# Analysis of Chlorinated Hydrocarbon Pesticides in Waters and Wastewaters

## Methods in Use in Water Quality Division Laboratories

A.S.Y. CHAU

INLAND WATERS BRANCH
DEPARTMENT OF THE ENVIRONMENT

CANADA

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# *Preface*

This manual outlines methods, procedures and techniques used in the Water Quality Division laboratories at Ottawa, Burlington, Moncton, and Calgary, for the analysis of chlorinated hydrocarbon pesticides in waters and wastewaters. The capability to measure the concentration of these pesticides in fish, mud and sediments has also been developed; these methods and procedures are not reported in this manual, but will be included in subsequent issues.

Published methods for pesticide-residue analysis were used extensively but modified for determining pesticides in waters and wastewaters; even while this manual was being written new procedures and techniques were being developed and put into use. New developments will be reported in later editions. It is intended to update this manual periodically in order that the newest methods are supplied to the Division's laboratories and other laboratories using these methods approved by the Water Chemistry Subdivision.

In conjunction with the commonly used gas-liquid-chromatography (GLC) and thin-layer-chromatography analysis, chemical derivation techniques followed by GLC examination of the derivatives are used extensively to confirm pesticide identities.

The manual "Methods for Chemical Analysis of Waters and Wastewaters in use in the Water Quality Division Laboratories", by W.J. Traversy, describes procedures for determining major ions, trace metals, nutrients and certain other parameters; this manual is also intended as a convenient working document containing all precedures, techniques and methods for use by laboratory staff.

Suggestion and comments to improve the usefulness of this manual would be appreciated.

J.P. Lively, Head,
Water Chemistry Subdivision

# *Introduction*

The possibility of contamination of water supplies from the use of pesticides has become a matter of major concern in recent years. The danger of contamination can arise from careless application of the pesticides, or from accidental or natural leaching from sprayed areas. In view of the stability and liability of many of these pesticides to long-term accumulation in our environment, organochlorinated hydrocarbons (o.c.) have attracted much attention. In order to assess and monitor the general levels of pesticide residues in our environment, sensitive and accurate analytical methods are needed to provide the necessary information. This manual describes methods used in laboratories of the Water Quality Division, Department of the Environment, for the analysis of some organochlorine pesticides in water.

Continual efforts are being made towards improving and refining the methods described in this manual; revisions will be added in future editions. It should be emphasized that the methods outlined in this manual serve only as guidelines; no universal methods exist that could be applied to samples from various sources and with different combinations of pesticides. As pointed out by Askew *et al* 1969, "the nature of co-extractives will undoubtedly vary with the location from which the samples are taken, and no completely comprehensive clean-up procedure" and analytical methods are available to meet with the problems encountered in each sample. Therefore, the individual analyst is encouraged to modify these methods in order to suit a particular situation.

The general approach to the analysis of pesticides encompasses some or all of the following steps:

1) Extraction of the sample.

2) Clean-up of the extract to eliminate or minimize co-extractives which often cause interference in the interpretation of the result. This is achieved by: (i) liquid-liquid partitioning and/or (ii) clean-up by absorption column chromatography or thin-layer chromatography (TLC) or both.

3) Gas Chromatographic (GLC) analysis and tentative identifications of peaks.

4) Confirmation by:

   a) multi-GLC column technique (Goulden, Goodwin, and Davies, 1963).

   b) thin-layer chromatography (TLC).

   c) chemical methods, wet (Sec. 4) or dry (Minyard and Jackson, 1965; Miller and Wells, 1969).

   d) spectroscopic analysis: mass spectrometry, infrared spectroscopy, nuclear magnetic resonance spectroscopy (NMR), and ultra-violet spectroscopy to a limited extent.

   e) physical methods: p-values (Bowman and Beroza, 1965).

   f) florisil column elution pattern – this fractionation step may be incorporated in the clean-up procedure.

Before describing procedures of actual o.c. analysis, it is advantageous to discuss related topics on pesticide analysis — such as purification of chemicals, solvents, and the awareness of sources of interferences. Understanding these topics will greatly assist the analyst in maintaining the reliability, accuracy, and reproducibility of analytical results. This manual was written with the technologist and the inexperienced analyst in mind. The manual is divided into ten sections, with the Appendix containing additional data and further readings to assist the worker in better understanding the difficult field of pesticide analysis.

# *Gas Chromatography*

The gas chromatograph, regardless of any special design, comprises four functional elements:

1. a carrier gas supply ($N_2$ for electron-capture gas chromatography using direct current),
2. injection port for the column, and
3. a detector connected to
4. the recorder.

The detector widely used for pesticide-residue analysis is an electron-capture detector (ECD), using either $^{3}H$ or $^{63}Ni$ as a radioactive source of β-particles. The $^{63}Ni$ detector, having a narrower linear dynamic range as compared to the $^{3}H$ detector, has the advantage of withstanding higher operation temperature and hence, has less chance of being contaminated. Since the column is the heart of a gas chromatograph, it is discussed in detail.

## 1.1. Column

The reports are numerous of different GLC column-packing materials for analysis. Basically, the liquid phase consists of QF-1, DC-11, SE-30, XE-60, DC-200, OV-17, etc., used singly or in combination. The solid supports are chromosorb W, gas chrom Q or Anakrom ABS, etc. Some of the columns used by other workers are summarized in the Appendix. The choice of column-packing materials depends on the type of sample being analyzed, the personal taste of the analyst, and the characteristics of the compounds to be analyzed. Generally, at least two columns of different polarity are used simultaneously for the analysis of one sample. This provides some preliminary confirmation of the identification of pesticides. It should be emphasized that even multiple-column chromatographic methods do not necessarily provide positive identity of an unknown pesticide (Robinson, 1967; Reynolds, 1968). The identity should be confirmed by a different test on the same sample (see confirmative tests, Sec. 4).

The relative polarity of some liquid phases is summarized below.

| Liquid Phase | Type | Polarity |
|---|---|---|
| SE-30 | Methyl silicone gum rubber<br>Silicone grease | non-polar |
| SE-52 | Silicone grease | intermediate |
| DC-11 | Silicone oil | non-polar |
| DC-200 | Silicone oil | non-polar |
| XE-60 | Cyanosilicone gum | intermediate |
| QF-1 | Fluorosilicone gum | fairly polar |
| OV-210 | Trifluoropropyl silicone | fairly polar |

Generally, the more polar the liquid phase, the less stable the column made from it. However, with the advent of the OV-series, this is not necessarily true.

Among some of the most widely used liquid phases (stationary phases) for routine application (i.e., SE-30, XE-60, QF-1), a column prepared from SE-30 has the longest life and is relatively unaffected by extraneous materials (Sissons, Telling and Usher, 1968). As a start, the widely used McCully and McKinley's column (1964), (Sec. 1.2.1.) SE-30 and QF-1 on chromosorb W and Burke and Giuffrida's column (1965) 10% DC-200 and 15% QF-1 on chromosorb Q, can be used. Although QF-1 has a relatively high bleed, it possesses good separation characteristics when used with SE-30. An attempt to replace QF-1 with a highly stable but similar characteristic liquid phase such as OV-210 is in progress.

## 1.2. Preparation of Column Materials

The importance of the preparation of column materials and column packing must be emphasized since a large part of success in gas-chromatographic analysis depends on the care taken in using proper techniques. There are several methods for the preparation of column materials (see Vol. I and II, Pesticide Analytical Manual, U.S. Department of Health and Welfare, Food and Drug Administration, U.S.A.). Two of these methods are described.

### 1.2.1. Mendoza's method (Mendoza, McCully, and Wales, 1968) for McCully and McKinley's column (1964).

Dissolve 0.8 gm SE-30 and 1.2 gm QF-1 in 20 ml ethyl acetate in a 500 ml round-bottom flask. Break the SE-30 into smaller pieces to hasten dissolution. With occasional swirling, gently heat the solution (Variac at 20-30V) for 1 hour or until the gum completely dissolves. Add chromosorb W, 80-100 mesh, (20 gm) to the silicone solution and *gently* reflux the mixture on a heating mantle for 4 hours. With periodic swirling of the contents, evaporate (Variac at 50) the ethyl acetate in a hood under a gentle flow of nitrogen. Gently tap the flask to dislodge adhering granules. Transfer the partially dried granules to a crystallizing dish and heat at 35°C until free of lumps. Complete the drying in an oven at 110°C for 2 hours. Precondition the coated material at 230°C for 4 hours before packing the column.

### 1.2.2. Semi-rotary evaporation method. (This method is modified from the standard rotary method and is also used in our laboratory for the preparation of GLC columns.)

Dissolve 1 gm of DC-11 and 1.5 gm of QF-1 in 140-150 ml (1+1) solution of chloroform and methylene chloride. After complete dissolution, add 22.5 gm chromosorb W (DMCS, acid washed) with occasional swirling and let the mixture stand for an hour. Evaporate the solvent using a gentle stream of nitrogen until the solvent just covers the slurry. Continue evaporation on a rotary evaporator (no vacuum) at about 40°C. Be sure to clamp the flask to the evaporator so it doesn't fall off. Periodically, disconnect the flask from the evaporator and swirl the contents gently in an irregular manner, gently tapping the flask to dislodge adhering granules. (The irregular swirling of the contents ensures thorough mixing, otherwise poor resolution and low sensitivity will result.) After the solvent is

totally evaporated, transfer the contents to an evaporating dish and heat at 110°C for 2-3 hrs. to complete drying. This material, free of lumps, is ready for packing into a column.

NOTE: If lumps were formed in the above process, gently remove them using a micro spatula. *Never break into pieces. Always handle the solid support very gently before and after coating or poor performance of the column will result.*

1.2.3. Column Packing

Our experience indicates that the popular vibration method, using a vibrator to pack columns, is hard to control and often produces columns with poor performance. The following methods used in this laboratory are recommended (Pesticide Analytical Manual, Sec. 1.2.).

a) *Suction method:* This method works well with coiled columns such as those in Varian Aerograph Hy-Fi models or Hewlett-Packard chromatographs; it is also satisfactory for U-shaped columns for Microtek 200. Using tweezers plug the exit end of the column with extra-fine silanized or preconditioned glass wool (explanation follows). By means of a rubber tubing, connect the exit end to the vacuum-release outlet A (Fig. 1). Dip the column outlet into an evaporating dish containing prepared column-packing materials. Turn on the aspirator, and by means of knob B adjust the vacuum to suck *gently* the column-packing material into the column. (Overly fast sucking will break some of the solid support.) Gentle tapping on the column may be necessary to settle the packing. After the column is packed, use tweezers to plug the outlet with silanized or preconditioned glass wool.

*Preconditioned glass wool (Pesticide Analytical Manual, Sec. 1.2.):* In a clean beaker, soak extra-fine, Pyrex-glass wool with pesticide-grade chloroform for a few minutes. Drain off excess solvent. Repeat this process two or three times. Heat the glass wool in 10% dimethyldichlorosilane in anhydrous hexane for 10 minutes. Rinse with anhydrous hexane and soak in anhydrous methanol for 10 minutes. Finally, rinse with methanol and dry in an oven at 100°C - 200°C.

b) *Gravitation method:* This method is suitable only for U-shaped or straight columns. Plug the exit end of the column with silanized-glass wool. Attach a funnel to the inlet (a commercial aluminum funnel for this purpose is available or it can be home-made with a small glass funnel and teflon tubing). Gently pour the packing material drop by drop through the funnel into the column in a 20-30° position to minimize breakage of particles as they fall to the bottom of the tube. Tap the column gently with a hard-rubber tubing or the eraser on a pencil to settle the packing. Add more packing material until the column is packed. Mild suction may be applied several times during packing to assist the packing material to settle evenly. After packing, plug the inlet end with approximately 2 or 3 inches of silanized or preconditioned glass wool for on-column injection. If a glass sleeve is used at the injection port, about 1/2 or 1 inch of glass wool is sufficient.

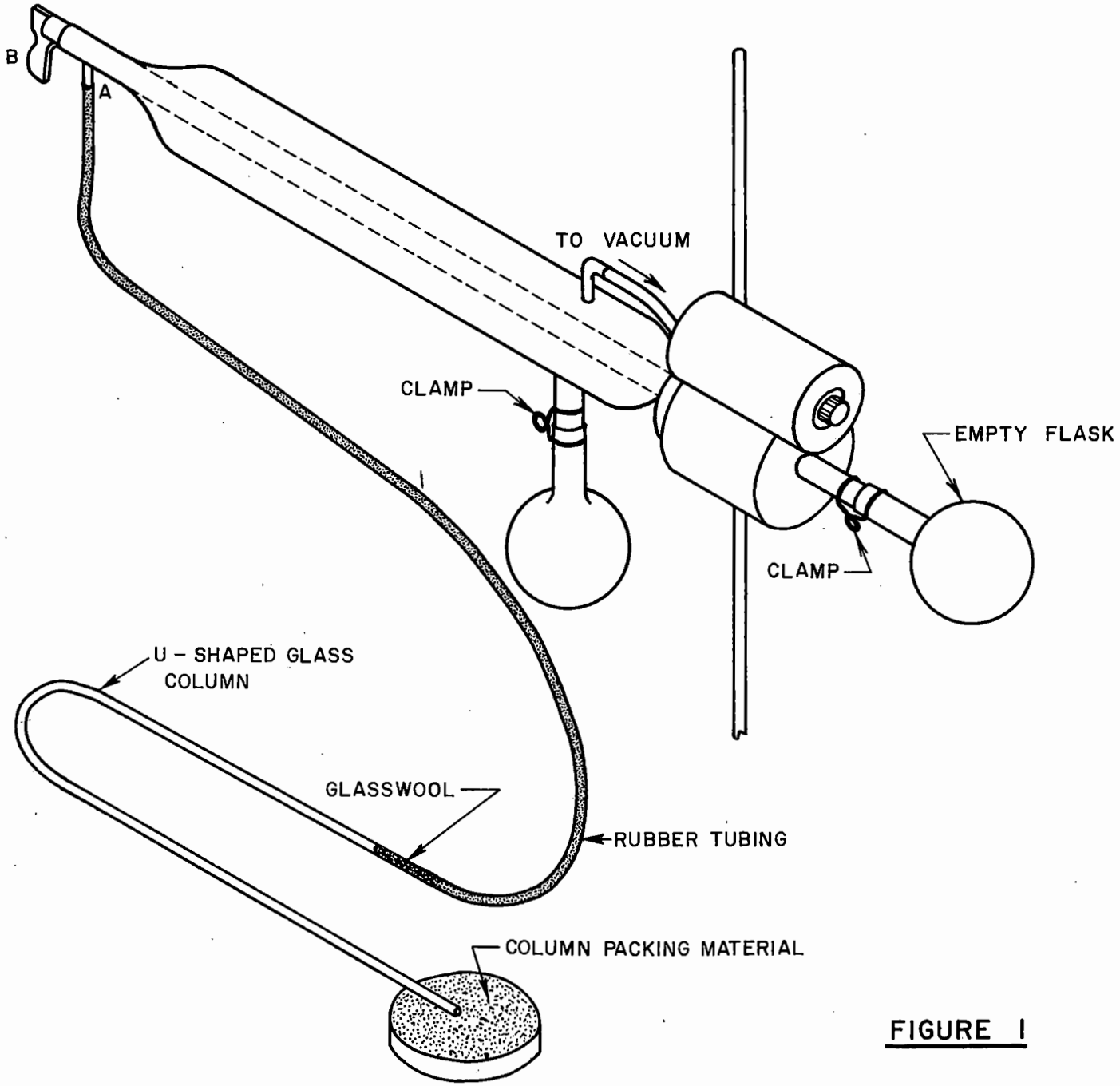
B
A
TO VACUUM
CLAMP
EMPTY FLASK
CLAMP
U – SHAPED GLASS COLUMN
GLASSWOOL
RUBBER TUBING
COLUMN PACKING MATERIAL

FIGURE I

### 1.2.4. Column Conditioning

With pure nitrogen flowing through the column, condition the column in the gas chromatograph *with detector disconnected* or in a column conditioner by heating the oven at 25-40°C above the operation temperature.

For a McKinley and McCully's column (1964) prepared according to the method of Mendoza, (McCully and Wales, 1968), conditioning over the weekend gives very good performance. (Mendoza's suggestion of only a few hours of conditioning, if this method is used, works well. However, it seems conditioning over the weekend gives even better performance). For DC-200 and QF-1 columns prepared by other methods, conditioning at 210°C-230°C for 4-7 days instead of the general recommended 24-78 hours period gives improved performance. All columns are conditioned with a nitrogen flow of 80-100 ml/min. The extended period of conditioning is particularly advantageous for coating materials such as QF-1 and DC-200 since these materials, particularly the polar QF-1, give relatively high bleeding. After conditioning by baking a column as described previously, the column is further conditioned by an injection of pesticide standards (1 mg/ml), types which are to be determined. (Be certain detector is disconnected). Repeat the injection up to 1-2 hours. Connect column to the detector and inject several picograms of standard of the same volume and observe if repeatable peak heights result. (This assumes that the analyst has already obtained good injection technique to reproduce his injections). If peak heights are not reproduced, disconnect the detector and repeat the injection of concentrated standard ( 1 µg/ml) as described previously. For polar compounds, particularly ones containing active hydrogen such as OH, SH etc., alternately injecting distilled water and concentrated solution will sometimes help.

