Removal of Chlorinated Insecticide Residues from Milk Fat by Molecular Distillation

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Added lindane, heptachlor, heptachlor epoxide, aldrin, DDT, DDD, and DDE were successfully removed from milk fat by a laboratory-scale molecular distillation. Removal of 95 to 99+% was achieved for levels of these pesticides which might be encountered in a severely contaminated sample of fat. A distillation temperature of 200° C. and pressure of 5×10^{-4} torr were required to obtain efficient removal. Pesticides undergoing distillation were recovered nearly quantitatively in apparently unaltered form. Aside from the possible application of molecular distillation for the removal of such pesticides on an industrial scale, this technique provides a method for concentrating residues prior to cleanup and analysis.

The presence of chlorinated insecticide residues in dairy products has been a subject of considerable concern in recent years. Precautions at the farm level to exclude contaminated feed and avoid the use of certain insecticides on the cows or in the milking area are the only currently available methods for reducing such residues. Some consideration, however, has been given to the removal or reduction of residues in dairy products through normal or modified processing procedures. Mann *et al.* (1950) found that pasteurization had little effect on the amount of DDT in milk. Langlois *et al.* reported the effects of processing and storage on the concentration of DDT and lindane (1964a) and of endrin, dieldrin, and heptachlor (1965) in various dairy products. They observed a decrease in the concentration of these insecticides during the spray or drum drying operation. As much as 50% of the heptachlor epoxide and dieldrin was lost during condensing. Butter and cheese in most cases contained less insecticide than milk on a fat basis, because some insecticide separated into the skim milk or whey. No detectable decrease in the concentration of insecticides was observed during the storage of cheese or ice cream. The authors concluded that most of the residues investigated are essentially stable to most of the normal processing and storage procedures to which dairy products are subjected.

Liska (1965) attempted to reduce the level of DDT in butter oil by treating cream with a surface active agent to break the emulsion. Varying success, ranging from 27 to 53% removal of DDT, was achieved.

The degradation of DDT in milk treated with hydrogen peroxide at levels used for pasteurization prior to Cheddar cheese manufacturing has been reported by Cardwell *et al.* (1966).

Gooding (1966) made the interesting observation that vegetable oils are essentially free of chlorinated insecticide residues following deodorization. All of the common residues, either adventitious or added, disappeared by the deodorization stage of refining. Hydrogenation alone eliminated chlordan, DDT, and DDD, but the fate of the various pesticides during such processing was not investigated.

Crosby (1965) indicated that little pertaining to the intentional removal of pesticide residues from food products had been published prior to October 1964. A limited amount of information is available concerning the removal of pesticides from fruits and vegetables during processing, but very little information can be found that pertains to animal products.

Ott and Gunther (1964) developed an analytical cleanup technique for milk fat based on the entrainment of chlorinated insecticides in a nitrogen stream. This work suggested that other physical methods based on the volatility of these compounds could likewise provide a means of removing them from the bulk of a lipid material.

The present study was initiated to determine the feasibility of reducing the level of chlorinated insecticide residues in milk by various physical treatments. According to Mann (1950), such residues are associated with the fat fraction. Thus the development of laboratory-scale procedures for removing chlorinated insecticides from milk fat appeared to be a logical point of departure.

Materials and Methods

Milk fat was prepared from fresh sweet-cream butter by liquefying the butter at 45° C. and decanting the fat layer. The milk fat was filtered to obtain a clear, homogeneous liquid and then held at 5° C. until used.

Analytical Grade insecticides were added to the milk fat prior to the distillations. The insecticides were dissolved in hexane, and an aliquot (5 ml. or less per 100 grams of milk fat) of the hexane solution was then added to the melted fat. In the initial studies, relatively high concentrations of insecticides were added to the fat to simplify and improve the accuracy of the analyses. In later studies, concentrations approximating those of severely contaminated milk fat samples were used.

Molecular distillations were carried out in an Arthur Smith Rota-film Model 50-2 molecular still. A trap packed with 3-mm. glass beads and cooled with liquid nitrogen was placed in the system adjacent to the molecular still to trap insecticides or other components not trapped on the cold finger of the still. Using a Leybold Model DO 121 diffusion pump, a pressure of 5×10^{-4} torr was easily attained during the distillations. A schematic drawing of the distillation assembly is shown in Figure 1. Distillation temperatures of 100°, 150°, and 200° C. were employed. The liquid fat was de-

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gassed through the degassing outlet of the feed flask until a pressure of about 10^{-3} torr could be established in the system. After degassing, the liquid fat was introduced into the still at a uniform rate requiring 3 hours for the passage of 100 grams of fat through the still.

