**Table V. Persisting $RL_{50}$ Values for Residues of Ethion on and in Peel of Field-Treated Lemons and Valencia Oranges**

| Fruit | Dosage[a], Pound/100 Gal. Water | $RL_{50}$, Days |
|---|---|---|
| Lemons | 1[b] | 25 |
| | 4[b] | 36 |
| | 1/4-pint E.C. + 1 3/4-gal. oil emulsion | 44 |
| Valencia oranges | 1[b] | 42 |
| | 4[b] | 43 |
| | 1/4-pint E.C. + 1 3/4-gal. oil emulsion | 25 |
| | 1 3/4-gal. proprietary formulation | 25 |

[a] For exact data, see Table IV. [b] 25% W.P.

stripping solutions were analyzed for ethion by the infrared technique described above.

Separate aliquots of fruit from the 4-pound dosage (Table IV) plots were hand-washed in a dilute Triton X-100 solution before processing to assess in the usual manner (*4*) the degree of adherence *vs.* rate of penetration of the fruit by ethion.

### Discussion

**Analytical Procedure.** Efficiencies of the unit cleanup procedures were determined separately in the absence and in the presence of citrus extractives. Selective adsorption of ethion on Florisil affords 84% recovery of ethion in the eluate; the fate of the remaining 16% was not investigated. From the partition ratios of ethion from *n*-hexane into acetonitrile (Table I), recovery of ethion from the partitioning steps is 89%. The over-all recovery of ethion from the combined steps is therefore 74%. Recoveries of ethion added to citrus peel extractives in *n*-hexane, reported in Table II, ranged from 60 to 74%.

Interference due to citrus peel extractives in *n*-hexane was not proportional to the amount of peel represented (Table III). As mentioned, stopcock grease seriously interferes with the infrared measuring step. Teflon plugs or water-lubricated plugs are recommended for separatory funnels.

A calibration curve for ethion in 0.3-ml. aliquots of carbon disulfide solutions conforms to Beer's law from 15 to 300 μg. of ethion both at 1017 $cm.^{-1}$ (slope, 17 μg. of ethion per 0.1 absorbance unit) and at 959 $cm.^{-1}$ (slope, 34 μg. per 0.1 absorbance unit) in a 5-mm. cavity cell. Upward extension of this range is achieved by diluting the residue with larger volumes of carbon disulfide.

**Residue Values.** Table IV lists residue values for ethion on and in the peel of field-treated lemons and Valencia oranges from replicated plots. Persisting $RL_{50}$ values (formerly referred to as half-life values) were calculated from the data in Table IV and are listed in Table V.

Residue values on those fruits which were hand-washed in dilute detergent solution prior to processing to simulate commercial practice demonstrate the rapid penetration of ethion into the fruit waxes and oils, and thus the impracticability of residue removal by washing at harvest time.


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**INSECTICIDE RESIDUES**

# Procedure for the Purification of Fat Samples Prior to Analyses for DDT, DDE, and Rhothane


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CONTROL LABORATORIES require clean-up techniques which are applicable to a wide range of foods. These must be efficient enough in the removal of pigments, waxes, fats, and other extraneous materials to allow the analyst to employ paper chromatography and other screening techniques and to study a number of pesticides simultaneously. They should be applicable to metabolites and degradation products, as well as to the parent compounds. Chlorinated pesticides find their way into milk and animal depot fat, and to detect trace quantities of these materials, a procedure which will handle large samples is imperative. A procedure developed in this laboratory (*1*) for the removal of waxes and pigments from plant extracts has been modified and applied to fat extracts containing as much as 100 grams of fat. The present article describes the technique and presents data on the determination of DDT, DDE, and Rhothane residues in a number of animal fats.

### Method

**Apparatus.** Cold bath. A tank which will hold acetone to a height of 7 to 8 inches and two or more 1000-ml. Erlenmeyer flasks is satisfactory. A stainless steel tank, insulated on the outside with 1/4 inch of powdered cork and 1/4-inch plywood has been used. Fisher utility clamps were screwed into the side of the box to hold the Erlenmeyer flasks. The bath was filled to a height of 7 to 8 inches with acetone or methanol and cooled to between −70° and −78° C. by adding dry ice directly to the cooling solvent. An excess of solid chunks of dry ice is left in the tank during the operation.