Conditioning the column by the described method will give sharper peaks, higher sensitivity and better reproducibility of peak heights of pesticides, especially those eluants such as dieldrin, endrin, methoxychlor, and DDT. In some cases, even with this additional conditioning step, a column may require several weeks to function at its maximum efficiency for some pesticides - notably DDT, methoxychlor, heptachlor and some polar metabolites. The use of commercial column conditioners such as Silyl-8 should be discouraged for columns containing polar liquid phases such as QF-1 and carbowax.

### 1.2.5. Decomposition of Pesticide on Gas Chromatographic Columns

Minimum contact of the vaporized pesticides with metal surfaces is necessary to avoid decomposition of pesticides on GLC columns. Therefore, glass columns with glass sleeves at the injection ports, or an on-column injection technique should be used. Stainless steel columns are undesirable for the analysis of o.c. since some o.c. such as DDT are dechlorinated to DDD and may be dehydrochlorinated to DDE in metal columns. Endrin is also known to isomerize thermally to a ketone, particularly in a metal column. It should also be noted that such thermal isomerization of endrin also occurs in glass columns particularly in the presence of co-extractives. This phenomenon is also observed in many laboratories for example, Reynolds and Sans. As noted by Burke and Giuffrida (1964, 1965), the chromatography of endrin is also influenced by the solid support. Two peaks, always with the same resolution and size relationship, are obtained when chromosorb P is used in the column and the injection temperature is varied from 175°C to 250°C. With a well conditioned Anakrom ABS column, only one peak is observed. In view of the possible decomposition of pesticide occurring in gas chromatographic columns, the analyst should be aware at all times of any anomaly observed in the chromatograms.

1.2.6. Injection Technique

Injection syringes must be cleaned immediately after use. It has been found that a highly contaminated needle needs to be cleaned many times (sometimes up to 40 or 50 times) by drawing acetone through the top of the barrel and pushing the acetone out of the needle to another container. Periodically, soak separately a needle and plunger overnight in acetone or ethyl acetate. It should also be emphasized that since some new syringes give GLC responses, they should be thoroughly cleaned and checked for background interferences.

Before injection, the residue should be noted since it varies from needle to needle. Because this amount of liquid generally goes into the gas chromatographic system,* the apparent volume as indicated on the needle is more than that injected into the gas chromatograph. For example, if one wishes to inject 5 µl into the gas chromatograph, and the residual volume is 0.6 µl, one would withdraw the solution to be injected to 5 minus 0.6 µl, i.e., 4.4 µl mark instead of the 5 µl mark. There should be no air bubbles in the liquid. To check the volume, withdraw the solution slightly up into the barrel. The volume is then the difference at both ends of the solution on the marking on the barrel. Push the needle into the septum in the gas chromatograph and push the plunger immediately into the barrel. Hold the plunger for a few seconds before withdrawing the needle from the septum. Each analyst will develop his own injection technique. Regardless of which he chooses, the injection should be performed in a consistent manner in order to reproduce results.

* Whether the residual solvent in the needle is completely evaporated in the gas chromatograph depends on the injection port temperature and the time that the needle is allowed in the injection port.

SECTION 2

# *Interferences*

Every effort should be taken to eliminate the major problems of laboratory contaminations or interferences, especially when using ultra-sensitive analytical techniques such as electron-capture gas chromatography. In some cases, these interferences cannot be eliminated completely; nevertheless, the analyst should be aware of their presence and take them into consideration when interpreting analytical results. Co-extractives, indigenous to the samples, are additional interferences and often difficult to eliminate. Since the nature, number, and type of interference varies among samples collected from different areas, no completely comprehensive clean-up procedure can be recommended. Hence, the approach to the elimination of interferences present in a sample is left to the judgement and experience of the analyst; the following discussions are to serve as a guide in handling these problems.

## 2.1. Interferences from Laboratory Origin

The most common source of interferences in the laboratory originates from glassware which has not been cleaned properly. Chemicals and materials not properly pretreated also contribute to this problem. Periodic checking of the cleanliness of glassware and the suitability of chemicals used in analysis is necessary to ensure low blank GLC responses and to enable proper action to be taken to eliminate or minimize these problems. Some common sources of interferences are discussed below.

### 2.1.1. Anhydrous Sodium Sulfate

Interfering materials can be encountered from using analytical-grade sodium sulfate during the analysis (Burke and Giuffrida, 1964; Lamar, Goerlitz, and Law, 1966). This chemical should be thoroughly washed in sequence with pesticide-grade benzene and methylene chloride followed by heating overnight or longer at 300°C to 600°C in a *clean* oven. There should be an oven used strictly for pesticide residue work. Severe contamination from sodium sulfate can be experienced after heating the chemical in an oven that has been used for other work.

### 2.1.2. Distilled Water (Lamar, Goerlitz, and Law, 1966)

Water should be obtained from an all-glass distillation system. Generally, commercial distillation units are designed to give low electrical conductivity rather than low organic content; therefore, minor provisions are made to eliminate organic constituents in water. Furthermore, the valves, connections, and plastic piping in most commercial distillation apparatus increase the organic constituents and are common sources of contamination. Hence, the highest quality of distilled water obtained from a distillation unit with glass and teflon parts should be redistilled over chromic acid in all-glass system to obtain organic-free water. However, not all organic constituents are deleterious to the analysis. Depending on the type of distillation apparatus and the source of water, redistillation over chromic acid may not be necessary.

2.1.3. Plastic Wash-bottles and Apparatus

During analysis, avoid contact with any plastic materials. For example, polyethylene bottles are known to give GLC and TLC responses similar to DDT-type compounds (Burke, 1965; Mestres, Barthes and Priu, 1966; Van Valin, Kallman, and O'Donnel, 1963).

2.1.4. Plastic Screw-caps

Plastic caps and the cap-lining used on sample bottles and reagent bottles often cause contamination (Burke, 1965; Lamar, Goerlitz and Law, 1966). Use a teflon sheet to cover the mouth of these bottles before screwing on the cap. Prewashed heavy duty aluminum foil may also be used. Cut aluminum foil into appropriate squares, soak well in ethyl acetate, and wash by decantation. Repeat soaking and washing three times. Finally, rinse with hexane and dry in an oven. Avoid holding the foil by hand during the cleaning process since these solvents dissolve oils from the hand and may cause interferences.

2.1.5. Solvents

All solvents should be pesticide grade or redistilled over 2 gm/l of silver nitrate ($AgNO_3$) and anhydrous potassium carbonate (20 gm/l) in an all-glass system through a fractionating column. Also effective is purification by passing the solvent through activated aluminum or florisil (Thornburg, 1966; Beckman and Guer, 1967). Since commercial pesticide-grade solvents (also called nanograde, toxigrade etc.) vary from batch to batch and from one manufacturer to another, they should be checked each time a new supply is received. Some batches of so-called pesticide-grade solvents have several interfering peaks. Both commercial pesticide-grade solvents or laboratory purified solvents should be checked against possible interferences as follows:

> Place in a rotary evaporator an amount of solvent equal to that used in the analysis. Concentrate solvent to 1 ml in a rotary evaporator and inject 8 µl into the gas chromatograph at lowest attenuation possible. If the extraneous peaks observed are below 0.5% of recorder response, the solvent is satisfactory; otherwise, it should be repurified again by distillation or passage through an alumina or florisil column.

2.1.6. Glassware

All glassware should be washed as soon as possible after usage since it is more difficult to clean when dry. The following procedures serve as a guide.

a) For previously cleaned or new glassware to be used in the future:

   1) Fill glassware (e.g. flasks) to 1/3 volume with pesticide-grade ethyl acetate; stop with clean glass-stopper and shake vigorously for a few seconds. (If shaking is not possible, rinse several times with this solvent).

2) Rinse again with ethyl acetate using a smaller volume (about half the volume of first washing). Also rinse the rim of the mouth of the flask.

3) Without drying, shake flask vigorously with pesticide-grade hexane, using the same glass-stopper.

4) Dry glassware with pure nitrogen and put overnight or longer in a clean oven at 300°C.

5) Rinse dried glassware with another few mls of hexane before use.

NOTE: When using nitrogen to dry glassware, make certain the outlet is clean and made of glass – a clean disposable pipette attached to a teflon or nylon tubing will suffice. Do not allow the tubing to come into contact with the flask.

b) For dirty glassware which will be used immediately:

1) Dispose of extract and content.

2) Using soap and water, shake vigorously or rinse several times with agitation.

3) Rinse well with hot water.

4) Rinse with distilled water.

5) Wash twice with washing acetone.

6) Without drying, shake vigorously with ethyl acetate (pesticide grade). Repeat.

7) Without drying, shake vigorously with hexane (pesticide grade).

8) Dry under nitrogen.

9) Rinse dried glassware with another few mls of hexane (pesticide grade) before use.

c) For dirty glassware for later use: Follow the above procedure (b) but omit step 8 and replace with 7A.

7A: Dry glassware in oven at 300°C until ready for use. Before use, rinse with hexane (pesticide grade) several times.

These detailed procedures may seem trivial; however, meticulous cleaning of glassware is absolutely necessary for water analysis. Contamination quite often results from improperly cleaned glassware. It may also be pointed out that a chromic acid bath is not satisfactory in cleaning glassware contaminated with organochlorine pesticides; most of these pesticides are stable in chromic acid at room temperature for many days (Chau, 1971; paper presented at Edmonton, Alberta). Some pesticides do react with chromic acid but they are not destroyed; for example, prolonged contact of heptachlor and aldrin with chromic acid will give heptachlor epoxide and dieldrin respectively (Chau, 1971; Sans, 1967).

An effective acid bath would be an almost boiling 3 to 1 mixture of concentrated sulfuric acid and nitric acid (Chau, 1971). Soaking dirty glassware in this solution for an hour and subsequently cleaning with soap and water will eliminate most of the interferences present. A brief washing with acetone will eliminate all interferences. However, this procedure is not generally used due to the extreme danger in using this mixture.

## 2.2. Interferences from Sample Co-extractives

Numerous co-extractives and artifacts are known to produce similar responses to the pesticides in question (Burke and Giuffrida, 1964; Lamar, Goerlitz, and Law, 1966; Mestres, Barthes, and Priu, 1966; Van Valin, Kallman, and O'Donnel, 1963; Goodwin, Goulden, and Reynolds, 1961; Pearson, Aldrich, and Stone, 1967; Chau and Cochrane, 1969a; Chau and Cochrane, 1969b; Glotfelty and Caro, 1970). These co-extractives may be intrinsic to a particular sample. For example, the artifact of dieldrin is known to be present in corn leaf extracts (Glotfelty and Caro, 1970) and aldrin artifacts are present in cabbage, turnip, and asparagus (McLeod and Chau). These artifacts probably resulted from sulfur compounds present in these crops. Sulfur has long been known to have the same GLC responses as aldrin (Pearson, Aldrich, and Stone, 1967). Some rubber and petroleum products also give GLC responses similar to dieldrin and DDT (Deubert, 1970). These products may also be present in surface water and are extracted during analysis. To complicate matters, two or more different pesticides or metabolites can give similar GLC or TLC responses (Chau and Cochrane, 1969a; Chau and Cochrane, 1969b; Osadchuk and Romach, 1968; Chau, 1969; Chau, 1970). For example, one of the degradation products of technical chlordane, 2-chlorochlordane and heptachlor, have similar GLC and TLC responses (Chau and Cochrane, 1969a; Chau and Cochrane, 1969b; Chau, 1970; Cochrane and Chau, 1970), whereas α-endosulfan and p,p'-DDE (Chau, 1969) have identical retention times in the gas chromatograph using many widely used columns. The list is almost endless of materials that give similar analytical responses to those pesticides concerned. It is very difficult to have a few clean-up procedures that solve all problems. There are undoubtedly many other unknown sources that can contribute to improper identification. The pairing of GLC and TLC, which was considered some time ago to be enough evidence for the identity of a particular compound, is no longer valid. Independent techniques such as mass spectroscopy, NMR, (nuclear magnetic resonance), infrared, and chemical confirmatory tests are necessary, particularly when the history of the sample is unknown, as in the case of water samples.

In this manual, no attempt is made to solve all the problems of improper identification due to artifacts since we are still faced with many unknowns. However, some solutions for positive identification are presented under Section 4 which deals with confirmation by chemical derivation and GLC techniques. Most, if not all, of those artifacts mentioned above which give similar responses to the pesticides in question can be differentiated. For example, sulfur and related compounds can be differentiated from aldrin by the chemical-derivation technique. Confusion due to p,p'-DDE, endosulfan, dieldrin and its artifacts can also be eliminated by this technique.

# *Purification of Solvents and Chemicals*

It is naive to think that if one uses pesticide residue grade solvents, one does not have to purify them. The decision to purify is based on two factors: compatibility of the manufacturer's specification to one's analytical conditions, and the variations in quality between batches.

It has been found that many commercial pesticide-grade solvents occasionally provide batches of poor quality. Therefore, when a new shipment is received it should be tested to assure its suitability for the analysis (Sec. 2.1.5.). Furthermore, specifications of quality vary among manufacturers, with background responses varying from parts per billion to parts per trillion. For some pesticides we often detect in our water analysis 0.005 ppb (5 parts per trillion) for one litre of water. Therefore, unless the background responses of the commercial solvents do not interfere with the pesticides to be analyzed, the solvents must be purified by one or more of the methods described in this section until the quality is satisfactory.

## 3.1. Distillation

For about one-half hour reflux the solvents (hexane, petroleum, ether, benzene, and other nonchlorinated solvents) with silver nitrate powder (2 gm per litre) and anhydrous potassium carbonate (20 gm per litre). Distill through a fractionating column packed with glass helices (an approximately 20 cm x 800 cm column is very satisfactory). Discard the first 300 ml and the last 500 ml (for a 5 litre batch). Check the purity according to Section 2.1.5.

## 3.2. Column

Pass solvents through prewashed and preactivated alumina or florisil (Sec. 3.3.). Discard first 10% of eluate. About 500 gm of solid adsorbent is sufficient for about 1-2 litre of solvent. This method, sometimes used in conjunction with distillation, is found to be satisfactory for all solvents including chlorinated ones.

## 3.3. Alumina - for solvent purification

Wash alumina (basic, acidic or neutral) by soaking and stirring in pesticide-grade methylene chloride, decant washing solution. Repeat twice with the same solvent. Transfer solution to a precleaned Buchner funnel with a precleaned filter paper. Apply suction until most of the solvent is removed from alumina. Release suction. Pour enough ethyl acetate to cover alumina. Apply suction until alumina is free of solvent. Heat alumina at 600°C-800°C in a *clean* oven overnight. Store in a clean glass container in a desiccator.

## 3.4. Florisil - for solvent purification

Follow method for alumina and heat in oven at 800°C overnight and store in a clean glass container in a desiccator.

Alumina and florisil, which contain no interfering peaks after elution with the solvents to be purified, can be activated in an oven without the prewashing steps described above.

# *Confirmatory Tests*

Our experiences with o.c. analysis show that GLC interpretation, even with the use of two or more different GLC columns, does not furnish positive evidence of the identity of an unknown pesticide residue. These findings echo those of other workers in this field (Robinson, 1967; Reynolds, 1968). Confirmation of a positive identity of a residue in samples, particularly those of unknown history, is necessary. Generally, the approach to the confirmation of identity comprises the following steps:

1. Tentative identification of pesticides based on GLC retention times on one GLC column.

2. Interpretation substantiated by GLC retention times from at least another column with different polarity.

3. Other confirmatory tests:

   a) Thin-layer chromatography (TLC).

   b) Spectroscopic and Spectrometric analyses:

      Nuclear magnetic resonance (NMR), infrared (IR) and mass spectrometry (M.S.)

   c) p-values (Bowman and Beroza, 1965).

   d) Chemical confirmatory tests - wet (see latter discussion) and dry techniques (Minyard and Jackson, 1965; Miller and Wells, 1969).

   e) Adsorption column elution pattern (this has been incorporated into some of the clean-up methods such as the widely used Mills (1963 and 1967) or Sans (1967) methods).

It is beyond the scope of this manual to discuss and compare the various available confirmatory tests. It is sufficient to mention that some confirmatory methods are limited in their application due to their sensitivity. At present, we use chemical derivation-GLC methods for confirmatory purposes. For many pesticides, this approach has the convenience and sensitivity suitable for routine analysis. The newly developed techniques in using mass spectrometry or NMR with C.A.T. (computer of average transients) connected to GLC would be very useful alternatives or additions.

In this section, chemical confirmatory tests are described. Section 5 will deal with another confirmatory test, namely, the widely used thin-layer chromatography.

## 4.1. Confirmation of p,p'-DDT by Dehydrochlorination

a) *Theory:* It has long been known that p,p'-DDT can be easily dehydrochlorinated with alkali to the corresponding olefin, p,p'-DDE. Similarly, o,p'-DDT can be converted to o,p'-DDE by alkali; however, this reaction is considerably slower than the dehydrochlorination of p,p'-DDT (Fig. 2).

