a muffle furnace and igniting at 550°C for 60 minutes. Allow to cool in a desiccator before weighing.

3. Attach the 400-ml chamber to an Omni or Sorvall mixer and blend for about 20 seconds. The sample should be fairly free flowing at this point.

4. Carefully transfer the sample to a chromatographic column. Rinse the mixer chamber with small portions of hexane adding the rinsings to the column.

5. Elute the column with 250 ml of 1:1 acetone-hexane at a flow rate of 3-5 ml/min into a 400-ml beaker.

6. Concentrate the sample extract to about 100 ml under a nitrogen stream and at a temp. no higher than 55°C. Transfer to a 500-ml separatory funnel containing 300 ml of distilled water and 25 ml of saturated sodium sulfate solution. Shake the separatory funnel for two minutes.

7. Drain the water layer into a clean beaker and the hexane layer into a clean 250-ml separatory funnel.

8. Transfer the water layer back into the 500-ml separatory funnel and reextract with 20 ml of 15% methylene chloride in hexane, again shaking the separatory funnel for two minutes. Allow the layers to separate. Discard the water layer and combine the solvent extracts in the 250-ml separatory funnel.

9. Wash the combined solvent extract by shaking with 100 ml of distilled water for 30 seconds. Discard the wash water and rewash the extract with an additional 100 ml of distilled water, again discarding the wash water.

10. Attach 10 ml evap. concentrator tube to a 250-ml Kuderna-Danish flask and place under a filter comprised of a small wad of glass wool and ca 1/2 inch of anhydrous Na_2SO_4 in a filter tube.

11. Pass the solvent extract through the drying filter into the K-D flask, rinsing with 3 portions of ca 5 ml each of hexane.

12. Attach Snyder column to top joint of K-D flask, immerse tube in 80°C water bath and concentrate extract to 5 ml.

13. Remove tube, rinsing joint with small volume of hexane. The sample is now ready for Florisil partitioning. This is conducted as described on page 3, Section 8,B of this manual.

V. CALCULATIONS:

1. Percent Dry Solids

$$\frac{\text{gm of dried sample}}{\text{gm of sample}} \times 100 = \% \text{ Dry Solids}$$

2. Percent Volatile Solids

gm of dried sample - gm of ignited sample = gm of volatile solids

$$\frac{\text{gm of volatile solids}}{\text{gm of sample}} \times 100 = \% \text{ Volatile Solids}$$

3. Concentration of Pesticide in Sediment

% dry solids x 50 gm = gm of dry sample extracted

$$\frac{\text{1 of sample extract injected}}{\text{1 of sample extract}} \times \text{gm of dry sample extracted} = \text{gm of dry sample injected}$$

$$\frac{\text{ng of pesticide}}{\text{gm of dry sample injected}} = \text{ppb of pesticide}$$

VI. SULFUR INTERFERENCE:

Elemental sulfur is encountered in most sediment samples, marine algae and some industrial wastes. The solubility of sulfur in various solvents is very similar to the organochlorine and organophosphate pesticides; therefore, the sulfur interference follows along with the pesticides through the normal extraction and cleanup techniques. The sulfur will be quite evident in gas chromatograms obtained from electron capture detectors, flame photometric detectors operated in the sulfur or phosphorus mode, and Coulson electrolytic conductivity detectors. If the gas chromatograph is operated at the normal conditions for pesticide analysis, the sulfur interference can completely mask the region from the solvent peak through aldrin.

This technique eliminates sulfur by the formation of copper sulfide on the surface of the copper. There are two critical steps that must be followed to remove all the sulfur: (1) the copper must be highly reactive; therefore, all oxides must be removed so that the copper has a shiny, bright appearance; and (2) the sample extract must be vigorously agitated with the reactive copper for at least one minute.

It will probably be necessary to treat both the 6% and 15% Florisil eluates with copper if sulfur crystallizes out upon concentration of the 6% eluate.

Certain pesticides will also be degraded by this technique, such as the organophosphates, chlorobenzilate and heptachlor (see Table 1). However, these pesticides are not likely to be found in routine sediment samples because they are readily degraded in the aquatic environment.

If the presence of sulfur is indicated by an exploratory injection from the final extract concentrate (presumably 5 ml) into the gas chromatograph, proceed with removal as follows:

1. Under a nitrogen stream at ambient temp., concentrate the extract in the concentrator tube to exactly 1.0 ml.

2. If the sulfur concentration is such that crystallization occurs, carefully transfer, by syringe, 500 µl of the supernatant extract (or a lesser volume if sulfur deposit is too heavy) into a glass-stoppered, 12-ml grad., conical centrifuge tube. Add 500 µl of iso-octone.

3. Add ca 2 µg of bright copper powder, stopper and mix vigorously 1 minute on a Vortex Genie mixer.

   NOTE: The copper powder as received from the supplier must be treated for removal of surface oxides with 6N HNO_3. After about 30 seconds of exposure, decant off acid, rinse several times with dist. water and finally with acetone. Dry under a nitrogen stream.

4. Carefully transfer 500 µl of the supernatant-treated extract into a 10-ml grad. evap. concentrator tube. An exploratory injection into the gas chromatograph at this point will provide information as to whether further quantitative dilution of the extract is required.

   NOTE: If the volume transfers given above are followed, a final extract volume of 1.0 ml will be of equal sample concentration to a 4-ml concentrate of the Florisil cleanup fraction.

Table 1. Effect of Exposure of Pesticides to Mercury and Copper

Compound	Percentage Recovery Based on Mean of Duplicate Tests	
	Mercury	Copper
BHC	81.2	98.1
Lindane	75.7	94.8
Heptachlor	39.8	5.4
Aldrin	95.5	93.3
Hept. Epoxide	69.1	96.6
p,p'-DDE	92.1	102.9
Dieldrin	79.1	94.9
Endrin	90.8	89.3
DDT	79.8	85.1
Chlorobenzilate	7.1	0
Aroclor 1254	97.1	104.3
Malathion, diazinon, Parathion, Ethion, Trithion	0	0

CONFIRMATORY PROCEDURES

B. THIN-LAYER CHROMATOGRAPHY

I. INTRODUCTION:

Thin-layer chromatography is primarily a qualitative tool which is useful in the identification of pesticides. It can be used to advantage as a confirmatory method in conjunction with gas chromatography. Thin-layer chromatography introduces a second physical basis for separation, that of adsorption chromatography.

Additional advantages of this technique, include simplicity, rapidity, low man-hour consumption, and its utility as a resolving, and cleanup procedure, for use with other methods of analysis.

The method is, in general, somewhat less sensitive than microcoulometry, being limited to about 10 Ng for easy visual inspection of most chlorinated pesticides and about 50 Ng of most organothiophosphates. Consequently, a macrosample is required. A stringent sample cleanup procedure is also required.

REFERENCES:

1. Kovacs, Martin, F., JAOAC. 46 (1963).

2. Kovacs, Martin, F., Ibid, 47 (1964).

3. Kovacs, Martin, F., Ibid, 48 (1965).

4. Kovacs, Martin, F., Ibid, 49 (1966).

5. Kovacs, Martin, F., Private Communication (1968).

6. Moseman, Robert, Private Communication (1968).

7. Pesticide Analytical Manual, U. S. Food & Drug Administration, Volume I, Sect. 410.

II. APPARATUS:

1. 8" x 8" glass plates, double strength window glass (Pittsburgh Plate Glass).

2. 3-1/4" x 4" clinical microslides (Arthur H. Thomas Co.).

3. Developing tank, Thomas-Mitchell, 8-1/2" x 4-1/2" x 8-1/2" deep (Arthur H. Thomas Co.).

4. Desaga/Brinkmann standard counting board.

5. Chromatographic chamber, 800 ml beaker.

6. Desaga/Brinkmann standard model applicator.

7. Desaga/Brinkmann drying rack, holds 10, 8" x 8" plates.

8. Spotting pipettes, 1, 5, and 10 µl, Kontes 763800.

9. Spray bottle, 8 oz., Thomas Co. #9186-R2.

10. Desaga/Brinkmann glass vacuum desiccator.

11. Desk blotter paper.

12. Ultra violet light source: 4-15 watt G. E. germicide lamps, shielded to protect operator, General Electric Co. G 15 T 8.

III. REAGENTS:

1. Aluminum oxide G. (Brinkmann or Warner-Chilcott).

2. N-heptane, chromatographic grade.

3. Methycyclohexane, practical, B.P. 100.5 - 101.5°C. (Matheson, Coleman, and Bell).

4. Tetrabromophenolphthalein ethyl ester (Eastman Organic Chemicals #6810).

5. Acetone, Reagent.

6. Silver Nitrate, Reagent.

7. Tetraethylenepentamine.

8. Citric Acid, granular, Reagent.

9. p-Nitrobenzyl pyridine.

10. Hydrogen peroxide, 30%, Reagent.

11. Ethyl ether, Reagent.

12. Acetonitrile, chromatographic grade.

13. Dimethylformamide, Reagent.

14. Preparation of reagent solutions.

A. Developing solvents

(1) For organochlorines

(a) 2% acetone in N-heptane (v/v) (mobile solvent).

(b) N-heptane (mobile solvent).

(2) For thio and nonthio organophosphates.

(a) Methylcyclohexane (mobile solvent).

(b) 15% or 20% dimethylformamide (v/v) in ether (immobile solvent).

B. Chromogenic reagents.

(1) For organochlorines.

(a) Dilute 0.1 gm of silver nitrate and 20 ml of 2-phenoxyethanol to 200 ml with acetone. Immediately add 3 drops of 30% hydrogen peroxide and mix. Keep stored in a cool dark place not longer than 1 week. Dark solutions should be discarded.

(2) For organothiophosphates.

(a) Stock dye solution.

Dissolve 1 gm of tetrabromophenolphthalein ethyl ester in 100 ml of acetone.

(b) Use concentration dye solution.

Dilute 10 ml of stock solution to 50 ml with acetone.

(c) Silver nitrate solution.

