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

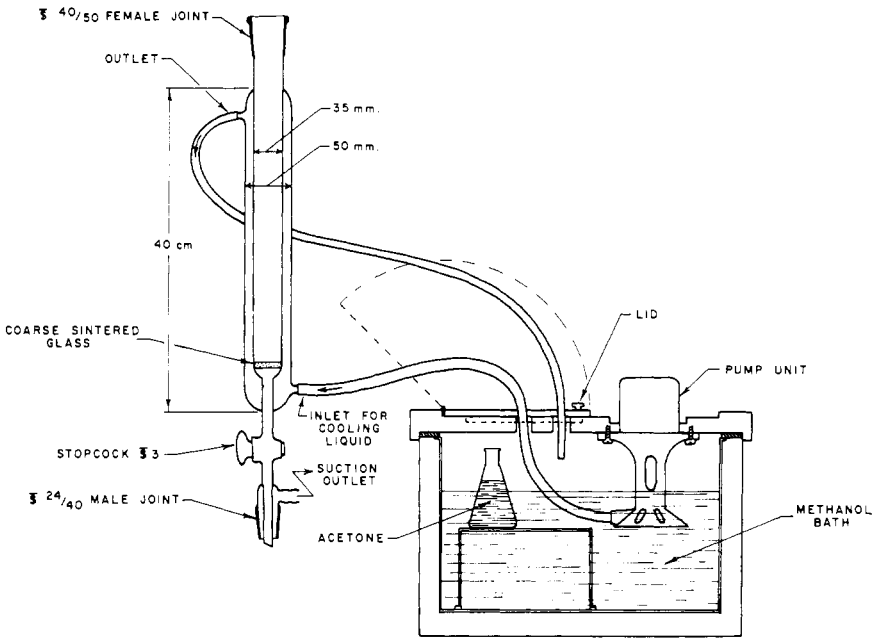

Figure 1. Cooling bath with pump and jacketed column

(The column shown is equipped for suction or pressure, but these were not used in this study)

outer box was made of plywood and the inner portion of stainless steel. A stand was inserted at one end of the box to hold the flasks containing the acetone used for eluting the column. A circulating pump (Williams-Anderson Co., Chicago) was attached to the cover of the box with the motor above and the pump below the cover to prevent methanol vapors from gaining access to the motor. The pump outlet was connected to the column with tygon tubing. The same tubing was used to join two or more columns and for the return to the box. Methanol was used as the circulating, cooling material rather than acetone, as the latter solvent attacks most types of pumps more readily than methanol.

Charcoal. Darco G60 (distributed by Brickmann and Co., Montreal, and manufactured by Atlas Powder Co., Wilmington, Del.).

Wood Cellulose. Solka Floc BW40 (Brown Co., Boston, Mass.) is shaken for one hour on a mechanical shaker with an excess of redistilled acetone. The excess acetone is poured off and the residue is re-extracted. The acetone must be redistilled and impurities removed from the Solka Floc to obtain satisfactory chromatograms.

C^{14}-labeled Dieldrin. 96% HEOD, main impurity approximately 3% aldrin, m.p. 147° to 150° C., specific activity 7.41 millicuries/millimole.

Standard No. 1. One tenth millicuries or 5128.28 μg. of C^{14}-labeled dieldrin made to 50 ml. with benzene (102.564 μg. per ml.).

Standard No. 2. Twenty milliliters of Standard No. 1 made to 100 ml. with benzene (20.513 μg. per ml.).

Standards containing from 0.0128 μg. per ml. to 0.8205 μg. per ml. were made by diluting Standard No. 2, and these were used for determining a straight line relationship between the number of counts of labeled dieldrin and the quantity in micrograms.

Procedure. Extraction and Partitioning. Butter from cows which had not been in contact with dieldrin was used throughout this work. The butter samples were cut into cubes and placed in a beaker in an oven at 60° C. As soon as the fat had melted and separated into layers, the oily layer was decanted off and filtered through a Whatman No. 1 filter paper. The sample was left in the oven during filtration to increase the rate of flow through the filter. The clean filtrate of butter oil was stored in the refrigerator until used.

Fifty grams of butter oil and a known quantity of Standard No. 2 dieldrin (usually 61.5 μg.) were dissolved in 500 ml. of *n*-hexane saturated with acetonitrile and transferred to a 3-liter separatory funnel. (The benzene was removed from the standard dieldrin prior to this operation.) Two hundred and fifty milliliters of acetonitrile saturated with *n*-hexane was added and served as the other partitioning solvent (*3*). The acetonitrile layer was drained off into a 1500-ml. beaker and the partitioning was repeated three times to give a volume of 1 liter of acetonitrile. The bulked acetonitrile was back extracted with 125 ml. of *n*-hexane saturated with acetonitrile and the hexane phase was discarded. It was not necessary to add anhydrous sodium sulphate to remove moisture from the acetonitrile phase with the samples employed in this study.