Büchner funnel, porcelain funnels with outside diameter 142 mm. and plate diameter 126 mm. Sintered glass (me-

A procedure is presented for the removal of pigments and fat from fat extracts, prior to determining residues of DDT, DDE, and Rhothane by qualitative paper chromatographic procedures or by a quantitative colorimetric method. Fats are precipitated from acetone in three stages: one at 5° and two at −70° C. Pigments and remaining traces of fat are removed on a Florisil column. The data show the percentage recovery of the three presticides from several types of animal fats.

dium porosity), jacketed funnels may be used. The funnels are cooled by circulating cold methanol from the bath with a suitable pump mounted on an insulated cover on the cold bath.

Chromatographic columns, 46 cm. in height and 4.5 cm. I.D. with coarse fritted glass disk and a stopcock.

Filter funnel, fritted glass disk 6 cm. in diameter, medium porosity.

Florisil, 60/100 mesh, a synthetic magnesium silicate prepared by the Floridin Co., Tallahassee, Fla. The Florisil is activated by heating at 300° C. for two hours and stored in a desiccator.

**Preparation of Fat Samples Prior to Purification.** BUTTER. Cut the butter sample into cubes and place in a beaker in an oven at 60° C. As soon as the fat has melted and separated into layers, decant off the oily layer and filter through a Whatman No. 1 filter paper. Leave the sample in the oven during filtration to hasten the rate of filtration. Store the clear filtrate of butter oil in a refrigerator until used.

PORK, BEEF, MUTTON FAT. Grind 240 grams of fat in a food chopper and transfer to a Waring blendor. Add approximately 720 grams of anhydrous sodium sulfate and blend. If the resulting mixture is not of a dry powdery consistency, add more anhydrous sodium sulfate until the proper consistency is obtained. Add 1200 ml. of $CCl_4$ and blend the whole mixture for three minutes. Filter the liquid layer through a medium fritted glass filter funnel. Evaporate the filtrate in a beaker under a stream of clean air in a water bath held at 60° C. Frequent stirring hastens evaporation and it is considered desirable to keep the fat at 60° C. for as short a time as possible. (Samples were stored in a refrigerator if they had to be left overnight at any step in the preparation.) Evaporation is considered complete when no smell of $CCl_4$ is detected when the contents of the beaker are stirred. Store fat in a refrigerator until used.

**Acetone Precipitation.** FIRST PRECIPITATION. Weigh 100 grams of fat into a wide neck, 1-liter Erlenmeyer flask. Add 750 ml. of acetone (reagent grade, redistilled) and mix thoroughly. If necessary, warm the mixture to dissolve the fat completely. Place the flask in a refrigerator at approximately 5° C. and let stand overnight. Cool the Büchner funnel and some acetone to 5° C. Mix the cooled sample thoroughly, and filter with suction through Whatman No. 1 filter paper in the Büchner funnel. During filtration pack the precipitate in the Büchner, using the top of a reagent bottle. Wash the Erlenmeyer and the precipitate with six 25-ml. portions of the cooled acetone. Pour the filtrate and washings into the Erlenmeyer flask. Rinse the filter flask with acetone and add these rinsings to the Erlenmeyer flask. Discard the precipitate

SECOND PRECIPITATION. Cool the bath to between −70° and −78° C. Place the Erlenmeyer flask, which contains the filtrate from the first precipitation, into the cold bath. Stir the mixture occasionally until the temperature of the contents of the flask reaches −70° C. and then leave the flask in the cold bath for an additional 15 minutes. Cool the Büchner funnel in the bath. Remove the funnel and filter the mixture as rapidly as possible using Whatman No. 1 filter paper and suction. A medium porosity, jacketed, sintered-glass funnel (H. S. Martin and Co., Evanston, Ill.) may be used, if the cooling liquid is circulated from the bath through the funnel. Pack the crystals in the funnel to prevent cracks forming in the precipitate cake during the filtration. Release the suction as soon as individual drops can be seen coming from the filter funnel. (Filtration cannot be carried to completion, since the precipitate melts as it warms during the filtration. The precipitate should melt as little as possible; therefore, filtration should be carried out rapidly).