Dissolve 0.5 $AgNO_3$ gm in 25 ml of distilled water and dilute to 100 ml with acetone.

(d) Citric acid solution.

Dissolve 5 gm citric acid in 50 ml of distilled water and dilute to 100 ml with acetone.

(3) For thio and nonthio organophosphates.

(a) 2% p-Nitrobenzyl pyridine in acetone (w/v).

(b) 10% Tetraethylenepentamine in acetone (v/v).

IV. PREPARATION OF T.L.C. PLATES:

8" x 8" plates

1. Add 30 g aluminum oxide G to 50 ml distilled water and shake for 30-45 seconds.

2. Pour into applicator and spread a 250 micron layer on glass plates. Make an arrow in corner of plate indicating direction of application.

3. Air dry for 15 minutes, then at 80°C for 45 minutes in a forced draft oven.

4. Remove, cool, and store plates in a desiccator.

3-1/4" x 4" micro slide plates

1. Preparation of adsorbent layer - Select five 8" x 8" and one 4" x 8" (calculated to cover the entire surface of the applicator board) photo-glass plates of uniform width and thickness. Wet the surface of the applicator board with a few ml of distilled water delivered from an eye dropper in the form of the letter "X", approximately the size of the plate to be mounted. Press each plate snugly into position on the applicator board, forcing out excess water under the plate to insure a tight fit. (Add enough water to the applicator board to prevent the appearance of air bubbles under the plate after it has been pressed into position).

2. Examine each 3-1/3" x 4" micro slide carefully by looking down each edge. To insure flatness of plates, use only slides that are visibly straight along all edges.

3. Mount the micro slides individually on the surface of the photoglass plates with their long axis perpendicular to the direction of layer application. With an eye dropper, place a few ml distilled water on the surface of the photoglass plate and mount micro slides. Force out the excess water so that no large air bubbles remain under the slide. The presence of a large air bubble indicates slide "bowing" due to an irregularity in the slide. When "bowing" is noted, discard the slide, and position another in its place.

4. Repeatedly slide an empty applicator across the series of mounted slides to force out excess water, and wipe surface of slides dry each time with a tissue. The empty applicator must ride smoothly and without effort across the series of slides. If not, re-examine the uniformity and positioning of micro slides.

5. To remove any remaining water, wipe the surface with a dry tissue, then with one soaked in 95% ethanol, and let dry.

6. Weigh 30 g Al_2O_3 G or MN-silica gel G-HR into a 250 ml ℨ Erlenmeyer flask. Add 50 ml distilled water to Al_2O_3 G or 60 ml to MN-silica gel G-HR, stopper flask, shake moderately for 15 to 20 seconds, and immediately pour slurry into applicator chamber. The time required for actual application should be approximately 10 seconds. Immediately after coating, grasp the applicator board at its ends, raise a few inches, then drop. This procedure which is repeated a few times, smooths out slight ripples or imperfections in the wet coating.

7. Let coated plates dry in position on the mounting board for 20 minutes. Mark each micro plate on the 3-1/4" edge farthest from the longitudinal center of the applicator board. This edge represents the top of each micro plate during subsequent development. The 3-1/4" edge of each micro plate at the center of the applicator board represents the end to be spotted. This is done because most of the coating irregularity occurs on the outer 3-1/4" edge of the plates.

8. Remove each plate individually with a spatula, and wipe the back side dry with a tissue. Place 4 micro slides on the surface of one 8" x 8" plate and place the plates in a rack for drying at 80°C for 1 hour in a forced draft oven.

9. After heating, cool the micro plates, and examine each individually in strong transmitted light for possible gross irregularities in the uniformity of the coating. Discard plate if gross irregularities are observed. Place 4 micro plates on the surface of each 8" x 8" plate, slide into a drying rack, and store in a desiccating storage cabinet until needed.

10. Sample spotting - Make a pencil mark at each side 1/2" above the bottom edge of the slide. The imaginary line between these points serves as the sample "spotting line." Draw an actual line across the slide 2-3/8" (about 6 cm) above the "spotting line." The actual line serves to mark the solvent front after development. Draw a pencil line along each side 1/4" in from the edge to prevent distortion of the solvent front during development.

11. Spot samples at 1/4" intervals along the imaginary "spotting line." Each micro plate will accommodate 10 application points as compared to 18 on a normal 8" x 8" plate. Spot samples and standards on the micro slide in the same manner as described later under SPOTTING.

V. SAMPLE PREPARATION:

1. The sample must be of sufficient size that when the extract from Florisil cleanup is concentrated to an appropriate volume, a 10 µl spotting volume will produce detectable compound spots. A serum extract from 50 grams concentrated to 100 µl should produce a visible spot of 2 ppb. An adipose tissue extract from 5 grams concentrated to 500 µl should give a readable spot at 10 ppb. These values assume the detection of chlorinated pesticides.

2. The extract from the 15% diethyl ether fraction contains far more lipids than are present in the 6% fraction. For this reason, some further cleanup is required. This is conveniently accomplished by spotting the equivalent of 5 grams of blood or 0.5 grams of fat on a 3-1/4" x 4" micro plate, developing with acetonitrile, and scraping off the alumina from the area at the solvent front. This is extracted in hexane and the resulting extract respotted on a standard 8" x 8" TLC plate.

VI. SPOTTING AND DEVELOPING:

1. Provide an imaginary spotting line across the plate by making a pencil mark 1-1/2" from the bottom edge of the plate on both sides.

2. Provide an imaginary solvent front by making a pencil mark 5-1/2" from the bottom edge of the plate on both sides.

3. With a micropipette, transfer a suitable amount of the extract to one of the spots, with repeated applications.

4. Spot standard solutions on the same plate. Standard concentrations should bracket the calculated amount of residue in sample.

5. Prepare chromatographic tank by placing 50 ml of developing solvent in the trough and 75 ml in the bottom of the tank.

6. Seal the tank and develop to the line scribed on the plate.

7. Remove plate and air dry in the hood.

VII. COMPOUND DETECTION:

1. Organochlorines

   a. Immediately after drying, spray plates with the chromogenic reagent.

   b. Air dry plates for 15 minutes.

   c. Expose plates to ultraviolet until the lowest concentrations of standards are visible.

2. Organothiophosphates

   a. Immediately after drying, spray plates with the "use concentration" dye solution. Spray moderately heavy.

   b. Overspray lightly with the silver-nitrate solution.

   c. After 2 minutes overspray the plate moderately with citric acid solution.

3. Thio and nothio organophosphates

   a. After drying, spray plate with p-Nitrobenzyl pyridine chromogenic solution and heat at 110°C for 10 minutes.

   b. Cool and overspray plate with tetraethylenepentamine solution.

   NOTES: 1. The color of solid p-Nitrobenzyl pyridine should be yellow. If there is any purple color, recrystallize from acetone. Oxidized solution will cause high background color and will reduce sensitivity.

   2. The tetraethylenepentamine should not have a deep color. If it does, decolorize and purify with charcoal.

4. Interpret results by comparing R_f values of sample spots against those of standard spots on the same plate.

VIII. GAS CHROMATOGRAPHY (E.C.) CONFIRMATION OF R.F. VALUES:

At times there may be reason to question the validity of a spot because of a slight shift in the position of the R. F. site or because of spot diffusion or a very faint appearing spot. When such doubt exists, GLC, EC examination of the material from the questionable R. F. site can serve to either confirm or negate the presence of the suspected compound.

Any of the pesticidal compounds (organochlorine) of lowest concentration in blood or fatty tissue such as β-BHC, heptachlor epoxide, o,p'-DDT and p,p'-DDD are frequently the most difficult to identify by customary GLC, EC. These compounds, if present, are generally in such low concentration that an extract aliquot equivalent to 5.0 grams of blood and 0.5 grams of fat is needed for spotting on the TLC plates.

1. Spot the TLC plate with 200 nanograms each of standards of the suspect compounds. Also spot the 6% diethyl ether fraction of the unknown extract in two places on the plate.

2. Develop the plate with n-Hexane until the solvent front has migrated 10 cm.

3. Cover with a small glass plate a portion of the plate containing one of the sample applications and spray the plate with the $AgNO_3$/2-phenoxyethanol reagent.

4. Develop the spots in the sprayed area in the usual manner and compute the R_f values for the standards.

5. Utilizing the standard R_f values, pin-point the elution sites for these compounds along the imaginary migration line of the unsprayed sample.

6. Scrape each sample elution with a flat edge spatula and transfer the alumina to separate centrifuge tubes.

   NOTE: Scrape another spot from an area of the plate lying outside the region of samples and standards and extract identically to the sample spots. This will serve as a reagent blank.

7. Add 1 ml of hexane which has been previously examined for assurance that it is free of contaminants which might contribute artifact peaks.

8. Stopper tube and shake vigorously one minute on a Vortex mixer.

9. Inject 5 µl of this extract into the gas chromatograph and observe the chromatogram for the presence or absence of the suspect compound peak. Adjustment of the injection volume may be required based on peak height resulting from the initial injection, provided of course, that there are any peaks.

IX. MISCELLANEOUS NOTES:

1. Thorough sample and extract cleanup must be employed.

2. Plates must be thoroughly washed.

3. All solvents, except ethyl ether, must be redistilled.

4. Prevent even minor contamination.

5. Isooctane tolerates more oil in the sample than other developing solvents.

6. For the detection of nanogram quantities it is imperative to use a source of U. V. radiation at least as intense as that provided by the specified equipment.

