STUDIES ON THE SPECIFIC DISTRIBUTION OF DIELDRIN IN MILK FRACTIONS

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CATHARINA YUNG - KANG WANG ANG

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This is to certify that the

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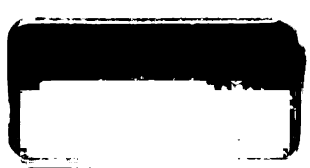

ABSTRACT

STUDIES ON THE SPECIFIC DISTRIBUTION
OF DIELDRIN IN MILK FRACTIONS

By

Catharina Yung-Kang Wang Ang

Most of the chlorinated hydrocarbon pesticides are lipophilic in nature, thus they are readily accumulated in fatty animal products. The present study was aimed to investigate the specific relationship between dieldrin and different lipid classes in the milk system. Milk was separated into various fractions by differential solvent extraction methods and by physical methods. The total lipid, phospholipid, and dieldrin contents in these fractions were determined.

Several extraction methods for pesticide residue analysis were compared. A method of the Food and Drug Administration, which provided good recoveries of pesticide as well as total lipids from milk was used in this research. The final determination of residue was carried out by electron capture gas chromatography.

Solvent extractions of milk containing added pesticide showed that the fat fraction extracted with non-polar

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hydrocarbon solvents, contained only about one-half of the total amount of dieldrin in milk. This incomplete recovery of pesticide with non-polar solvents might be due to the low recovery of solvents used for the extraction or due to the same factor that depressed the total recovery of solvents from milk. On the other hand, the concentrations of residue in this fraction was much higher than in the fraction which was extracted with ethanol and non-polar solvents after the initial extraction. Therefore, it was shown that dieldrin was more readily extracted with hydrocarbon solvents than were the milk lipids. However, all residue could not be extracted unless all lipid was extracted.

The distribution of dieldrin was quite similar in physically separated fractions of milk containing added pesticide and milk containing physiologically incorporated pesticide. This observation suggests that the results of experiments involving distribution of dieldrin in milk from either source might be the same. The relative amounts of dieldrin in various fractions closely resembles the relative amounts of total lipids in these fractions and both of them are significantly varied with the separation techniques used. Several independent milk collections and separations were made. Approximate quantities of dieldrin in these fractions were: unwashed cream, 85-87%; skim milk, 10-15%; refined skim milk, 1-3%; washed cream, 65-80%; butter, 52-78%; butteroil, 51-78%; buttermilk, 0.4-2.0%; and butter serum, 0.05-0.08%.

Comparable concentrations of dieldrin on a fat basis were found in whole milk, skim milk, cream, washed cream, butter and butteroil. Lower levels of residue were observed in buttermilk and butter serum, and lowest values were found in refined buttermilk, refined butter serum and fat globule membrane pellets. Refined skim milk contained higher levels of pesticide on a fat basis than other fractions. These data reflect that different factors may influence the distribution of pesticide in milk. The lower levels of residue in membrane materials (buttermilk, butter serum and membrane pellets) were probably related to the higher levels of phospholipid content or high-melting glyceride content, or both, in these fractions. The greater quantities of residue observed in refined skim milk containing extremely low amounts of lipids might be due to the slight solubility of this pesticide in the serum of milk.

It is not certain whether dieldrin is less preferentially deposited in the lipids of buttermilk and butter serum in the natural original milk system or such a finding is a consequence of re-orientation during separation processes. Nevertheless, dieldrin apparently has a tendency to be distributed with the neutral lipids or free fats in milk.

Analysis of control milk fractions did not lead to any firm conclusions since the residue levels in these samples were too low.

STUDIES ON THE SPECIFIC DISTRIBUTION OF DIELDRIN IN MILK FRACTIONS

By

Catharina Yung-Kang Wang Ang

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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INTRODUCTION

Pesticide chemicals have been widely used in the past two decades for various purposes and have brought about significant developments in food and agricultural production. However, the use of such chemicals has also generated residue problems in our environment. Numerous reports have indicated the occurrence of these compounds in a variety of foodstuffs and biological materials, and caused some unintentional effects in non-target living organisms.