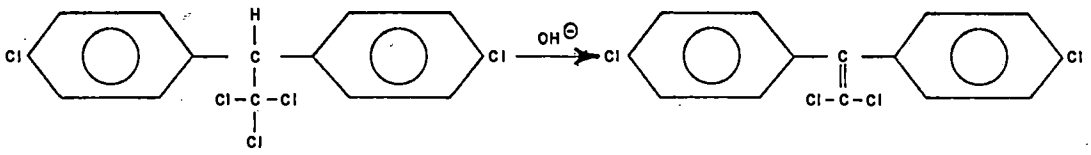

FIGURE 2

b) *Procedure:* Concentrate an aliquot of sample extract containing suspected p,p'-DDT in a graduated centrifuge tube to 0.1 ml - 0.2 ml under a gentle stream of nitrogen (approx. 40°C). To the residue add 1 ml of 0.1 N sodium methoxide/methanol ($NaOCH_3/CH_3OH$) solution. Heat mixture for 1/2 hr. at 50-60°C. Add 5-6 ml distilled water and 1 ml pesticide-grade hexane, shake a few times and after the hexane layer separates, draw the hexane into a syringe and inject into a GLC. Drying the hexane layer with anhydrous sodium sulfate is not necessary if care is taken not to draw any aqueous layer into the needle.

NOTE: For o,p'-DDT increase reaction time to 3/4 hr. at 60°C to ensure complete conversion.

c) *Sensitivity:* 0.01 ppb for p,p'-DDT and o,p'-DDT.

4.2. Confirmation of p,p'-DDD by Dehydrochlorination (Klein and Watts, 1964; Haller, 1945)

a) *Theory:* p,p'-DDD can also be dehydrochlorinated to an olefin (DDMU) by alkaline treatment. DDMU, 2-chloro-1, 1,-bis-(p-chlorophenyl)-ethylene, is also one of the metabolites of p,p'-DDT and p,p'-DDD found in nature in some biological systems (Peterson and Robinson, 1964; Hoffman and Rathkamp, 1968), (Fig. 3).

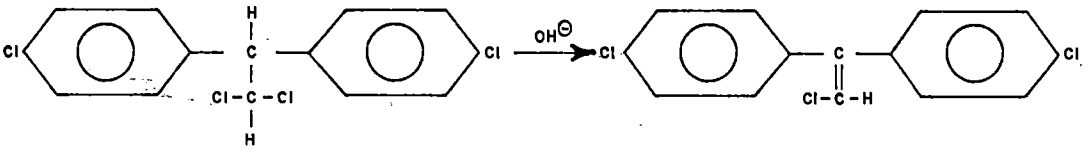

FIGURE 3

b) *Procedure:* The same procedure is used for confirmation of p,p'-DDT except the reaction time is 1 hr. at 60°C.

c) *Sensitivity:* Above 0.01 ppb for p,p'-DDD.

4.3. Confirmation of Heptachlor

4.3.1. Reaction with Silver Carbonate Followed by Silylation

a) *Theory:* The allylic chlorine atom is very active as compared with chlorines in other parts of the molecules. For a heptachlor level above 0.5 ppb and with a well conditioned column, the silylation step can be omitted; for lower levels of heptachlor, silylation enhances the level of detectability, (Fig. 4).

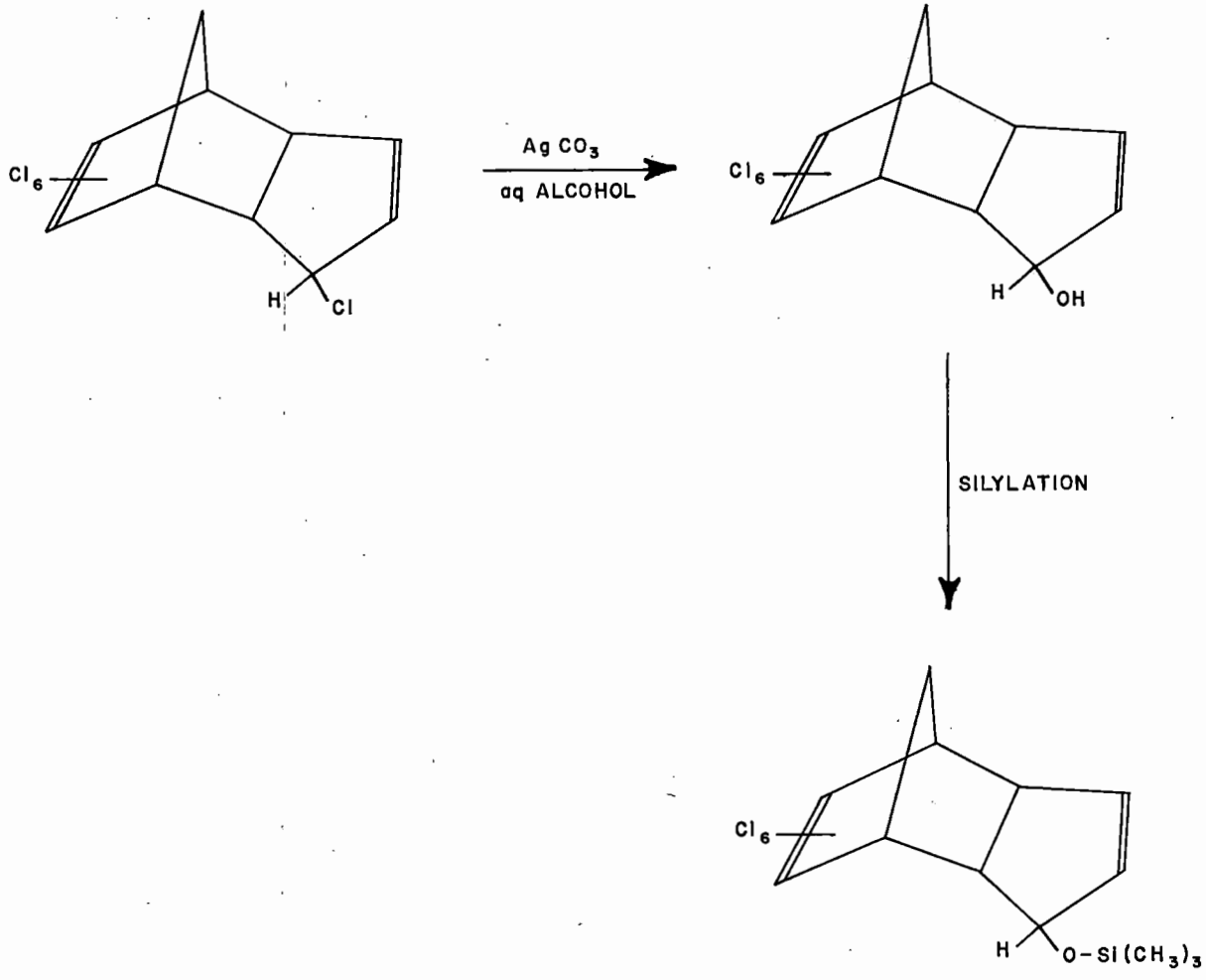


FIGURE 4

b) *Procedure:* Transfer a suitable aliquot of sample extract to a K-D graduated tube (12 ml). Evaporate just to dryness with a gentle stream of nitrogen. Add 2 ml of 50% aqueous ethanol and about 100 mg silver carbonate, reflux on a K-D concentrator for 3/4 hr. using a Snyder column. Add 6-8 ml distilled water to the reflux mixture. Remove refluxing column. Add 1 ml hexane, close tube with a glass stopper and shake vigorously for a few seconds. Allow hexane layer to separate. The hexane extract can be injected if level of heptachlor is high. For low level, withdraw hexane layer to a clean tube having a glass stopper. Repeat extraction three times with 1 ml portions of hexane, and combine hexane extract. Dry the hexane by adding 500 mg anhydrous sodium sulfate to the tube. Shake briefly. Transfer dried hexane extract into another tube. Wash sodium sulfate twice, each time with 2 ml ether. Combine washing solution and hexane extract. After concentrating to 1 ml under nitrogen, add about 20 drops prepared silylation reagent by mixing 1.5 ml hexamethyldisilazane and 0.5 ml trimethylchlorosilane in 10 ml dry pyridine. (This silylation agent can be kept a few weeks in the refrigerator under anhydrous condition). Stopper the tube and put in a sand bath at 55-60°C for $\frac{1}{2}$ hr. Add 6 ml distilled water. Shake contents. After organic layer separates, inject into GLC for analysis.

c) *Discussion:* After silver carbonate reaction, heptachlor is converted to 1-hydroxychlordene. The presence of the alcohol peak and the absence of a heptachlor peak after the reaction is positive evidence of heptachlor in the sample. Dependent on their type, the artifacts may or may not disappear after the reaction but only heptachlor will give a peak corresponding to 1-hydroxychlordene. For low level heptachlor, the 1-hydroxychlordene peak does not show up in the gas chromatograph due to low sensitivity of the electron-capture detector (ECD) towards this compound. Silylation will greatly enhance its level of detectability.

d) *Sensitivity:* 0.5 ppb - without silylation, 0.02 ppb - with silylation.

4.3.2. Reaction with Silver Acetate - Acetic Acid (Cochrane and Chau, 1968a).

a) *Theory:* See Fig. 5.

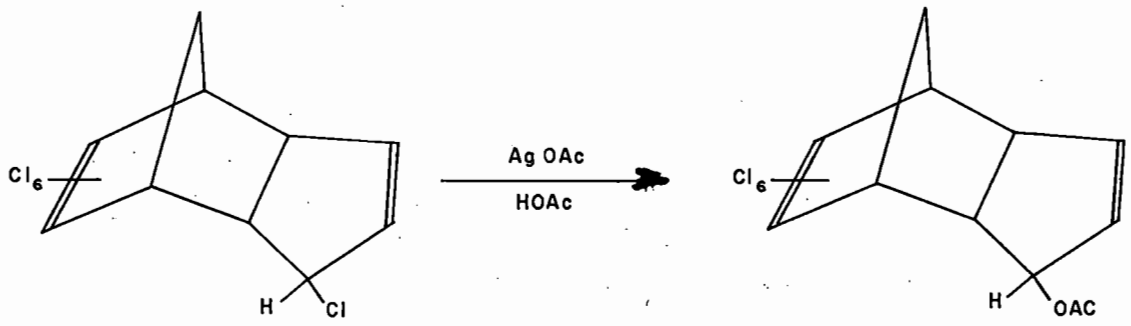


FIGURE 5

b) *Procedure:* Evaporate an aliquot of sample extract in a Konte graduated tube as described previously. Add 1.0 ml glacial acetic acid and 100 mg silver acetate (AgOAc). Reflux for 30 min. Cool, add drop by drop with agitation, 1 ml saturated potassium hydroxide (KOH) solution. Cool in running tap water if necessary. Then add 5-6 ml distilled water. Add concentrated sodium carbonate ($Na_2CO_3$) drop by drop until evolution of carbon dioxide subsides. Shake contents vigorously with 1 ml hexane. After organic layer separates, inject it into GLC for interpretation.

c) *Discussion:* After this reaction, heptachlor will be converted to 1-acetoxychlordene with a different retention time. Thus, after this reaction the presence of 1-acetoxychlordene peak and the absence of a heptachlor peak confirms the presence of the latter compound in the sample.

d) *Sensitivity:* 0.05 ppb.

4.3.3. Chromous Chloride Reduction (Chau, 1970; Cochrane and Chau, 1970).

a) *Theory:* See Fig. 6.

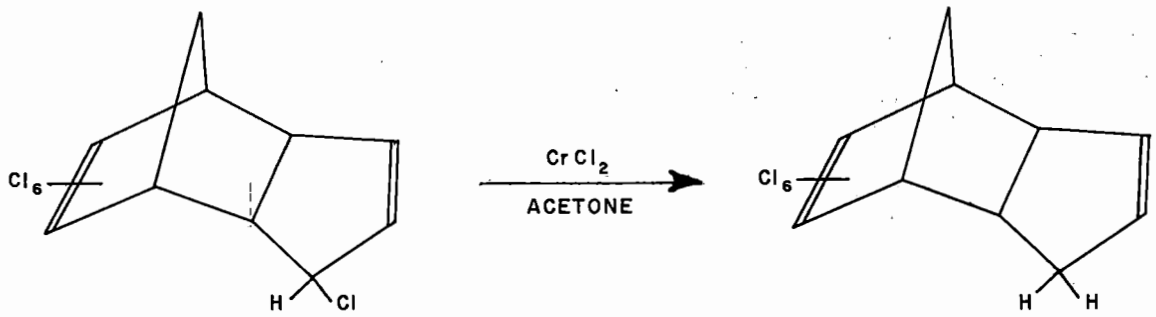


FIGURE 6

This dechlorination reduction takes advantage of the active allylic chlorine atom which is selectively reduced by chromous chloride ($CrCl_2$) without reducing the other stable chlorines.

b) *Procedure:* To an evaporated aliquot of sample extract, add 1 ml acetone and 2 ml chromous chloride solution under nitrogen. This can be performed in a dry box under nitrogen atmosphere or by adding the chromous chloride solution to a tube containing 1 ml acetone and sample extract, with a gentle flow of nitrogen flowing over the surface of the acetone solution. Stopper the tube immediately after adding the chromous chloride. The resulting solution should be sky blue or at least mainly blue. (If it turns green, most of the chromous chloride has been oxidized by atmospheric oxygen and more chromous chloride solution and acetone must be added).

After securing the glass stopper to the mouth of the tube with masking tape or a spring-loaded clamp, transfer the tube to a water bath at 55°C - 60°C for 40 min. Remove stopper, add 6-7 ml distilled water and 1 ml hexane; shake for a few seconds. Inject the supernatant liquid into the GLC for interpretation.

c) *Discussion:* This method can also be applied to the confirmation of endrin (4.7.1.). If endrin and heptachlor are present together in a sample, they can be converted to their respective dechlorinated products with different retention times; thus, these two pesticides can be confirmed simultaneously by this method without ambiguity.

d) *Sensitivity:* 0.005 ppb.

4.3.4. Reaction with Potassium Tert-butoxide (t-BuOK) and Tert-butanol (t-BuOH) followed by Silylation (Chau and Cochrane, 1969a, 1969b)

a) *Theory:* Reaction of heptachlor with potassium tert-butoxide and tert-butanol gives the same derivative, 1-hydroxychlordene, as obtained from the reaction with $Ag_2CO_3$. The 1-hydroxychlordene can be either acetylated or silylated (Fig. 7).

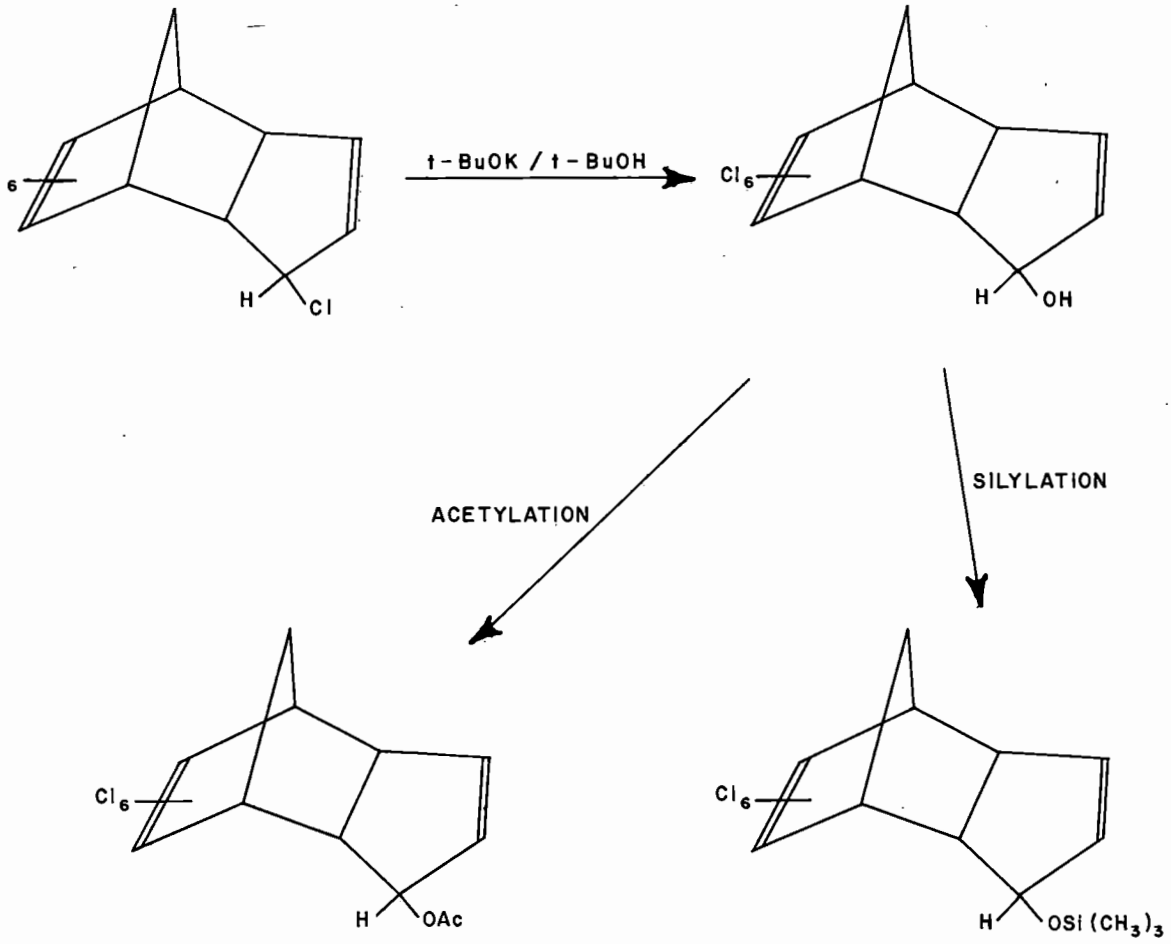


FIGURE 7

b) *Procedure:* Place an aliquot of sample extract in a K-D tube. Evaporate just to dryness under a gentle stream of nitrogen. Add 2 ml of tert-butanol (t-BuOH) and approximately (ca) 100 mg potassium tert-butoxide (t-BuOK). Put tube on a Snyder condenser. Reflux the solution for 30 min. Add a few drops of t-BuOH to maintain the reaction volume at 1.5 to 2 ml. Cool reaction mixture by immersing tube in cool water. Add ca 6-8 ml distilled water and 1 ml hexane. Shake mixture, let settle a few minutes; if the level of heptachlor in the sample is high, inject a few microlitres of the hexane extract.

