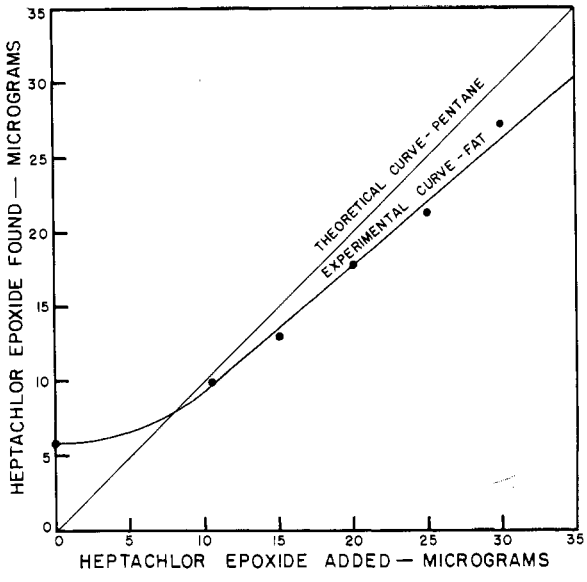

Figure 4. Recovery curve, uncorrected data

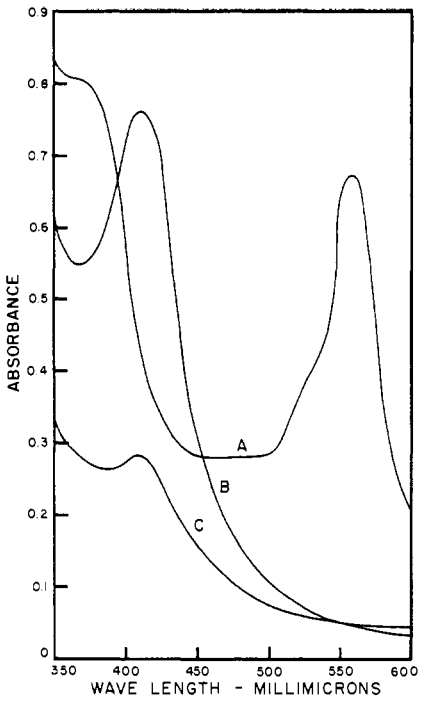

Figure 5. Absorption curves of heptachlor and heptachlor epoxide mixtures

A. Heptachlor (Polen-Silverman reagent)
B. Heptachlor epoxide (Polen-Silverman reagent)
C. Heptachlor epoxide (Davidow reagent)

chlor is present in samples to be analyzed for heptachlor epoxide, an enhancing effect will be exerted. This is illustrated in Figure 5. It is possible to analyze these mixtures in a single color development by applying calculations to measurements at the two characteristic peak wave lengths. A procedure involving chromatographic separation and separate color development is more reproducible and, hence, is preferred.

If a large amount of heptachlor is present in the sample, part of it may appear in the epoxide fraction. Figure 5 shows that the influence of heptachlor upon the heptachlor epoxide determination is significant, and erroneous results will be obtained if the proper corrections are not applied. A standard two-wave-length method for treating a two-component system must be used to make this correction (*4*).

As a general rule, heptachlor is absent under normal circumstances. When this fact has been indicated for a particular set of determinations, it will usually be more convenient to use saponification in preference to acid treatment in the cleanup. Slightly lower absorbance values result from acid treatment. By using acid treatment in the calibration, however, the appropriate correction to 100% recovery will yield the final analytical results. All things considered, the saponification step appears more convenient where a choice is available.

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INSECTICIDE RESIDUES

Procedure for Cleanup of Plant Extracts Prior to Analyses for DDT and Related Pesticides

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Pigments and waxes are extracted with pesticide residues from plant material, and interfere in quantitative and qualitative methods of analysis. Therefore, a cleanup procedure is needed, which will efficiently remove pigments and waxes from the organic solvent without removing or destroying the pesticides. Recently (*8*) stress has been placed on more efficient techniques for extracting pesticide residues from fruits and vegetables. These more vigorous extraction methods require more efficient cleanup procedures, because more pigments and waxes, as well as pesticides, are present in the extracts. Many cleanup procedures described in the literature are applicable in respect to one type of crop or one specific pesticide. A cleanup procedure was sought which would remove essentially all

A method is presented for the removal of pigments and waxes from plant extracts prior to determining residues of the DDT group of pesticides. The waxes are precipitated from an acetone solution of the extract at −70° C. and the pigments are removed by passing a benzene solution of the extract through a Florisil column. The method is applicable to a variety of plant materials, including leafy vegetables, brassic crops, and citrus and waxy fruits. Data show the recovery of DDT and other related chlorinated hydrocarbon pesticides following this cleanup procedure. The procedure is compared with others mentioned in the literature.

pigments and waxes from a heavily contaminated extract from any type of plant material and yet provide a good recovery of a wide range of pesticides.