Chlorinated hydrocarbon insecticides account for most of the residue problems. This group of chemicals is very persistent in normal conditions and very hydrophobic as compared to other kinds of pesticides, such as those in the organophosphate and carbamate groups. Consequently, they are more readily found, especially in fatty food products like egg, milk and meat.

Detectable amounts of chlorinated hydrocarbon residues in milk and dairy products have been reported frequently. The possible routes of contamination in milk are through the feed ingested by cows, the direct application of these chemicals to animals and their barns, or by accidental contamination during milking and handling.

Since milk is one of the major sources of human food, especially for infants and certain invalids, the presence of any persistent toxic chemicals in these products is of great concern.

High levels of residues in milk can be prevented effectively through the control of feed contamination, whereas small quantities of these persistent chemicals are almost unavoidable in milk and other food products. Another approach to reduce the pesticide levels is the application of food processing procedures to alter residue concentrations in the finished products. A number of routine dairy processing techniques and other physical treatments have been evaluated for this purpose.

The aim of the present research was to study the distribution properties of dieldrin, a commonly used chlorinated hydrocarbon insecticide, in milk, which was either from cows which had ingested dieldrin or had dieldrin added to it after milking. Chemical and physical separations of fluid milk into various fractions were made and their lipid and dieldrin contents were determined. This study may assist in perceptiveness of the characteristic deposition of such a toxicant in similar biological materials and a possible relation to the toxification mechanism. It may further provide a rational basis for research on the improvement of processing techniques to reduce the amounts of trace residues in milk and milk products.

REVIEW OF LITERATURE

Pesticide Residues in Milk and Other Foods

Shortly after the introduction of DDT for insect control, residues of DDT were found in milk from cows which had ingested DDT (Wilson et al., 1946; Carter, 1947). Since that time, many other chlorinated hydrocarbon insecticides, such as dieldrin, BHC, lindane, aldrin, endrin, chlordane, and haptachlor and some of their derivatives have also been found in market milk in various levels as reported by Marth and Ellickson (1959c), Clifford et al. (1959), Heineman and Miller (1961), and Duggan (1967).

A number of studies on the contamination of residues in food and total diet have been reported in the United States (Mills, 1963; Williams, 1964; Campbell et al., 1965; Duggan et al., 1966, 1967) and in other countries (Robinson and McGill, 1966; Abbott et al., 1969). Duggan and co-workers in the Food and Drug Administration (FDA) have conducted a series of surveys on the residues in food and feed items (Duggan, 1967, 1968; Duggan and Dawson, 1967; Lipscomb, 1968), in the total diet samples (Duggan et al., 1966, 1967; Martin and Duggan, 1968; Corneliussen, 1969), and the dietary intake of pesticide residues (Duggan and Weatherwax, 1967; Duggan and Lipscomb, 1969).

The FDA studies showed that about one-half of the food items and all of the diet samples examined contained pesticide residues. The major residual substances in foods are the chlorinated hydrocarbon insecticides. Animal products contained generally higher concentrations of residues as compared to the dietary fruits and crops. More than 50% of the total daily intake of chlorinated residues by the general population were from products of the dairy and meat food classes. Dairy products accounted for about 13.6% of the total daily intake.

Since milk is an essential food item for infants and many milk products are important for human diet, the tolerance levels of all chlorinated hydrocarbon chemicals in milk had been set at "zero" by 1961 (Moffitt, 1963). At that time, the lowest reliable determination level for DDT and DDT-like substances was 2.5 ppm on a fat basis by colorimetric methods. The analytical methods have been improved significantly in the last ten years, especially since the development of electron capture gas chromatographic techniques. It has become possible to detect much lower quantities of pesticide chemicals, and many milk samples have been found to contain residues at levels greater than "zero." Although the tolerance levels were maintained at "zero," a series of actionable levels for various pesticide residues were announced by the FDA during late 1963 to 1964; for example, this actionable level for combined DDT and its derivatives was 1.25 ppm on a fat basis or 0.05 ppm in whole

milk, and for aldrin and dieldrin was 0.25 ppm on a fat basis or 0.01 ppm in whole milk (Henderson, 1965). In 1967, the tolerance for DDT, DDE and DDD residues combined was established at the previous actionable level (Duggan, 1967; Laben, 1968).