The acetonitrile was evaporated off by placing the beaker in a fume cupboard and allowing a gentle stream of clean air to flow over the surface of the solvent at room temperature. A residue of 500 to 1000 mg. of fat remained after careful partitioning.

To remove the butter oil from creams, a volume of cream containing approximately 50 grams of butter fat (depending on whether it is whipping cream or coffee cream) was measured into a centrifuge bottle and three volumes of redistilled acetone were added. The mixture was stirred occasionally at room temperature for 20 minutes. The mixture was spun in a centrifuge for 30 minutes at 2000 r.p.m. and the supernatant and butter oil poured off. Redistilled acetone, 100 ml., was added to the casein precipitate and the mixture stirred to break up the lumps of casein. The mixture was centrifuged again and the supernatant added to the supernatant from the first extraction. The precipitate was extracted with 100 ml. of a 1 to 1 mixture of ethyl and petroleum ether and the supernatant added to the acetone extracts. The extract was filtered through a medium porosity, sintered-glass funnel.

The solvent was removed in a flash evaporator at 60° C. and the fat removed from the flask with a 1 to 1 mixture of ethyl and petroleum ether. One extraction with 100 ml. of the ether mixture followed by three extractions, using 50 ml. for each extraction, should remove all the fat. Approximately 75 grams of anhydrous sodium sulfate was added to the extract and the mixture refrigerated at 5° C. for 30 minutes, with occasional stirring. The cold mixture was filtered through a medium porosity sintered glass filter and the precipitate washed with 100 ml. of the ether mixture.

The ether was removed in a stream of filtered air or nitrogen and the resulting butter oil weighed and partitioned in the manner described for the butter oil from commercial butter.

Column Chromatography. Thirty-five grams of extracted Solka-Floc and 7 grams of Darco G60 charcoal were mixed thoroughly in a beaker. Ten and one-half grams of the mixture was removed and the remainder was slurried with acetone and added to the column (Figure 1). When the transfer was complete, the stopcock was opened until the acetone level had reached the upper surface of the column material. The residual fat from the partitioning was dissolved in 100 ml. of acetone and added to the remaining 10.5 grams of the column material. The mixture was slurried and added to the column. The beaker was rinsed with two 50-ml. volumes of acetone, and these were added to the column. This transfer was carried out with the stopcock closed.

The cold bath (Figure 1) was filled with methanol and sufficient dry ice was added until the temperature had dropped to −78° C. The circulating pump was turned on, and the methanol was circulated until the solvent passing around the column had dropped to −70° C. The acetone above the column was stirred during the cooling period. The stopcock was opened and the flow rate adjusted to 80 to 100 drops per minute. The first 100 ml. was discarded, the next 400 ml. was collected separately, and a portion of this fraction was used for paper chromatography. Precooled acetone was used for elution.

PAPER CHROMATOGRAPHY. The 400 ml. of eluate was placed in a beaker and the solvent was removed in a fume cupboard, using a current of air at room temperature. The residue was dissolved in 1 ml. of ethyl ether (peroxide-free) and transferred to a 5-ml. test tube. The beaker was washed with three more 0.5-ml. volumes of ethyl ether to complete the transfer. The ether was removed under a gentle stream of nitrogen gas and 0.1 ml. of ethyl acetate was added just prior to the application of 6 μl. of ethyl acetate solution to the chromatographic paper.

Chromatography was carried out according to the procedure described in an earlier publication (*5*) with 4% mineral oil as the immobile phase on the washed paper and 70% aqueous acetone as the mobile phase.

Quantitative Recoveries. Duplicates of 0.5 ml. of a series of five standards containing from 0.0128 μg. to 0.8205 μg. per ml. were plated into stainless steel planchets which were placed in the dark for one hour to evaporate the solvent. Samples were counted to 4000 using a gas flow counter with an ultra thin window. The average number of counts per minute per microgram was recorded.

Two milliliters of each 100 ml. of eluate up to the tenth fraction (1 liter of eluate) was plated into stainless steel planchets and counted in the same manner as the standard.