The Jones-Riddick (*7*) and the Burchfield-Storrs (*1*) methods efficiently remove waxes from extracts but, with the more efficient extraction techniques, they do not remove the pigments completely.

Hoskins *et al.* (*6*) have described a polyethylene-alumina column for the removal of waxes. Unfortunately, however, the brand of polyethylene suggested was not available and a substitute had to be used. Under these conditions the method was unsatisfactory, as the polyethylene was slightly soluble in the eluting solvent.

McKinley and Mahon (*9*) have used a Florisil column with petroleum ether–ethyl ether as the eluting solvent for the isolation of pesticides from extracts of fat. A Florisil column with benzene as eluting solvent was found efficient for the removal of pigments but not waxes from plant extracts.

Fairing and Warrington (*4*) used acetone at − 15° C. to separate methoxychlor from the unsaponifiable portion of fats and waxes.

The present paper describes a cleanup procedure in which the waxes are removed by precipitation from an acetone solution of the extractives at − 70° C. and the pigments are removed by Florisil column chromatography. This procedure is compared for efficiency of removal of extraneous material and recovery of chlorinated hydrocarbon pesticides with the Jones-Riddick and Burchfield-Storrs procedures when combined with Florisil chromatography.

Method

Apparatus. Cooling Bath. A 1-quart Mason jar filled with dry ice and acetone serves as a cooling bath. It may be wrapped in cork sheeting for insulation. On top of the jar is placed a sheet of cork 11 × 11 × 0.4 cm. This cork sheet has two holes, each 2.5 cm. in diameter, through which are inserted 20 × 2.4 cm. test tubes. Through another hole, 0.7 cm. in diameter, is inserted a thermometer calibrated to −70° C. and kept upright by two elastic bands wrapped around the stem.

Büchner funnels with medium porosity fritted disks 4.0 cm. in diameter.

Chromatographic columns, 2.5 cm. in inside diameter, with coarse fritted-glass disks and stopcocks to regulate the rate of flow.

Reagents. 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 2 hours; then it is stored in a desiccator.

Anhydrous sodium sulfate.

Procedure. Evaporate the extract of the plant material to approximately 10 ml. in a water bath at 60° C. under a stream of oil-free air. Transfer the entire residue to a 20 × 2.4 cm. test tube, using benzene to rinse the beaker. Continue the evaporation until 3 ml. of solvent remains in the test tube. Add 20 ml. of acetone and mix thoroughly. Place the test tube in the cooling bath at −70° C. and fill the other test tube in the bath with acetone to be used for rinsing. Immerse the Büchner funnel in another bath of dry ice and acetone. After the solutions and funnel have cooled for 15 minutes, remove the funnel, place it under suction, and rinse with 10 ml. of cold acetone. Pour the sample into the funnel and filter with suction. Proceed as quickly as possible, once filter and sample are removed from the dry ice–acetone bath. Rinse the test tube with two 3-ml. portions of cold acetone and replace the sample test tube in the bath between washings. Filter the rinsings through the residue in the funnel. Wash the residue with another 3 ml. of cold acetone. Pour the filtrate into a 100-ml. beaker; rinse the filter flask with two 5-ml. portions of acetone and add rinsings to beaker. Add 5 grams of anhydrous sodium sulfate and stir. Place the beaker under a stream of air and evaporate with occasional stirring until a dry powder remains.

Make a slurry of activated Florisil and benzene and add to the chromatographic columns until the Florisil column is 8 inches high. Adjust the stopcock so that the column runs at approximately 80 to 100 drops per minute. Add 5 grams of anhydrous sodium sulfate to the top of the column. When the benzene has just entered the sodium sulfate layer, add the dry, powdered sample. Place a 200-ml. volumetric flask under the column to collect the eluate. Rinse the 100-ml. beaker four times with 5-ml. portions of benzene. Add the rinsings to the column while allowing benzene to enter the sodium sulfate layer between each addition. Rinse the sides of the column with 5 ml. of benzene and then add 150 ml. of benzene. Allow the column to run to dryness and make eluate up to 200 ml.

