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UNITED STATES DEPARTMENT OF AGRICULTURE
CONSUMER AND MARKETING SERVICE
MEAT AND POULTRY INSPECTION PROGRAM

WASHINGTON, D.C. 20250

August 3, 1971

TO: LABORATORY DIRECTORS

You may, by now, have heard through the public press of the crisis that has developed because of PCB contamination of animal feeds. Owners of flocks and herds who are affected may be asking your laboratory for help. If you have had considerable experience analyzing for pesticide residues, we assume you could also analyze for PCB's.

If you should accept samples for analysis, we recommend you use the enclosed method. To the best of our knowledge, the PCB in question was Monsanto's Aroclor No. 1242. If you wish, we will be glad to furnish some of this material to you as a standard; please telephone Mr. Anthony J. Malanoski at 301, 447-4800, extension 596 or 597. He will also answer any questions regarding methodology.

Your results will be monitored by a split sample procedure by one of our Federal laboratories. Your reports, consisting of analytical findings, sample numbers and identification of growers should be air mailed daily to Mr. O. L. Bennett, Head, Chemistry Group, Laboratory Services Division, C&MS, USDA, Washington, DC 20250.

Sincerely,

R Paul Elliott

R. Paul Elliott, Chief
Chemistry & Microbiology Branch
Laboratory Services Division

Enclosure

What will B/C 5000

DSW 378310

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Detection of Polychlorinated Biphenyls (PCB's) in Adipose Tissue

The acetonitrile partition, described in the attached method, requires a minor modification to insure a quantitative recovery of the PCB's.

At the end of the acetonitrile partition, the petroleum ether extract should be concentrated to 25 ml and transferred to the florisil column after the column has been washed with 150 ml of petroleum ether. (Discard wash as soon as the petroleum ether extract is placed on the column).

If multiple unknown peaks are present on the final chromatogram, PCB's may be present.

Detection Limit

Assume — 5 g sample

— 25 ml final vol

— 5 ul injected

— abs min 1×10^{-9} g

$\frac{1 \times 10^{-9}\ g}{5\ ul} \times \frac{10^3\ ul}{ml} \times 25\ ml \times \frac{1}{5} \times 10^3\ g/kg \times 10^3 \frac{mg}{g}$

$= PPM = 1$

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Quantitation of Polychlorinated Biphenyls (PCB's) in Adipose Tissue

The attached chromatograms indicate the method which should be used for quantitation. As shown, 12 peaks from sample 1 were matched (same retention time) with 12 peaks from the PCB 1242 standard, and the peak height of each one was measured.

The sum of the heights of the identifiable peaks from the sample was divided by the sum of the heights of the corresponding peaks from the 5 nanogram standard. Since the injection represented 1 mg. of sample, the concentration of PCB 1242 was 1.9 ppm in this sample.

You may or may not be able to match as many peaks as are shown on the attached chromatograms. For proper quantitation, however, you should use as many peaks as can be properly matched.