Transfer the filtrate to a 1000-ml. beaker and rinse the filter flask with acetone. Add the rinsings to the filtrate. Place the beaker under a stream of filtered air in a water bath maintained at 60° C. Wash the precipitate back into the Erlenmeyer flask, using acetone to complete the trasnfer. Add acetone until the total volume is approximately 750 ml. Dissolve the precipitate, warming the flask if necessary.

THIRD PRECIPITATION. Replace the Erlenmeyer flask in the acetone bath and repeat the procedure used for the second precipitation. This time rinse the Erlenmeyer and the precipitate in the Büchner funnel with 25 ml. of acetone which is cooled to −70° C. Discard the precipitate. Combine the filtrate with the filtrate from the second precipitation and continue the evaporation of the solvent until approximately 10 ml. of liquid remains. Add 100 ml. of benzene and 150 grams of anhydrous sodium sulfate. Stir the mixture immediately and place the beaker in a refrigerator for 15 minutes. Stir occasionally during the cooling to prevent the formation of large lumps of hydrated sodium sulfate. Break up any lumps which may have formed and filter the mixture, using a filter funnel with fritted glass disk of medium porosity. Rinse the beaker with three 20-ml. portions of benzene and pour the rinsings through the $Na_2SO_4$ in the filter funnel. Rinse the $Na_2SO_4$ with four additional 20-ml. portions of benzene. Transfer the filtrate and washings to a beaker and evaporate to approximately 5 ml. It is expedient to carry four to six samples through the acetone precipitation step at one time.

**Florisil Chromatographic Step.** Prepare a Florisil column 20 cm. in height by slurrying Florisil with benzene and pouring the slurry into the chromatographic column. Add enough anhydrous $Na_2SO_4$ to make a layer 2 cm. deep on top of the column. Allow the column to run at the rate of 80 to 100 drops per minute. When the benzene has just entered the sodium sulfate layer, place a 500-ml. volumetric flask under the column to collect the eluate. Add the residue which remains after the sample has been carried through the acetone precipitation step. Rinse the beaker with several small portions of benzene and add these rinsings to the column allowing the solvent just to enter the $Na_2SO_4$ layer between each addition. Add benzene and continue the elution until 500 ml. of eluate is collected. Use this eluate for qualitative and quantitative analyses of the pesticides.

### *Results and Discussion*

This procedure was compared with other clean-up techniques for plant extracts (*1*) and was considered superior to the other methods for the isolation of DDT, Rhothane, methoxychlor, and Kelthane. It was discovered later that the major fraction of Kelthane was not being recovered. Technical Kelthane contains several minor components which react in the Schechter-Haller (*4*) method, and these are eluted quantitatively from a Florisil column with benzene, but

**Table I. Effect of Bath Temperature on Efficiency of Fat Removal from 100 Grams of Butter Oil**[a]

| | Bath Temp. (sample held at this temp. 30 min.) | | |
|---|---|---|---|
| | −40° to −45° C. | −55° to −60° C. | −65° to −70° C. |
| Fat precipitated (g.) at 5° C. (refrigerator) | 19.5 | 17.8 | 20.4 |
| Fat precipitated at temp. of cold bath (g.) | 64.2 | 74.4 | 76.0 |
| Residue in filtrate (before column chromatography (g.) | 15.7 | 7.4 | 3.7 |
| Residue in eluate (after column chromatography) (g.) | 4.6 | 0.22 | 0.012 |
| % recovery of 700 μg. DDT added to butter oil | Analysis not possible | 96.8 | 103.4 |

[a] Statistical analyses were not carried out as most samples were analyzed only in duplicate.

**Table II. Effect of Cooling Time on Efficiency of Fat Removal from 100 Grams of Butter Oil**[a]

| | Time Sample Held at −70° C. | | |
|---|---|---|---|
| | 1 min. | 15 min. | 30 min. |
| Wt. residue in filtrate (before column chromatography) | 6.0 g. | 3.9 g. | 4.2 g. |

[a] Same as in Table I.

**Table III. Weight of Residue Remaining following Purification**[a]

| Type of Fat | Weight Sample, Grams | Weight Residue, Grams | % Purification |
|---|---|---|---|
| Butter oil | 100 | 0.0168<br>0.0172 | 99.98 |
| Beef | 100 | 0.0215<br>0.0216 | 99.98 |
| Pork | 100 | 0.0233<br>0.0210 | 99.98 |
| Mutton | 100 | 0.0163<br>0.0143 | 99.99 |
| Human | 30 | 0.0221<br>0.0232 | 99.93 |

[a] Same as in Table I.