For low levels of heptachlor, transfer hexane layer into a dry tube by means of a clean disposable pipette. Repeat hexane extraction of the reaction mixture three times with 2 ml hexane. Dry the combined hexane extracts over anhydrous sodium sulfate. Transfer the hexane extract into another glass-stoppered tube. Wash the sodium sulfate twice with ca 3 ml ether. Combine ether washing solution with hexane extract, evaporating to about 1 ml. At this stage, the hexane concentrate can be silylated as described previously (Sec. 4.3.1.) or can be acetylated in the following manner.

Add 1 ml of acetylating reagent (1 part dry pyridine and 2 parts redistilled acetic anhydride) to the hexane concentrate. Let mixture stand at least 45 min. at 60°C. (At this stage, reaction mixture can be left at room temperature overnight in the dark without affecting the result). Add ca 4 ml distilled water and neutralize the reaction to litmus paper with 10% sodium hydroxide. Add 1 ml hexane, shake and analyze the organic layer by GLC.

c) *Sensitivity:* 0.02 ppb.

4.4. Confirmation of Aldrin

4.4.1. Halogenation of Aldrin

a) *Theory:* Aldrin contains an active double bond which can be easily halogenated by bromine or chlorine in carbon tetrachloride ($Br/CCl_4$ or $Cl_2/CCl_4$), (Fig. 8).

b) *Procedure:* Evaporate an aliquot of sample extract just to dryness under nitrogen in a glass-stoppered tube; add 1 ml of freshly prepared bromination reagent by adding 1 drop bromine in 25 ml carbon tetrachloride. Stopper the reaction tube and agitate contents. Leave the tube in the dark for 10 minutes. Evaporate carbon tetrachloride under a stream of nitrogen. Add 1 ml hexane and approximately (ca) 2 ml of 50% aqueous sodium bisulfate or thiosulfate solution. Shake vigorously. Allow organic layer to settle and analyze the hexane layer by GLC. For chlorination, use 1 ml chlorination reagent by bubbling chlorine gas through 25 ml carbon tetrachloride for 10 min. and follow the steps for bromination.

c) *Sensitivity:* 0.05 ppb for bromination chlorination.

4.4.2. Epoxidation of Aldrin by Peracids (Chau and Cochrane, 1969a; Noren, 1968; Osadchuk and Wanless, 1968)

a) *Theory:* The active double bond of aldrin can be epoxidized to its corresponding epoxide and dieldrin by peracids such as peracetic

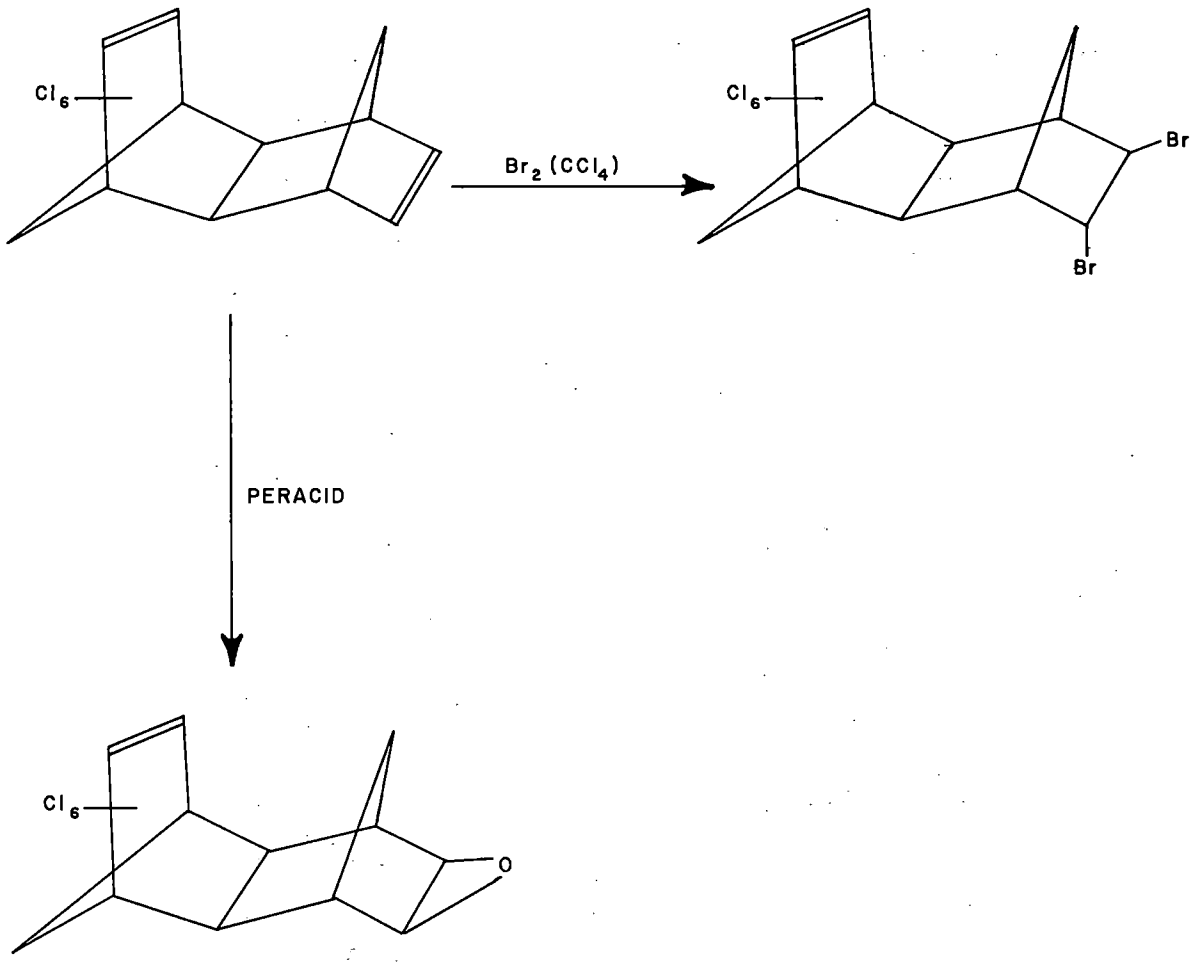


FIGURE 8

acid, monoperphthalic acid, m-chloroperbenzoic acid, and perbenzoic acid, (Fig. 8).

b) *Procedure:* Evaporate an aliquot of sample extract as described previously under nitrogen. Add 1 ml ethereal monoperphthalic acid solution (at least 5% solution or a 5% m-chloroperbenzoic acid solution in chloroform). Agitate contents, stopper the tube and let stand at room temperature for 20-25 min. before evaporating under nitrogen until dry. Add 2 ml of 10% sodium sulfate solution and 1 ml hexane. Shake a few times before injecting an aliquot of the supernatant liquid into gas chromatograph.

c) *Discussion:* Chloroform or other chlorinated solvent should be evaporated completely when used, otherwise a broad solvent peak will appear on the chromatogram. Monoperphthalic acid is not available commercially but can be prepared in the laboratory (Chau and Cochrane, 1969a). It has an advantage over m-chloroperbenzoic acid

in that the latter gives some responses in GLC if it is not removed completely by alkali.

4.5. Confirmation of Heptachlor Epoxide (Chau and Cochrane, 1969a; Cochrane and Chau, 1968b)

a) *Theory:* The oxirane ring in heptachlor epoxide can be opened by a strong base. The resulting alcohol can be silylated or acetylated to increase sensitivity of its detectability by ECD (Fig. 9).

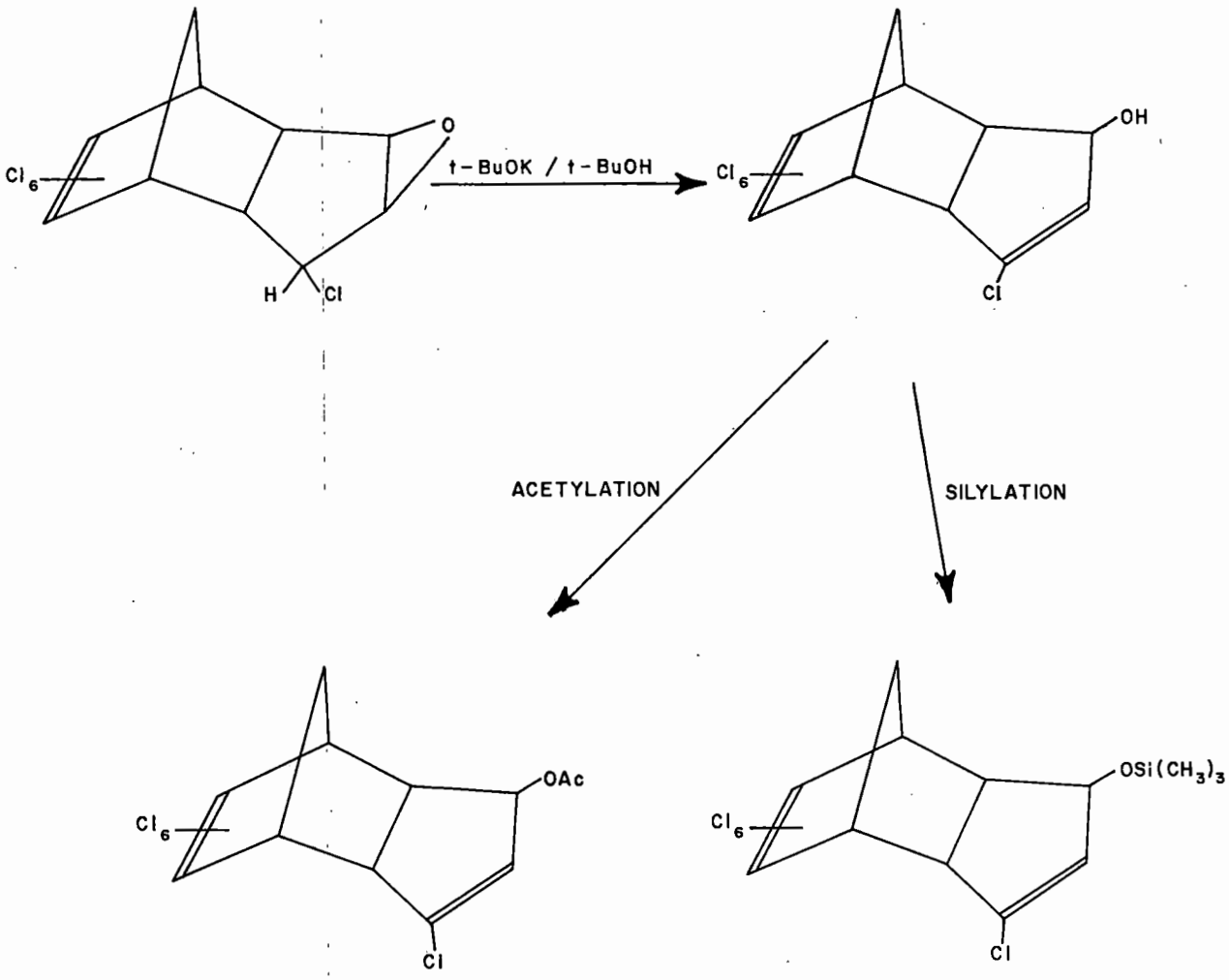


FIGURE 9

b) *Procedure:* Procedures for potassium tert-butoxide (t-BuOK and tert-butanol (t-BuOH) treatment and subsequent silylation or acetylation are identical to those described for the confirmation of heptachlor (4.3.4.).

c) *Sensitivity:* 0.02 ppb.

d) *Discussion:* Since the alcohols and the corresponding silyl ether or acetoxyl derivatives obtained from heptachlor and heptachlor epoxide respectively have different GLC retention time, this t-BuOK/t-BuOH method can be used for the simultaneous confirmation of heptachlor and heptachlor epoxide if they are present together in a sample extract.

4.6. Confirmation of α- and γ- Chlordanes (Chau and Cochrane, 1969a, 1969b)

a) *Theory:* α-chlordane (cis-chlordane) and γ-chlordane (trans-chlordane) can be dehydrochlorinated by a strong base such as potassium tert-butoxide in tert-butanol. These two compounds are the major constituents of technical chlordane. Together they comprise over half the weight of the total constituents in technical chlordane (Fig. 10).

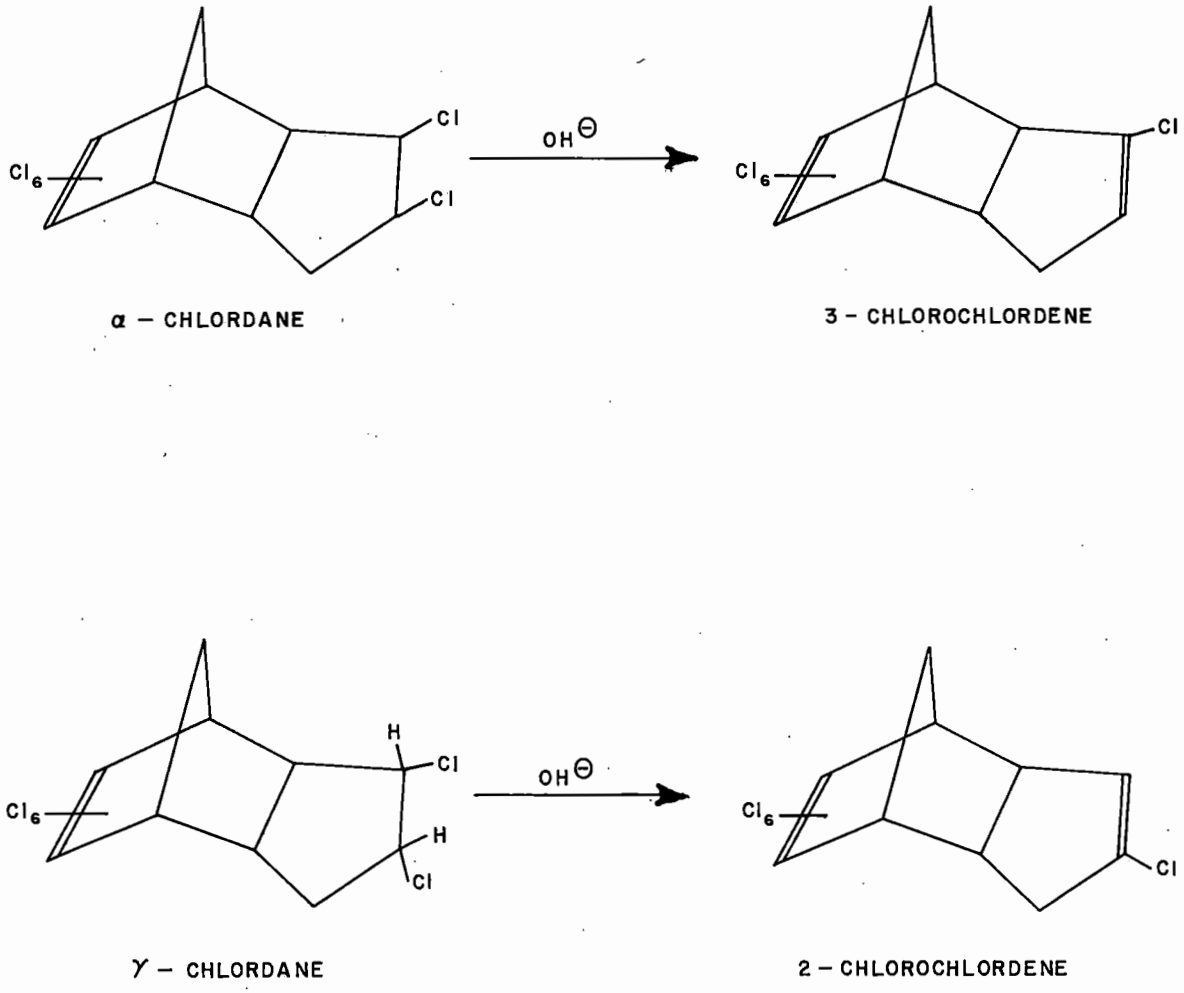


FIGURE 10

b) *Procedure:* After evaporating an aliquot of sample extract, add potassium tert-butoxide and tert-butanol in the manner described for heptachlor (4.3.4.).

c) *Discussion:* If heptachlor and heptachlor epoxide are not present in the sample extract, the silylation or the acetylation procedure can be omitted. The method of t-BuOK/t-BuOH followed by silylation and acetylation can simultaneously confirm heptachlor, heptachlor epoxide, α-chlordanes and γ-chlordanes.

d) *Sensitivity:* 0.02 ppb for α-chlordane and γ-chlordane respectively.