7. Always spray the chromogenic agent in a direction perpendicular to the direction of solvent flow (side to side).

n-Heptane Solvent System

Adsorbent	Al_2O_3 G (Merck), 250 μ thick, air dried 72 hr at room temperature
Plate Size	8"x8"
Front Travel	10 cm
p,p'-TDE Travel	3.9 cm
Developing Tank	9"x9"x3.5", saturated
Visualization	$AgNO_3$, UV exposure
Temperature	24-26°C
Amount Spotted	80-200 ng

Pesticide	R_{TDE}
Hexachlorobenzene	2.7
Aldrin	2.1
p,p'-DDE	2.0
Heptachlor	2.0
Chlordane (tech)	2.0, 1.8, 1.4, 1.2*
o,p'-DDT	1.9
PCNB	1.8
Perthane olefin	1.8
p,p'-TDE olefin	1.8
TCNB	1.7
Telodrin	1.7
Toxaphene	1.7, 1.2*
Strobane	1.7, 1.2*
p,p'-DDT	1.6
o,p'-TDE olefin	1.6
Chlorbenside	1.3 (grey)
BHC (tech)	1.3, 1.1, 0.27, 0.10
α -BHC	1.3
Perthane	1.3
Lindane	1.1
o,p'-TDE	1.1
p,p'-TDE	1.0
Endosulfan	0.88, 0.07
Ronnel	0.85
Heptachlor epoxide	0.71
Endrin	0.71
Dieldrin	0.52
Carbophenothion	0.42 (yellow)
Methoxychlor	0.33, 0.27
β -BHC	0.27
Ovex	0.18
Dichlone	0.16
Dyrene	0.15 (grey)

Pesticide	R_{TDE}
Tetradifon	0.11
δ -BHC	0.10
Delta Keto "153"	0.09
Kelthane	0.06
Sulphenone	0.00 (large and fuzzy)
Captan	0.00 (sharp edged grey)
Chlorobenzilate	0.00 (light)
Monuron	0.00 (large and dark)
Diuron	0.00
Endrin aldehyde	0.00 (very small)
Endrin alcohol	0.00

Most intense spot underlined
*Leaves a streak with these major spots

2% Acetone in n-Heptane Solvent System

Adsorbent	Al_2O_3 G (Merck), 250 μ thick, air dried 72 hr at room temperature
Plate Size	8"x8"
Front Travel	10 cm
p,p'-TDE Travel	5.7 cm
Developing Tank	9"x9"x3.5", saturated
Visualization	$AgNO_3$, UV exposure
Temperature	24-26°C
Amount Spotted	80-200 ng

Pesticide	R_{TDE}
Hexachlorobenzene	1.7
Perthane olefin	1.4
PCNB	1.4
Aldrin	1.4
p,p'-DDE	1.4
Chlordane (tech)	1.4, 1.3, 1.2, 1.1*
p,p'-TDE olefin	1.4
Telodrin	1.4
Heptachlor	1.4
TCNB	1.3
o,p'-TDE olefin	1.3
Toxaphene	1.3, 1.2*
Strobane	1.3, 1.2*
o,p'-DDT	1.3
p,p'-DDT	1.2
Chlorbenside	1.2 (fuzzy grey)
Perthane	1.2
BHC (tech)	1.1, 0.92, 0.72, 0.25
α -BHC	1.1
Ronnel	1.1
Endrin	1.0
Carbophenothion	1.0 (fuzzy yellow)
Heptachlor epoxide	1.0
p,p'-TDE	1.0
o,p'-TDE	0.95
Lindane	0.92
Endosulfan	0.92, 0.24
Dieldrin	0.90
Tetradifon	0.82
Methoxychlor	0.79
Ovex	0.76
β -BHC	0.72
Dichlone	0.72, 0.00

Pesticide	R TDE
Dyrene	0.51
Sulphenone	0.31
Kelthane	0.28
∂-BHC	0.25
Delta Keto "153"	0.23 (very small)
Captan	0.09
Chlorobenzilate	0.05
Monuron	0.00
Diuron	0.00 (dark spot)
Endrin aldehyde	0.00 (very small)
Endrin alcohol	0.00

Most intense spot underlined
*Leaves a streak with these major spots

R_f Values

Adsorbent	Al_2O_3 G
Mobile solvent	Methylcyclohexane

Pesticide	R_f Value	
	Immobile Solvent	
	15% DMF	20% DMF
Dimethoate	0.01	0.01
Azinphosmethyl (Guthion)	0.09	0.06
Imidan	0.09	0.07
Methyl parathion	0.17	0.11
Coumaphos	0.23	0.15
Malathion	0.34	0.22
Dioxathion	0.37	0.24
Parathion	0.41	0.27
Demeton (thiol)	0.44	0.32
EPN	0.49	0.33
Methyl carbophenothion	0.50	0.36
Sulfotepp	0.69	0.55
Carbophenothion	0.74	0.59
Ronnel	0.76	0.62
Ethion	0.77	0.63
Demeton (thiono)	0.79	0.67
Phorate	0.81	0.71
Disulfoton	0.82	0.72
Diazinon	0.86	0.78

(1) Presence of chloride in adsorbent layer reacts with $AgNO_3$ and prevents coupling of dye and pesticide to form characteristic blue or purple spot. Some aluminum oxide coatings do not have to be prewashed to remove chloride. If, however, maximum compound sensitivities of 0.05 μg cannot be achieved with unwashed Al_2O_3 coating, prewashing is recommended.

(2) Chromogenic spray reacts only with sulfur-containing phosphate esters. The following compounds do not react; oxygen analog of parathion, dichlorvos, naled, mevinphos, Phosphamidon and trichlorfon.

(3) The following minimum amounts of sulfur-containing phosphate esters can be detected: 0.05 μ g diazinon, demeton (thiono), carbophenothion, parathion, malathion, ronnel, dioxathion, EPN, coumaphos, sulfotepp, and ethion; 0.1 μg azinphosmethyl, methyl parathion, and demeton (thiol). The lower limits of detectability of dimethoate, Imidan, methyl carbophenothion, phorate, and disulfoton were not determined. At 0.5 g, or greater, the thio-phosphate esters vary as to color produced with the chromogenic reagents. Carbophenothion, parathion, EPN, coumaphos and diazinon appear vivid blue. Ethion, azinphosmethyl, sulfotepp, dioxathion, and malathion appear purple. Ronnel and methyl parathion appear dull blue while both thiol and thiono demeton appear bluish purple.

MICRO SCALE ALKALI TREATMENT FOR USE IN PESTICIDE RESIDUE CONFIRMATION AND SAMPLE CLEANUP

(Reproduction of manuscript in press, 1972 by
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Procedures involving alkali treatment for dehydrochlorination of certain organochlorine pesticides and saponification of fats have long been employed in pesticide residue analysis. In 1942 Brand and Busse-Sunderman (1), and in 1946 Soloway et al. (2), studied rates of dehydrochlorination of DDT. Mills (3) used refluxing alcoholic KOH in the cleanup of fatty foods for paper chromatographic detection of alkali-stable organochlorine pesticides. Klein and Watts (4) used alcoholic NaOH to dehydrochlorinate o,p'- and p,p'-DDT, p,p'-TDE, and Perthane prior to gas chromatographic separation of the respective olefins. These investigators called attention to several earlier uses of alkali dehydrochlorination in pesticide residue chemistry. Recent literature contains numerous references to application of this treatment in pesticide residue analyses, including an adaptation for use in a pre-gas chromatographic (GLC) column (5).

In spite of the knowledge of this reaction, we have observed that it is not fully and effectively utilized by residue laboratories for forming derivatives, gaining information for identity confirmation, or obtaining better cleanup of troublesome extracts. This is probably because the procedure has not been described in detail for simple micro scale application in multi-residue analysis.

The purpose of this work was (1) to arrive at optimum and convenient parameters of the alkali treatment in order to obtain rapid dehydrochlorination resulting in product solutions suitable for analysis by GLC; (2) to obtain complete yield and recovery of olefins from a number of bis(phenyl) chloroethane pesticides; (3) to determine the effect of the treatment on several important pesticide and industrial chemicals; (4) to describe in detail the procedure for routine application in the residue analytical laboratory.

METHOD

Reagents and Apparatus

(a) Potassium hydroxide - Anhydrous pellets.
(b) Ethanol - USP 95%.

(c) Hexane - Suitable for use with electron capture gas chromatography (Burdick and Jackson Laboratories, Inc. 1953 S. Harvey St., Muskegon, Mich. 49442).
(d) Micro condenser - 19/22 ST; K-569250 (Kontes Glass Company, Vineland, N. J. 08360).
(e) Concentrator tube - Mills type, 19/22 ST with stopper, 10 ml graduated in 0.1 ml up to 1.0 ml; K-570050 (Kontes Glass Company).
(f) Alkali dehydrochlorination reagent - Dissolve 2 g KOH in 100 ml ethanol.
(g) Ethanol - water, 1+1 - Combine equal parts by volume of distilled water and ethanol.
(h) Gas chromatograph - Equipped with electron capture detector and 6' x 4 mm id glass column containing either (1) 10% DC-200 or (2) 1:1 mixture of 15% QF-1 + 10% DC-200 on 80-100 mesh Chromosorb W(HP).

Operating conditions: N_2 flow 120 ml/min; temperatures, column and detector 200°C, injector 225°C; concentric design electron capture detector operated at DC voltage to cause 1/2 full scale recorder deflection for 1 ng heptachlor epoxide when full scale deflection is 1×10^{-9} amp.

Procedure

Accurately pipet, into a 10-ml Mills tube, 2 ml of a petroleum ether solution of sample extract [6% or 15% Florisil eluate (6)] containing concentrations of pesticides suitable for subsequent GLC analysis. Add 1 ml of 2% ethanolic KOH and a few carborundum chips and fit the tube with a micro condenser. [Note: Avoid getting alkali on the ground glass joint: light greasing of joint with silicone lubricant may prevent sticking.] With a test tube clamp, hold the tube over an opening in the steam bath in such a manner that gentle boiling occurs. When the volume has been reduced to about 1 ml, insert the tube completely into the steam bath opening and heat vigorously for 15 minutes or until the volume reaches 0.2 ml. Remove tube from the steam. If a precipitate has formed, as is often the case with extracts containing fatty substances, add a few drops of 2% ethanolic KOH and warm gently in steam with swirling until the precipitate dissolves. After the solution has cooled slightly, add about 2 ml ethanol-H_2O (1 + 1). Allow solution to reach room temperature and pipet 1 or 2 ml hexane into tube. Stopper tube with ground glass, invert, shake vigorously for about 30 seconds, and allow solvent layer to separate sharply. With microliter syringe, carefully withdraw aliquot of upper layer for determination by GLC. [Note: Separation of phases should be sharp so that solution withdrawn for GLC analysis will be free from alkali.]