**Table IV. Recovery of Pesticides Following Purification**[a]

| Pesticide Added | Percentage Recovery of Added Pesticide | | | | |
|---|---|---|---|---|---|
| | Butter oil | Beef fat | Pork fat | Mutton fat | Human fat |
| Technical DDT | 91 | 91 | 86 | 78 | 77 |
| | 92 | 102 | 95 | 78 | 86 |
| | 91 | 102 | 95 | 86 | ... |
| | 96 | ... | .. | .. | ... |
| Rhothane | 80 | ... | .. | .. | ... |
| | 89 | ... | .. | .. | ... |
| DDE | 100 | ... | .. | .. | 99 |
| | 109 | ... | .. | .. | 103 |

[a] Same as in Table I.

**Table V. Elution of Kelthane from Florisil Column**[a]

(2.5 cm. in diam. and 20 cm. high)

| Eluting Solvent | Fraction of Eluate in Which Major Component is Found |
|---|---|
| Benzene, ml. | No elution by 650 |
| 0.5% acetone in benzene, ml. | 50–150 |
| 0.5% acetonitrile in benzene, ml. | 50–150 |

[a] Same as in Table I.

the main fraction is held on the column. The larger columns used in the present study did not allow a quantitative recovery of methoxychlor. Dieldrin is not eluted with benzene but can be eluted when small quantities of acetone or acetonitrile are added to the benzene, but the eluates are not clean enough for paper chromatographic analyses.

The procedure can be used for purification of 100 grams of fat prior to analyses for DDT, DDE, and Rhothane. It has been used on butter oil, beef fat, pork fat, mutton fat, and human fat. The purified extract can be analyzed qualitatively by paper chromatography using the mineral oil–acetone system (*3*) and quantitatively by the Downing and Norton (*2*) modification of the Schechter-Haller (*4*) method.

Butter colors yellow AB, yellow OB, β-carotene, and Annatto do not interfere, as they are held on the Florisil column. DDT, DDE, and Rhothane may be eluted and separated from dieldrin and other pesticides. The dieldrin and other pesticides may be eluted with benzene containing either acetone or acetonitrile, and this eluate may be cleaned up on another type of column. A mixture of wood cellulose and Darco G 60 carbon is being studied for this purpose.

To remove the fat from samples as large as 100 grams without loss of the pesticide residues three precipitations must be carried out and the fat must be stirred while precipitation is taking place. Table I shows the effect of temperature on the removal of the fat. Approximately 19 ± 1.5 grams of fat precipitates from 100 grams of butter oil at 5° C. if the sample is allowed to stand at this temperature for 30 minutes. Of the remaining fat, 64.2 grams was precipitated in a bath held between −40° and −45° C. leaving 15.7 grams of residue in the benzene solution which was added to the Florisil column. The Florisil column does not remove fat efficiently, as 4.6 grams of fat was eluted from the column and the whole eluate could not be analyzed. However, when the bath was held between −55° and −60° C., the precipitation was more complete and only 0.22 gram of fat remained in the eluate. Analysis of the sample by the Schechter-Haller procedure is possible when this temperature is used for DDT at 7 p.p.m. However, when the eluate is concentrated for the detection of a pesticide at 0.1-p.p.m. level, there is sufficient fat present to interfere. A more complete removal of the fat is obtained when the temperature of the cooling bath is between −65° and −70° C. The recovery values for DDT added to the butter oil were quantitative and extraneous fat did not interfere when paper chromatography was employed for analyses of the eluate when precipitation was carried out at the lower temperature.

The time required to obtain maximum precipitation of the fat at −70° C. was investigated. Considerably less fat remained in the filtrate, if the sample was allowed to remain at −70° C. for 15 minutes than for one minute (Table II). Allowing the fat to remain at −70° C. for 30 minutes does not appear to be more efficient than for 15 minutes. However, if larger samples were used, it might be necessary to allow a longer time for precipitation.

Since the temperature and time factors were established using butter oil (Table I and II) and depot fat from animals was to be analyzed, it was necessary to determine if the procedures were satisfactory for other animal fats.

Table III shows the percentage purification for butter oil, beef, pork, mutton, and human fat. In every case, over 99.9% of the fat from a 100-gram sample was removed when the three-stage procedure followed by Florisil chromatography was employed for precipitation of the fat.