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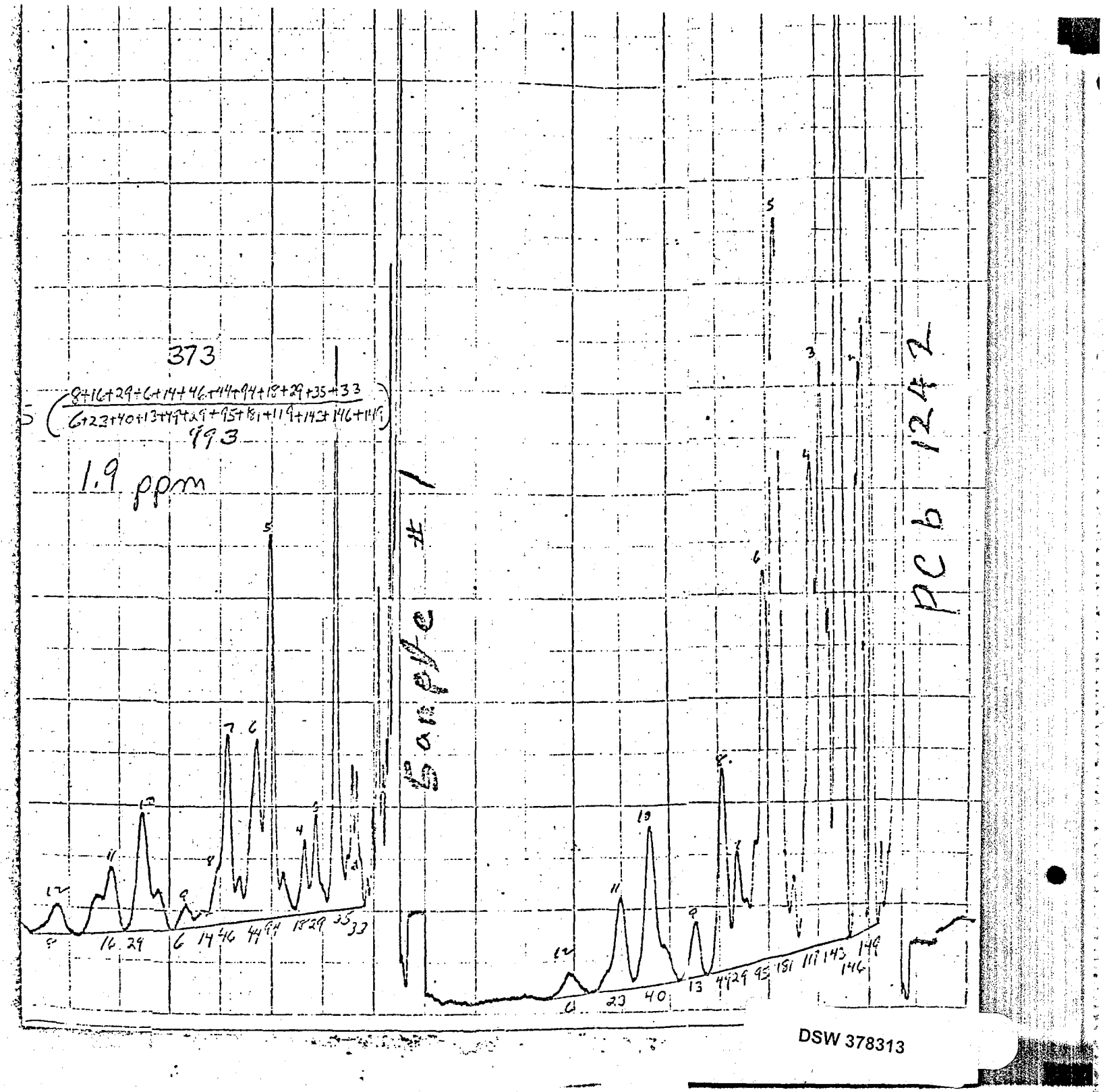

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44 CHLORINATED HYDROCARBON ANALYSIS

Since chlorinated hydrocarbon pesticides are stored in a fat, or fatty tissue, all such analyses are reported on the basis of the extracted or rendered fat.

THEORY

The sample is dissolved in petroleum ether and extracted with acetonitrile. The acetonitrile extracts are diluted with water to partition the pesticide residues into petroleum ether. The residues are purified by chromatography on a Florisil column, eluting with mixtures of petroleum and ethyl ethers. Residues in the concentrated eluates are measured quantitatively by gas-liquid chromatography or semi-quantitatively by thin layer chromatography. Identification is by a combination of gas-liquid and thin-layer chromatography.

APPARATUS

(a) Chromatographic columns. With teflon stopcocks and coarse fritted plate, 22mm id x 300 mm.

(b) Alternate column - Chromatographic columns without stopcocks. With coarse fritted plate, 22mm id x 300 or 400 mm - (when this column is used, it is topped with anhydrous sodium sulfate powder for flow control).

(c) Filter tubes. Approximately 22mm x 200mm with a short delivery tube and a coarse fritted plate (a glass wool plug can also be used).

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(d) Kuderna-Danish Concentrators. Five hundred ml with 5 or 10 ml volumetric receiving flasks or graduated tubes (Kolmer or Mills types; Kontes Glass Company, Vineland, New Jersey 08360, Catalog No. K-570000, K-621400, or equivalent.)

(e) Alternate concentrator. If the solutions can be made to a volume of 25 ml, an erlenmeyer flask with a 24/40 S joint with a three ball Snyder column may be used.

(f) Separatory funnels. Teflon stopcocks - 500 ml and 125 ml.