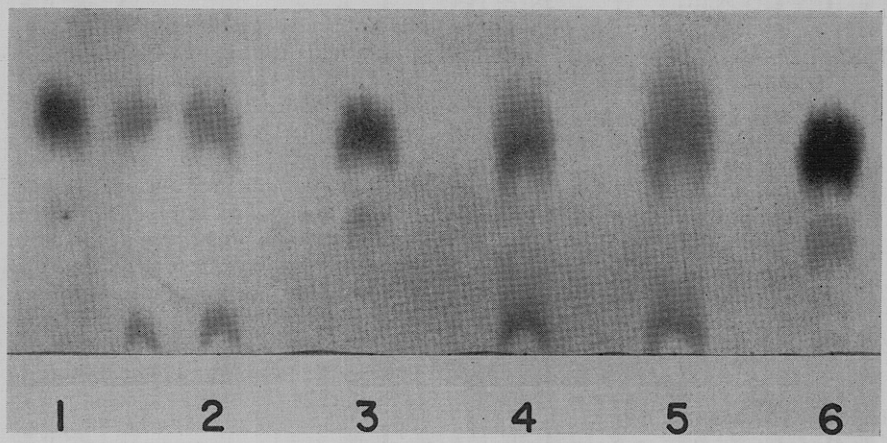

Figure 2. Chromatogram developed in acetone—mineral oil system

Samples 1, 3, and 6 represent 1.64, 3.28, and 6.56 μg. of technical dieldrin. The sample between 1 and 2, samples 2, 4, and 5 represent 2, 4, 8, and 10 μg. of the concentrated column eluate. The dark spots farthest away from the numbers are dieldrin spots, while those near the point of application represent trace quantities of impurities which are present in most fats and give the silver nitrate reaction on paper

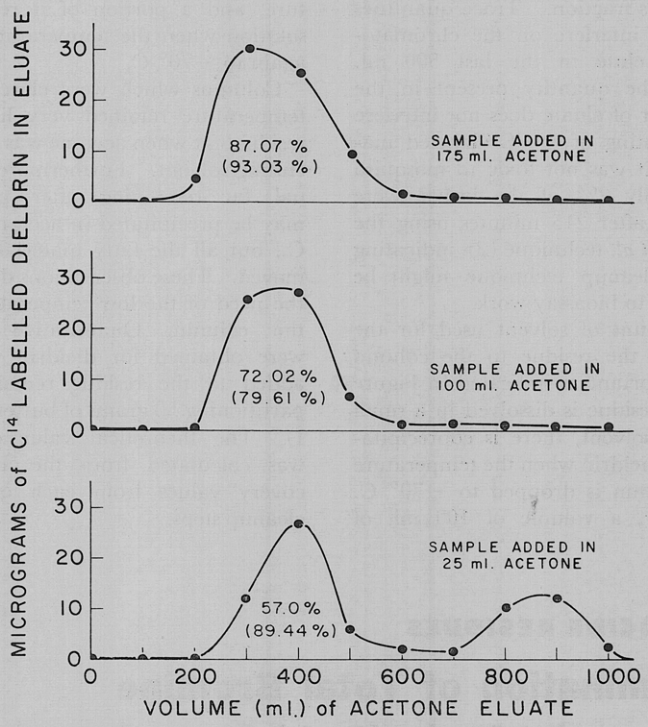

Figure 3. Counts of C^{14}-labeled dieldrin in each 100 ml. of acetone eluate

(The figures under each curve in brackets are percentage recovery in 1000 ml. of eluate and the figures not in brackets are percentage recovery in the first 500 ml. of eluate)

Results and Discussion

The results of counting the five duplicate standards were plotted against the amount of dieldrin (micrograms) in each standard and a straight line relationship was obtained with no indication of self-absorption. Ten counts of 4000 each, using the five standards, averaged 4552 counts per minute per microgram with a standard deviation of 168.6. This figure was used in calculating the recoveries in the eluates in subsequent analyses.

The benzene solution containing the dieldrin was placed in a dark cupboard while the benzene was evaporating to prevent losses of the pesticide. Samples which were plated and stored in the dark for one hour averaged 198.3 c.p.m. Samples stored in the dark overnight gave an average count of 165.0 c.p.m., while those stored in the light overnight gave an average count of 67.7 c.p.m. with a wide variation between individual values. These observations indicate the necessity of avoiding contact with light while removing the solvent. This is particularly important when counting samples which are free of all extraneous material, such as the column eluates. (The chlorinated insecticide DDT is even more susceptible than dieldrin.) A counter with a thin window is essential, when drying times are reduced, because of the danger of small quantities of solvent entering the open type of counting tube.

Figure 2 demonstrates the utilization of this technique for the analyses of column eluates. The chromatographic paper should be washed thoroughly and carefully (*5*) no more than a day or two before use to avoid a dark background.