Recovery data for pesticides added to the depot fat prior to extraction at the rate of 7 p.p.m. are given in Table IV. Recoveries of technical DDT from butter, beef fat, and pork fat are good. A few values for DDT from mutton fat and human fat were below 80%, but this was not considered to be serious when such large samples were used. Rhothane was recovered from butter oil and DDE from butter oil and human fat.

The volume of eluate required to elute the three pesticides was investigated by quantitatively analyzing each 50 ml. of eluate until 1000 ml. of eluate for DDT, DDE, or Rhothane was collected (Table V). The three pesticides studied quantitatively were eluted in the first 350 ml. of benzene eluate. Methoxychlor, technical Kelthane, and dieldrin were not eluted in the volumes indicated in Table V. Later studies show that these three compounds may be eluted by adding small quantities of acetone or acetonitrile to the benzene, but these eluates require further clean-up before they can be analyzed by paper chromatographic procedures.

The acetone precipitation–Florisil chromatographic procedure (*1*) has been utilized for extracts of large samples of fat. The clean-up is efficient enough to allow one to employ the eluates for both quantitative and qualitative analyses.

DDT and related pesticides are not stable in strong alkali while dieldrin and related pesticides are unstable in strong acids, thus restricting the use of procedures such as saponification or sulphonation of fats as general procedures. The procedure presented here does not require the use of strong acids or base and should be applicable to both groups of pesticides.

Dieldrin and other pesticides are not eluted from the florisil with benzene, but other solvents can be used for elution. This should be an advantage in making the technique more specific. The objective is to make this procedure as general as possible and present studies are designed for this purpose.

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**INSECTICIDE RESIDUES**

# Procedure for Cleanup of Butterfat prior to Analyses for Dieldrin Residues


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**A procedure is presented for the isolation of dieldrin from 50-gram samples of butter oil. The dieldrin is free enough from extraneous fatty material to be identified by paper chromatography. The procedure consists of partitioning the fat between acetonitrile and *n*-hexane followed by elution from a Darco G60–Solka Floc column with acetone**


THE CHLORINATED INSECTICIDE DIELDRIN contains 85% hexachloroepoxyoctahydro-endo, exo-dimethanonaphthalene (HEOD) and is used quite extensively for the control of grasshoppers and other insects. This type of control means that crops are sprayed which may be ingested by animals used for milk production or for food. Dieldrin, if ingested, may find its way into dairy products and animal depot fat.

The method of analysis in the Shell Chemical Co. Series (*6*) requires saponification of the fat and isolation of the dieldrin from the nonsaponifiable material on a column of magnesium oxide and celite. The method is reasonably specific and sensitive, but time-consuming for routine analyses. The eluates are not clean enough from this cleanup, in the authors' experience, for paper chromatographic analyses.

Control laboratories require a technique which is sensitive, specific, and rapid. Paper chromatographic techniques are useful as screening procedures as they usually meet these requirements and allow analyses of samples for several different pesticides simultaneously. However, to use paper chromatographic techniques for fat analyses, the cleanup step must be extremely efficient. Saponification or sulfonation of the fat usually adds specificity to a quantitative method by eliminating other insecticides. Cleanup procedures for screening methods should have the potential value of being applicable to a number of pesticides.

Fat may be precipitated without appreciable loss of dieldrin with acetone at −70° C. (*1*), but the dieldrin—unlike DDT, DDE, and DDD (Rhothane)—cannot be eluted from a Florisil column using benzene as the eluting solvent. Benzene containing 0.5% of either acetonitrile or acetone, or acetone alone, will elute the dieldrin from the Florisil column, but the residual fat is not retained. Acetonitrile will not elute the fat, but only about 60% of the dieldrin is recovered in an acetonitrile eluate.

A method is described which will allow quantitative recovery of dieldrin from large samples of butter oil and be free enough from extraneous material that it can be chromatographed for identification. In practice, only a small percentage of the samples which contain an appreciable residue requires further analyses by the more precise quantitative method (*6*).

### Method

**Apparatus and Reagents.** Chromatographic columns. As shown in Figure 1.

Cold bath. A double-walled box, insulated with cork (internal dimensions, 24 × 30 cm.) was constructed. The