Discussion

Development of Method

Initial experimentation was performed to establish the reaction conditions which would give complete and rapid dehydrochlorination of p,p'-DDT, o,p'-DDT, p,p'-TDE, o,p'-TDE, methoxychlor, and Perthane and which would permit complete recovery of the respective olefins. Work was done to determine the effect on olefin formation of fatty substances not removed during sample cleanup and the capacity of the reaction to eliminate fatty substances.

Several considerations found necessary for practical and reliable use of the alkali treatment have been incorporated into the method and are briefly discussed. The steam bath was chosen as a source of heat because of its ready availability and convenience. The Mills reaction tube was fitted with a micro condenser to eliminate losses due to volatilization, which often occurred in the absence of the condenser. Both KOH and NaOH have been used to the satisfaction of previous investigators. The more frequent use of KOH by other workers and its higher solubility in ethanol made it the choice for this work. An alkali concentration of 2% has been widely used, and was found ideal for treatment of aliquots of cleaned-up sample extracts containing quantities of bis(phenyl) chloroethane pesticides ranging from a few nanograms to 100 µg. In order to provide sufficient reflux time and temperature, it was necessary that the initial volume of ethanol be in excess of 0.5 ml. When smaller volumes were used, dehydrochlorination was usually incomplete. Emulsions often occurred during extraction of the olefin into hexane after saponification of fatty substances. The use of ethanol + water (1+1) instead of water as the diluent resulted in a sharp separation of hexane and aqueous layers. Recoveries of olefins were not adversely affected if the ethanol content was less than about 70%. Less than 30% ethanol did not adequately enhance separation of the two layers. The non-volatile fatty substance transferred to acetonitrile by partitioning a petroleum ether solution of butterfat with acetonitrile was used in tests to evaluate the effects of fatty substances on the reaction. Experiments in which varying volumes of 2% ethanolic KOH were used to saponify 350-mg portions of this butterfat showed that each 1 ml of 2% KOH would saponify about 50 mg of the fat. When the weight of fatty substances exceeded about 50 mg, complete dehydrochlorination of Perthane (40 µg) and methoxychlor (4.0 µg) was not obtained. However, p,p'-DDT (8.0 µg) was completely dehydrochlorinated in the presence of 100-120 mg of fat. Additional experimentation showed that complete dehydrochlorination of methoxychlor and Perthane did not occur until the fat was completely saponified; these were the bis(phenyl) chloroethanes most resistent to dehydrochlorination. Quantities of Perthane and p,p'-DDT up to 100 µg in the presence of not more than 50 mg butterfat were readily dehydrochlorinated with 1 ml of 2% KOH at steam bath temperature; dehydrochlorination of larger amounts of pesticide was not attempted. Hexane, because of its higher boiling point and greater ease of drawing into a microsyringe, was used

instead of petroleum ether, to extract the olefin after reaction in order to avoid possible errors in quantitation.

Effect on Selected Chemicals

The dehydrohalogenation reaction, as described under "Method", was applied to p,p'-DDT (0.8 µg), o,p'-DDT (0.8 µg), p,p'-TDE (2.0 µg), o,p'-TDE (0.4 µg), methoxychlor (4.0 µg), and Perthane (40 µg). Quantities shown in parentheses were chosen for ease in GLC determination. The pesticides were treated individually in petroleum ether, in 6% ethyl ether/petroleum ether Florisil eluates (6) containing the equivalent of 6 g of kale, and in petroleum ether containing 30-60 mg of fatty substances extracted from butter by partitioning (6) between petroleum ether and acetonitrile. Gas chromatography with electron capture detection, operated as described under "Method", was used for all determinations.

Each pesticide was completely altered in each of the solution types, i.e., none of the parent compound remained after treatment as described under "Method". Percent recoveries of the respective olefins were calculated according to the following equation:

$$\frac{\text{wt. olefin compound determined by GLC}}{\text{wt. parent compound represented in aliquot to GLC}} \times \frac{\text{wt. parent compound/mol. wt.}}{\text{wt. olefin compound/mol. wt.}} \times 100$$

Recoveries of olefins approximated 100% and ranged from 86% for p,p'-DDE and o,p'-TDE olefin in petroleum ether to 110% for p,p'-DDE in the extract from butter. Two olefin derivatives, the cis and trans isomers, are formed from o,p'-TDE (7). These have identical retention times on the two GLC columns used in this work and were quantitated as a single compound.

Several additional common organochlorine pesticides and polychlorinated biphenyls (PCB) were subjected to the described alkali treatment. All tests were made with petroleum ether solutions of the chemical under study. The quantity of each chemical was chosen for ease of determination by GLC and is given in parentheses.

Polychlorinated biphenyls ranging from 21 to 60% average chlorine content were stable to this treatment. Complete recoveries were obtained for the commercial PCB mixtures, Aroclors 1221 (16 µg), 1232 (16 µg), 1242 (16 µg), 1254 (8 µg), and 1260 (8 µg).

Recoveries of unchanged aldrin (0.4 µg), dieldrin (0.4 µg), and endrin (0.4 µg) ranged from 70 to 90%. No alteration products were detected.

Heptachlor (0.4 µg) and heptachlor epoxide (0.4 µg) were markedly affected; recoveries of the original compound ranged from 30 to 50%. Minor GLC peaks were observed on the 10% DC-200 column at retention times relative to aldrin of 1.63 after treatment of heptachlor epoxide and 0.59 and 0.93 after treatment of heptachlor.

The alkali treatment completely eliminated lindane (0.2 µg) and the alpha (0.2 µg), beta (0.2 µg), and delta (0.2 µg) isomers of BHC. Following the reaction, only small early eluting gas chromatographic peaks, presumably from trichlorobenzenes, were observed.

About 40% of mirex (4.0 µg) remained unchanged after reaction; sometimes a minor GLC peak appeared at a retention time relative to aldrin of 1.83 on the 10% DC-200 column.

Endosulfan I and II, treated separately, were completely eliminated. Each isomer gave a single alteration product with retention time relative to aldrin of 1.82 on the 10% DC-200 column and 2.23 on the 1:1 10% DC-200/15% QF-1 column. The peak height of the alteration product was approximately one-tenth the peak height of the parent compound. A structure for this derivative has been proposed (8).

Endosulfan sulfate also was completely eliminated. Two alteration products were obtained with retention times relative to aldrin of 0.28 and 0.38 on the 10% DC-200 column.

Dicofol (1.0 µg) was completely eliminated, but only 65% of the major alteration product, 4,4'-dichlorobenzophenone, was recovered. A minor peak was observed in the chromatogram at a retention time relative to aldrin of 1.71 on the 10% DC-200 column. The 4,4'-dichlorobensophenone (2.0 µg) was not affected by treatment with alkali.

The products resulting from alkali treatment of toxaphene (10.0 µg) gave a multicomponent chromatogram but consisting of components with earlier retention times than toxaphene itself.

The electron capture GLC responses to sulfur (20 µg), frequently encountered in residue analysis at retention times relative to aldrin of 0.23, 0.55, and 1.13 on the 10% DC-200 column, were eliminated by the alkali treatment.

Application of Method

The most obvious use of the alkali treatment is to form the olefins of bis(phenyl) chloroethane pesticides for confirmation of residue identity. The complete yield and recovery of the olefin derivative makes possible quantitative

confirmation of a residue of the parent pesticide. For example, a residue of p,p'-DDT can be quantitated before alkali treatment and verified by quantitation as p,p'-DDE after treatment.

In addition, the GLC retention time region of the reacted compound can be examined for presence of peaks from unreacted and presumably interfering substances. We have found the alkali treatment especially useful in connection with the real or suspected presence of residues of PCB. In this case the characteristic olefin derivatives of p,p'-DDT,o,p'-DDT, and p,p'-TDE can be formed and the GLC retention time region underlying the parent compounds can be examined. The stability of PCB to alkali, with no change in the GLC pattern, is a characteristic which can be readily utilized in confirmation of the identity of this complex residue. The high recovery of unreacted dieldrin, endrin, and aldrin following alkali treatment likewise can be of value in the confirmation of identity of these pesticides.

Cleaned-up extracts of some samples may contain non-pesticidal substances which give rise to electron capture response. Other extracts may contain fatty substances, not removed by the cleanup, which can prohibit application of some tests, e.g., thin layer chromatography. This is particularly true of the 15% ethyl ether/petroleum ether Florisil column eluate (6) for non-fatty samples such as carrots and fatty samples such as some fish. Electron capturing substances present in carrots must be eliminated before determination of dieldrin and/or endrin residues. Treatment with alkali serves well for this purpose. Extracts of fatty samples may require treatment to eliminate both electron capturing substances and non-volatile fatty substances. In many instances thin layer chromatography or microcoulometric GLC cannot be accomplished prior to alkali treatment. Electron capture responses to sulfur, often a source of annoyance to the residue chemist, are also eliminated by this treatment.

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DETERMINATION OF METHYL MERCURY IN FISH, BLOOD, BRAIN AND URINE

I. INTRODUCTION:

During the past year, mercury has been found to be a frequent contaminant of fish in North America. Much effort has been spent in improving or developing methods for analyzing commodities for total mercury content, usually by colorimetric (1) or atomic absorption procedures (2). However, it has been shown (3) that most, if not all, mercury found in fish is present as methyl mercury, a compound thought to have much greater chronic toxicity than other forms of mercury. Therefore, appropriate methodology, specific for methyl mercury, is desirable. (The form in which mercury may be present in other contaminated commodities varies and is not necessarily methyl mercury).

