Forced Volatilization Cleanup of Butterfat for Gas Chromatographic Evaluation of Organochlorine Insecticide Residues

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A procedure is presented for the completely physical cleanup of butterfat prior to analysis for organochlorine insecticide residues. Two versions of a new device employing a forced volatilization principle have been developed and are described for the cleanup step; subsequent analysis is by means of microcoulometric gas chromatography. The entire method requires about an hour for a 2-gram sample, and readily responds to about 0.5 p.p.m. each of seven possible organochlorine insecticide residues in butterfat; methoxychlor is demonstrable at about 10 p.p.m.

A MAJOR PROBLEM in pesticide residue evaluations is the cleanup required to segregate the residue from the foodstuff in form suitable for ultimate qualitative and quantitative analysis. This is particularly true in the analysis of fatty materials, such as butter, which are among the most difficult materials from which to isolate pesticide residues. Several recent methods (*1*, *2*, *5*, *9*, *10*, *13*, *17*) now supplement the one method (*12*) previously and generally accepted as workable for this purpose. Each of these has merit but is in one way or another limited in usefulness. A few procedures have been described for rapid estimation of organochlorine residues by determining total organically bound chlorine in the sample (*6*, *7*, *15*). Missing is an all-inclusive method which consumes only small amounts of common reagents and is rapid, quantitative, and sensitive while offering some specificity among the greatest possible number of pesticide residues. Almost concurrently with the present work, which is an attempt to fulfill the stated requirements, has been the work of Langlois *et al.* (*8*).

Described here are two versions of a new device designed especially for the cleanup of organochlorine insecticide residues in butterfat. Employing a forced voltatilization principle (*3*, *4*), the device physically separates volatile materials from those which are either nonvolatile or considerably less volatile at a given operating temperature. The more volatile materials, including many insecticide residues, are thus isolated from the bulk of the butterfat. In this form these residues are ready for direct analysis by microcoulometric gas chromatography.

The method readily responds to about 0.5 p.p.m. each of the eight compounds tested, exclusive of methoxychlor, in 2.0 ml. (1.8 grams) of butterfat or to about 0.02 p.p.m. each in the equivalent 45 grams of original milk. Methoxychlor can be detected at about 10 p.p.m. in butterfat.

Materials and Methods

Special Apparatus. Either a self-contained forced volatilization apparatus (FVA) (Figure 1) or a heater-tape forced volatilization apparatus (HT-FVA) (Figure 2).

High-efficiency scrubber (Figure 3) for trapping emergent volatiles from either HT-FVA or FVA.

Microcoulometric gas chromatograph (MCGC), Dohrmann Instruments Co., San Carlos, Calif. The instrument should be equipped with removable quartz insert and the T-200S titration cell for halides.

Miscellaneous Apparatus. Flowrator Tube, calibrated precision bore, No. 02-F 1/8-12-5/35 (Fisher & Porter Co., Hatboro, Pa.) or equivalent. It should be calibrated at a given head pressure (5 p.s.i. is adequate) for flow rate of water-pumped nitrogen at laboratory temperatures with a wet test meter or by displacement of water with the gas effluent from the top of the trap system of the FVA or HT-FVA filled with the recommended amount of redistilled acetone. One liter of nitrogen per minute is the recommended flow rate.

Hypodermic needle, 20 outer gage, 12 inches long (Hamilton Co., Inc., Whittier, Calif.).

Powerstat, or equivalent variable transformer, 7.5 amp., 0- to 135-volt output.

Spring grip clamp, size No. 7-B (A. H. Thomas Co., Philadelphia, Pa.). Remove one jaw, the thumb push rod, and the knurled nut and its bolt.

Waste flask (for FVA only), 50-ml. suction with ⅋ 19/38 outer joint and a gas-tight stopcock on the side arm.

Reagents. Hexadecane, 99%, b.p. 288° C. (Matheson Coleman & Bell) as a stable refluxing bath liquid for the FVA (for the isolation of most of the organochlorine compounds of interest).