(g) Thin layer apparatus and accessories. Desaga/Brinkman apparatus for thin layer, or equivalent.

(h) Chromatographic tank and accessories. Arthur H. Thomas Co., Vine Street at 3rd, Philadelphia, Pa. 19105, No. 3106-F05, or equivalent, with metal troughs.

(i) Ultra violet light source. Four germicidal lamps, 15 watt, 18" long mounted in a metal cabinet 20" x 10" x 10" to accommodate two glass plates.

(j) Gas liquid chromatograph. Barber Colman Model 5000 or equivalent with electron capture detector, power supply, electrometer and 5 mv recorder.

(k) Columns. Six feet x four mm id (approximately) packed with 10 percent DC-200 on 100-120 mesh Anakrom ABS, (Analabs Inc., Post Office Box 501, North Haven, Connecticut 06473) or 3 percent JXR on 100-120 mesh Gas Chrom Q, (Applied Science Laboratories, State College, Pennsylvania 16801). The column should be conditioned at 250°C with a nitrogen flow of 100 to 120 ml/min until endrin emerges as a single peak.

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(1) Alternate column - 6' x 4 mm (approximately) packed with 3 percent QF1 and 1 percent DC-200 on 100-120 mesh Gas Chrom Q. The column should be conditioned at 200°C. QF1 bleeds excessively at higher temperatures. (If this column is used, the 6 percent and 15 percent elutions may be combined).

SOLVENTS

Some solvents, sold as suitable for use as received, are available from many manufacturers, and are usually identified as Nanograde or chromatographic grade. All solvents, however, must be tested prior to use to determine whether purification is required. This test is to be performed as outlined on the following page. Those solvents requiring purification must be distilled in an all-glass apparatus. However, if Nanograde or chromatographic grade solvents are found to require purification, they should be returned to the supplier with a request for replacement, since their high cost is predicated upon purity.

CAUTION

Ethers (excluding petroleum ether) containing unsafe concentrations of peroxides can detonate when they are distilled or concentrated. No quantity of ether, therefore, should be so treated before the following test is performed:

"Shake one ml of a freshly prepared saturated solution of KI with nine ml of ether in a 25 ml glass stoppered cylinder. Any yellow color indicates a concentration of peroxide greater than 0.005 percent."

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Such a concentration is dangerous, and ether containing this level of peroxide should be discarded, or returned to the supplier with a request for an exchange.

Purity Test

Electron capture gas chromatography requires the absence of substances causing any detector response from the following test: Place 300 ml of solvent in the Kuderna-Danish Concentrator fitted with a three-ball Snyder column and a calibrated collection tube and evaporate to five ml. Inject five ul of concentrate into the gas chromatograph using operating conditions described in the paragraph, "Residue Detection Method - Gas Chromatography." Concentrate must not cause recorder deflection $>$ one mm from base line for 1 - 60 minutes after injection. If thin layer is used for semi-quantitative analysis, 50 ul of the concentrate is spotted on the plate, developed, aprayed and exposed to UV light as described in the paragraph, "Thin-layer chromatography." There should be no spots visible.

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REAGENTS

(a) Acetonitrile. Practical Eastman Organic Chemicals (our experience has been that it seldom requires purification). If purification is necessary, purify as follows:

To four liters of acetonitrile add one ml of H_3PO_4, 30 grams P_2O_5, a few boiling chips and distill in all glass apparatus at 81-82°C. DO NOT EXCEED 82°C.

(b) Acetonitrile saturated with petroleum ether - Saturate CH_3CN with petroleum ether (g). A small layer of petroleum ether should just be visible on the CH_3CN.

(c) Eluting solvent, 6 percent - Dilute 60 ml ethyl ether (e) to one liter with petroleum ether (g).

(d) Eluting solvent, 15 percent - Dilute 150 ml ethyl ether (e) to one liter with petroleum ether (g).

(e) Ethyl ether - Reagent or ACS grade. (See Caution under Solvents).

(f) Florisil - Pesticide Residue grade (60-100 mesh) Floridin Company, 2 Gateway Center, Pittsburgh, Pa. 15222.