The most widely used method for methyl mercury analysis is that described by Westöö (3,4,5,6). This method has been used successfully for the analysis of fish, red blood cells, plasma and brain of hamsters fed methyl mercury. The method described herein represents a modification of the procedures described by Westöö in her papers and private communications.

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7. Laboratory Information Bulletin, FDA, October, 1970 DETERMINATION OF METHYL MERCURY IN FISH AND ANIMAL TISSUES BY GAS LIQUID CHROMATOGRAPHY, Kamps, L., and Malone, B.

II. PRINCIPLES:

In this procedure, methyl mercury chloride (CH_3HgCl) is formed by addition of hydrochloric acid to homogenized tissue, and the organic salt is extracted into benzene. The methyl mercury is extracted from the benzene solution by partitioning it with aqueous cysteine solution, which layer is then acidified and the salt reextracted back into benzene. The final benzene layer is analyzed by gas-liquid chromatography.

III. APPARATUS:

1. Gas chromatograph fitted with column 6' x 4 mm i.d. of 5% HIEFF-10B on Chromosorb W, H.P., 80/100 mesh and with an electron capture detector.

2. Sorval homogenizer with 50-ml cups. Available from Ivan Sorval Co., Norwalk, Conn.

3. Centrifuge tubes, 35 ml, conical, Kimble #45201 or the equivalent.

4. Centrifuge bottles, 250 ml, Corning #1280, or the equivalent.

5. Centrifuge with adjustable speed up to 3,000 r.p.m. with carrier heads appropriate for tubes and bottles listed above in 3 and 4.

6. Cylinder, grad., 100 ml.

7. Separatory funnels, 30, 60 and 250 ml with Teflon stopcock.

8. Pipets, transfer, 2,4,6 and 50 ml.

9. Pipets, measuring 1 and 2 ml, grad., in 0.01 ml.

IV. REAGENTS AND SOLVENTS:

1. Benzene, pesticide quality. Background contamination must be checked by injecting 5 µl of a 15 to 1 concentrate. No peaks should appear in the area shortly after the solvent front.

2. Methyl mercuric chloride*. Available from K & K Laboratories, stock number 23308. Stock Standards of:

   a. 1 mg/ml in benzene.

b. 1 mg/ml in ethanol-water (1:1).

Dilute stock solutions a. and b. as needed for recovery trials and GLC working standards.

NOTE: The stability of CH_3HgCl dilute standard solutions is questionable. Stock solutions, 1 mg/ml in benzene or in (1:1) alcohol/H_2O should be stable indefinitely. It is suggested that dilute solutions in H_2O be made fresh every 2 or 3 days and dilute solutions in benzene every 2 months.

*Some of the mercury standards will be available from the Perrine Repository.

3. Ethyl mercuric acetate. Available from Ventron, P.O. Box 159, Beverly, Mass. 01915, stock #87301.

Prepare benzene solution of 1 mg/ml.

4. Phenyl mercuric acetate. Available from K & K Laboratories, stock #17412.

Prepare benzene solution of 1 mg/ml.

5. Methyl mercuric iodide. Available from K & K Laboratories, stock #2508.

Prepare benzene solution of 1 mg/ml.

6. Column conditioning mixture: Combine equal portions of foregoing items 2a, 3, 4 and 5 and set aside for column conditioning.

7. Cysteine hydrochloride-monohydrate. Aldrich Chemical Co., stock #C 12, 180 - 0.

8. Sodium acetate $.3H_2O$, Mallinckrodt #7368 or the equivalent.

9. Sodium chloride, reag. grade, fine crystal.

10. Sodium sulfate, reag. grade, anhydrous.

NOTE: Contaminant peaks are sometimes observed from this material. Each batch should be checked by injecting a benzene extract into the gas chromatograph.

11. Cysteine Solution - Dissolve 1.00 g cysteine hydrochloride, 0.775 g sodium acetate. (3 H_2O) and 12.5 g anhydrous Na_2SO_4 in distilled H_2O and dilute to 100 ml. Prepare fresh every 3 days.

12. Mercuric Chloride Solution - Dissolve 5.0 g $HgCl_2$ and 17.0 ml conc. HCl in distilled H_2O and dilute to 100 ml. Shake 4 times (3 min. each) with 50-ml portions of benzene to remove impurities.

13. Hydrochloric acid, conc., reagent grade.

14. 6.0 N Hydrochloric acid - Prepare by diluting 185 ml conc. HCl to 1 liter with dist. water.

    NOTE: It has been found that reagent grade HCl may occasionally be contaminated with materials that interfere with the chromatographic determination of CH_3HgCl. It is suggested that the acid be triple washed with equal volumes of pesticide quality benzene in a sep. funnel.

15. Column packing - 5% HIEFF-10B (phenyldiethanolamine succinate). Available from Applied Science Laboratories, Stock #09120. Coat on 80/100 mesh Chromosorb W, H.P. After the 6-ft. column is packed, conditi conditioning should be carried out as follows: Place in conditioning oven in the manner described in Section 4,A,(2), raise the temperature to 210°C and apply a carrier flow of ca 25 ml/min. Maintain conditioning for 4 days. During this period, the organic mercury mixture (Subsection IV,6) is injected into the column in three sessions during each day, each injection session consisting of three 10 µl injections spaced one or two minutes apart. Therefore, at the end of the 4-day period, the column should have received a total of 36 injections of the mixture (see MISC. NOTE 1).

V. PROCEDURE:

All glassware used in the procedure shall, after washing in the usual manner, be rinsed successively with NH_4OH, distilled water and ethanol (see MISC. NOTE 2). The following procedure, unless otherwise noted, is intended for fish containing 0.1 ppm or more of CH_3Hg:

Extraction and Partitioning

A. Fish, Blood & Brain

1. Weigh 10 grams of sample into 50-ml homogenization cup. Measure 55 ml of dist. water in a 100-ml grad. cylinder and add a sufficient amount to just cover sample. Homogenize at medium speed for 3 minutes.

2. Transfer sample to a 250-ml sep. funnel rinsing with the water remaining in the cylinder. Add 14 ml of conc. HCl, 10 grams of NaCl and mix.

   NOTE: In the analysis of red blood cells, plasma or brain, add at this point 2 ml of the impurity-free $HgCl_2$ solution (Subsection IV,12).

3. Add 70 ml of benzene, stopper funnel and shake vigorously for 5 minutes (a reciprocating mechanical shaker is preferable if available).

4. Transfer sample to a 250-ml centrifuge bottle, cover with foil and centrifuge at 2000 r.p.m. for 10 minutes.

   NOTE: The initial 10-minute centrifuging may not adequately separate the aqueous-organic phases. The solid material at the organic-aqueous interface must be quite compact to allow easy removal of 50 ml benzene. If recentrifugation is necessary, stir the sample vigorously with a glass rod before centrifuging. Should the benzene layer appear cloudy, centrifuge for an additional 10 minutes.

5. With a vol. pipet, carefully transfer 50 ml of the benzene layer into a 60-ml sep. funnel. Take care not to disturb the solids collected at the benzene-water interface.

6. Add 6.0 ml of the cysteine solution to the 60-ml sep. funnel, stopper and shake vigorously for 2 minutes.

   NOTE: To increase the limit of detectability, the volume of cysteine solution should be reduced to 1.2 ml, applied with a 2-ml Mohr pipet. Further modifications in this connection are given in NOTE form below.

7. Allow to stand 10 minutes, then drain aqueous layer emulsion into a 35-ml centrifuge tube. Centrifuge at 3000 r.p.m. for 20 minutes. If emulsions persist, stir vigorously with a glass rod and repeat centrifuging as necessary.

8. With a volumetric pipet, transfer 2 ml of the cysteine solution from the centrifuge tube to a 30-ml sep. funnel. Add 1.2 ml of 6N HCl and 4.0 ml benzene, stopper and shake vigorously for 2 minutes.

NOTE: For lower detectability levels, transfer 0.80 ml (or as much as possible to remove and measure) of the cysteine solution, then reduce the addition volumes of 6N HCl and benzene to 0.6 and 2.0 ml, respectively.

B. Procedure for Urine

1. Pipet 15 ml of urine into a 60-ml separatory funnel. Add conc. HCl until a pH of 1 is obtained, and mix slowly to avoid loss of sample due to foaming.

2. Add 30 ml of benzene and shake vigorously for 5 minutes (a reciprocating mechanical shaker is preferable if available).

3. Transfer sample to a 100-ml centrifuge bottle, cover and centrifuge at 2000 r.p.m. for 10 minutes.

4. Volumetrically transfer 25 ml of the benzene layer to a 60-ml sep. funnel.

5. Add 5 ml of cysteine solution to the 60-ml sep. funnel, stopper and shake vigorously for 2 minutes.

6. Drain aqueous layer emulsion into a 35-ml centrifuge tube. Centrifuge at 3000 r.p.m. for 10 min. If emulsions persist, stir with a glass rod and repeat centrifugation as necessary.

7. Carefully transfer 4 ml of the cysteine solution to a 30-ml sep. funnel. Add 1.2 ml of 6N HCl and 5 ml of benzene, stopper and shake vigorously for 2 minutes.

8. Allow to stand for 10 minutes, discard aqueous layer and dry benzene by adding a pinch of anhydrous Na_2SO_4 prior to GLC.

Gas Chromatography

Set column temperature at 170°C and adjust carrier flow to 120 ml per minute. Set attenuator to obtain a peak height of 60-80% f.s.d. resulting from the injection of 1.6 ng of methyl mercuric chloride in benzene. If column is properly prepared and conditioned, the peak should be of reasonable symmetry with minimal tailing as illustrated in Figure 1. Make a sufficient number of repetitive standard injections to assure response reproducibility before making sample injections.

It has been observed that all methyl mercury salts elute at the same retention time. These include the Cl, Br and I salts of methyl mercury, as well as Panogen (methyl mercury dicyandiamide).

