The purpose of this report is not only to describe a new cleanup device designed especially for butterfat, but also to point out its utility in a rapid and complete method, as presented, for the detection and determination of organochlorine insecticide residues in butterfat.

Time may be saved by two slight procedural modifications. Direct transfer of trapping solution with *n*-hexane rinses to a miniaturized K-D apparatus incorporating a conical 2.5-ml. graduated centrifuge tube, followed by quick evaporation of most of the trapping solvent, efficiently retains the sought butter volatiles directly in this tube. Also, the use of a short, nonresolving column (*16*) in the MCGC is recommended, thereby employing the entire method as a true screening test for the detection and estimation of organohalides as a group in butterfat. Indeed, employment of this technique may well be the major emphasis this project should receive, as based on a report (*14*) which pointed out that, in the first nine months of 1962 in California, pesticide residues found in milk products were mostly DDT, DDD, and DDE, while only a few samples showed lindane, toxaphene, or methoxychlor. If toxaphene is indeed an offender, the only known way to achieve its analysis by gas chromatographic means is the short-column MCGC technique of Witt *et al.* (*16*). This technique, employed in the present over-all method, should also increase sensitivity for the detection of organohalides in butterfat since in any gas chromatographic analysis a fast eluting sharp peak is more readily detectable than an equivalent amount eluting more slowly with a broadened peak.

For an entire method starting from milk, the rapid Waring Blendor extraction procedure (*5*) is suggested to isolate the butterfat prior to forced volatilization cleanup.

Although designed for use on butterfat, the application of a forced volatilization device to other oily materials, and to other separation problems, should be apparent. Exact choice of bath medium for the FVA depends upon the temperature desired for a given problem.

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INSECTICIDE RESIDUES

Rapid Cleanup of Dairy Products for Analysis of Chlorinated Insecticide Residue by Electron Capture Gas Chromatography

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THE DEVELOPMENT of the electron capture detector by Lovelock and Lipsky (*4*) gave the chemist a valuable tool for detection of trace amounts of chlorinated insecticide residues. Watts and Klein (*8*) reported the detection of nanogram quantities of chlorinated insecticides and recovery data of trace amounts of DDT, aldrin, and BHC added to collards and kale. They also reported results on limited numbers of butter, cheese, and vegetable oil samples. Subsequently, Klein *et al.* (*2*) reported recovery data of DDT added in the 0.1 to 1.0 p.p.m. range to 1.0-gram samples of butter and refined vegetable oils. Their average recovery was approximately 90%. The procedure of sample cleanup was similar to that of the one used by Mills (*5*). The method of sample preparation, insecticide extraction, and measurement described here is equally efficient and, moreover, time saving.

Moats (*6*) reported on an improved, one-step Florisil-column cleanup procedure for detection of insecticide residues in butterfat by using paper chromatography. In this method, insecticide residues are separated from butterfat by elution from a partially deactivated Florisil column with 20% methylene chloride in petroleum ether. In some research in this laboratory, the Babcock test (*1*) was used for extraction of butterfat and selected chlorinated insecticides from dairy products prior to cleanup on a florisil column (*3*). With this method, impurities were removed so that optimum column stability and electron capture detector sensitivity were maintained. However, the extraction and cleanup of butterfat from dairy products other than butter still required two separate procedures.

This paper presents a technique for combining the extraction and separation of trace quantities of selected chlorinated insecticide residues from dairy products prior to analysis by electron capture gas chromatography.

Methods

Reagents. Reagent-grade methylene chloride and technical-grade petroleum ether, 30° to 60° C., were redistilled be-

An extraction and cleanup technique for dairy products prior to analysis for selected insecticide residues by gas chromatography has been developed. Using this procedure, one technician can analyze 25 to 35 samples per 8-hour day and detect nanogram quantities of the insecticides. Recoveries of added insecticides were more than 90%. The technique described should be a valuable screening procedure for analysis of dairy products for chlorinated insecticide residues.

fore use. The Entomological Society of America insecticide reference standards used were obtained from Nutritional Biochemical Corp. The standards and unknown samples were prepared in hexane, 65° to 67° C., and stored at 2° to 5° C. Florisil, 60- to 100-mesh, activated at 650° C. was obtained from the Floridin Co., Tallahassee, Fla. This was heated at 140° C. for 12 to 14 hours. After the Florisil cooled to room temperature, 5% water was thoroughly mixed with it, and the mixture was held in an air-tight container for 48 hours before use. The eluant was a mixture of 20% methylene chloride in petroleum ether.