4.7. Confirmation of Endrin

4.7.1. Acid-catalyzed Isomerization (Chau and Cochrane, 1969a)

a) *Theory:* In the presence of acid, endrin is isomerized to a half-cage ketone by transannular cyclization (Fig. 11).

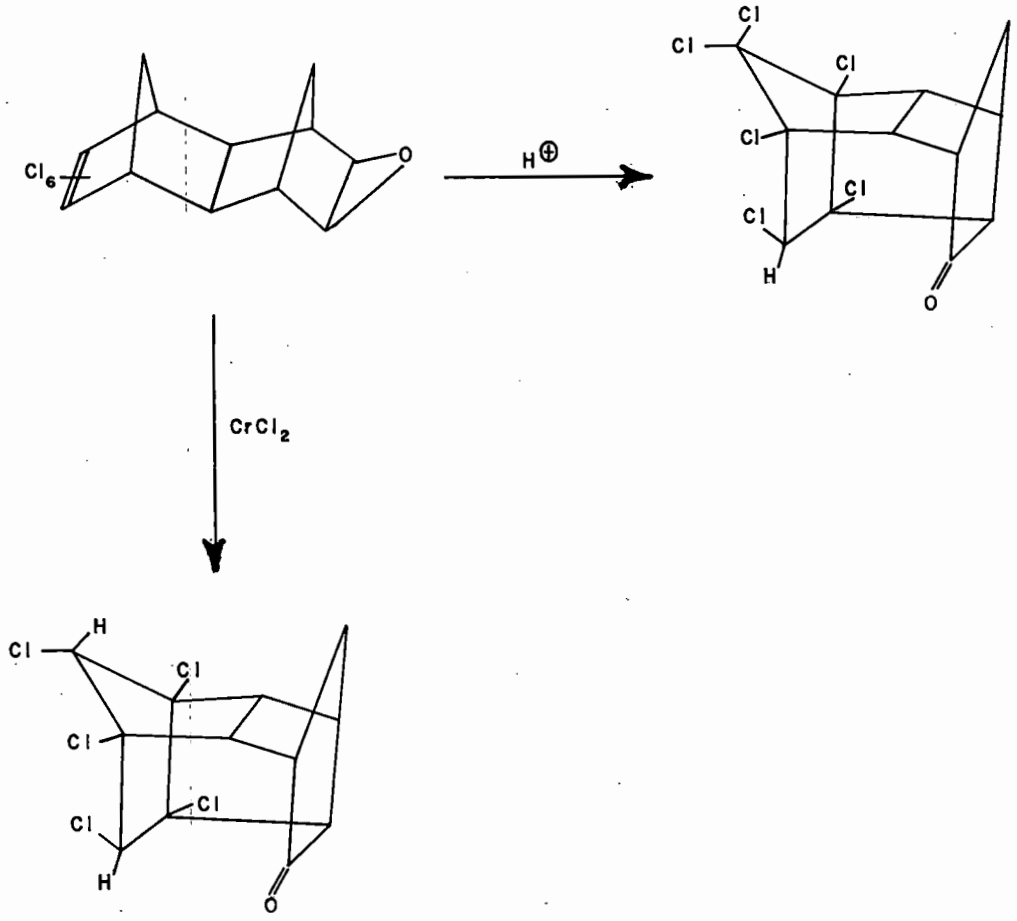


FIGURE 11

b) *Procedure:* After evaporating an aliquot of sample extract just to dryness, add 1/2 ml of concentrated sulfuric acid, rotating contents so that all the residue is wet. After 15-20 min., cool the reaction tube in an ice bath, carefully add ca 2 ml water dropwise and with swirling down the side of the tube. Then add dropwise 3 ml ice-cold saturated potassium hydroxide solution followed by 1 ml benzene. Shake contents and analyze benzene layer by GLC.

c) *Discussion:* This half-cage ketone is a photolyzed product and is found in nature. It is also observed in some GLC columns when endrin is injected because of the thermal isomerization of endrin to this compound.

4.7.2. Chromous Chloride Reduction (Chau, 1970; Chau, Won, and Cochrane, 1971; Chau and Cochrane, 1971).

Follow the same procedure as for heptachlor (4.3.3.), (Fig. 11).

a) *Sensitivity:* 0.4 ppb endrin in sample.

4.8. Confirmation of α- and β-endosulfan

4.8.1. Reduction Followed by Silylation (Chau, 1969).

a) *Theory:* Both isomers of endosulfan, i.e., α-endosulfan and β-endosulfan, can be reduced by lithium aluminium hydride to the same diol. Greatest sensitivity will be achieved by silylation of this diol (Fig. 12).

b) *Procedure:* Concentrate an aliquot of sample extract to 0.1 ml - 0.2 ml. Add 2 ml anhydrous tetrahydrofuran (THF) and ca 50 mg lithimum aluminium hydride (LAH) and flush contents with dry nitrogen. Stopper tube at once and heat for 1 hr. in a 60°C sand bath. Cool tube 5 min. in ice bath and destroy excess LAH with the addition dropwise of 2 ml ice water followed by 1 ml 5% hydrochloric acid that was precooled 5 min. in the ice bath. After $H_2$ evolution subsides, add 4 ml ether and shake mixture. Pipette the ether extract into a clean glass-stoppered test tube and repeat extraction until total volume is ca 10 ml. Vigorously shake ether extract with finely powdered anhydrous sodium carbonate. Transfer ether extract, with rinsing solution, to glass-stoppered tube and evaporate to dryness at ca 30°C. Add 1 ml silylation reagent (4.3.1.); flush tube with dry nitrogen, and let mixture stand 30 min. in 55°C-60°C. sand bath. Cool tube at 5°C-10°C to room temperature; add 2 ml water and 1 ml hexane, and shake. Inject an aliquot of the hexane layer into GLC for interpretation.

c) *Sensitivity:* 0.4 ppb of endosulfan in sample.

4.8.2. Acetylation (Chau, 1969), (Fig. 12).

Evaporate an aliquot of sample extract. Add 1 ml acetylation reagent (0.2 ml conc. $H_2SO_4$ in 60 ml acetic anhydride and 40 ml glacial acetic acid; store in brown bottle). Heat tube 45 min. at 100°C. Cool tube to 5°C-10°C (ca 5-10 min. in ice bath). Add 1 ml cold saturated KOH solution drop by drop while shaking; slowly add 10% $NaHCO_3$ until evolution of carbon dioxide subsides. Add 1 ml hexane, shake, and analyze the supernatant layer by GLC.

*Sensitivity:* 0.4 ppb of endosulfan in sample.

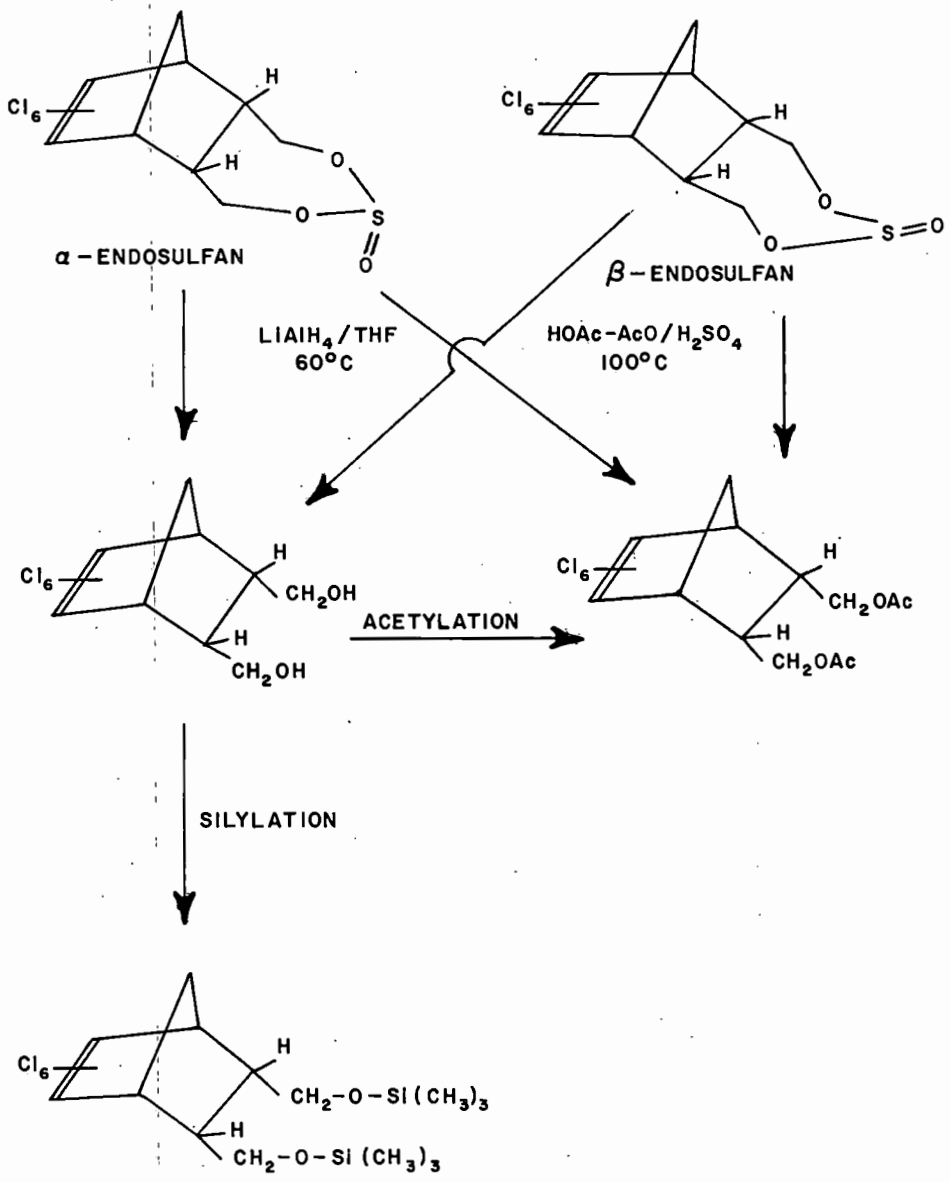


FIGURE 12

4.9. Confirmation of Dieldrin (Hamence, Hall, and Caverly, 1965)

a) *Theory:* The oxirane ring in dieldrin can be opened with strong acid and in the presence of acetic anhydride ($AC_2O$) a derivative with different retention time from the parent pesticide is formed, (Fig. 13).

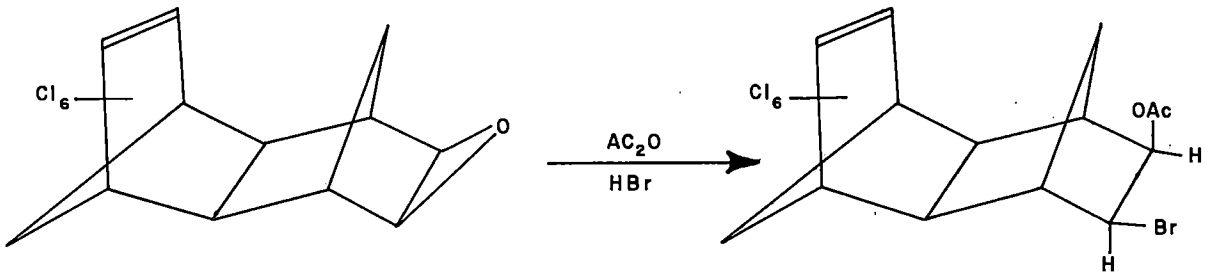


FIGURE 13

b) *Procedure:* To 10 ml of 48% hydrobromic acid in a glass flask, cooled in ice, carefully add dropwise 20 ml of acetic anhydride. Stopper the flask, mix and allow to stand for 30 min. Evaporate a suitable aliquot of sample extract in a glass-stoppered glass tube as described above. Add 0.5 ml of the hydrobromic acid-acetic anhydride mixture prepared above. Rotate contents so that all the sample residue is wetted by the reagent. Set the mixture aside for 1/2 hr. Add 2 ml of distilled water; add drop by drop with agitation, saturated sodium carbonate solution until evolution of carbon dioxide stops. Add 1 ml hexane. Stopper the tube and shake. After organic layer separates, analyse by GLC.

c) *Sensitivity:* 0.1 ppb.

d) *Discussion:* The product from this reaction has been found to be a transbromoacetate of dieldrin by NMR analysis (Chau, 1971).

SECTION 5

# *Thin-Layer Chromatography (TLC)*

Due to its simplicity and reasonable specificity TLC is popular in pesticide-residue analysis and is sometimes used as a clean-up procedure. Unfortunately, since its sensitivity is much lower than that of the electron-capture detector, the application of TLC in detecting low levels of pesticide residues is limited. In this section, conventional TLC technique for pesticide-residue analysis is discussed. A modified procedure developed a few years ago and used occasionally by this author is also discussed.

## 5.1. Coating of Thin-layer Plates (Modification of Stahl's Procedure, 1958).

The alumina and silica gel used for the preparation of thin-layer plates contains calcium sulfate as a binder. Since the slurry prepared from these materials becomes too viscous to spread in less than 5 min., all equipment and materials should be ready for the coating operation before the slurry is prepared.

Clean the glass plates (200 mm x 200 mm, or other chosen size) with soap and water; rinse with acetone so that they are free from oily materials and other contaminants (oily glass plates give uneven thin-layer plates). Dry the glass plates in air at room temperature or in a warm oven. Arrange 5 clean plates in a spreader with a smaller plate on each end, making sure all the plates are of uniform thickness and width.

In a glass-joint conical flask (250 ml), add 30 gm silica gel followed by 50 ml distilled water (free of chloride). Alternatively, silica gel can be ground with water in a porcelain mortar. Stopper the flask and shake evenly for 30-60 sec., gentle tumbling works fine. Vigorous shaking produces bubbles and results in uneven plates. Immediately after shaking, pour the slurry into the trough of a Stahl TLC applicator sitting on the glass plates which are arranged on a spreader. Apply a layer 0.25 mm thick on the 5 glass plates with rapid but even movement. (Air bubbles or lumps in the slurry must be avoided if good TLC plates are to be prepared).

Allow the plates to dry at ambient temperature for 10-20 min. and transfer the coated plates to a rack. Activate the plates in a vertical position for 1 hr. at 110°C. Immediately store plates in a desiccator -- before use a plate can be activated again for a few minutes or used as is. To prevent inconsistent results the same drying and storage procedures should be followed each time a new set of coated plates are made. It should also be noted that during hot humid weather results vary from plate to plate when exposed to humidities of greater than 50%. For this reason, the plates should be used in a dehumidified room if possible and activated for a fixed period (e.g., 30 min.) each time they are used in hot humid weather. In any case, unnecessary long exposure of plates to the atmosphere must be avoided.

## 5.2. Spotting

Evaporate a suitable aliquot of clean-up sample extract to a very small volume (ca 100 µl). Using a spotting template as a guide, apply the sample extract on one side and the standard on the other by means of a

micropipette or a microsyringe (25 µl or 10 µl is convenient for most applications) at a distance of 10 cm from each spot and 25 mm from the bottom edge of the plate. Although as many as 18 spots can be put on a single 200 cm x 200 cm plate for standard solutions, a maximum of 10 to 12 spots are recommended to avoid overlapping and slanting in some samples.

5.3. Development and Visualization

Preparation of development tank: For 200 cm x 200 cm TLC plates use a rectangular tank, such as Canlab C5432-3C or equivalent, containing 150 ml to 200 ml of developing solvent or solvent mixtures, (see 5.3.1. on choice of developing solvents). Line both sides of the tank with two pieces of filter paper, arranging the tank so that the filter papers are well soaked with solvent. After the papers are moistened, press the papers against the side of the tank. Cover the development tank tightly (silicone grease applied on tip of the cover) and let stand at room temperature for a few minutes to allow saturation of the solvent vapor within the chamber. These steps are necessary to avoid the "edge effect" that would result from inadequate chamber solvent saturation (Demole, 1958). This phenomenon is indicated by substances near the edge of the plate migrating higher than the same substances in the centre.

5.3.1. Developing Solvents

The choice of developing solvent or mixture of solvents depends on the polarity of compounds. A general rule is the more polar the compounds, the more polar the development solvent(s) used. The polarity of solvents is given in the elutropic series of solvents below (Stahl, 1969).

| | |
|---|---|
| n-Hexane | Lowest polarity |
| Heptane | |
| Cychlohexane | |
| Carbon Tetrachloride | |
| Toluene | |
| Benzene | |
| Chloroform | |
| Tetrahydrofuran | Intermediate |
| Ethyl Ether | |
| Ethyl Acetate | |
| Pyridine | |
| Acetone | |
| n-Butanol | |
| n-Propanol | |
| Ethanol | |
| Methanol | |
| Water | |
| Acetic Acid | Highest |

NOTE: Several authors have slightly different versions of this series; however, this variation does not appreciably affect the results on the choice of solvents.

5.3.2. Visualization

After development (i.e., the solvent moves up to a previously marked height, ca 15 cm from top of plate), transfer the developed plate to a fumehood to evaporate the solvent (approx. 5 min.). In the fumehood, spray the whole plate evenly 2 or 3 times with an appropriate chromogenic agent.