VI. CALCULATIONS:

If 50 ml of benzene and 2.0 ml of cysteine are recovered during the procedure for 10 g samples, the amount of sample per ml of final benzene solution is 0.595 g. This calculation is as follows:

$$g/ml = A \times \frac{C}{B} \times \frac{E}{D} \div F \quad \text{where}$$

A = of sample analyzed.

B = ml benzene added (normally 70 ml).

C = ml benzene recovered (normally 50 ml).

D = ml cysteine solution added (normally 6.0 ml).

E = ml cysteine solution recovered (normally 2 ml).

F = ml benzene added for final partition (normally 4.0 ml).

This calculation may be applied to all variations of the CH_3HgCl method. It is readily apparent that sample size affects the limit of detectability; however, the analysis of samples larger than 10 g is not recommended. Results in terms of ppm CH_3HgCl can be converted to ppm Hg by multiplying by a conversion factor of 0.80.

VII. MISCELLANEOUS NOTES:

1. Obtaining and maintaining acceptable gas chromatography of methyl mercury is difficult. Chromatography has been tried on the follow-

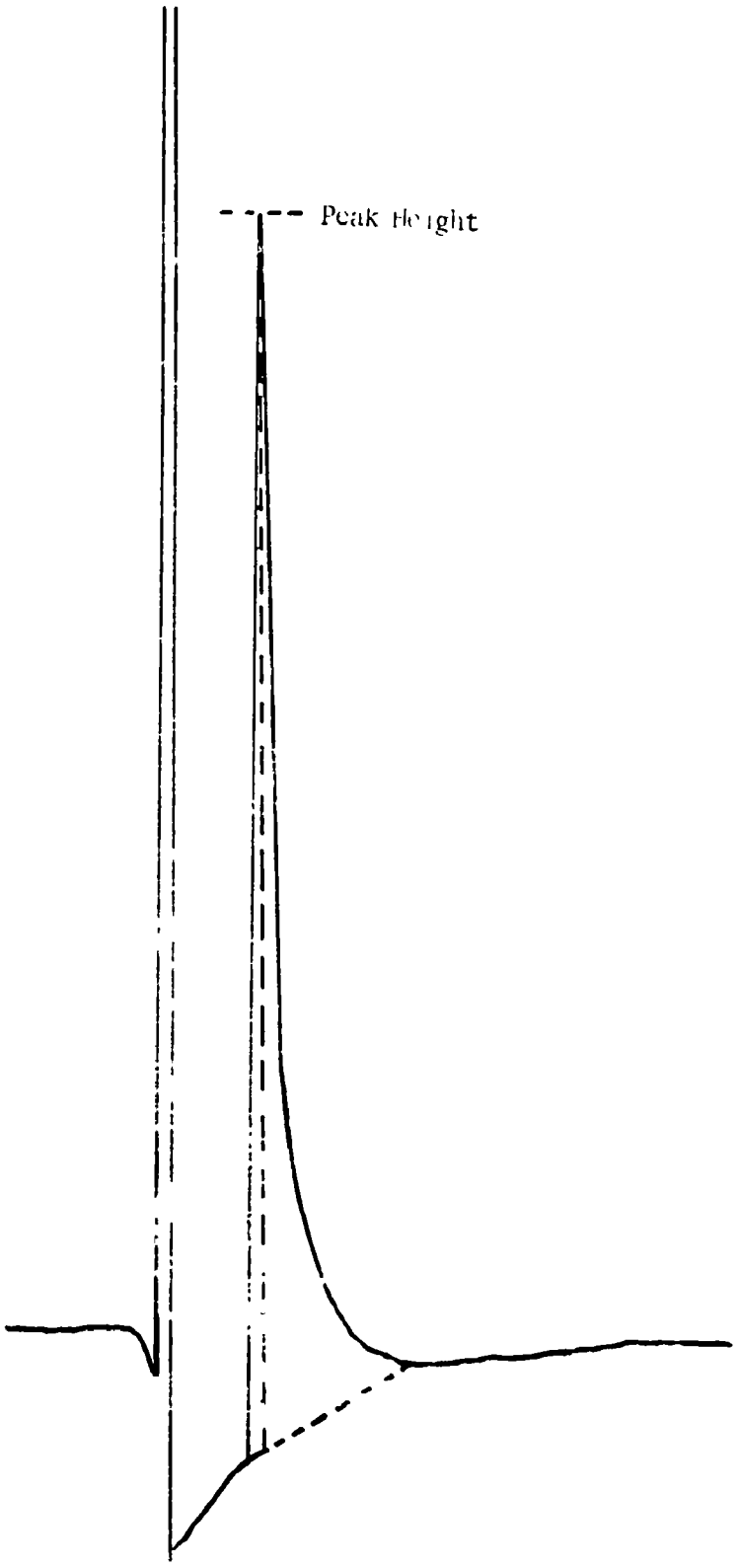
ing liquid phases: 10% Carbowax 20 m, 20% Carbowax 20 m, 15% DEGS (stabilized), 5% HIEFF-10B [Phenyldiethanolamine Succinate (PDEAS)]. Acceptable chromatography was not obtained on a 15% DEGS column. To date 5% HIEFF-10B has produced the best chromatography. Proper pre-conditioning of the column is very critical. Provided that column is properly conditioned, it should be possible to obtain a peak of at least 50% f.s.d. resulting from the injection of 0.150 nanograms of CH_3HgCl. If the sensitivity should fall short of this, further conditioning with repeated injections of the concentrated mixture may be indicated.

2. Glassware such as pipets which come in contact with fairly concentrated solutions of CH_3HgCl should be rinsed with NH_4OH, distilled H_2O, and C_2H_5OH, but it is probably not necessary to rinse the rest of the glassware with anything but distilled H_2O.

3. Benzene solution of CH_3HgCl cannot be concentrated without significant loss of CH_3HgCl. Limit of detectability is therefore restricted, as discussed above in Subsection VI.

4. The analysis should not be stopped overnight at any point except when the sample is in benzene solution. Prolonged contact with cysteine solution results in low recoveries.

Figure 1. Typical chromatogram of 1.6 ng of CH_3HgCl on 5% HI-EFF-10B at 170°C. and carrier flow of 120 ml/minute.



5. Masterflex Pump: With electronic speed control. Any peristaltic pump capable of delivering 1 liter of air per minute may be used.

6. Flowmeter: Capable of measuring an air flow of 1 liter per minute.

7. Aeration Tubing: A straight glass frit having a coarse porosity. Tygon tubing is used for passage of the mercury vapor from the sample bottle to the absorption cell and return.

8. Drying Tube: 6" x 3/4" diameter tube containing 20 grams of magnesium perchlorate. The apparatus is assembled as shown in Figure 1.

   NOTE: In place of the magnesium perchlorate drying tube a small reading lamp with 60W bulb may be used to prevent condensation of moisture inside the cell. The lamp is positioned to shine on the absorption cell maintaining the air temperature in the cell about $10^{o}C$ above ambient.

9. Bottles, B.O.D., 300 ml, with ℨ glass stoppers. Fishers #2-926, or the equivalent.

10. Pipets, transfer - 1, 2 and 5 ml.

11. Pipets, measuring - 1,2 and 5 ml.

IV. REAGENTS:

1. Sulfuric acid, conc., reagent grade.

2. Sulfuric acid, 1.0 N. Dilute 28.0 ml of conc. H_2SO_4 to 1000 ml with distilled water.

3. Sulfuric acid, 0.5 N. Dilute 14.0 ml of conc. H_2SO_4 to 1000 ml with distilled water.

4. Nitric acid, conc., reagent grade, of zero or low Hg content.

   NOTE: If a high reagent blank is obtained, it may be necessary to distill the HNO_3.

5. Stannous sulphate, reagent or purified grade. Add 25 g of the reagent to 250 ml of 0.5 NH_2SO_4. This mixture is a suspension and requires continuous stirring during use.

   NOTE: Stannous chloride or hydroxylamine hydrochloride may be substituted for the stannous sulphate.

6. Sodium chloride - hydroxylamine sulfate solution. Dissolve 12 g of each compound in distilled water and dilute to 100 ml.

7. Potassium permanganate, cryst., reag. grade. Dissolve 5 g in 100 ml of distilled water for a 5% w/v solution.

8. Potassium persulfate, reagent grade. Dissolve 5 g in 100 ml of distilled water for a 5% w/v solution.

9. Mercuric chloride, reag. grade, cryst., A.C.S.

   a. Stock mercury solution - dissolve 0.1354 grams of $HgCl_2$ in 100 ml of 1.0 N H_2SO_4. 1 ml = 1 mg Hg.

   b. Working mercury solution - Make two successive 1/100 dilutions of the stock solution to obtain a final working standard containing 0.1 µg per ml.

      NOTE: The stock solution is relatively stable and may be held, tightly stoppered, for periods up to a month. The working solution should be prepared weekly.

V. PROCEDURE:

Calibration

1. Transfer 1, 2 and 5 ml aliquots of the working Hg solution to 300-ml BOD bottles and add distilled water to each bottle for a total volume of 100 ml.

   NOTE: In additional BOD bottle, add 100 ml of distilled water and carry through all successive steps as a reagent blank.

2. Stopper and shake bottles vigorously for complete mixing.

3. Add 5 ml of conc. H_2SO_4, mix by swirling and then 2.5 ml of conc. HNO_3 to each bottle and, without stoppering, mix gently by swirling.

   NOTE: Loss of Hg may occur at elevated temperatures. However, with the specified volumes of acids, the temperature should rise only 13°C and no Hg losses have been observed under these conditions.

ANALYTICAL QUALITY CONTROL

INTERLABORATORY

I. BACKGROUND:

The term "quality control" may connote to some a system of controlling the quality of a manufactured product. Truly, the phrase is widely applied in this role, but it also can be aptly applied to a "level of performance". It is in the latter sense that the term is applied in the chemical analysis for pesticide residues. A program of quality control is a means of assuring the output of reliable and valid analytical data.