Activate not more than a two-day supply of Florisil by heating at 650°C for two hours (caution: DO NOT EXCEED 650°C). Transfer while hot (use beaker tongs, asbestos gloves and funnel) to erlenmeyer flasks, stopper loosely with aluminum foil wrapped corks and cool in a desiccator stored in the dark.

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Standardization of Florisil

Reagents

Indicator. Dissolve 1 gm of phenolphthalein in ethyl alcohol and dilute to 100 ml.

Ethyl alcohol, neutralized to phenolphthalein.

Lauric acid solution. Dissolve 10.000 gms lauric acid in distilled hexane and dilute 500 ml.

0.05N NaOH - Dissolve 2.000 gms sodium hydroxide in distilled water and dilute to one liter.

Standardized NaOH solution - Pipet 10.0 ml lauric acid solution (200 mg) into a 125 ml erlenmeyer flask. Add 50 ml of neutralized ethyl alcohol, three drops of indicator and titrate with 0.05N NaOH to a permanent end-point. Calculate mg lauric acid per ml 0.05N NaOH.

Procedure

Transfer 2.000 g activated Florisil to a 25 ml ℨ erlenmeyer flask. Add 20 ml lauric acid solution (400 mg), stopper and shake occasionally for 15 minutes. Let adsorbent settle and pipet 10.0 ml of supernatant into 125 ml erlenmeyer flask. Avoid inclusion of any Florisil. Add 50 ml neutral ethyl alcohol and three drops of indicator solution; titrate with 0.05N NaOH to a permanent end-point.

Calculate amount of lauric acid adsorbed on Florisil, (Lauric Acid Value), as follows: Mg Lauric Acid/g Florisil = 200 — (ml required for titration x mg Lauric Acid/ml 0.05N NaOH.)

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A lauric acid value of 110 is the acceptable value; i.e., 20 g of Florisil would fill a 22mm i.d. column to a depth of 110mm. To obtain the equivalent quantity of Florisil, divide 110 by the Lauric acid value for that batch and multiply by 20:

$\frac{110 \times 20}{LA\ V}$ = g Florisil required.

The columns are prepared as in the clean up technique, and prewashed with 100 ml petroleum ether. A 1 ml aliquot of mixed pesticides containing 0.2 ug lindane, 0.4 ug heptachlor epoxide, 0.6 ug dieldrin and 0.6 ug endrin is placed on the column. The columns are eluted also as in the clean up technique. The eluates are concentrated, transferred to 25 ml volumetric flasks and made to volume with petroleum ether. One ml of the mixed pesticides is also diluted to 25 ml with petroleum ether in a volumetr flask. Inject 5 ul of the standard and of the two eluates into the gas chromatograph. The recovery of the pesticides when compared to the standard must be at least 95 percent and lindane and heptachlor epoxide must be in the six percent elution while dieldrin and endrin must be in the 15 percent elution.

(g) Petroleum ether 30°- 60°C, boiling range.

(h) Sodium sulfate - anhydrous granular; anhydrous powder.

(i) Aluminum oxide - Al_2O_3G available from Warner-Chilicott Laboratories, Instruments Division, 200 South Garrad Blvd., Richmond, California 94801. Al_2O_3G neutral, available from Merck & Co., Rahway, New Jersey 07065.

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(j) Developing solvents - (1) n-heptane, commercial grade (no purification required)

(2) n-heptane, commercial grade, containing 2 percent acetone, reagent grade.

(k) Chromogenic agent - Dissolve 0.100 grams $AgNO_3$ in 1 ml H_2O, add 20 ml 2-phenoxyethanol (Practical, Eastman Organic Chemicals), dilute to 200 ml with reagent grade acetone, add one very small drop of 30 percent H_2O_2 and mix. Store in dark over night and decant into spray bottle. Discard after four days.

(l) Standard solutions for thin-layer chromatography.

(1) Stock solutions A_1 and A_2 (mixture of aldrin, lindane heptachlor epoxide and methoxychlor). Weigh 0.1 gram of each pesticide into same 10 ml glass stoppered volumetric flask. Repeat with 0.05 grams of each into a second volumetric flask. Dissolve in ethyl acetate, dilute to 10 ml and mix. One ul of each solution contains 10 ug and 5 ug, respectively, of each pesticide.

(2) Stock solutions B_1 and B_2 (mixture of BHC, DDE, DDD, DDT, dieldrin, and endrin). Prepare solutions of 10 and 5 ug/ul as in (1).