This eluate represents the extract, free from pigments and waxes. Aliquots may be used for quantitative and qualitative analyses.

Results and Discussion

To test and compare the efficiency of cleanup, plant extracts containing large amounts of extractives other than pesticides were prepared by the method of Gunther and Blinn (*5*). This is a vigorous extraction method, and any procedure which would clean this extract would be likely to clean extracts prepared in other ways.

Procedure for Extract Preparation. Blend 500 grams of plant material for 5 minutes in a large Waring Blendor with 500 ml. of isopropyl alcohol. Add 1000 ml. of benzene, and continue the blending for an additional 5 minutes. Filter the mixture through cheesecloth. Pour the benzene layer off and wash four times with a volume of warm water equal to the volume of benzene. The water wash removes isopropyl alcohol from the benzene layer, and the volume of benzene recovered is an aliquot of the benzene added. Any pesticide present on the plant material is considered to be evenly distributed in the total volume of benzene. To check for losses of pesticides during the cleanup procedure, add known quantities of the pesticides to aliquots of the washed extract.

A cleanup procedure applicable to a wide variety of crops was being sought; therefore it was decided to test the procedure on extracts of apples, cabbage, lettuce, and oranges. These crops represent the waxy fruits, brassic crops, leafy vegetables, and citrus fruits, respectively.

Waxes from plant extracts were much less soluble in acetone than in other solvents such as *N,N*-dimethylformamide, ethyl acetate, *n*-hexane, pentane, isooctane, acetonitrile, and methyl ethyl ketone. Cooling the acetone to − 70° C. decreases the solubility of the waxes still further. From 1000 ml. of an apple extract, 0.0234 gram of wax was precipitated at 10° and 0.0792 gram was precipitated at −70° C.

Price or brand stamps had to be cut from the surface of the plant material

Table I. Efficiency of Removal of Extractives

Cleanup Procedure	Weight Residue, Grams				% Removal of Extractives			
	Orange	Apple	Lettuce	Cabbage	Orange	Apple	Lettuce	Cabbage
No cleanup	1.3275	1.2199	0.8167	1.1398				
Florisil chromatography	0.0949	0.0745	0.0114	0.0751	92.8	93.9	98.6	93.4
Acetone precipitation	0.6920	0.3793	0.4351	0.4266	47.9	68.5	46.7	62.5
N,N-Dimethylformamide–*n*-hexane partitioning	0.0463	0.0664	0.0307	0.0623	96.5	94.6	96.2	94.5
Acetonitrile–*n*-hexane partitioning + Florisil chromatography	0.0193	0.0028	0.0016	0.0029	98.5	99.8	99.8	99.7
N,N-Dimethylformamide–*n*-hexane partitioning + Florisil chromatography	0.0021	0.0017	0.0001	0.0033	99.8	99.9	100.	99.7
Acetone precipitation + Florisil chromatography	0.0370	0.0078	0.0136	0.0084	97.2	99.4	98.3	99.3

prior to extraction. Some of the dyes used in these stamps were not removed during the cleanup procedure.

Comparison of Cleanup Procedures. The efficiency of cleanup of the acetone precipitation–Florisil chromatographic method was compared with the methods of Jones and Riddick (*7*) and Burchfield and Storrs (*1*).

A slight modification of the Jones and Riddick method (*7*) has been used in this laboratory for some time and was used in the work described here. The plant extractives were dissolved in 25 ml. of *n*-hexane and extracted with four 10-ml. portions of acetonitrile. The combined acetonitrile extracts were back-extracted with 5 ml. of *n*-hexane. The acetonitrile solution now contained the pesticides; some of the plant pigments and waxes had been removed.

In the *N,N*-dimethylformamide–*n*-hexane partitioning procedure, the plant extractives were dissolved in 30 ml. of *n*-hexane and extracted first with 30 ml. of *N,N*-dimethylformamide and then with 10 ml. of *N,N*-dimethylformamide. The combined dimethylformamide extracts were diluted with 40 ml. of water, and any *n*-hexane separating was removed under a stream of air. The aqueous dimethylformamide was extracted with 20 ml. of *n*-hexane. This hexane contained the pesticides partially freed from extraneous material.