Equipment. Chromatographic columns were 20-mm. o.d. × 600-mm., borosilicate glass tubes plugged at one end with glass wool. Analytical columns were $^1/_8$-inch o.d. × 4-feet, borosilicate glass packed with 2.5 or 5.0% Dow 11 Silicone on 60- to 80-mesh hexamethyldisilazane-(HMDS) treated Chromosorb W. The analytical instrument was a Wilkens Aerograph Hi-Fi Model 600 gas chromatograph with an electron capture detector containing a 250-mc. tritium ionization source operated with a 90-volt potential across the detector. The recorder was a 1-mv. Leeds and Northrup Model H with a disk integrator unit.

Cleanup and Analysis. Dairy product samples containing not more than 1 gram of butterfat were ground with 25 to 30 grams of Florisil to form a free-flowing powder. Twenty-five grams of florisil were poured into a chromatographic column and prewashed with 50 ml. of an equal mixture of methylene chloride and petroleum ether. The washings were discarded. Next, the sample-Florisil mixture was poured into the chromatographic column to form the top layer. From 150 to 650 ml. of the eluant mixture were used depending on which insecticide residues were being eluted. The eluant was evaporated to dryness from a beaker in a water bath at 50° to 60° C. The residues were transferred from the beaker to a calibrated test tube and made up to a 5- or 10-ml. standard volume with hexane.

Analysis. The analytical column was operated in the range of 185° to 195° C. with a nitrogen (High Purity) carrier gas flow of 60 to 80 ml. per minute. Standard insecticide solutions were analyzed before and after each series of unknown samples. Depending upon the quantity of insecticide present, from 5 to 25 μl. of unknown samples in hexane were used for analysis.

Results and Discussion

The sample size for dairy products varied because of differences in butterfat content. Sample sizes were 1 gram for butter; 2.0 grams for cheese, dried milk, and cream; 5.0 grams for evaporated milk; and 10.0 grams for whole milk. These sizes ensured that the amount of butterfat placed on the Florisil was less than 1 gram. If a sample contained over 1 gram of butterfat, some butterfat was usually eluted from the cleanup column with the insecticide residue, resulting in poor stability of the analytical column and loss of detector sensitivity. As evidence that the cleanup technique was effective, when the proper sample size was used, the same analytical column could be used for approximately 500 analyses.

The extraction and cleanup technique has been used for five selected chlorinated insecticides added to dairy products. Table I contains recovery data for these insecticides. Recovery of the selected insecticides from dairy products was consistently over 90%. Only 150 ml. of eluant were necessary to eluate DDT, DDE, or lindane. Also, these insecticides could be eluted from Florisil which was not previously deactivated with 5% water. For dieldrin, heptachlor, heptachlor epoxide, and endrin, the use of activated Florisil was necessary. Elution of heptachlor and heptachlor epoxide required 250 ml. of eluant. Dieldrin was eluted with 550 ml. of eluant, and endrin with 650 ml. of eluant. The time required for cleanup ranged from 30 to 90 minutes depending on the volume of eluant required.

The quantity of insecticide in a sample injected into the chromatograph must be adjusted so that the analysis was performed within the linear range of the instrument. Adjustments were possible in original product sample size, final standard volume used, and size of aliquot injected on the chromatograph. A final standard volume in hexane of less than 5 ml. usually caused some baseline shift. This is no doubt due to a concentration of some impurities from the product sample passing through the Florisil column.

With the instrument used in this work, it was not necessary to use less than a 5-ml. final volume in hexane to achieve the desired sensitivity. When a 10-gram sample of milk, a final sample volume of 5 ml. of hexane, and a 10-μl injection for analysis were used, the sensitivity of the method is 0.01 p.p.m. for lindane, heptachlor, heptachlor epoxide, DDE, and dieldrin. Under the same conditions, the method has a sensitivity of 0.05 p.p.m. for DDT and endrin. Increased sensitivity can be obtained by using larger product sample size and a larger Florisil column for extraction and cleanup prior to analysis. This was not pursued because sensitivities achieved were sufficient for the research planned. If used as a screening technique on product samples, increased sensitivity would be desirable.

Table I. Recovery of Insecticides Added to Various Dairy Products

Insecticide	Per Cent Recovery[a,b] 0.1 p.p.m. added	1.0 p.p.m. added
DDT	94.0	96.0
Lindane	94.0	94.0
Heptachlor	94.5	94.5
Dieldrin	95.0	95.0
Endrin	90.0	91.0

[a] Represents average of 12 samples.
[b] Standard deviation = ±2%.