(3) Dilutions of stock solutions: From the stock solution prepare dilutions containing 0.2, 0.1, 0.05, 0.02, 0.01, 0.005 and 0.002 ug/ul.

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(m) Individual Standards. Separate standards of each insecticide should be prepared at 0.1 ug/ul concentration so that the migration of each insecticide can be determined and the insecticide can be identified in the mixtures.

(n) Gas liquid chromatography. The individual standards from (m) are diluted so that a five ul injection will contain approximately 0.25 nanogram of heptachlor epoxide and will give approximately 50 percent of full scale deflection on the recorder. The concentrations of all other insecticides should be adjusted accordingly: Lindane and BHC should be approximately 1/2 of the heptachlor epoxide; dieldrin, endrin and DDE should be 1-1/2 times; DDD should be about twice, DDT about 4 times and methoxychlor about eight times. This is approximate, and must be determined for each gas liquid chromatograph used.

(o) Gas liquid chromatography mixture standards. After retention time and response have been determined for the separate insecticides, prepare mixtures so that the compounds will not interfere with each other, i.e., BHC, DDE, DDD and methoxychlor in one solution; lindane, aldrin, heptachlor epoxide, dieldrin and endrin in another. The concentrations should such that a 5 ul injection produces a 1/2 scale recorder deflection.

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DETERMINATION: Sample Preparation

Fatty tissue. Pass chilled sample through food chopper twice. If dry ice is available, add small quantities to fat to maintain a low temperature. Plug a powder funnel moderately tight with glass wool; place approximately 100 grams of the comminuted fat in the funnel; position the funnel in a beaker and render at 125°C until the fat ceases to drop.

Muscle tissue. Pass sample through food chopper three times. Grind suitable quantity with anhydrous Na_2SO_4 to combine with moisture in sample. Transfer granular mixture to centrifuge bottle, add 100 ml petroleum ether, shake vigorously and centrifuge about 5 minutes at about 1500 rpm. Pour off solvent layer into beaker and re-extract residue twice with 50 ml portic of petroleum ether. Evaporate combined extracts to obtain fat.

All chlorinated hydrocarbon residues are reported on the basis of the rendered or extracted fat.

Acetonitrile Partitioning

Weigh 5 grams of melted fat into a 50 ml beaker, add 10 ml of petroleum ether and transfer to a 125 ml separatory funnel. Use an additional 15 ml in small portions to rinse the beaker for a quantitative transfer of the fat Add 25 ml of acetonitrile saturated with petroelum ether and shake vigorously for 1 minute. Let layers separate (adequate time must be allowed to obtain a good separation). It is a good idea to plan to use the time during the separation for a subsequent operation - i.e., during the first extraction, the preparation of the 500 ml separatory funnels containing the

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petroleum ether and 2 percent Na_2SO_4. Drain the bottom (acetonitrile) layer into a 500 ml separatory funnel containing 300 ml of 2 percent Na_2SO_4 and 100 ml of petroleum ether. Extract the petroleum ether solution in the 125 ml separatory funnel with 3 additional portions of acetonitrile saturated with petroleum ether, shaking vigorously 1 minute each time. Combine all extracts in the 500 ml separatory funnel. Stopper, invert the 500 ml separatory funnel, vent pressure, and shake gently 1 minute to transfer the pesticides to petroleum ether. Let layers separate cleanly and discard lower aqueous layer. Wash the petroleum ether in 500 ml separatory funnel with two 50 ml portions of 2 percent Na_2SO_4 (after addition of 2 percent Na_2SO_4, stopper, invert and vent. Occasionally, excessive pressure will build up). Discard washings and draw off petroleum ether layer through a 2" column of anhydrous granular Na_2SO_4 in the filter tube into a 250 ml erlenmeyer flask. Rinse the separatory funnel and the column with two 10 ml portions of petroleum ether. (The total elapsed time for the acetonitrile separation should be approximately 2 hours).