A large number of samples of milk and dairy products from different geographical areas in the United States have been examined by the FDA. Duggan (1967) reported that 57% of the samples contained residues. Most frequently found chemicals were DDE, dieldrin, DDT, heptachlor epoxide, DDD, BHC, lindane, aldrin, heptachlor and methoxychlor. They accounted for 99.3% of the residues. The organophosphate compounds are rarely found in milk, since they are readily hydrolyzed by the rumen fluid of animals (Cook, 1957; Marth and Ellickson, 1959b). The average concentration of each kind of chlorinated residue in milk and dairy products was about one-tenth the tolerance or actionable level. Dieldrin content in dairy products was approximately 0.042 ppm on a fat basis.

Sources of Contamination and Excretion of Residues in Milk

The major source of pesticide residues in milk has been found to be the contaminated feed consumed by dairy cows. The feed may become contaminated in several ways, such as the direct application of chemicals to the forage crops, the drift of pesticides from adjoining fields, or the

crops may have been grown in contaminated soil (Henderson, 1965; Kiermeier, 1968).

Numerous reports have indicated the transfer of chlorinated insecticides from feed into milk of dairy cows. Wilson et al. (1946) detected DDT in milk from cows fed DDT-treated pea vine silage. Mann et al. (1950) further indicated that DDT was found in various products made from contaminated milk. App et al. (1956) observed the presence of dieldrin in the milk from cows fed on dieldrin treated pasture. Gannon and associates (1959a, 1959b, 1960) and Marth and Ellickson (1959b) conducted several experiments by feeding various chlorinated pesticides including dieldrin, DDT, methoxychlor and heptachlor to cows at different dosage levels for varied periods of time. These workers noted that the rate of appearance, the rate of accumulation from continuous feeding and the concentration of residues in milk varied among pesticide chemicals. They (Gannon and Decker, 1960) found that heptachlor in feed was converted by dairy cows to heptachlor epoxide in milk. The conversion of aldrin to dieldrin in dairy cows was reported by Ivey et al. (1961). The transfer of endrin, Kelthane and chlordane from feed to milk were demonstrated by Johnsen et al. (1961), Zweig et al. (1963) and Westlake et al. (1963), respectively.

Williams et al. (1964) found that DDT, DDD, and DDE were present in milk from cows fed rations containing DDT. The concentrations of DDT and DDD in milk were increased

according to the feeding levels of DDT, but DDE concentration apparently was not affected.

Dairy cows may become contaminated through the direct spray of chemicals to the animals or in their barns. It has been shown that some pesticide compounds can enter into cows through the skin.

Carter and Mann (1949) detected DDT in milk from a cow sprayed with DDT. Marth and Ellickson (1959a) indicated that highest levels of DDT in milk generally appeared shortly after spraying, in some instances DDT appeared in milk up to four months, whereas methoxychlor declined from milk quite rapidly. Radeleff et al. (1960) found dieldrin in milk following the spray treatment of cows with dieldrin. Claborn et al. (1960) further reported that DDT and dieldrin were found in higher levels than methoxychlor and toxaphene in milk two days after the cows had been sprayed with these chemicals.

Other studies involving the feeding to or the spraying of pesticides onto dairy cows have been reviewed by Marth (1962) and Henderson (1965). The chlorinated hydrocarbon pesticides were also found to be deposited in body fat of cows or other animals through a spray treatment or after oral ingestion (Gannon et al., 1959a; Radeleff et al., 1960; Claborn et al., 1960). A close relationship of the residues in the body fat and in the milk fat has been observed.

Comparatively more studies have been made for DDT than other residues in cows fed contaminated feed. The concentration of residue in milk fat as well as in body fat was proportional to the residue level in feed, and to the total intake of this compound by the cows. Laben et al. (1966) found that an equilibrium of DDT content in milk was achieved after eighteen to twenty-one weeks of continuous feeding to lactating cows, but there was no sufficient data showing an equilibrium between the total intake and the storage of DDT in body fat of cows (Laben, 1968).