To determine the amount of each insecticide removed by a given distillation, an aliquot of the milk fat sample containing the added insecticides was removed prior to distillation and analyzed concurrently with the portion subjected to the distillation treatment. Normally, only the untreated aliquot and the stripped fat after distillation were analyzed, but in one instance the distillates which had been collected at the cold finger of the still and at the trap cooled with liquid nitrogen were also analyzed to demonstrate the nearly quantitative recovery of unaltered insecticides.

The Florisil column cleanup technique as proposed by Langlois *et al.* (1964b) and by Moats (1963) was employed, with minor modifications, prior to analysis by gas-liquid chromatography (GLC). Florisil (Fisher Scientific Co., 60 to 100 mesh) was heated at 135° C. for 14 hours and then partially deactivated by the addition of 5 ml. of water per 100 grams of Florisil. Chromatographic columns, 2 × 50 cm., equipped with Teflon stopcocks were employed. The column packing, in order of addition, consisted of 3 cm. of tightly packed glass wool, 11.5 cm. of Florisil, and 2.5 cm. of anhydrous sodium sulfate. The eluting solvent was methylene chloride–petroleum ether (1:4, v./v.). The column was packed dry, then washed with 50 ml. of the eluting solvent. The fat sample to be analyzed was dissolved in hexane and added directly to the top of the column; 5 ml. of the hexane solution containing not over 500 mg. of fat was applied. The sample was then washed into the column with three 5-ml. portions of eluting solvent before filling the upper portion of the column with solvent. Quantitative recovery of the insecticides involved in this study was achieved by collecting 300 ml. of eluate from the column. Solvents employed throughout the analysis were either high purity (Mallinckrodt, Nanograde Solvents) or were redistilled and checked for impurities giving a response at the electron-capture detector.

Excess solvent was evaporated prior to analysis by introducing the eluate into a 250-ml. beaker which was then placed on a hot plate preheated to 80° C. A current of air (30 liters per minute) was directed over the top of the beaker during evaporation. Boiling of the solvent was eliminated by rapid surface evaporation, and adequate concentration was achieved in approximately 30 minutes. This method of concentration was quantitative for the insecticides investigated.

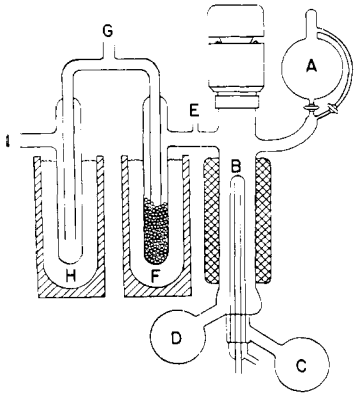

Figure 1. Molecular distillation apparatus

A. feed flask; *B.* cold finger; *C.* receiving flask for material trapped at cold finger; *D.* receiving flask for stripped fat; *E.* outlet to Pirani gage; *F.* trap packed with beads and cooled with liquid N_2; *G.* outlet to ion-discharge gage; *H.* safety trap; *I.* outlet to diffusion pump is series with forepump

The concentrated eluate from the Florisil column was made up to final volumes of either 10 or 50 ml. depending upon the concentrations of added insecticides.

A measured volume of a solution containing an internal standard compound was incorporated in the final dilution for each analysis. The inclusion of an internal standard at this stage of the analysis provided a simple means of correcting for minor variations in injection size and GLC recorder response between injections. Dieldrin served as an internal standard for analyses involving lindane, heptachlor epoxide, and DDT, and heptachlor epoxide served as the internal standard for the analyses involving aldrin, heptachlor, DDE, and DDD. Internal standards should prove useful in certain pesticides analyses, provided the internal standard compound is absent in the sample.

GLC analyses were carried out with an F and M Model 810 chromatograph equipped with an electron-capture detector. The column consisted of a glass tube (120 cm. × 4-mm. i.d.) packed with 3.8% silicone rubber SE-30 on Diatoport S.

Results and Discussion

The removal of lindane, heptachlor epoxide, and DDT was initially studied at different distillation temperatures with pressure held constant. The efficiency of removal improved greatly from 100° to 150° C., and the removal of about 95% or greater of each insecticide was achieved at 200° C. On the basis of this finding, all subsequent distillations were carried out at 200° C.

The per cent removal of insecticides for the various distillations is summarized in Table I. Similar percentage removals were obtained when the concentration of added pesticides was decreased approximately 50-fold. These data suggest that a significant reduction in the concentration of these residues can be attained for initial levels ordinarily found in severely contaminated samples of natural origin.