Clean Up Technique Florisil Column

Prepare 22 mm id florisil column, containing 100 mm after packing of activated florisil topped with about 1/2" of anhydrous sodium sulfate (110 mm should pack to 100 mm). Transfer petroleum ether extract to column, letting it pass through the column at a rate not to exceed 3 ml/min (optimum rate is approximately 2½ ml/min). When the last of the petroleum ether has sunk into the column, change receivers and elute with 110 ml of 6 percent

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ethyl ether in petroleum ether at the same rate. Change receivers and elute with 110 ml of the 15 percent ethyl ether in petroleum ether. Concentrate each eluate to 5 ml using the Kuderna-Danish or alternate concentrator. Transfer each eluate quantitatively to a 25 ml volumetric flask and made to volume with petroleum ether. When the elution from the florisil column is held to the low rate specified, the insecticides are eluted quantitatively from the column and both eluates are suitable for thin-layer or gas-liquid chromatography.

First eluate (6 percent) contains aldrin, BHC, DDE, DDD(TDE), o,p'- and p,p'-DDT, heptachlor, heptachlor epoxide, lindane and methoxychlor. Second eluate (15 percent) contains dieldrin and endrin.

Residue Detection Method Gas Chromatography

Operating conditions for 10 percent DC-200 or 3 percent JXR column. Injection temperature 220°C, column temperature 185° to 200°C, detector temperature 210°C maximum, carrier gas flow 120 ml/min nitrogen. Detector voltage 24 to 40 volts; exact voltage must be determined for each chromatograph to obtain the maximum sensitivities.

Operating conditions for mixed column - 3 percent QF1 and 1 percent DC-200. Injection temperature 200°C, column temperature 170° to 185°C, detector temperature 200°to 210°C. Carrier gas flow 100-120 ml/min nitrogen. Detector voltage 24 to 40 volts; exact voltage must be determine for each chromatograph.

Check the linearity of the system. Determine the individual retentio times of the insecticides. Make calculations only from peaks that are in the linear range.

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Inject a suitable aliquot (3-8 ul) of the eluates from the 25 ml volumetric flask into the chromatograph using a 10 ul syringe. If peaks are out of the linear range, re-inject using a smaller aliquot or dilute to bring the response into the linear range. Tentatively identify residue peaks on the basis of retention time. Measure areas under the peak or peaks from the unknown and compare to the areas obtained from known quantity of the standard insecticide. For most accurate measurement, unknowns and standards should be of similar size and in the linear range.

Chromatograph the standard pesticide immediately after the sample. Alternatively, it is possible to use only peak heights with a slight loss in accuracy, but good judgement is required, and it is very important that all measurements be in the linear range of the detector.

Thin-layer Chromatography

A. Preparation of adsorbent Layer

Select five 8" x 8" double strength window glass plates of uniform width and thickness and smooth off the corners and edges with a file or other suitable instrument. Before coating, wash the plates in hot soapy water and thoroughly rinse with distilled water. Press the plates snugly into position on the mounting board which has a retaining ledge on one side and one end. The plastic board is mounted so that its long side with raised ledge faces the operator while the short side with ledge is

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to the right of the operator. Before coating, the surface of the plates are wiped with a few ml of 95 percent ethanol on a tissue to remove fingerprints or other adhering material.

Position the applicator, trough open, with the left edge 1/4" in from the edge of the first plate to be coated.

To coat 5 plates, weigh 30 gms Al_2O_3G into a 250 ml S erlenmeyer flask. Add 50 ml distilled water, stopper flask and shake moderately for 45 seconds. Violent shaking produces bubbles resulting in a "pock-marked" layer.

Note: Suspensions which contain adsorbent with binders set rapidly and the entire procedure from the preparation of the slurry to the final coating must be completed within 2 minutes.

After shaking, immediately pour the slurry into the applicator chamber. Rotate the chamber by turning the large level handle through 180°C. After a few seconds, the slurry begins to flow out of the exit slit. Grasp the applicator with both hands and pull it manually with a steady motion across the series of plates. Approximately 5 seconds are required for the actual coating procedure. Immediately after application, tap the edge of the mounting board or shake the entire board gently to smooth out slight ripples or imperfections in the wet coating.

Let coated plates dry in position on mounting board for 15 minutes. Then dry plates in a forced draft oven at 80°C for 30 minutes. Remove plates and cool.

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Examine the plates carefully in transmitted and reflected light for imperfections or irregularities in the coating. Plates should be discarded if extensive rippling or mottling of the layer is observed.