For alumina plates: Use Mitchell's spray (1961) - 0.1 gm of 2-phenoxyethanol, and dilute to 200 ml with acetone. Then add 1 drop of 30% hydrogen peroxide, $H_2O_2$, for preservative. Keep in the dark.

For silica gel plates: Use Morley's spray* - 1.7 gm of $AgNO_3$, 10 ml of $H_2O$, 5 ml of $NH_4OH$ and 185 ml of acetone. Mix and keep in the dark.

After spraying, allow the sprayed TLC plate to dry in the air in the fumehood for 10-15 min. and expose to strong ultraviolet (U.V.) radiation for 10-20 min. or until dark spots of pesticides have appeared on a light grey background. An U.V. source of four or five General Electric germicidal lamps of 15 watts placed 8-12 in. from the TLC plate is found sufficient to produce dark spots.

NOTE: Longer exposure of a TLC plate to U.V. light increases slightly the sensitivity of detection. Mitchell's spray can be kept longer in the dark than Morley's spray. Generally, these can be kept up to a week. Discard the solution if a black or silver mirror is formed.

5.4. TLC-GLC Method

Spot a TLC plate with an appropriate standard mixture, an aliquot of sample extract, and the same standard mixture as shown in Fig. 14. Develop the plate as usual. Cover the sample portion (B part) with a piece of cardboard, taking care not to scratch the coating. Spray with an appropriate chromogenic reagent (see previous discussion for choice). After drying, irradiate the covered sample portion with U.V. lights until dark spots appear on the standard portion. Draw a line 1/2 cm above and below each of the standard spots. Use suction or scrape the area on the sample portion into a tube containing 1 ml hexane. Shake, let settle, and inject supernatant liquid into GLC.

This method is a modification of Chiba and Morley's TLC clean-up method (1964). It is used here not for clean-up but for confirmation purposes. Instead of spraying and irradiating the whole plate for visualization, GLC is used to "visualize" an area which corresponds to a particular standard. Visualization of spots by spraying and U.V. irradiation is useless for samples of pesticides below the detection limit of TLC, but "visualization" by GLC works quite well.

For samples that contain a complicated mixture of pesticides of different polarity, two plates are generally used. One with low polarity solvents (e.g. 0.5 ml acetone in 200 ml hexane), the other with a higher polar solvent (1.0 ml-2.0 ml of acetone in 200 ml hexane). A better separation of pesticides results. For two pesticides with close rf values, the area scraped and extracted with hexane will contain both pesticides which does not hamper the interpretation.

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* Morley, H.V., private communication.

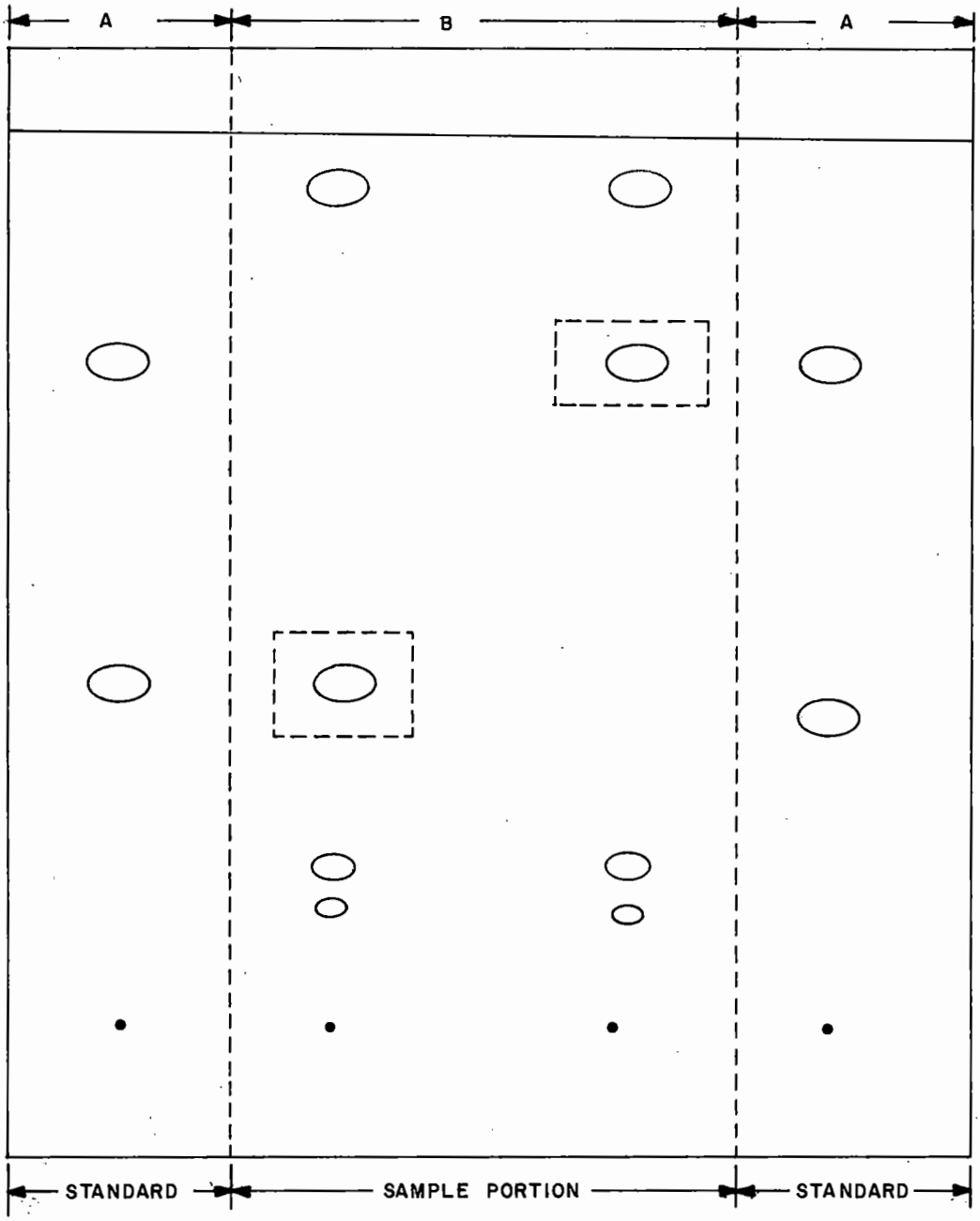


FIGURE 14

5.5. Discussion

TLC is widely used for confirmation of identity after preliminary GLC analysis. Its applicability is greatly hampered by its relatively low sensitivity as compared to GLC-ECD; however, this difficulty is resolved with the present pairing of TLC-GLC. Although TLC in general and TLC-GLC in particular are useful tools for confirmation, one should still be careful

in interpreting results based on these techniques; many compounds are known to have very similar or identical rf value, e.g. sulfur and aldrin, 2-chlorochlordene and heptachlor, DDT isomers and metabolites.

Silica gel G-TLC plates cannot be sprayed with Mitchell's chromogenic agent because the chloride content in commercial silica gel produces a dark-grey or dark background, thus decreasing sensitivity or obscuring detection. One can prewash the TLC plate (Kovacs, 1963) or use Morley's spray to overcome this problem.

Developing solvents for TLC varies from worker to worker, due to conditions varying from one laboratory to another. One must find the right solvents for the developing of TLC plates. We use 0.2 ml to 2.0 ml acetone in 200 ml hexane as a developing solvent mixture. The amount of acetone used depends on the activities of the plates and the pesticides in question. Benzene and hexane mixtures can also be used.

Several important points must be emphasized here:

1) Due to the high adsorptive capacity of silica gel plates, considerable care must be exercised in handling and storage to avoid adsorption of vapors of interfering substances (Kovacs, 1963).

2) The activity of prepared TLC plates changes with the length of storage and amount of handling, even if kept well in a desiccator. The solvent system for these plates found applicable for a certain compound may be unsatisfactory after a few months for the same compound. Therefore, after long storage it is advisable to reactivate the plates before use and check the suitability of the solvent system on standards before doing samples which do not have enough material for repetition.

3) Developing-solvent mixtures should not be reused after developing TLC plates unless they contain one kind of solvent. This is particularly important in a solvent mixture containing a small percentage of one solvent in another.

4) Values of pesticides change in the presence of some co-extractives. For example, starch as a co-extractive in certain vegetation modified the rf values of some pesticides (Chau, unpublished observation on changes of rf values of carboxyl and α-naphthol in potato extract). For this reason, the extracts of samples with which the analyst is unfamiliar should have pesticide standards added to them and applied on a TLC plate. A comparison should then be made with a standard in order to observe any changes in rf values of pesticides due to co-extractives.

# *Purification and Synthesis of Standards*

In many cases, only technical-grade standards are available. It is desirable and necessary to purify these standards by a combination of techniques such as recrystallization, adsorption column chromatography, preparative thin-layer chromatography, preparative gas chromatography, vacuum sublimation, and vacuum distillation. In some cases, the only method of obtaining standards is by synthesis in the laboratory.

It is impossible to have one or two universal procedures for purifying purposes since the chemical and physical characteristics of pesticides are different. Therefore, it is left to the chemist's initiative to devise or adapt suitable procedures for such purposes. However, examples follow of purification and synthesis of pesticide standards.

6.1. Purification of Technical Dieldrin (≈78%)*

Dissolve one gram of technical dieldrin (a yellowish solid) in a minimum of hot hexane. While hot, transfer to a column containing 30-40 gm florisil (60-100 mesh, not activated). Elute with a 9.5:0.5 hexane-ether mixture (500 ml - 800 ml) until only a small amount of solid elutes out. (This can be easily checked by adding a few drops of eluate from the column to a piece of micro-slide and evaporating the solvent with blowing air. The amount of solid left on the glass will give an idea of how much is being eluted). Pure GLC dieldrin standard results from the evaporation of eluates under vacuum and the recrystallization three times from a hexane-ether (9:1) mixture.

6.2. Purification of Technical Aldrin*

The procedure for dieldrin is used.

6.3. Purification of Technical Endrin*

The procedure for dieldrin is used, but use hexane-benzene (9:1) as the eluent and solvent to dissolve endrin.

6.4. Purification of Technical Heptachlor*

Dissolve 1 gm with hexane and let it pass through decolorizing charcoal (20 gm) under suction. Elute with 200 ml of hexane. Concentrate the filtrate under vacuum to 3-5 ml and transfer to a florisil column (60-100 mesh, not activated) and elute with hexane as described under Sec. 6.1. Recrystallize 3 or 4 times from a hexane-ether mixture (8:2) at 0°C.

6.5. Purification and Separation of Endosulfan Mixture (Chau, 1969)

The pure isomers of α-endosulfan and β-endosulfan are not available commercially. They can be separated by column chromatography (Chau, 1969; Lindquist and Dahm, 1957) or preparative GLC (Zweig and Archer, 1960).

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* Sec. 6.1, 6.2, 6.3, 6.4: Chau, unpublished result, 1970.

Dissolve 2 gm technical endosulfan (brown amorphous solid, 96%) which contains both α-endosulfan and β-endosulfan, in 30 ml benzene and under suction pass through decolorizing charcoal (Sec. 6.4) with 200 ml benzene. Evaporate eluate to dryness and redissolve in minimum benzene-hexane (1+1) (6 ml-8 ml). Place this solution on a florisil (60-70 gm) column and elute with 500 ml hexane to obtain α-endosulfan (mp 108-109°C). Continue elution with hexane until solid no longer comes out. This fraction contains mainly α-endosulfan with some β-isomer. This can be saved for further separation. Elute column with 250 ml benzene-hexane (1:9) and discard this fraction. Elution with benzene (500 ml) gives mainly β-isomer; concentrate this fraction to about 10 ml and allow to crystallize. Four or five recrystallizations from benzene-hexane (8:2) gives pure β-endosulfan (mp 208-210°C).

NOTE: Care should be taken in eluting the β-endosulfan fraction. Variation of the activity of florisil will not give a clear cut separation; thus, rechromatographing may be necessary.

6.6. Synthesis of Endrin Ketone (Chau, Won, and Cochrane, 1971)

Endrin ketone is found in nature and is also the isomerization product in most GLC columns. Although at present we do not carry out analysis for this product, confusion is eliminated by knowing its behaviour and relative retention times to its parent compound and other pesticides we look for. Endrin ketone can be prepared by photolysis but chemical synthesis is more convenient.

Using a pestle and mortar, grind 1 gm of pure endrin with 20 ml of 90% sulfuric acid solution which has been precooled for 15-20 min. in an ice-salt bath. After grinding for 5 min., allow the mixture to stand at room temperature for 1 hr. with occasional grinding. Pour with caution the contents into 200 ml of ice-cold water. Extract the resulting mixture 6 times with 125 ml portions of chloroform. Wash the combined chloroform extracts with 10% sodium hydroxide solution and twice with 200 ml of distilled water. Dry the extract by passing rapidly through 100 gm of anhydrous sodium sulfate and then concentrate under vacuum to approximately 30-40 ml. Add an equal volume of benzene and then pass through a 50 gm decolorizing charcoal column under suction. Wash the column with an additional 50 ml of benzene. Combine eluates, and concentrate them to 10-15 ml. Allow the solution to stand at 10°C-15°C overnight in order to obtain pure crystals (0.8 gm). Recrystallization from boiling chloroform produces a pure sample with mp of 280°C.

SECTION 7

# *Preparation of Standard Solutions*

7.1. Solvents

Hexane, iso-octane, and benzene are suitable solvents in the preparation of standard solutions for injection into GLC. Chloroform, carbon tetrachloride, ethyl acetate, acetone, methylene chloride and other halogenated hydrocarbon or polar solvents are not suitable for this purpose since they either give a broad solvent peak or shorten the life of the GLC columns or both. The use of benzene has been discouraged by some workers (Analytical Methods for Pesticide Residue in Foods; Food and Drug Directorate, Ottawa, Ontario); however, we found that benzene does not give a deleterious effect to ECD. This author has used benzene interchangeably with hexane for a long period on a GLC column with satisfactory results.

The pesticides presently being analyzed are lindane; heptachlor; aldrin; heptachlor epoxide; dieldrin; p,p'-DDE; p,p'-DDD; p,p' - methoxychlor; α-and β-endosulfan; and endrin. All these pesticides are soluble in benzene and slightly less soluble in hexane at the concentration specified in Section 7.1.1.

7.1.1. Stock Solution

Weigh exactly 100 mg of pure pesticide standard and make up to 100 ml in a volumetric flask with benzene or hexane. Shake well and keep in refrigerator.

7.1.2. Nanogram Solution

From the stock solution (at room temperature) withdraw exactly 100 µl using a 100 µl syringe and transfer to a clean 100 ml volumetric flask. Dilute to the 100 ml mark with hexane or benzene. Shake flask well and keep in refrigerator when not in use.

7.1.3. Picogram Solution

Withdraw 100 µl of nanogram solution (at room temperature) and make up to 10 ml with benzene or hexane in a 10 ml volumetric flask. The resulting solution, equivalent to 10 pg/µl, is suitable for injection into GLC.

7.1.4. Mixed Nanogram Standard A

From individual stock solutions (7.1.1.), withdraw the specified amount as indicated in the table following and transfer to a 10 ml volumetric flask. Dilute to the mark with hexane or benzene. Shake flask well.

The resulting concentration of the pesticide standards will be 10 ng/µl for the first six, 20 ng/µl for DDD and 40 ng/µl for DDT.

7.1.5. Mixed Picogram Solution A

From the mixed nanogram solution (7.1.4.) withdraw 10 µl and dilute to 10 ml. The resulting solution contains 10 pg/µl of pesticides except DDD and DDT which have the concentration of 20 and 40 pg/µl respectively.

| Individual Stock Solutions | Amount to be Withdrawn |
|---|---|
| Lindane | 100 µl |
| Heptachlor | 100 µl |
| Aldrin | 100 µl |
| Heptachlor Epoxide | 100 µl |
| Dieldrin | 100 µl |
| p,p'-DDE | 100 µl |
| p,p'-DDD | 200 µl |
| p,p'-DDT | 400 µl |

It may be convenient to have at least two different mixed picogram solutions - one with the concentration of pesticides as discussed previously (Sec. 7.1.4.), and the other with half the concentration.

7.1.6. Mixed Nanogram Solution B

Prepare as A, except with the following standards and concentrations.

| Standards | Concentrations of Stock Solution |
|---|---|
| Endrin | 400 µl |
| Methoxychlor | 500 µl |
| α-Endosulfan | 100 µl |
| β-Endosulfan | 200 µl |

The resulting mixed nanogram solution contains 40 ng/µl of endrin, 60 ng/µl of methoxychlor, 10 ng/µl of α-endosulfan, and 20 ng/µl of β-endosulfan.

7.1.7. Mixed Picogram Solution B

From mixed nanogram solutions B (7.1.6.), transfer 10 µl and dilute to 10 ml with benzene or hexane. The resulting solution contains 40 pg/µl of endrin, 60 pg/µl of methoxychlor, 10 ng/µl of α-endosulfan and 20 ng/µl of β-endolsulfan.

Two picogram solutions, each with a different concentration, may be necessary - one solution of half the above concentration (5 µl of mixed nanogram solution in 10 ml) and the other double the above concentration (20 µl in 10 ml). The picogram solutions are for GLC standards whereas the nanogram solutions are for TLC standards.

SECTION 8

# *Procedures for Water Analysis*

## 8.1. Scope and Application

This method covers the qualitative and quantitative gas chromatographic determination of twelve organochlorinated pesticides (o.c.) in water and wastewater. With modifications to this method, other o.c., their metabolites and degradation products can also be determined.