Neither method (*1*, *7*) removed all the pigments from the extract. Accordingly, these procedures were combined with the Florisil chromatographic procedure.

To compare the methods, large volumes of extracts were prepared as described. One thousand milliliters of each extract was carried through each cleanup procedure and, after cleanup, the solvent was evaporated in a tared flask and the weight of residue was determined. Another 1000 ml. of each extract was evaporated to dryness and the weight of residue was taken as the weight of extractives present before cleanup. Table I shows the result of this comparison. All procedures—viz., acetonitrile–*n*-hexane partitioning plus Florisil chromatography, *N,N*-dimethylformamide-*n*-hexane partitioning plus Florisil chromatography, and acetone precipitation plus Florisil chromatography—cleaned the extracts very satisfactorily. Only very small amounts of colorless residue were obtained.

Table II. Recovery of DDT

Cleanup Procedure	% Recovery of Added DDT	
	Apple extract	Lettuce extract
Acetonitrile–*n*-hexane	80	92
+ Florisil chromatography	80	92
	73	95
	63	97
N,N-Dimethylformamide–*n*-hexane +	30	54
	40	55
Florisil chromatography	47	64
	51	68
Acetone precipitation +	91	92
Florisil chromatography	97	95
	97	96
	97	96

These three procedures were then compared with respect to recovery of technical DDT. Large volumes of lettuce and apple extracts were prepared for this purpose. Technical DDT was added to the extracts at the level of 7 p.p.m. on the plant material, or 3.5 mg. of DDT per 1000 ml. of extract. One thousand milliliters of each extract was carried through each of the three cleanup procedures and the DDT present in the cleaned extract was determined by a method essentially the Downing and Norton modification (*3*) of the Schechter-Haller (*10*) DDT procedure (Table II). These figures show that the best recovery of DDT was obtained from extracts cleaned by the acetone precipitation–Florisil chromatographic procedure. The low recovery of DDT from apple extract using the acetonitrile-*n*-hexane partitioning procedure may be due to DDT trapped in the waxy material, which did not dissolve in either of the organic solvents during partitioning. When no plant extractives were present, technical DDT was recovered satisfactorily following any of the three cleanup procedures.

In the acetone precipitation–Florisil chromatographic procedure, the acetone precipitation always precedes Florisil chromatography. In the other two cleanup procedures, the order varies with the type of extract. In the case of a heavily pigmented extract, such as lettuce, the Florisil column step must precede the partitioning in order that the interphase between the two solvents can be seen. With waxy fruits, such as apples, the partitioning step must precede the column chromatography; otherwise some undissolved waxy material will settle on top of the column and interfere with the flow rate of the column. Difficulty is encountered often with emulsion formation during partitioning. It is a distinct advantage to be able to follow an identical procedure with all types of extract.

Table III. Recovery of Chlorinated Hydrocarbon Pesticides Following Acetone Precipitation–Florisil Chromatography

Pesticide	Level of Pesticide Addition, P.P.M.	No. of Analyses	% Recovery (Range)
p,p'-DDT	7	2	100–101
o,p'-DDT	7	2	93–96
Technical DDT	7	2	91–97
Rhothane	7	4	92–102
Methoxychlor	14	2	90–92
Kelthane	3	2	93–94

Recovery of DDT and Related Chlorinated Hydrocarbon Pesticides Following Acetone Precipitation–Florisil Chromatographic Cleanup. The recoveries of *p,p*-DDT, *o,p*-DDT, methoxychlor, Rhothane, and Kelthane following acetone precipitation–Florisil chromatographic cleanup were checked. These pesticides react quantitatively in the Downing and Norton (*3*) modification of the Schechter-Haller (*10*) DDT procedure, as shown in Figures 1 and 2; so this modified procedure was used for the quantitative analyses following cleanup. Figure 1 represents the absorbances obtained when 112 γ of the pesticides are nitrated and the color is developed in a total volume of 6 ml.