Five more 8"x8" plates may be prepared while the first set is drying. Be sure the applicator is thoroughly cleaned and dried before reusing. The 10 coated and dried plates may be prewashed immediately.

B. Prewashing of Adsorbent Layer

Scrape 1/2" of adsorbent off the edge of the plate with a razor blade. Then pour 15 ml 50 percent aqueous acetone into a metal trough inside the chromatographic tank. Cut out a 3/4" x 8" strip of Whatman No. 1 filter paper, wet with solvent and place over the scraped off portion with 1/4" overlapping the adsorbent layer. Place the plate in the chromatographic tank, seal tank with masking tape and develop with 50 percent aqueous acetone to within 1-1/2" from the top of the plate (75-90 minutes). Remove the plate from the tank, remove filter paper wick, invert the plate, and dry in the hood for 5 minutes. Dry the plate at 80°C for 45 minutes. Remove plate from oven, cool and store in desiccator until needed. The prepared plates should be used within one week after preparation.

C. Sample Spotting

A pencil mark is made 1-1/2" from the bottom of the plate at both sides. An imaginary line between the two points indicates the sample spotting or origin "line". Draw a line (which removes the coating

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completely across the plate 5-1/2" from the bottom edge. This line represents the solvent front after development. On the lower edge of the adsorbent starting 3/4" in from the left edge of the plate make 18 marks with a pencil at 6/16" intervals. (Fewer marks with greater intervals may be used if desired). The marks serve as horizontal guides to sample application. The identity of samples and standards may be etched directly into the adsorbent layer above the solvent front line and in alignment with these marks.

The imaginary spotting "line" is actually a shadow line cast by a strong light source from a wooden ruler supported 1" above the plate. The ruler shadow is alinged on the two 1-1/2" marks on either edge of the plate. The shadow line and 18 marks respectively serve as vertical and horizontal guides for sample application.

For optinum semi-quantitative estimation, adjust aliquot of sample spotted to give residue spot within the range .005 ug to 0.1 ug. Concentrations of standards and standard mixtures spotted should be .002, .005, .01, .02, .05, .1 and .2 ug. Sample spots greater than .2 ug are difficult to estimate quantitatively and less than .005 ug may be difficul to distinguish. The volume of sample extract spotted should not exceed 10 ul if possible and spotting should be done repeatedly with a 1 ul Konte spotting pipet, or equivalent. Standard and sample solutions should be spotted with the same pipet, but the pipet must be cleaned between spottir For the best results keep the size of spotted sample as small as possible. All 6 percent extracts are spotted on one plate and 15 percent extracts are spotted on another plate.

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Spot standard solutions of pesticides (mixtures A&B or single compounds) in different positions on the same plate. The choice of pesticides to be used for identification may be governed by the pesticide residues to be expected in the sample examined. For semi-quantitative estimation, varying amounts of knowns may be chromatographed from spots adjacent to samples.

D. Development, Spraying and Exposure of Plates

1. Development

Place liners and metal trough in tank. Presaturate liner by pouring 75 ml developing solvent into bottom of tank prior (30 minutes or longer) to developing plate. Presaturation decreases development time and improves uniformity of Rf values.

For plates spotted with 6 percent extracts, pour 50 ml n-heptane into the trough. Place the lower edge of the plate in the metal trough with the top of the plate leaning against the side of the tank. Place the glass cover plate on the tank and seal with masking tape.

For plates spotted with 15 percent extracts use 2 + 98 acetone-heptane as developing solvent.

When the solvent front just reaches the pencil line 10 cm above the spotting "line", remove the plate and dry in hood for 5 minutes.

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2. Spraying

Support plate on one side and spray fairly heavily with the chromogenic reagent, using lateral motions of the spray bottle perpendicular to the direction of solvent flow.

Spray until plate appears translucent or soaked with reagent. Under-spraying will result in poor sensitivity. After spraying, dry the plate in the hood for 15 minutes, then place immediately under ultraviolet light source.

3. Exposure

Expose the plate to ultraviolet light until the spot for the standard of lowest concentration appears. Five nanogram quantities of most of the chlorinated organic pesticides should be visible after 15-20 minutes exposure. Exposure times in excess of 30 minutes will not harm the plates. For best results place plates 3" from the botton edge of the germicide tubes.

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