The partitioning procedure using acetonitrile and *n*-hexane leaves a residue of from 500 to 1000 mg. from 50 grams of butter oil, and it is this fraction of the

Table I. Percentage Recovery of Dieldrin from Butter Fat[a]

After Partitioning Only	Through the Column Only	Net Recovery (Two Steps) Actual	Theoretical
82.32	93.03	69.11	
79.23	87.07	73.42	
79.95	90.99	71.36	
80.32	99.43	81.55	
81.67	94.07	86.02	
Av. 80.70	92.91	76.29	74.89

[a] Each value in the table was obtained on a different sample of butter.

fat which is difficult to remove efficiently. The first 500 ml. of eluate from the column is almost completely free of all traces of this fraction. Trace quantities which may interfere on the chromatogram will elute in the last 500 ml. However, the quantity present in the entire 1 liter of eluate does not interfere in the counting of the C^{14}-labeled material, and it was not toxic to mosquito larvae. Only 2% of the larvae were inactivated after 215 minutes using the Burchfield *et al.* technique (*2*), indicating that this cleanup technique might be useful prior to bioassay work.

The amount of solvent used for the addition of the residue to the column is very important as indicated in Figure 3. If the residue is dissolved in a small volume of solvent, there is coprecipitation of the dieldrin when the temperature on the column is dropped to −70° C. If, however, a volume of 100 ml. of solvent is used for the transfer, the recovery value is increased from 57.0% in the first 500 ml. of eluate to 72.0%. The increase in the amount of dieldrin in the earlier fractions of eluate takes place without any shift of the peak. The lower graph in Figure 3, showing 57.0% recovery for the first 500 ml. was allowed to warm up to room temperature overnight with approximately 25 ml. of solvent above the upper surface of the column. The column was cooled again to −70° C. and another 500 ml. of eluate was collected. Another portion of the dieldrin came off the column with a peak after 300 ml. had passed through the column. This suggests that the dieldrin, which had coprecipitated in the fat during the first drop in temperature with only 25 ml. of solvent present, must have gone back into solution after the column had warmed to room temperature, and a portion of it remained in solution when the temperature dropped again to −70° C.

Columns which were eluted at room temperature retained very little of the residual fat when acetone was used as the eluting solvent. Furthermore, the residual fat remaining after partitioning may be precipitated in acetone at −70° C., but all the fatty material is not removed. These observations demonstrate the need of the low temperature and of the column. Quantitative recoveries were obtained for dieldrin which was added to the residue remaining after partitioning 50 grams of butter oil (Table I). The theoretical value of 74.89% was calculated from the average recovery values from each of the two cleanup steps.

One hundred grams of lettuce containing added C^{14}-labeled dieldrin was extracted by the Klein procedure (*4*) to obtain a large portion of the pigments and waxes. The solvent was removed, and the residue was added to the column described without partitioning and with the column operating at room temperature. Eluates were clean enough for paper chromatographic analyses, and quantitative recoveries were obtained, suggesting that this cleanup procedure may be applicable to extracts of fruit and vegetables as well as butter fat.

Acknowledgment

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NEMATOCIDE RESIDUES

Determination of Total Bromine Residues in Agricultural Crops by Instrumental Neutron Activation Analysis

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VARIOUS ORGANIC BROMO-COMPOUNDS are employed in the field to combat the destructive action of plant-parasitic nematodes. Examples of such soil fumigant nematocides are methyl bromide, ethylene dibromide, propargyl bromide, and 1,2-dibromo-3-chloropropane (DBCP). It is of interest and concern to determine the extent of the resulting nematocide residues in edible crops. Residue tolerances that have been set for some of these organic bromides are based upon analysis for total bromine. Existing chemical methods for such total bromine determinations in crop materials are generally fairly sensitive and accurate, but quite time consuming. The present study was initiated to explore the possibilities of a much faster technique, that of instrumental neutron activation analysis (*3–5*). Check (untreated) plant samples and samples of plants grown in soil treated with DBCP were analyzed. The method appears to be very useful.

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Theory of Method

The theory of neutron activation analysis has been discussed previously (*1, 3–7, 9*). However, the application of the theory to bromine is apropós. Reference is made below to the basic equation of activation,

$$A = Nf\sigma\left(1 - e^{\frac{-0.693t_i}{t_{0.5}}}\right) \qquad (1)$$

The Br^{82} disintegration rate that would result from irradiation of 1 μg. of bromine for 1 hour at a neutron flux of 10^{12} neutrons per sq. cm. per second may be computed as follows. Normal bromine consists of 50.6% Br^{79} and 49.4% of Br^{81} (*2*). The cross section for the $Br^{81} \xrightarrow{n} Br^{82}$ nuclear reaction is 2.6×10^{-24} sq. cm. per nucleus (i.e., 2.6 "barns")