Procedure for Preparation of FVA. When thoroughly clean and dry, the FVA as shown in Figure 1 is to be insulated and protectively wrapped. Mount it temporarily in an inverted position by clamping the two end projections to solid bench supports. Cover its openings except the ⅋ 19/22 joint with 2 or 3 layers of aluminum foil held in place with rubber bands. Plug the ⅋ 19/22 joint with lint-free tissue. Wrap the body of the FVA with 2 layers of aluminum foil, shiny surface inward; secure with thread. Wrap water-soaked asbestos tape over the aluminum foil, smoothing the latter in front of the working area. Continue wrapping with workable lengths of moistened tape until approximately $^1/_4$-inch of this insulation is applied. Smooth the asbestos fibers uniformly over the apparatus and to thin layers around all projects and orifices. Allow to air dry; then with a sharp scalpel cut a viewing window $^1/_4$-inch wide by 11 inches long through the insulation on front and back sides of the FVA. Protect the squared cuts by a single layer of $^3/_4$-inch wide self-adhesive, glass-cloth tape (Mystik Tape Products of California, Los Angeles, Calif.).

Clamp the FVA permanently and solidly in an upright position, with the ⅋ 19/38 joint about 6 inches above the bench; the clamp should be positioned on the FVA near the bottom, under one of the strong electrode projections, and provide the only support. Another clamp should be fitted loosely around the ⅋ 24/40 joint to maintain perpendicular stability.

Pour enough hexadecane down the upper arm to cover the heating element.

Lubricate the ⅋ 24/40 joints of a short (about 10 inches) air condenser with a few drops of the bath medium and insert. Fill a ⅋ 24/40 drying tube with indicating Drierite (W. A. Hammond Drierite Co., Xenia, Ohio), held in place with plugs of glass wool, and insert into the air condenser.

Slowly increase the voltage across the

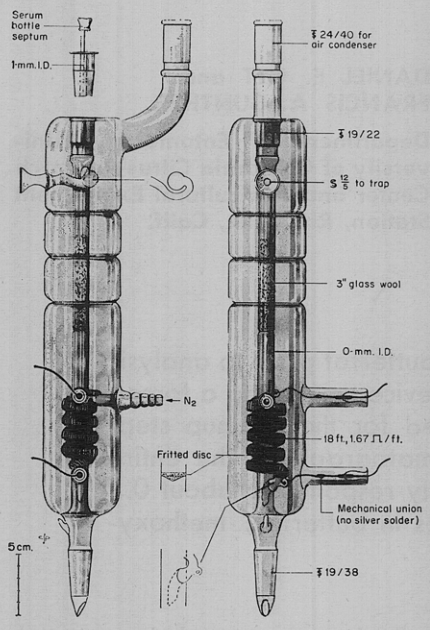

Figure 1. Basic unit of the all-glass forced volatilization apparatus (FVA)

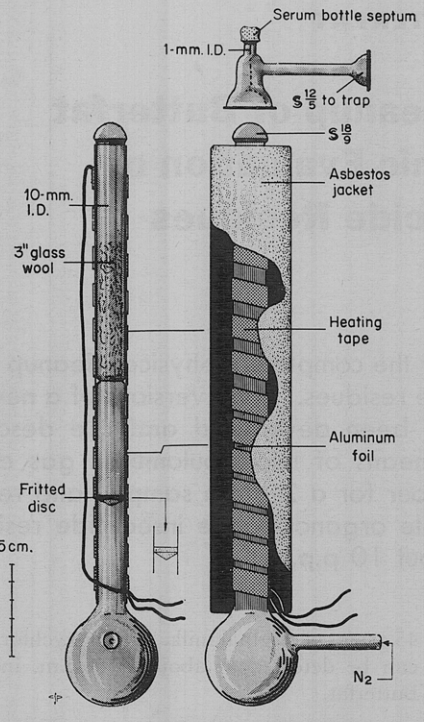

Figure 2. Heater-tape forced volatilization apparatus (HT-FVA)

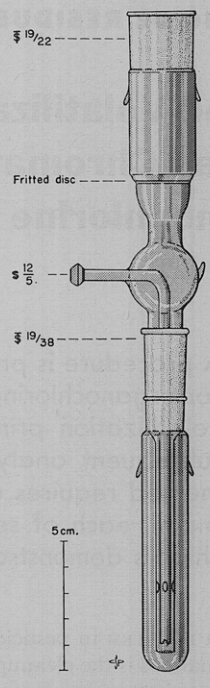

Figure 3. Scrubber used for trapping emergent volatiles; essentially the same as earlier described by Gunther *et al.* (3,4)

heater terminals, by using the variable transformer, until the bath begins to boil, then hold the voltage at a setting which will maintain vigorous boiling.