There are basically two general types of analytical quality control, INTERLABORATORY and INTRALABORATORY. The former term indicates the involvement of two or more laboratories, providing a mathematical basis for the confidence placed in the results of sample analyses and an insight into analytical areas needing attention. An intralaboratory program provides some systematic guidelines within a laboratory to assist in the prevention of a sizable amount of inaccurate analytical data.

II. THE INTERLABORATORY PROGRAM:

The prime objectives of this program are:

A. To provide a measure of the precision or reproducibility of methods of analysis.

B. To measure the precision and accuracy of analytical results between laboratories.

C. To identify weak methodology.

D. To detect training needs.

E. To upgrade the overall quality of laboratory performance.

The Perrine Technical Services Section functions as the coordinating unit for an interlaboratory quality control program which has been operating since 1966. The program takes in all the Community Pesticide Studies and National Monitoring Laboratories, operating under contract with the United States government to conduct analytical chemical monitoring for pesticidal residues in man and in the environment.

The quality control program includes the following areas of activity:

A. An interlaboratory check sample program.

B. The operation of an electronic facility for the repair and calibration of laboratory instruments.

C. A repository to provide analytical grade pesticide standards.

D. Providing uniform analytical methods.

E. Furnishing materials of uniform standard quality such as precoated GLC column packings and cleanup adsorbent.

F. Providing abbreviated, informal on-the-job training for specific requirements.

G. Assisting with problems related to analytical methodology by phone, mail or on-site consultations.

The services provided by above items B, E and F are confined to laboratories within the U. S. Environmental Protection Agency and those under contract with E.P.A.

The pesticide analytical standards repository currently stocks about 400 standards which are available to federal, state, and municipal laboratories, U. S. universities and international agencies such as World Health Organization. The standards are listed in a separate manual available on request to qualified recipients.

Currently, 35 laboratories participate in the interlaboratory check sample program (Item A above). Sample materials are selected which relate to the routine work of the majority of the participants. Substrates which have been used include fat, blood, brain, liver, standard pesticide mixtures in solvent, and simulated air samples (ethylene glycol spiked with pesticides at concentration levels found in actual air samples).

After selection of the substrate, a bulk sample is thoroughly homogenized and transferred to the vials in which the samples will be mailed to participants. One of the Perrine chemists with experience in the appropriate analytical method then runs a series of four or five analyses, in duplicate, using the GLC detection mode(s) which will be suggested to the participants. Confirmatory techniques such as TLC, p-values, derivatizations are applied as required for assurance of compound identifications. All data are examined to establish potential reproducibility of analysis from vial to vial and to determine approximate quantitative values.

When samples are mailed, a covering letter is sent to all participants providing any special instructions for handling the sample.

Generally, the time allocated for analyzing and reporting results approximates that which would be considered normal for processing a routine sample. The participants are requested to report their results on forms supplied. The supplemental data requested includes information on sample size, extent of sample extract concentration, all instrumental operating parameters, identity of GLC columns used, and numerical data upon which quantitative calculations are based. The participants are requested to include with their report all pertinent chromatograms, fully identified so that material on chromatograms may be easily related to numerical data on report forms.

When the report from each laboratory is received at Perrine, the quantitative results are entered on a SUMMARY OR RESULTS sheet. If any result appears to be completely erroneous, the laboratory is contacted at once and requested to review their work and advise us promptly if they see fit to change their reported value. There have been occasional instances of a misplaced decimal point or failure to apply a divisor or multiplier dilution factor, or simply a transcription error. When all laboratory results have been recorded, the statistical workup is made, computing the mean standard deviation and relative standard deviation for each pesticide reported by the majority of the laboratories. A relative performance or ranking chart is then made up establishing a numerical ranking level for each laboratory. This is based on (1) extent of deviation of each reported pesticide from the calculated mean (in the case of spiked sample, the formulation value is the base of reference), (2) false identification of compounds not present in the sample, and (3) failure to identify compounds known to be present.

Upon completion of the performance calculations, the data sheets and chromatograms of the laboratories demonstrating relatively poor performance are subjected to detailed examination to try and determine the potential factors responsible for the relatively inferior performance. A detailed critique is then written, each such laboratory is contacted by phone to immediately apprise them of their performance problem, and to make such suggestions as may be appropriate so that corrections can be initiated at once.

The reports from all the rest of the laboratories are then scanned for any irregularities that might lead to future problems. A general letter is drafted, a copy of which is mailed to all laboratories. In this letter, mutual analytical difficulties experienced by several laboratories are discussed, and suggestions are offered which appear to apply broadly. Included with the letter to all laboratories are (1) a copy of the SUMMARY OF RESULTS with each laboratory identified by code number, (2) a copy of the RELATIVE PERFORMANCE TABLE, and (3) a private critique to each laboratory where indicated.

From information obtained from the interlaboratory check sample program, a number of actions have been taken towards standardization. For example, bulk lots of Florisil are purchased on specification directly from the manufacturer for distribution in smaller units to the laboratories; similarly, bulk lots of GLC column packing are purchased on very rigid specifications; standard analytical methods have been distributed and are updated frequently.

The overall success of the quality control efforts is well illustrated in the following tables. Of greatest significance is the continuing improvement in interlaboratories precision over a four-year period.

Tables showing progression of interlaboratory precision from 1968 thru 1972:

FAT ANALYSIS

Interlab Check Sample Number	Year	Number of Labs	Number of Compounds	Mean Rel. * Std. Dev. %
9	1968	21	7	38
11	1969	19	7	24
21	1972	16	7	19

BLOOD SERUM ANALYSIS

6	1968	22	6	36
10	1969	20	5	29
16	1970	22	4	21
17	1971	20	4	17
22	1972	17	4	14

* Formerly called "Coefficient of Variation"

ANALYTICAL QUALITY CONTROL

INTRALABORATORY*

There are a number of factors in every laboratory of which any one or combination, if not carefully considered, can lead to inaccurate analytical data. This discussion is intended to highlight a number of these factors and to suggest a program for the avoidance of these pitfalls. There is no intent to dwell on any specific points of methodology in the preparative or determinative areas of analysis as these points are covered in detail in the appropriate section of this manual. Some of the more important broad factors include:

1. Sensitivity and accuracy of analytical balances.
2. Purity of reagents such as solvents, distilled water, Na_2SO_4, NaCl, compressed air or nitrogen, etc.
3. System of cleaning glassware.
4. Accuracy of analytical standard solutions.
5. Condition of gas chromatograph and accessories: recorder, electrometer, detector, carrier gas system, columns and heating components-tempsets, variacs, programmers.
6. Deviations from analytical methods adopted for use in program.
7. Demonstrated capability of personnel to obtain satisfactory recoveries.
8. Training and experience of personnel.

A. ANALYTICAL BALANCES:

In the pesticide laboratory, the most critical weighings will be those of the primary analytical standards. Obviously, if the balance is not sufficiently sensitive and precise, the accuracy of the final standard solutions will be open to question. The balance should be capable of sensitivity of 0.05 mg and should be checked no less than yearly by a qualified balance specialist.

B. PURITY OF REAGENTS, SOLVENTS AND DISTILLED WATER:

This is an area of extreme importance, particularly in those laboratories working with substrates having residues in the parts per billion range. Reagents or solvents from certain manufacturers are often found to yield excessive background contamination whereas those from other manufacturers yield minimal background. In general, pesticide

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*Some of the procedural instructions written specifically for a given network of laboratories.

quality solvents, distilled in glass, will give the least trouble. However, each lot from any source should be checked before use, evaporating a portion by as great a concentration factor as would ever be employed in any method for which the solvent is used. See Section 3, C.

Any reagent or material used in a preparative method is a potential source of contamination which can result in extraneous chromatographic peaks. It is good practice and requires little time and effort to soxhlet extract such materials as sodium sulfate, glass beads, NaCl, glass wool, etc., using as the extracting solvent(s) that or those used in the method.

Reagent blanks must be run constantly for each of the analytical procedures, with final extracts being reduced to the same concentration level normally used for the sample material.

Distilled water can be troublesome, particularly in a procedure where a large volume is used. Such a procedure is the Mills, Onley, Gaither cleanup method for adipose tissue where 700 ml of water is used in the acetonitrile partitioning step. The final extract concentration may be as little as 5 ml so that a potential contaminant concentration factor of 140 x must be considered. Since the source of extraneous peaks in laboratory water is organic in nature, distillation, even though an all-glass still, is not necessarily sufficient cleanup. Co-distillation of organic material with water is a well documented phenomenon. The use of an activated charcoal filter just before the distillation procedure has been found to significantly improve the water quality. If deionization of the water is desirable, the charcoal filter should be installed between deionizer and the distillation equipment for trapping the organic materials eluting from the polystyrene matrix of the deionizing column before the water enters the still.

C. CLEANING OF GLASSWARE:

There are a few areas of analytical chemistry where this operation is any more critical than in the residue laboratory. This job is frequently done by lower grade personnel with little or no technical training. Therefore, the responsibility falls on the chemist to maintain close control over cleaning procedures to be certain that the glassware will be entirely free of contamination. In general, the cleaning operation should include (1) soaking and washing in high temperature bath of synthetic detergent in water, (2) rinsing with tap water, (3) rinsing with distilled water, and (4) rinsing with acetone. In the cleaning of glassware to be used in sample concentrations, (such as evaporative concentrator tubes) the tap water rinse should be followed by a soak in hot chromic acid cleaning solution. This step insures removal of any traces of organic material. This soak is followed by thorough rinsing with tap and distilled water, and finally with acetone and hexane.

H. TRAINING, EXPERIENCE AND RESPONSIBILITIES OF PERSONNEL:

A number of the routine functions of the residue laboratory can be carried out by a technician who has been given careful on-the-job training by the chemist. This relieves the chemist to devote his efforts to the more complex problems of instrumentation and interpretation.

In the small laboratory of three to six people, the responsibility rests with each chemist to sufficiently acquaint himself with his analytical instruments to perform routine adjustments and preventive maintenance. Such duties as module replacements, replumbing and simple servicing should be considered as the normal responsibility of the chemist. The more complex electronic servicing is generally the function of the electronics specialist.