The following o.c. are determined by this method:

1) Lindane
2) Heptachlor
3) Heptachlor Epoxide
4) p,p'-DDE
5) Aldrin
6) Dieldrin
7) p,p'-DDD
8) p,p'-DDT
9) Endrin
10) p,p'-Methoxychlor
11) α-Endosulfan
12) β-Endosulfan

## 8.2. Principle of Method

The water sample is extracted by an organic solvent after which the solvent is concentrated to an appropriate volume for GLC analysis. Preliminary identification of the pesticide is based on GLC retention time on the column used for the analysis. Confirmation is based on multi-column techniques and a chemical confirmative test, or TLC.

## 8.3. Interferences

Other pesticide residues and their degradation metabolites, as well as many organic compounds other than o.c. (Sec. 2), may interfere in the analysis. As most of these interferences cannot be separated from the o.c. in question, differentiation by the analyst is required.

## 8.4. Sampling Procedure and Storage

Water samples should be collected and stored in an all glass system at below room temperature to retard degradation of some o.c. A clean sheet

of teflon should cover the mouth of the sample bottle before putting on the cap. This avoids the risk of the sample (water) coming in contact with the plastic cap and the glue lining it. Alternatively, a pre-cleaned heavy-duty alumina foil can be used to replace teflon. NEVER USE PLASTIC UTENSILS DURING ANALYSIS FOR STORAGE OR TRANSFERRING.

8.5. Apparatus

All glasswares must be washed with heavy-duty soap and water, rinsed well, firstly with pesticide grade acetone, secondly with large quantities of pesticide grade hexane. If possible heat glassware in an oven at 600°C (at least overnight). Rinse again with hexane prior to use. It should be emphasized that heating alone cannot remove all the organic constituents.

8.5.1. GLC with good sensitivity, equipped with electron-capture detectors (either $^{63}Ni$ or $^{3}H$ foils); e.g. Microtek 220 or equivalent.

8.5.2. Disposable pipettes.

8.5.3. Graduated centrifuge tubes 15 ml with glass stoppers, or their equivalent.

8.5.4. Volumetric flasks of various sizes, e.g., 1 ml, 5 ml, 10 ml, 100 ml, etc.

8.5.5. Hamilton micro-syringes, 10 µl for injections and other sizes such as 25 µl, 100 µl, 250 µl, etc. for preparation of standard solutions.

8.5.6. Kuderna-Danish Evaporator and glassware.

8.5.7. Oven at 650°C.

8.6. Reagents

All solvents must be of pesticide grade. All chemicals must be of highest purity and, if applicable, should be pretreated to eliminate artifacts or interferences.

8.6.1. Acetonitrile

8.6.2. Acetone

8.6.3. Hexane or Petroleum Ether

8.6.4. Benzene

8.6.5. Methylene Chloride

8.6.6. Chloroform: Pesticide grade chloroform is available from: MC & B, Manufacturing Chemists, Norwood, Ohio, U.S.A. Analytical-graded solvents or a poor batch of pesticide-grade solvent can be redistilled in an all-glass system as described in Sec. 3.

8.6.7. Alumina, pretreated (Sec. 3).

8.6.8. Anhydrous sodium sulfate, pretreated (Sec. 3).

8.6.9. Florisil, 60-100 mesh, calcined at 650°C (factory treated) and stored in a desiccator until used. Before the use and standardization of florisil (Sec. 8.10), check any contaminations or interferences by passing 200 ml of benzene through a 15 gm florisil column and analyze the concentrated eluate (1 ml) by GLC. The amount and type of solvent used for this test should be the same as used in the analysis. In cases where more than one kind of solvent in column clean-up is used, always use the more polar solvent for this test. Thus, if hexane and benzene are used as eluants in the analysis, as in Sans' column procedure, use benzene for this test.

8.6.10. Pesticide standards and standard solutions: See Sections 6 and 7 for the purification and preparation of solutions.

8.6.11. Chemicals for confirmatory tests:

a) Chromous chloride aqueous solution, from Fischer Scientific Company. Use without purification but run a blank before using.

b) Acetic anhydride: Distill once in an all-glass column over anhydrous sodium acetate (10 gm/120 ml) and redistill through a fractionation column packed with precleaned glass helix. Don't collect the first and last 200 ml.

c) Acetic acid: Distill once over chromic acid through a fractionation column and redistill again without chromic acid. In some cases, ordinary analytical grade can be used, provided the blank has no peaks interfering with the confirmatory test.

d) Chromic acid ($CrO_3$): Use without purification.

e) Pyridine: Redistill over potassium hydroxide pellets through a fractionation column and store over potassium hydroxide pellets in a dark bottle. Collect only the fraction that distills between 113°C-117°C.

f) Sulfuric acid ($H_2SO_4$), Analytical grade: Use without treatment.

g) Bromine Analytical grade: Use without treatment.

h) Silylation agent: Mix 1.5 ml hexamethyldisiliazane, 0.5 ml trimethyl chlorosilane, and 10 ml dry pyridine in a glass-stoppered tube while flushing with dry nitrogen. Stopper immediately and keep in a refrigerator.

i) Silver carbonate: Wash well with hexane and acetone. Dry in a vacuum desiccator or in an oven.

j) Silver acetate: Follow the same treatment as for silver carbonate.

l) Potassium tert-butoxide: Use without prior treatment; however, test for interfering peaks before use. If necessary, sublime under vacuum in an all-glass system using an air condenser.

m) Chlorine solution: Bubble pure chlorine gas through $CHCl_3$ or $CCl_4$ (pesticide grade) until light yellow. Stopper solution and keep in a fumehood. Discard when colorless.

n) Ethanol Pesticide grade: This is available from MC & B, Manufacturing Chemists, U.S.A. Absolute ethanol can be used.

8.6.12. GLC column packing materials:

a) SE-30 and ultra-phase SE-30 (methyl silicone).

b) QF-1 (fluoro silicone).

c) DC-11

d) DC-200

e) OV-101 (dimethyl silicone).

f) OV-210 (trifluoropropyl silicone).

g) Gas-chrom Q, 100-120 mesh size.

h) Chromosorb W, acid washed 60-80 mesh size, 80-100 mesh size.

i) Chromosorb W, acid washed and DMCS treated 100-120 mesh size.

j) Anakromb ABS, 100-110 mesh size.

8.6.13. GLC columns:

a) McKinley and McCully's column (1964): 4% SE-30 and 6% QF-1 on 60-80 (or 100-120) mesh size. Chromosorb W, acid washed and/or DMCS treated.

b) 6% QF-1 and 4% DC-11 on 100-120 mesh size on Chromosorb W, acid washed and DMCS treated.

c) Burke and Giuffrida's column (1964): 15% QF-1 and 10% DC-200 on 100-120 mesh size Gas Chrom-Q.

d) Column developed in this laboratory (Chau and Wilkinson, 1972): 4% OV-101 and 6% OV-210 on Chromosorb W, acid washed, HDMS treated 80-100 mesh size.

8.7. Procedure for Extraction of Samples

8.7.1. Method A: Shake vigorously one litre of water sample with 8 ml of benzene in the original sample bottle for at least 30 complete turns. To avoid having the solvent come in contact with the plastic cap and lining, cover the mouth of the bottle with a piece of heavy-duty aluminium foil which has been soaked and cleaned with pesticide-grade methylene chloride, acetone and hexane, in that order.

8.7.2. Stir the mixture with a magnetic stirrer so that the vortex formed at the surface almost reaches the bottom of the bottle. After 3/4 of an hour, allow the layers to separate. If an emulsion forms, add a few drops of saturated sodium sulfate solution and agitate the organic layer. The sodium sulfate must be prewashed and the solution extracted with hexane (Sec. 3), unless no GLC interferences appear when extracting the sodium sulfate solution with hexane.

8.7.3. Add distilled water to the mixture so that the organic layer is at the narrow neck of the bottle. Transfer the organic layer by means of a cleaned disposable pipette to a clean 15 ml centrifuge tube, making sure that the solvent does not come in contact with the rubber bulb. Repeat twice the extraction and stirring procedures, using 3 ml benzene for each procedure.

8.7.4. Evaporate the organic extract by a gentle stream of nitrogen to 8 or 9 ml. Dry the combined organic extract by adding pre-treated anhydrous sodium sulfate into the organic extract. Stopper the tube and shake for a few seconds.

CAUTION: Do not let the organic layer remain with $Na_2SO_4$ for an extended period of time (more than 1 hr.). Losses of pesticides by adsorption may result.

8.7.5. Transfer the organic layer to another cleaned 15 ml centrifuge tube (use a cleaned, disposable pipette). Wash the sodium sulfate and the side of the tube with 2 ml pesticide-grade petroleum ether; transfer by the same pipette to the above organic extract. Repeat the washing twice with 1 ml of petroleum ether.

8.7.6. Under a gentle stream of pure nitrogen, evaporate the combined and dried organic extract to about 8-9 ml. Complete the concentration step to the desired volume (1 or 2 ml) in a Kuderna-Danish evaporator with a Synder column.

8.7.7. Method B: Transfer all the water from the sample bottle, recording exact volume, to a 1 or 2 litre separatory funnel. (The funnel size depends on the volume of water extracted. For 1 litre of water, a 2 litre funnel is used). Rinse the sample bottle once with 50-60 ml distilled water and twice with 60 ml hexane. Add the rinsing solution to the separatory funnel.

8.7.8. Shake contents vigorously and allow the organic layer to separate. If emulsion foams, add a few drops of one of the following: saturated sodium sulfate solution, methanol, isopropanol or 2-octanol. All chemicals used are of highest quality and are tested for minimum or absence of extraneous peaks.

CAUTION: Don't add too much alcohol, otherwise a large solvent peak may result; 5 to 10 drops should be sufficient.

8.7.9. Transfer aqueous layer back to the sample bottle and dry the organic layer under rapid suction through a short column of anhydrous sodium sulfate (50 gm). Use a 500 ml round-bottom flask as a receiver. Apply gentle pressure by means of purified air or nitrogen to push the organic solvent through the tube. A Buchner funnel and suction flask can be used instead of an anhydrous sodium sulfate column. If so, make certain the filter paper is well soaked and washed with pesticide acetone and then hexane.

8.7.10. Transfer the water layer so that almost every drop is back to the separatory funnel and twice repeat extraction (steps 8.7.7 to 8.7.9.) with 35 ml hexane. After the last extraction, rinse the separatory funnel twice with 20 ml of hexane and pass the hexane wash through anhydrous sodium sulfate into the 500 ml flask as described previously.

8.7.11. Concentrate the combined hexane extracts under vacuum to approximately 3 ml. During evaporation the water bath temperature should not exceed 40°C. When the extract is concentrated to 10-12 ml, finish the concentration step by letting the flask rotate in air away from the water bath. This step is critical. Severe loss of pesticide will result if the extract is allowed to dry, particularly if the water bath is too warm.

8.7.12. Using a disposable pipette, transfer the hexane concentrate to a florisil column prepared as follows:

a) In a 20 cm x 400 cm chromatographic column with coarse sintered disk near the bottom, filled with hexane (3/4 full), add 2 gm pretreated anhydrous sodium sulfate, followed by 15 gm of florisil in portions (60-100 mesh, 660°C factory treated). Tap the column gently while adding the florisil into the column; this prevents channelling. Drain some hexane to settle the florisil layer. Add 3 gm of pretreated sodium sulfate ensuring minimum disturbance on the florisil layer.

b) Pre-wash the column with 50 ml benzene, followed by 2 successive additions of 75 ml hexane. Allow column to drain and discard eluates.

c) Quantitatively transfer the hexane extract to the column. Allow the extract to sink just to the surface of the sodium sulfate layer. Wash the round-bottom flask with 3 ml hexane and transfer the washing solution to the column. Let the extract run down as before. Repeat twice with 3 ml hexane.

d) Pour 100 ml of hexane into the round-bottom flask, swirl the flask and pour into the column. (Pour carefully; do not disturb the florisil layer).

e) Run the eluate into a 200 ml round-bottom flask and concentrate under vacuum to approximately 3 ml. Transfer contents with the hexane-rinsing into a Konte evaporation tube; concentrate to the desired volume as described in Section 8.7.6.

f) Elute the same column with 100 ml of 5 to 1 benzene - hexane, catching the eluate in a second 250 ml flask and concentrate as in (e).

NOTE: The hexane fraction from the column will elute aldrin, p,p'-DDE, p,p'-DDT, heptachlor. The benzene-hexane fraction will elute dieldrin, α-endosulfan, endrin, heptachlor epoxide, methoxychlor and p,p'-DDD.

8.8. Calculation

The concentration of a particular pesticide is proportional to the peak area or, if the peak is sharp, to the peak height of an injection. However, non-linearity between peak area (or peak height) and concentration exists due to the limitation of ECD, the electrometer, the column condition and recorder responses. ECD detector is very sensitive to co-extractive in the samples and depending on the type of extracts injected, the co-extractives

will affect the ECD response linearity even on the same day. Therefore, a standard calibration curve should be done daily and used with extreme caution. For practical purposes, pesticide concentration determined by direct comparison with the standard is satisfactory, providing the recorder pen deflection is below 70% full scale and the peak area (or peak height) of the sample is very close to that of the standard. The following calculation is based on extraction of a water sample:

Hsam = Peak height (or area) of a response in the sample.

Hstd = Peak height (or area) of the corresponding standard.

Wstd = Weight in pg of standard for giving Hstd response.

Wsam = Weight (or volume) of water used for extraction.

Vinj = Number of microliters of extract injected to give a peak height of Hsam.

$\chi$ = Volume of sample extract in ml.

$$\text{Microgram/}\ell \text{ (ppb)} = \frac{\text{Hsam}}{\text{Hstd}} \times \text{Wstd} \times \frac{1}{\text{Wsam}} \times \frac{1}{\text{Vinj}} \times \chi \times 10^3$$

8.9. Note on Standardization of Florisil in Using Sans' Column* (1967)

Factory-calcined florisil (at 650°C) varies in activity from batch to batch. It is necessary to standardize a new batch when it is received; the activity should be checked periodically to ensure it does not change upon storage. A large batch of florisil should be subdivided quickly into smaller portions (a portion is taken out and then subdivided) in a dehumidified room and each portion stored in a tightly capped brown bottle in a desiccator; in this way, changes in activity of florisil are greatly minimized.

NOTE: Do not unnecessarily expose florisil to the atmosphere.

*Standardization* - Use the mixed nanogram solution A (Sec. 7) containing 10 ng/µl each of lindane, heptachlor, aldrin, heptachlor epoxide, p,p'-DDE, 20 ng/µl of p,p'-DDD and 40 ng/µl of p,p'-DDT. Prepare a florisil column containing 15 gm florisil as described in Section 8.7.12 a and b. Dilute 50 µl of the mixed standard to approximately 1 ml in a tube and transfer the solution to the column. Allow the solution to sink just to the surface of the sodium sulfate layer. Wash tube with 2-3 ml hexane and transfer to the column. Repeat with 2-3 ml hexane. After the solvent sinks just to the top of the $Na_2SO_4$ layer, elute column with 125 ml hexane or petroleum ether and collect eluate in a 200 ml round-bottomed flask. Then elute the column with 125 ml of benzene - hexane mixture (5:1). Collect eluate in another 200 ml round-bottomed flask. Concentrate each eluate to 10 ml and examine by means of a gas chromatograph.

NOTE: Elution rate should be adjusted to 5-6 ml/min. The first fraction (hexane or petroleum ether) should contain: heptachlor, aldrin, DDE,

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* Sans, W.W., Standardization of florisil, private communication.

DDT. The second fraction should contain: dieldrin, heptachlor and DDD. If some of the pesticide standard in the second fraction is partially eluted out in the first fraction, the activity of florisil has decreased. If some of the first fraction is eluted in the second fraction (or the second fraction is only partially eluted), the activity of florisil is too active; in this case use the same amount of florisil but add 0.2 to 0.5 ml ethanol (depending on the degree of activity, judged by the quantity of pesticide standards eluted) in benzene when washing the column, (Sec. 8.7.12. b). If florisil decreases in activity, use 17 gm instead of 15 gm; if necessary adjust the amount by adding 1 gm each trial until fractionation is complete.