With stainless steel springs attach the waste flask to the bottom joint of the FVA. By means of clean rubber tubing, series-connect the nitrogen inlet of the FVA, the calibrated Flowrator Tube, and the needle valve on a pressure regulator attached to a nitrogen tank. A simplified operational flow diagram is:

$$N_2 \longrightarrow \text{Flowmeter} \longrightarrow \begin{matrix}\text{FVA}\\ \text{or}\\ \text{HT-FVA}\end{matrix} \longrightarrow \text{Trap}$$

After at least 15 minutes of vigorous boiling of the bath medium with nitrogen flowing through the FVA at 1 liter per minute, temperature equilibrium should be achieved. Place a one-hole cork over the top central orifice and insert a long stem 360° C. thermometer through the cork and down the column of the FVA until the bulb almost touches the fritted disk. Allow 15 minutes for temperature equilibrium; the reading should be within 10° C. of the actual boiling point of the bath medium. Raise the thermometer until the bulb is level with the nitrogen outlet; after 30 minutes this reading should be at least 190° C. for removal of DDT from butterfat. If the reading is below 190°, increase the heater input voltage cautiously as there may be bumping if the voltage is raised too high too quickly. Once proper temperature characteristics are established, the setting for the particular variable transformer used should be recorded.

Remove the thermometer and insert the glass injection port fitted with a rubber serum-bottle septum and lightly lubricated. If the final analysis does not involve infrared, a silicone stopcock grease may be used; otherwise, a small amount of control butterfat is recommended. The injection port is conveniently held in place with a spring-loaded pressure plate made from one half of a spring grip clamp (see Miscellaneous Apparatus); the tip of the flat inner side of the remaining jaw is lowered against the rim of the injection port and clamped in place.

Procedure for Preparation of HT-FVA. The essential preliminary step is the attainment of desired temperature and carrier gas flow characteristics. With nitrogen flowing through the column of the HT-FVA at 1 liter per minute, measure the temperature inside the column near the disk as described for the FVA. This temperature should be a constant 275° C., adjusted by a variable transformer connected to the heater tape. (The heater tape, $^1/_2$-inch wide by 24 inches long, is wrapped in a single layer with turns spaced further apart at the top so as to give a slight temperature gradient along the length of the tube.)

Lubricate the ground glass joints of the HT-FVA as for the FVA.

Procedure for Butter Samples. VOLATILIZATION. Warm the butter to about 50° C., stir until thoroughly mixed; then, while keeping it warm, allow the aqueous phase to separate.

Hypodermically insert 12 ml. of trapping solvent in the conventional Kuderna-Danish (K-D) tube of the trap. Insert the top part of the trap and secure it to the tube with springs. Reduce nitrogen flow through the volatilization device from 1 liter per minute to about 300 ml. per minute. Lubricate the spherical joint of the trap and clamp it to the volatilization device. Gently raise a 600-ml. beaker filled with ice and water around the tube of the trap. Inject 6 ml. of trapping solvent onto the fritted disk in the trap. For highest recoveries, a two-ball Snyder column is fitted to the joint at the top of the trap, and trapping solvent is permitted to drip into the top of the column from a separatory funnel to provide a continuous supply of fresh trapping solvent; the rate of addition is adjusted to approximate the rate of solvent evaporation loss.

The butterfat in the sample should now be transparent. Draw slightly more than 2 ml. into a 2-ml. Luer-Lok tip syringe fitted with a short 18-gage needle. Hold the syringe with the needle up, draw all butterfat out of the needle, and replace it with the long 20-gage needle; adjust the volume of butterfat to the 2-ml. mark while tapping the syringe to dislodge entrained bubbles. With a tissue wipe the excess butterfat from the tip of the needle. Hold the plunger of the syringe securely in position with slight lateral forefinger pressure. Increase nitrogen flow through the volatilization device to about 500 ml. per minute. With the free hand and the tissue force the tip of the needle through the septum by applying most of the force with this hand holding and guiding the needle. After the puncture, guide the needle

Table I. Per Cent Recoveries as Determined by Microcoulometric Gas Chromatography of Organochlorine Compounds from Fortified Butterfat (2.0 ml. or 1.8 grams) after Forced Volatilization Cleanup