The laboratory administration must recognize the need and importance of continuing training for the residue chemist. There are few areas of analytical chemistry more complex or challenging than that of pesticide residue analysis of biological or environmental media. Any skilled analyst appreciates all too well that the pathways are liberally strewn with booby traps. In the administration of the interlaboratory check sample program over the years, many instances have been observed where the analyst was obviously not sufficiently trained to recognize certain pitfalls. A few large laboratories may have sufficient personnel to conduct in-house training programs. However, the average smaller laboratory of three to five people cannot divert personnel to conduct the type of intensive training required. Furthermore, technological advances in instrumentation dictate the need for even the most skilled chemists to take refresher courses from time to time.

I. SUMMATION:

The type of program discussed above requires an expenditure in man hours and/or money. Total work output is somewhat reduced. The question should rightfully be asked "Is it worth the effort?" This may be answered in terms of the type of pesticide laboratory involved. In a laboratory primarily concerned with law enforcement, the analytical chemist may be called at any time as an expert witness to defend in court the accuracy and precision of his work on a given sample. Revelation of the lack of an active program of analytical quality assurance provides the opposition with valuable ammunition for discrediting the laboratory's case.

Pesticide laboratories concerned with the development of analytical

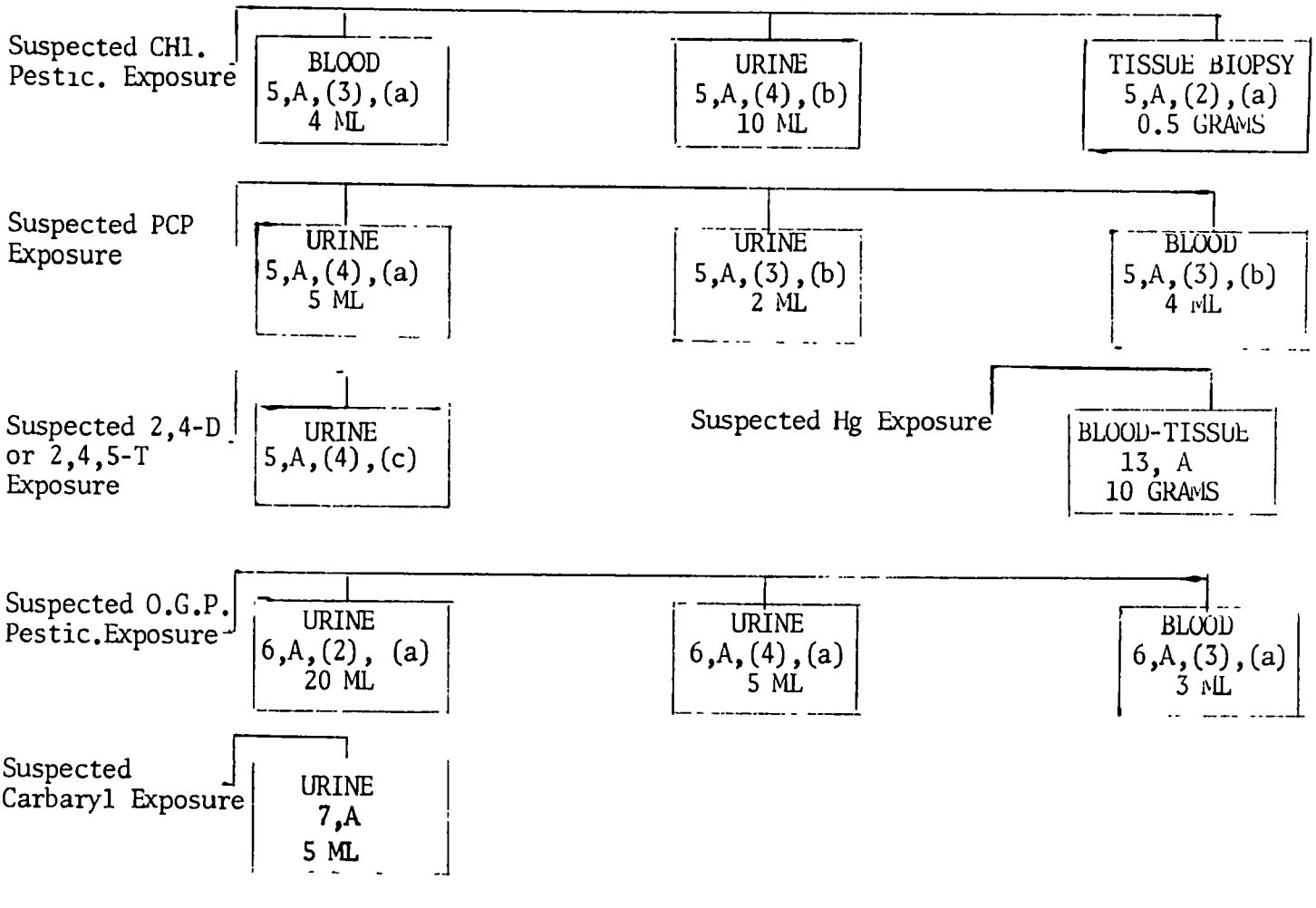
data of a survey nature have a pressing responsibility to insure quality analytical performance. Generally, such laboratories are part of a network feeding data to a central point for processing. Erroneous data from one or more of the component laboratories of the network can lead to false conclusions concerning the pesticide profile of the sample material in the areas assigned to those errant laboratories. For example, let us assume that a laboratory assigned to the analysis of general population blood in a given area is consistently, but erroneously, finding higher pesticide levels than is being reported by laboratories in other areas. Plotted on a national scale, it would then appear that the people of this area contain higher levels of pesticides than the rest of the national population.

We think it appropriate to reiterate here the importance of periodic checking of pesticide profiles in substrates by confirmatory techniques. The reporting of pesticides not actually present is just as misleading as failure to identify pesticides which are present.

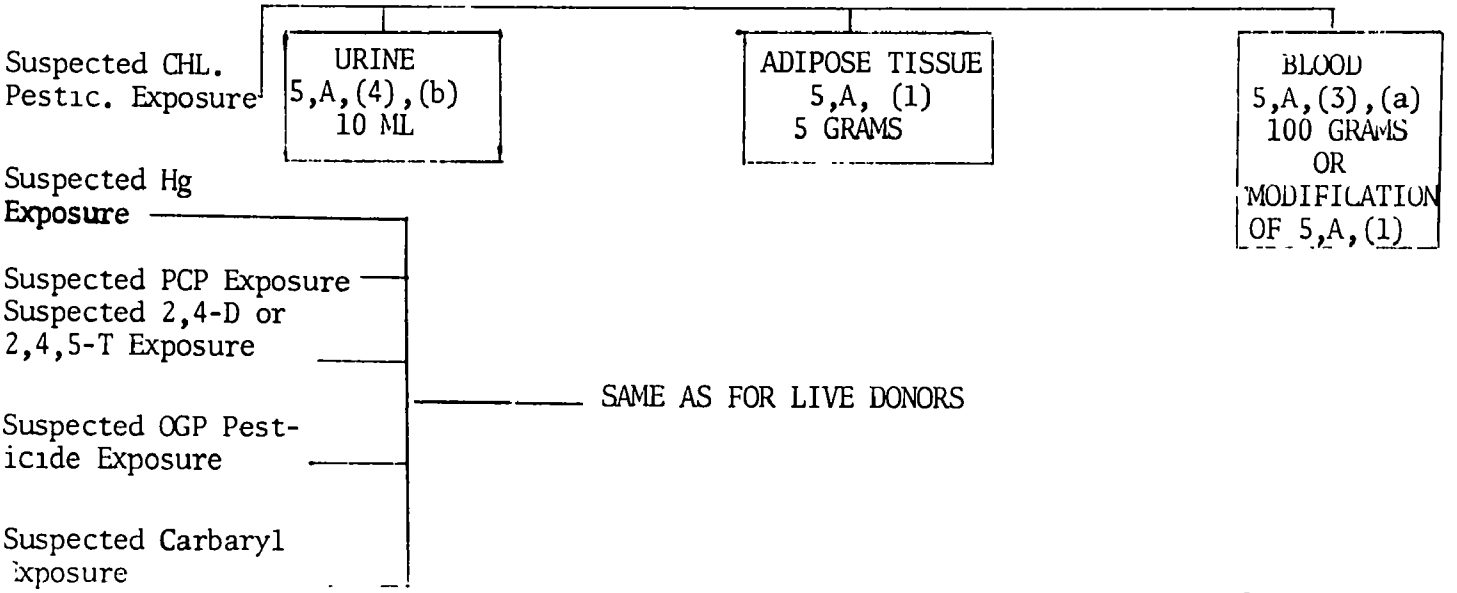
A properly functioning intralaboratory quality control program greatly reduces the possibility of any laboratory straying into a pattern of continuing error since the periodic recovery checks serve to flash a warning. The lack of such a program inspires little confidence in the routine data from any laboratory. When the analytical work program of a laboratory is planned, definite consideration must be given to the allowance of sufficient time to conduct a quality assurance program. A low total output of reliable analytical data is at least useful. An impressively high volume of uncontrolled data, however, is worse than none at all because of the distorted and misleading profile that could result.

TENTATIVE TISSUE, EXCRETA AND METHOD SELLCTION FOR ABNORMAL PESTICIDE EXPOSURE CASES; BLOCK DIAGRAM

LIVE DONORS



AUTOPSY SAMPLES



Sample size given represents a maximum, number and letter designations refer to method number as listed in the TABLE OF CONTENTS.

PESTICIDE ANALYTICAL STANDARDS

The Perrine Primate Laboratory operates a repository for pesticide reference standards. The current standards index lists approximately 400 pesticidal compounds which are available in limited quantity to governmental, university, and commercial laboratories holding government contracts for pesticide residue analyses.

The complete standards index, recently revised, is available to qualified recipients and a copy may be obtained by writing to:

Technical Services Section
Perrine Primate Laboratory
P. O. Box 490
Perrine, Florida 33157