At 200° C., approximately 7% of the total lipid material was distilled to the cold finger. This fraction would consist largely of lower molecular weight glycerides. The compounds which contribute to the flavor of milk fat were also removed during the distillation, and the stripped fat had little odor after pesticide removal.

The quantitative recovery of lindane, heptachlor epoxide, and DDT was attempted following one distillation at 200° C. The distillate in the trap cooled with liquid nitrogen was taken up in hexane and analyzed separately as was the material collected at the cold finger in the still. The per cent of the total added pesticides recovered from these two fractions and from the stripped-fat fraction is presented in Table II. Over-all recoveries of 98.3% lindane, 93.6% heptachlor epoxide, and 92.8% DDT were achieved. The inability to re-

Table I. Per Cent Removal of Insecticides from Milk Fat Subjected to Molecular Distillation[a]

Insecticide	Treatment 1 200° C.	2 200° C.	3 200° C	4 200° C.	5 150° C.	6 100° C.
Lindane	94.7		96.0	95.3	71.4	24.4
Heptachlor epoxide	97.8		98.1	98.2	90.2	42.2
DDT	98.3		94.7	99+	90.6	49.3
Heptachlor		94.6				
Aldrin		94.8				
DDE		98.0				
DDD		99+				

[a] The concentrations in parts per million of insecticides employed in the various treatments were as follows: Treatments 1 and 2: lindane, 0.8; heptachlor epoxide, 1.6; DDT, 2.4; heptachlor, 0.8; aldrin, 0.8; DDE, 1. 6; DDD, 1.6. Treatments 3, 4, 5 and 6: lindane, 40.0; heptachlor epoxide, 80.0; DDT, 120.0.

cover 100% of the added insecticides following distillation might have resulted from incomplete trapping or, more probably, from a portion of the pesticides distilling to parts of the still such as rubber gaskets which could not be washed with solvent for recovery. At any rate, no evidence of degradation was noted since the GLC pattern for the fractions subjected to distillation revealed only symmetrical peaks with retention times in agreement with the original compounds.

Preliminary investigations of other laboratory-scale techniques for removing chlorinated insecticides from milk fat were not fruitful. Steam distillation at reduced pressure was of little value; approximately 19% lindane and 5% heptachlor epoxide were removed by such a treatment. Washing 100 ml. of milk fat with 25 liters of water at 80° C. over a period of 24 hours in a liquid-liquid extraction procedure was completely ineffective. Recrystallization of milk fat from ethanol or ethanol-hexane mixtures yielded a product with a decreased concentration of lindane, heptachlor epoxide, and DDT. The percentage removal by recrystallization from solvent varied from 53 to 77, 49 to 76, and 38 to 70, respectively. The difficulty of quantitatively recovering the fat from a solvent was extreme, and this approach was soon abandoned.

Aside from its possible application as a means of removing pesticide residues from edible fats and oils, the potential value of molecular distillation for concentrating low levels of chlorinated insecticide residues in fats and oils prior to analysis should be evident. As an analytical step, molecular distillation can make possible the collection of over 90% of such residues in a small volume of distillate. Thus, the possibility of detection of these residues at very low concentration levels by analysis of the distillate resulting from as much as several liters of fat or oil may provide another means of increasing the sensitivity of analysis. The results of such a study will be the subject of a subsequent report.

At present, the dairy industry is legally bound to a zero tolerance level of pesticide residues in its products. In practice, however, it appears impossible to exclude completely such residues with even the best management practices. The concept of zero tolerance is unrealistic, but minimizing the pesticide residue content of foods will continue to be a desirable goal until definite acceptable levels based on toxicological studies

Table II. Disposition of Insecticides Following Molecular Distillation at 200° C. and 5 × 10^{-4} Torr

Insecticide	Per Cent of Added Insecticide Recovered: Cold finger	N_2 cooled trap	Stripped milk fat	Total
Lindane	87.2	6.5	4.7	98.4
Heptachlor epoxide	87.8	4.0	1.8	93.6
DDT	92.8	0.0	0.0	92.8

are established. Minimal exposure of the plant or animal to pesticides represents one method of maintaining low levels of residues in foods. The development of pesticides persistent enough to accomplish the job of pest destruction but degradable enough to disappear before human consumption represents another approach. The removal of residues during the processing of foods represents a third approach. The latter approach may be physically impossible or economically unsound for many food items. Nevertheless, it is desirable to investigate all methods capable of reducing residue levels, since the acceptable level of many pesticide residues in foods is not yet known with certainty.

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