A large volume of apple extract was prepared and to 1000-ml. aliquots of this extract the pesticides were added in amounts which would be found on 500 grams of plant material if the pesticides were present at the tolerance

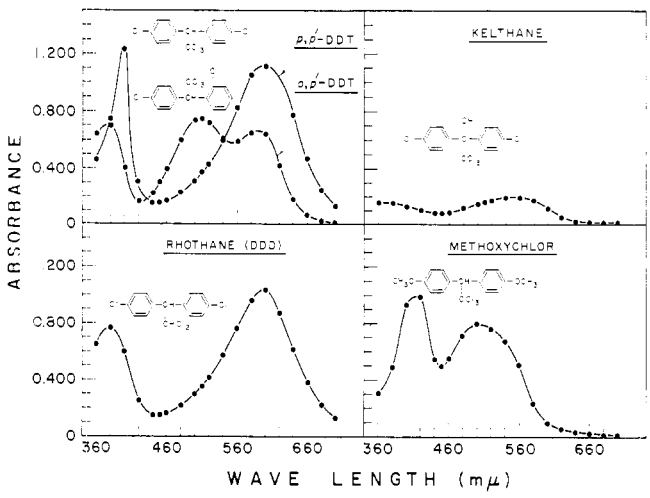

Figure 1. Absorption curves of nitrated chlorinated hydrocarbon pesticides

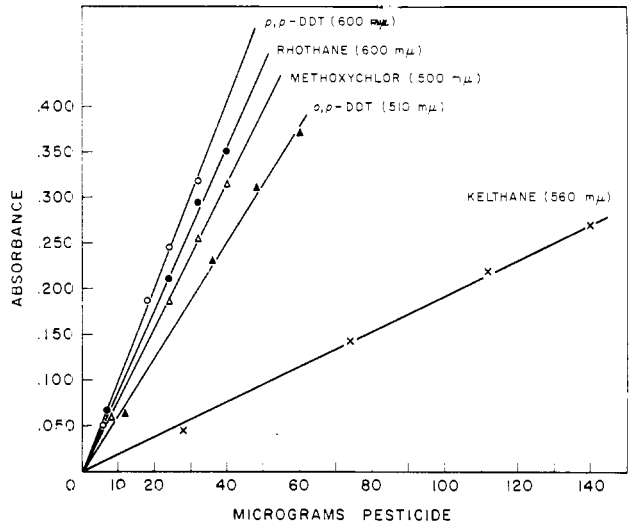

Figure 2. Calibration curves of nitrated chlorinated hydrocarbon pesticides

level permitted by the Canadian Food and Drug Regulations (*2*). The extracts were then carried through the acetone precipitation–Florisil chromatographic procedure and analyzed quantitatively. Colorimetric measurements were made at the following wave lengths: *p,p'*-DDT, 600 mμ; *o,p'*-DDT, 510 mμ; Kelthane, 560 mμ; methoxychlor, 500 mμ; Rhothane, 600 mμ.

As the extract had been prepared from apples purchased on the market, pesticides may have been present and therefore a "blank" determination was made. Two 1000-ml. aliquots of the extract were carried through the procedure without the addition of pesticides and absorbance measurements were made at each of the above wave lengths. These "blank" absorbance measurements were subtracted from the absorbance measurements of the fortified samples.

Data in Table III show that the recoveries for all this group of pesticides were 90% or better. It is planned to check the recoveries of other groups of pesticides following the acetone precipitation–Florisil chromatographic procedure.

Conclusion

The acetone precipitation–Florisil chromatographic procedure is recommended for routine use in the cleanup of plant extracts prior to determination of DDT and related pesticides. It is a general procedure, applicable to a wide variety of crops; it removes pigments and waxes efficiently from extracts prepared by even the most vigorous extraction techniques; the recoveries of DDT and related chlorinated hydrocarbon pesticides are good (much better than with the other methods investigated); and no extremely toxic solvents are involved. This efficient cleanup procedure permits the utilization of available chemical methods of analyses and may open the way for the use of methods not yet thoroughly explored for pesticide analyses, such as infrared and ultraviolet spectrophotometry and gas chromatography.

A routine procedure for determination of pesticide residues on plant material with unknown spray history might proceed as follows: extraction, acetone precipitation–Florisil chromatographic cleanup, qualitative analysis by the chromatographic procedure of McKinley and Mahon (*9*), and quantitative determination of the pesticides shown to be present. Many chlorinated hydrocarbon pesticides, when present singly in an extract, can be determined quantitatively by the method of Downing and Norton (*3*). This provides a further generalization in routine analyses.

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