P.P.M. Added	Lindane	Aldrin	Heptachlor Epoxide	Dieldrin	DDE	DDD	p,p'-DDT	Methoxychlor
In admixture from FVA[a]								
1.1, 11.1	82 ± 12	84 ± 6	94 ± 2	93 ± 3[b]		84 ± 4		33[c]
Singly from FVA								
0.6, 1.1, 2.8, 5.6, 11.1	—[d]	—	—	101 ± 12	—	—	55 ± 11	—
					(8 ± 7)	(12 ± 6)	(75 ± 10)[e]	
In admixture from HT-FVA[a]								
0.6, 11.1	89 ± 13	—	96 ± 14	—	—	—	85 ± 14	—
0.6, 1.1, 11.1	—	84 ± 10	—	73 ± 14	—	95 ± 24	—	87 ± 9[c]
1.1, 2.8, 5.6, 11.1	87 ± 8	82 ± 5	89 ± 6	92 ± 9		98 ± 13		42[c]
Singly from HT-FVA								
0.6 to 11.1	89 ± 6	93 ± 3	98 ± 5	87 ± 13	92 ± 11	—	72 ± 9	—
					(2 ± 2)	(6 ± 4)	(80 ± 13)[e]	
1.1, 11.1	—	—	—	—	—	—	86 ± 6	—
						(3 ± 2)	(89 ± 6)[f]	
11.1	—	—	—	—	—	78 ± 13	—	37 ± 4
								(61 ± 11)[g]

[a] Quartz wool in the cleanup device. [b] Compounds shown connected were unresolved in the gas chromatographic procedure; therefore from the total area an average value was taken for each. [c] Value from determination only at the 11.1 p.p.m. fortification level. [d] — means compound not added to the butterfat sample. [e] Total value for DDT calculated by including the percentage of apparent breakdown to other compounds (assumed to be DDE and DDD, see text) indicated in parentheses. Borosilicate glass wool in the FVA. [f] Same as footnote *e*, except quartz wool in the HT-FVA. [g] Some apparent breakdown to unknown compound (assumed to be methoxychlor-ethylene). Data recorded as for DDT in footnote *e*.

with the tissue until the tip comes within an inch of the disk in the column. With a slow, steady thumb pressure expel the syringe contents. Withdraw syringe and needle while holding the septum down; at the same time wipe the hot needle with tissue. Increase nitrogen flow to 1 liter per minute and note the time. Check for possible leaks in the total system, as detected by soap or other bubble solution; usually leaks at this stage are self-evident from dense aerosol generation by the hot butterfat.

Immediately rinse syringe and needles several times with hexane followed by acetone, and air dry.

After 20 minutes, nitrogen flow is reduced to only a slight positive flow as evidenced by a slow bubbling through the trap. The trap is removed and the joint between top of trap and tube is immediately broken so liquid above the disk will drain into the tube. Insert a short strip of folded aluminum foil in the joint to keep it open.

The volatilization device is now back-flushed with nitrogen as follows. The side-arm on the waste flask is opened to permit the bulk of the butterfat to drain into the flask. The nitrogen inlet tubing is removed and attached to an inner-member 12/5 spherical joint which, in turn, is attached by a pinch clamp to the normal gas outlet of the volatilization device. Nitrogen at 1 liter per minute is back-flushed through the system for 10 minutes. A vacuum line should be placed near the waste flask outlet to draw off the vapors of hot butterfat.

Further Treatment. For some types of final determination, including use of the MCGC, it is sufficient merely to evaporate the trapping solvent from the trapped butter volatiles, followed by adjustment to a standard volume with redistilled *n*-hexane. The top of the trap is removed from its tube, the walls of the tube joint are rinsed with about 1 ml. of *n*-hexane, and the tube is fitted onto a 500-ml. K-D flask. A small funnel is placed in the neck of this flask, and the top of the trap including the two-ball Snyder column are rinsed over it with about 10 ml. of *n*-hexane. The combined trap and rinse solution in the K-D apparatus is then evaporated in a steam bath in the usual manner at a maximum temperature of 80° C. If necessary, last traces of solvent are evaporated from the tube under a gentle jet of filtered air while warming the tube in a water bath to about 40° C.