Both the feeding and spraying studies demonstrated that once the dairy cows were exposed to chlorinated pesticides, the residues in the body tissue could be translocated and secreted into milk for a long period of time. When the source of contamination had been stopped, residues in milk as well as in beef tissue were reduced relatively rapidly in an initial stage and this was followed by a slower reduction period. Moubry et al. (1968a) indicated that the reduction rate of total DDT and its analogues in the milk fat was much more rapid as compared to the decline of these compounds in the body fat of cows.

Chlorinated hydrocarbon pesticides may also be found in milk when the cows have inhalated these chemicals in the barn after spray treatment (Heineman and Miller, 1961; Witt et al., 1966b). However, only traces of residues were attributed to this route of contamination.

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In recent years, the incidence of high levels of milk contamination have been reduced effectively through educational programs and legislative regulation (Laben, 1968). Today, it is rare to find contaminated milk as a result of the improper use of pesticides directly on cows, the use of treated seeds as feed or from accidental contamination during milking and handling (Fries, 1970). However, low levels of residues in milk are relatively difficult to avoid, due to the fact that lots of feed may have been unintentionally contaminated with pesticide chemicals (Laben, 1968; Fries, 1970).

Forage crops may become contaminated through the absorption of pesticide residues from the soil which has been previously treated with these compounds. Cole et al. (1966) found that more than 50% of the forage and grain samples contained DDT at levels from traces to 0.33 ppm, and those samples were from farms where no DDT had been used for at least three years. Some chlorinated hydrocarbon pesticides applied to soils may persist a number of years under normal conditions (Hardee et al., 1964).

The uptake and translocation of dieldrin and other pesticide residues by plants have been studied by Mumma et al. (1966), Wheeler et al. (1967), and Nash (1968). Their results suggested that the transfer of these substances from soil to plant could be the source of contamination in food crops and in the milk from cows which had not been fed with any treated feed.

The United States Department of Agriculture recently announced that DDT and other persistent chlorinated pesticides will be used only in preventing or controlling diseases or other "essential uses" by the end of 1970 (Anonymous, 1969). There should not be much residue problem in the future. However, because of the heavy use worldwide of these chemicals in the past, and the high stability of these compounds, residues have been and will be found in our environment for a long period of time. Feed and forage crops may still be found to contain these residues as a consequence of absorption from soil and water, and some traces of residues in milk apparently will not be totally prevented for many years.

Animal Decontamination

Some of the recent studies were focused on the possibility to decontaminate dairy cows after they had been exposed to pesticide chemicals. Once the contaminated feed is removed, pesticide residues in milk and body fat of cows will decrease gradually as described above. This natural decontamination process is usually slow (Moubry et al., 1968b).

Since most of the chlorinated hydrocarbons are deposited in fatty tissues of cows, it was thought that alteration of body fat mobilization or storage might be effective in accelerating the secretion of residues from body fat. Fries et al. (1969) found that the rates of elimination of

DDT and DDE from milk and dairy cows were not related to the loss of body weight. Only the concentration and total amount of DDD in milk were affected by the body energy loss. However, Miller (1967) reported that when dairy cows were on a low energy diet, the rate of decline for DDT in milk was increased.

Feeding thyroprotein did not significantly affect the concentration of DDT or dieldrin in milk fat from contaminated cows (Braund et al., 1966; Miller, 1967; Stull et al., 1968). Later, Braund et al. (1969) stated that varying dietary energy did not speed the rate of dieldrin reduction, but feeding thyroprotein decreased the time for dieldrin to reach 1.0 ppm in milk fat. They also showed that the concentration of dieldrin in whole milk was not affected when the milk fat was decreased by feeding pelleted hay to cows.

Another approach, which has been suggested, is the use of drugs to induce liver microsomal enzymes to accelerate the detoxification mechanism. Street et al. (1966) found that heptobarbital was effective in reducing the storage of dieldrin in rats. Oral administration of phenobarbital was also effective in reducing the concentration of DDD in milk of dairy cows, and increased DDD excretion in the urine of young bulls (Alary et al., 1968). Fries (1970) thought that only DDD retention was affected by hexobarbital in rats, and by either hexobarbital or pentobarbital in cows, whereas DDT and DDE were essentially unchanged.