With 2.5% Dow 11 Silicone on HMDS-treated, 60- to 80-mesh Chromosorb W at 185° C. with a 60- to 80-ml. per minute carrier gas flow, and analysis for the 5 insecticides on the gas chromatograph was completed in less than 10 minutes. DDE and *p,p'*-DDT were eluted as separate peaks from the analytical column. Heptachlor and heptachlor epoxide were separated as two peaks. The isomerized products of endrin appeared as reported by Phillips *et al.* (7). There was very little breakdown of *p,p'*-DDT on the column when a borosilicate glass injector liner and a borosilicate glass column were used. Without a borosilicate glass injector liner, the use of a stainless steel column resulted in a 25% breakdown of *p,p'*-DDT to a product which shifted into the DDD peak at 185°–195° C. Increasing the Dow 11 Silicone liquid phase to 5% resulted in even better separation of insecticides, but retention time and therefore analysis time were increased by 3 or 4 minutes.

At present, this method has not been tried for other chlorinated insecticides.

It has been used to extract and clean up other animal product samples containing fat prior to analysis for the five insecticides. Work on the use of this method for cleanup of animal blood, eggs, chicken fat, tallow, lard, and ground meat will be reported at a later date.

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INSECTICIDE RESIDUES

Dilan Residue Determination by Microcoulometric Gas Chromatography

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A cleanup procedure using either activated carbon or nonactivated Florisil was successfully applied to extracts of pears for the residue analysis of Dilan. Preliminary survey analysis of Dilan was accomplished by gas chromatography with programmed temperature and a thermal conductivity detector. Residue analyses sensitive to 1 to 2 μg. of Dilan were made by microcoulometric gas chromatography. The analysis of commercial Dilan formulations by programmed temperature gas chromatography is feasible.

DILAN is a mixture of Bulan [2-nitro-1,1 - bis(*p* - chlorophenyl)butane], Prolan [2-nitro-1,1-bis(*p*-chlorophenyl)-propane], and related compounds. The commercial mixture is composed of 53.3, 26.7, and 20.0%, respectively, of the above components. The related compounds are principally the *o-p'* isomers similar to those associated with DDT (*7*).

Dilan is reported to have insecticidal properties and has been used for the control of pear psylla in California in conjunction with studies on the pear decline disease. The psylla was implicated as a possible agent in the transmission of this disease. As a result of treatments to control this pest, residue data were required before California recommendation could be made for the use of this pesticide. Cooperative work between university entomologists and this laboratory are required to develop the necessary control measures and recommendations for the use of the pesticide based on insecticide performance, safety, and residue levels.

Two methods of analysis for Dilan have been reported. Mitchell (*8*) described a paper chromatographic procedure for the separation of the components of Dilan, and the subsequent location and measurement of the spots on the paper. Jones and Riddick (*6*) described a colorimetric procedure involving the conversion of the aliphatic nitro groups to the aci-form. The converted product is complexed with ferric chloride in an acid medium to form a colored product. In the latter method, quantities less than 50 μg. were not reliable and quantities less than 10 μg. were not detectable. The best results were obtained with 100- to 500-μg. quantities of Dilan which thus would involve sample quantities as large as 500 grams to achieve the desired sensitivity. Blank problems were reported, which may have been partially caused by the absence of any cleanup measures.

A cleanup procedure and method of analysis utilizing a gas chromatograph with a microcoulometric detector has been developed for the analysis of Dilan in pears. Residues as low as 1 to 2 μg. can be detected.

Experimental

Preliminary investigations on Dilan were made with a programmed temperature gas chromatograph using a thermal conductivity detector. A commercial mixture was resolved into two distinct peaks (Figure 1), indicating a 75:25 component ratio. A sample of purified Dilan containing a mixture of *p,p'* isomers of Prolan and Bulan showed a similar chromatogram. Also, a formulated product made from a technical

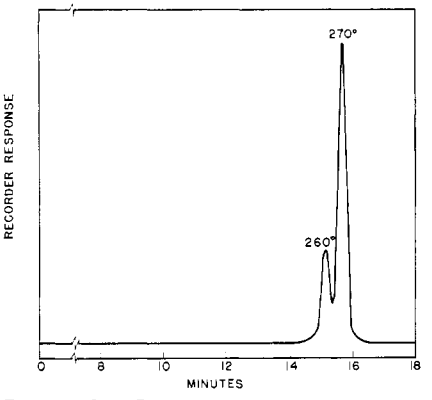

Figure 1. Programmed temperature gas chromatogram

25 μg. Dilan in 25 μl. injected on an F and M Model 500 gas chromatograph, thermal conductivity detector; initial temperature 100° C., program rate 11° per min.; 2-foot stainless steel column containing 20% General Electric SE-30 silicone rubber gum on 40- to 50-mesh Chromosorb P, Prolan response at 260° C. and Bulan response at 270° C.