Increasing Efficiency of Cleanup. The degree or efficiency of cleanup may be determined by comparing weight of butterfat sample injected to weight of butter volatiles collected. Observed ratios have varied from 11:1 to 70:1. The latter value was achieved with a loose plug of borosilicate glass wool in the column of the FVA. (Quartz glass wool is recommended to inhibit breakdown of DDT.) With a stirring rod push a loose 3-inch plug of glass wool into the column to the constriction shown in Figure 1 or Figure 2. The top of this plug should be about 2 inches below the gas outlet. Carefully clean glass wool particles from the injection port. When necessary, the glass wool plug is withdrawn with a hooked length of wire.

Maintenance of Apparatus. A volatilization device at an elevated temperature, except when it is thoroughly clean, should always be under slight nitrogen purge. When cool, it may be cleaned by copious rinsing with hexane from a syringe with long needle, followed by rinses with acetone. Occasionally more thorough cleaning is necessary due to the gradual accumulation of intractable deposits in the column: repeatedly and carefully draw hot chromic-acid cleaning solution through the column, then rinse it copiously with water, distilled water, and acetone.

Butterfat Fortification for Standardization. Draw a given volume of butterfat supernatant from melted commercial butter into a large hypodermic syringe fitted with a large-bore needle. Expel most of it into a glass-stoppered graduated cylinder. While the butterfat is still molten, add by microsyringe exactly that amount of analytical grade organochlorine compound in *n*-hexane solution (1.0 μg. per μl.) which will give the level of fortification desired, in terms of μg. of compound per ml. of butterfat. The butterfat in the graduated cylinder is adjusted to volume by addition from the large syringe, over the microsyringe needle to rinse it, followed by thorough mixing by shaking.

Analytical Method. The analysis consists basically of conventional microcoulometric gas chromatography.

A removable insert is used—a 2-inch length of 52-mesh platinum gauze (*11*) is coiled one turn inside the quartz insert. With an injection block temperature of 200° C., much of the extraneous volatile material from butterfat is entrained as a thin film on the gauze and never reaches the chromatographic column. The insert in the instrument is exchanged for a clean one after each sample injection. A wooden applicator stick facilitates removal of the silicone rubber septum without damage.

Organohalides are separated on a 4-foot, 1/4-inch O.D. aluminum column packed with 5% Dow 200 silicone oil

(12,500 c.s.) on acid-washed 30- to 60-mesh Chromosorb P, at 205° C., 20 p.s.i. back pressure, and a nitrogen flow of 115 ml. per minute.

The coulometer is fitted with a precision resistor to provide impedance match with a 1-mv., high-impedance recorder and is operated at the highest net resistance (51.2 ohms) of the range switch control.

Butterfat samples from a forced volatilization device are adjusted to 0.4 ml. with *n*-hexane in calibrated 2.5-ml. conical centrifuge tubes with ⅌ 9 stoppers. Aliquots from 10 to 80 μl. are injected by microsyringe into the MCGC. For large aliquots, peak broadening is minimized by greatly reducing nitrogen carrier gas flow before injecting the sample, then resume the normal carrier gas flow rate.

To determine efficiency of the volatilization apparatus, integrator peak areas of the recorded gas chromatograms of fortified butterfat samples are frequently compared with peak areas achieved from standard solutions of the organochlorine compounds. Unfortified control samples are run through the total procedure.

Results and Discussion

The results in Table I are expressed in terms of per cent recoveries achieved with the indicated purified organochlorine compounds added singly and in mixes to butterfat. In most instances, each fortified sample was replicated through the entire method. Appropriate compensation of the peak areas of fortified samples due to background in control chromatograms was employed. It was common to find 0.1 to 0.3 p.p.m. of apparent DDE and also of DDT in commercial butterfat. Values in Table I are averages over the indicated fortification range. No complete explanation is available for the high variability of DDD recoveries from 0.6 to 11 p.p.m. when in admixture with other compounds. However, it should be pointed out that for very precise residue analysis one would determine recovery values for each of several fortification levels for a given compound so that an average value could be compared with an "unknown" at approximately the same level for quantitative evaluation. The precision between several replicates of a standard at a given level of fortification would in most cases be better than those indicated in Table I, where for the sake of simplicity an average was given to encompass a wide range of fortifications.