# *Appendix*

9.1. The following list of different GLC columns for o.c. pesticide residue analysis, by no means exhaustive, illustrates the variety of columns used in this field.

| ITEM | COLUMNS | REFERENCE |
|---|---|---|
| 1 | 1.3% Cyanosilicone GE-XE-60 and 0.13% Epikote Resin 1001 on Chromosorb G (DMCS, AW 70-80 mesh) | Goulden *et al*, 1963 |
| 2 | 1.3% Apierzon L grease and 0.2% Epikote Resin 1001 on Chromosorb G (DMCS, AW, 70-80 mesh) | Goulden *et al*, 1963 |
| 3 | 10% DC-200 (12,500 cst) on Anakrom ABS (80-90 mesh) | Shuman and Collie, 1963<br>Burke and Giuffrida, 1964 |
| 4 | 5% Acetate-fractionated DOW corning high vacuum silicone grease on Chromosorb W (AW, 80-100 mesh) | Burke and Giuffrida, 1964 |
| 5 | 5% DC-11 on Chromosorb W (Non-AW, 60-80 mesh) | Dimick and Hartmann, 1963 |
| 6 | 0.15% Carbowax 20 M and 1.5% DC-200 on Chromosorb G (AW, 60-70 mesh) | Lamar *et al*, 1966 |
| 7 | 0.1% Carbowax 20 M and 1.0% QF-1 on Chromosorb G (AW, 60-70 mesh) | Lamar *et al*, 1966 |
| 8 | 4% SE-30 on Chromosorb A (80-100 mesh) | Saha, 1966 |
| 9 | (1 + 1) mixture of 15% QF-1 and 10% DC-200 on Chromosorb Q (80-100 mesh) | Reynolds, private communication<br>Sans, private communication<br>Burke and Holswade, 1966 |
| 10 | (1 + 1) mixture of 13% QF-1 and 8% DC-200 on gas Chrom Q | Pearson *et al*, 1967 |
| 11 | 1% Epon 1001 and 0.5% Viton A on Chromosorb W (DMCS, AW, 100-120 mesh) | Parke and Bruce, 1968 |
| 12 | 3% SE-30 on Chromosorb W (DMCS, AW, 80-100 mesh) | Bevenue and Yeo, 1969 |
| 13 | 3% DC-200 on gas Chrom Q (100-120 mesh) | Purdue *et al*, 1969 |
| 14 | 10% QF-1 on Chromosorb G (100-120 mesh) | Purdue *et al*, 1969 |

| ITEM | COLUMNS | REFERENCE |
|---|---|---|
| 15 | 10% Carbowax 20 M on Chromosorb W | Grice, Yates and David, 1970 |
| 16 | 10% DC-200 on gas Chrom Q (80-100 mesh) | Klein and Link, 1970 |
| 17 | 5% QF-1 on High Performance Chromosorb W (DMCS, AW, 80-100 mesh) | Radamski and Rey, 1970 |
| 18 | 5% OV-101 on gas Chrom Q (80-100 mesh)<br>5% OV-17 on gas Chrom Q (80-100 mesh)<br>5% OV-210 on gas Chrom Q (80-100 mesh)<br>5% OV-225 on gas Chrom Q (80-100 mesh) | Bowman and Beroza, 1970 |

9.2. Retention Time

9.2.1. Retention Time for Semi-Polar Column 1

| PESTICIDES | RETENTION TIME (IN ARBITRARY UNIT) |
|---|---|
| Lindane | 3.75 |
| Heptachlor | 5.35 |
| Aldrin | 6.60 |
| Heptachlor Epoxide | 9.60 |
| α-Endosulfan | 12.2 |
| p,p'-DDE | 12.4 |
| Dieldrin | 14.6 |
| o,p'-DDT | 16.3 |
| Endrin | 16.7 |
| p,p'-DDD | 17.8 |
| β-Endosulfan | 18.8 |
| Endrin Pentacylic Ketone | 19.3 |
| p,p'-DDT | 21.8 |
| p,p'-Methoxychlor | 25.4 |

INSTRUMENT PARAMETERS

*Column:* Glass, 1/4" O.D., 6 feet packed with 1 + 1 mixture of 15% QF-1 and 10% DC-200 on gas Chrom Q (100-120 mesh).

*Carrier Gas:* Nitrogen flow rate at 100 ml/min.

*Temperatures:* Injection 200°C; Column over 200°C; Detector ($^{63}Ni$) 250°C.

9.2.2. Retention Time for Semi-Polar Column 2

| PESTICIDES | RETENTION TIME (IN ARBITRARY UNIT) |
|---|---|
| Lindane | 1.9 |
| Heptachlor | 2.65 |
| Aldrin | 3.2 |
| Heptachlor Epoxide | 4.7 |
| α-Endosulfan | 6.05 |
| p,p'-DDE | 6.05 |
| Dieldrin | 7.20 |
| Endrin | 8.30 |
| p,p'-DDD | 8.80 |
| β-Endosulfan | 9.55 |
| p,p'-DDT | 10.7 |
| Methoxychlor | 16.1 |
| Endrin Ketone | 20.5 |

INSTRUMENT PARAMETERS

*Column:* Glass 1/2" O.D., 6 feet packed with 6% SE-30 and 4% QF-1 on chromosorb W (AW, DMCS treated, 100-120 mesh size).

Carrier gas and operating temperature identical to 9.2.1.

9.2.3. Retention Time for Semi-Polar Column 3

| PESTICIDES | RETENTION TIME (IN ARBITRARY UNIT) |
|---|---|
| Lindane | 1.85 |
| Heptachlor | 2.60 |
| Aldrin | 3.20 |
| Heptachlor Epoxide | 4.65 |
| o,p'-DDE | 4.75 |
| γ-Chlordane | 5.0 |
| α-Chlordane | 5.5 |

| PESTICIDES | RETENTION TIME (IN ARBITRARY UNIT) |
|---|---|
| α-Endosulfan | 5.80 |
| p,p'-DDE | 5.90 |
| o,p'-DDD | 6.50 |
| Dieldrin | 7.00 |
| Endrin | 7.7 |
| o,p'-DDT | 7.85 |
| p,p'-DDD | 8.30 |
| p,p'-DDT | 10.35 |
| p,p'-Methoxychlor | 15.2 |
| Endrin Pentacylic Ketone | 18.8 |

INSTRUMENT PARAMETERS

*Column:* Glass 1/2" O.D., 6 feet packed with 4% OV-101 and 6% OV-210 on chromosorb W (AW, HDMS treated, 80-100 mesh).

Carrier gas and operating temperature identical to 9.2.1.

9.2.4. Retention Time for Semi-Polar Column 4

| PESTICIDES | RETENTION TIME (IN ARBITRARY UNIT) |
|---|---|
| Lindane | 1.30 |
| Heptachlor | 1.75 |
| Aldrin | 2.10 |
| o,p'-DDE | 3.15 |
| γ-Chlordane | 3.3 |
| Heptachlor Epoxide | 3.15 |
| α-Chlordane | 3.6 |
| p,p'-DDE | 3.9 |
| α-Endosulfan | 3.95 |
| Dieldrin | 4.75 |

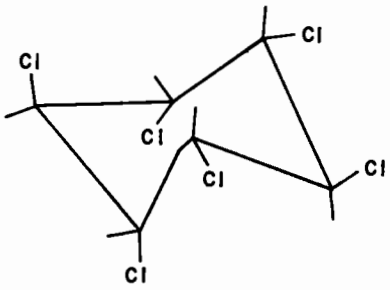
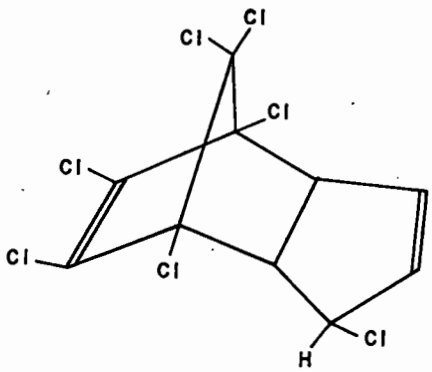
| PESTICIDES | RETENTION TIME (IN ARBITRARY UNIT) |
|---|---|
| o,p'-DDT | 5.15 |
| Endrin | 5.4 |
| p,p'-DDD | 5.75 |
| β-Endosulfan | 6.20 |
| p,p'-DDT | 6.85 |
| p,p'-Methoxychlor | 10.05 |
| Endrin Pentacyclic Ketone | 13.3 |

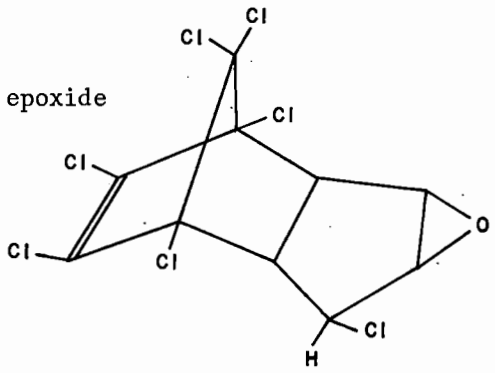
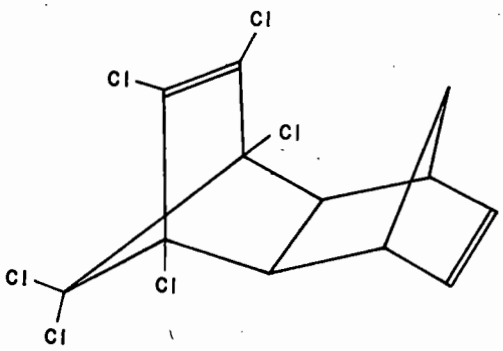
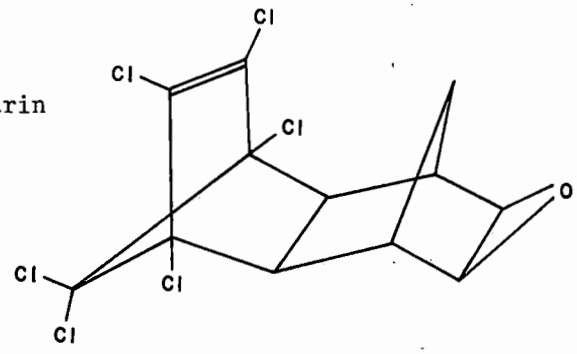
INSTRUMENT PARAMETERS

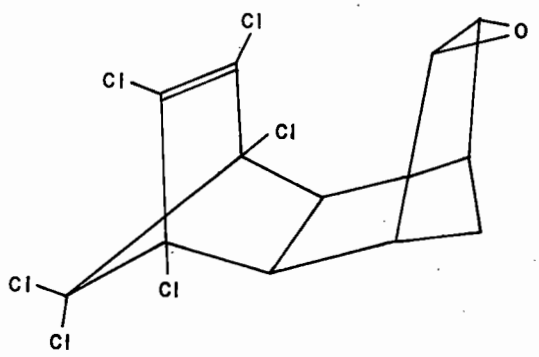
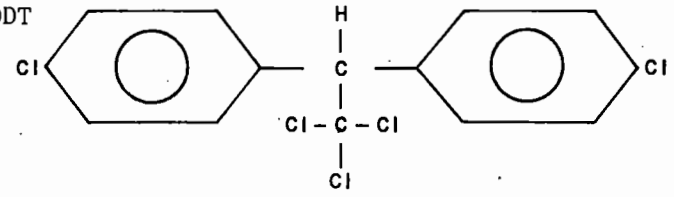
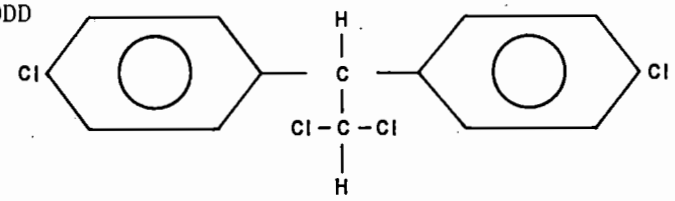
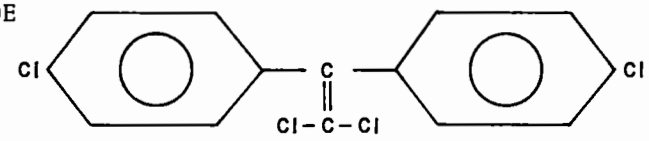
*Column:* Glass 1/2" O.D., 6 feet packed with 6% QF-1 and 4% DC-11 on chromosorb W (AW, DMCS treated, 100-120 mesh size).

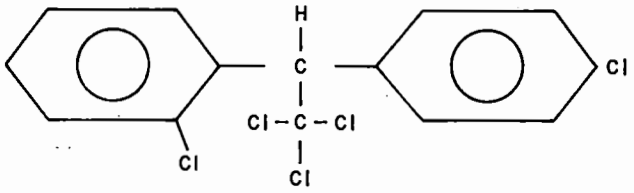
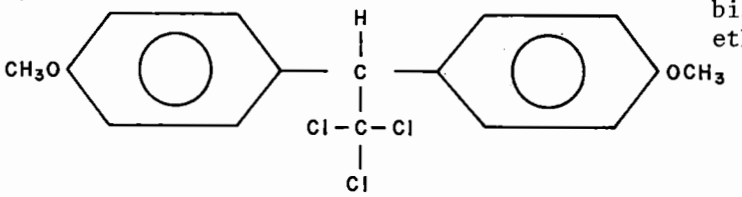
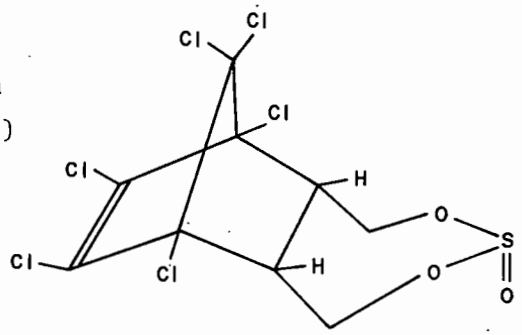
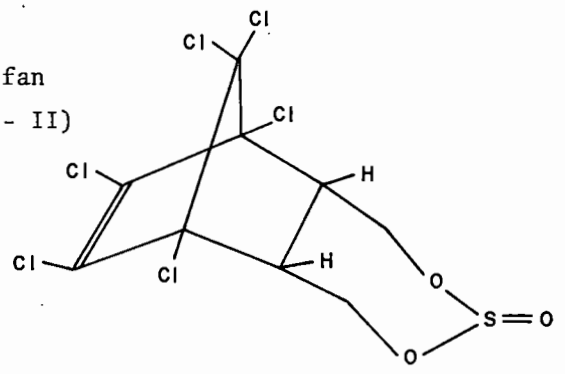
Carrier gas and operating temperature identical to 9.2.1.

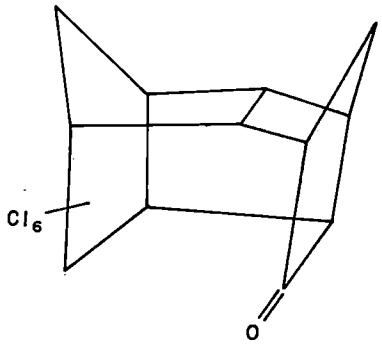
9.3. Chemical Structure and Nomenclature of Some O.C. Pesticides

| COMMON NAME | STRUCTURE | CHEMICAL NOMENCLATURE |
|---|---|---|
| Lindane (γ-BHC) |  | γ-1,2,3,5,6 - hexachlorocyclohexane |
| Heptachlor |  | 1,4,5,6,7,8,8 - heptachloro -3a,4,7,7a - tetrahydro -4,7- methanoindene |

| COMMON NAME | STRUCTURE | CHEMICAL NOMENCLATURE |
|---|---|---|
| Heptachlor epoxide |  | 2,3 - Epoxy,1,4,5,6,7,8,8, - heptachloro - 3a,4,7,7a - tetrahydro - 4,7 - methanoindene |
| Aldrin |  | 1,2,3,4,10,10 - hexachloro - 1,4,4a,5,8,8a - hexahydro - 1,4 - endo, exo - 5,8 - dimethanonaphthalene |
| Dieldrin |  | 1,2,3,4,10,10 - hexachloro - exo - 6,7 - epoxy - 1,4,4a,5, 6,7,8,8a, octahydro - 1,4, - endo, exo - 5,8, dimethano-naphthalene |

| COMMON NAME | STRUCTURE | CHEMICAL NOMENCLATURE |
|---|---|---|
| Endrin |  | 1,2,3,4,10,10 - hexachloro - exo - 6,7 - epoxy - 1,4,4a,5,6, 7,8,8a - octahydro - 1,4 - endo, endo - 5,8 - dimethanonaphthalene |
| p,p'-DDT |  | 1,1,1 - trichloro - 2,2 - bis (p - chlorophenyl) ethane |
| p,p'-DDD (TDE) |  | 1,1, - dichloro 2,2, - bis (p - chlorophenyl) ethane |
| p,p'-DDE |  | 1,1 dichloro - 2,2 - bis (p-chlorophenyl) ethylene |

| COMMON NAME | STRUCTURE | CHEMICAL NOMENCLATURE |
|---|---|---|
| o,p'-DDT |  | 1,1,1 - trichloro - 2 - 0 - chlorophenyl - 2 - p - chlorophenlethane |
| Methoxychlor |  | 1,1,1, - trichloro - 2,2 - bis (p-methoxyphenyl) ethane |
| α-Endosulfan (Thiodan - 1) |  | 6,7,8,9,10,10 - hexachloro - 1,5,5a,6,9,9a - hexahydro - 6,9, - methano - 2,4,3, - benzodioxathiepin - 3 - oxide, α isomer |
| β-Endosulfan (Thiodan - II) |  | β-isomer |

| COMMON NAME | STRUCTURE | CHEMICAL NOMENCLATURE |
|---|---|---|
| Endrin Pentacyclic Ketone |   | 1,8,9,10,11,11 - hexa - chloropentacyclo - $(6.2.1.0^{2,7}.1^{3,6}.0^{5,9})$ - dodecan - 4 - one |

9.4. Suggested Reading

For general and specific references, the following publications are recommended:

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2) Pesticide Analytical Manual, Volume 1 and 2, U.S. Department of Health, Education and Welfare, Food and Drug, Administration, U.S.A.

3) FWPCA Methods for Chlorinated Hydrocarbon Pesticides in Water and Wastewater, U.S. Department of the Interior, U.S.A.

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