Preliminary use of borosilicate glass wool plugs in the cleanup devices demonstrated that *p,p'*-DDT in fortified butterfat underwent partial breakdown in this step to respectable amounts of two other compounds as shown by gas chromatograms. The breakdown of DDT by the particular gas chromatograph used was either undetectable or insignificant. By retention time criteria, one breakdown product was assumed to be DDE; the other product could have been *o,o'*-DDT, *o,p'*-DDT, or DDD. Of these three candidates, DDD seemed the only choice from quantitative considerations coupled with the improbability of isomerism. The formation of DDD from DDT under these conditions does not seem to be a realistic assumption either; however, work in progress will positively identify these products. Until identification is made, DDE and DDD are the assumed products, and the per cent recovery values in parentheses in Table I were included solely to indicate total recovery for DDT, with these two assumed products added in the calculation. The last entry for DDT at 86% represented quartz wool in the HT-FVA, with chromatograms indicating only 3% breakdown to assumed DDD, with no other product detectable; total calculated recovery for DDT in this instance is thus 89%.

Table II. Per Cent Recoveries as Determined by Infrared Assay of DDT from Fortified Butterfat (2.0 ml.) Subjected to HT-FVA[a] and Florisil Column Cleanup

P.P.M. DDT Added	Recovery,[b] %
10.9	71
27.2	81
43.5	83

[a] Borosilicate glass wool employed. [b] Assumed to be all DDT as there was no detectable DDE. Some DDD may have been present as it is difficult to distinguish from DDT by infrared assay.

Other methods of determination were also utilized. Infrared analysis is suitable for some organochlorine compounds in butterfat at high levels of fortification. For example, a lower limit of 10 to 15 μg. of DDT is detectable in 0.3 ml. of carbon disulfide in a 5-mm. cavity cell. Cleanup beyond forced volatilization is also necessary for quantitative infrared assay of DDT from butterfat. Typical results are shown in Table II.

Extra cleanup for this purpose can be achieved either by several concentrated sulfuric acid washes of an *n*-hexane solution (*5*) of butter volatiles or by Florisil column chromatography essentially as described by Mills (*12*). Both extra cleanup steps have certain disadvantages besides the time involved. Not all pesticides will survive this harsh acid-wash treatment. While very effective in removing infrared-interfering extraneous volatile materials, the Florisil column procedure does not permit quantitative elution of all insecticides.

Gas chromatography using electron-affinity detection indicated no advantage over the MCGC directly on butterfat samples cleaned up by forced volatilization. Upon injection of diluted aliquots large enough to gain more sensitivity than the MCGC offered, the electron-affinity detector was simultaneously overloaded with too much background material. Extra sensitivity can be achieved for both types of detectors by Florisil column cleanup after the forced volatilization procedure.

Increased sensitivity cannot be achieved by increasing butterfat sample size injected into the present forced volatilization device. Scaling up the size of such a device must be accompanied by correspondingly increased nitrogen flow rates per unit cross-sectional area.

Important factors for best cleanup, besides flow rate characteristics, are length of column, and temperature and temperature-gradient characteristics of column. Given a cleanup device closely approximating the size of that shown in Figures 1 or 2, nitrogen flow rate, temperature of column, and temperature gradient through the column may have to be varied independently from the corresponding values described herein until a compromise between efficiency of cleanup and per cent recovery of sought compounds, satisfactory to suit the needs of the problem at hand, has been achieved. Once conditions have been set and per cent recovery of a given compound has been established, operation of the cleanup device becomes routine for analysis of that compound in "unknown" butterfat samples. A competent technician should be able to operate three of these devices concurrently, affording about 20 analyses per day.

Empirical observations dictate the needs for cleaning a forced volatilization device. It is thoroughly acid cleaned if obviously dirty; it is cleaned by solvent rinses when: between given samples it is known or suspected that there is a change to another pesticide or group of pesticides, or it is known or suspected that there is a change from a sample with relatively high pesticide concentration to a sample of relatively lower concentration, the efficiency of the cleanup becomes unacceptable, or the greatest accuracy in the final analysis is desired.

Both versions of the volatilization apparatus appear to be equal in effectiveness with butterfat. However, the heater-tape version is slightly more convenient to use.

Of the eight compounds tested, all except DDT and methoxychlor are determined almost quantitatively and reproducibly down to about 0.5 p.p.m. in butterfat. This statement holds for DDT if its breakdown products, when produced in the cleanup device, are added in. This partial breakdown can be minimized by use of quartz rather than borosilicate glass wool in the volatilization apparatus.

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-