A report by Wilson (1970) showed that phenobarbital fed to cows, either during the contamination period or in the postcontamination period, did significantly reduce the levels of dieldrin in both the body fat and milk fat of dairy cows, although the fate of possible metabolites was not investigated.

Recycling of dieldrin in ruminants was suggested by Cook et al. (1967). Wilson et al. (1968) and Wilson (1970) demonstrated that the administration of activated charcoal to sheep, goats or cows, which were either ingesting or had ingested dieldrin, would increase the excretion of dieldrin in feces. The efficiency of removal was greater when charcoal and dieldrin were fed at about the same time. Somewhat contrarily, Fries (1970) reported that activated charcoal by itself was not effective in removal of either stored DDT in rats, or the concentrations of dieldrin, DDT and DDD in the milk from previously contaminated cows.

Metabolic Transformation of Dieldrin

The literature offers abundant evidence of the conversion of DDT to DDE, DDD or DDA in various microorganisms, rats, ruminants, humans, and plants. It has also been shown that aldrin and heptachlor are readily metabolized to dieldrin and heptachlor epoxide in biological systems, respectively. Excellent studies on the metabolism of various pesticides have been reviewed by Hayes (1965), Lykken and

Casida (1969), and Brooks (1969). Only those reports regarding the biotransformation of dieldrin will be presented.

Ludwig and associates (1964) fed rats with labeled aldrin at a 0.2 ppm level in the daily diet, and found that after eight weeks, the total excretion of aldrin and dieldrin in the urine and feces was equivalent to the daily intake, and almost all of the aldrin was eliminated from the body twelve weeks after the feeding was stopped. However, Quaife et al. (1967) questioned the validity of the equilibrium since only one feeding level was tested, the rats used had grown rapidly, and the equilibrium was only maintained for a short time.

Datta and co-workers (1965) observed an unusual toxic and alkali-unstable metabolite in the urine of male rats fed dieldrin and aldrin in the diet. The chemical structure and properties of this metabolite have been evaluated by Damico et al. (1968) and Klein et al. (1968). They found that aldrin and dieldrin yielded almost the same metabolite, and which was two to three times more toxic to flies than were aldrin and dieldrin. Structural determinations showed that this metabolite was an oxidized, dechlorinated compound that no longer has an intact dieldrin system. The chemical name suggested for this compound is listed in the Appendix, referred to as dieldrin metabolite I. At about the same time, Richardson et al. (1968) also identified a metabolite from rat urine, and this metabolite showed similar properties and structural groups as dieldrin

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metabolite I, although Richardson et al. proposed a somewhat different chemical name for their compound (dieldrin metabolite I').

Richardson et al. (1968) also isolated a metabolite from rat feces and identified it as the 6-chloro-monohydroxy derivative of dieldrin. The chemical name suggested for this fecal metabolite is referred to as dieldrin metabolite II in the Appendix.

Korte and Arent (1965) found a different metabolite in the urine of rabbits after oral administration of C^{14} labeled dieldrin. They identified this compound as trans-6, 7-hydroxydihydroaldrin, i.e., a 6-chloro, trans-diol formed by hydrolytic cleavage of the epoxide ring. The full chemical name is given in the Appendix as dieldrin metabolite III. The toxicity of this compound to mammals was reported as 1/12 to 1/16 that of dieldrin.

Matthews and Matsumura (1969) reported that, from in vivo and in vitro studies, dieldrin was attacked by three enzyme systems in rat, and the most important degradation route being a glucuronic conjugation system upon one of the oxidation products of dieldrin in the liver. They found some other metabolites in addition to the three metabolites mentioned above.

In an experiment with C^{14}-dieldrin, Hedde et al. (1970) determined that about 80% of the radioactive dose was deposited in the carcass of sheep, 9-10% recovered from feces, and 5-9% in the urine over a period of three to four

days. The radioactivity in the urine was contained in six metabolites, four of which were hexane-extractable. Two of these four metabolites were further identified by Feil et al. (1970): one appeared to be the trans-6, 7-dihydroxydihydroaldrin (dieldrin metabolite III), and another one appeared to be identical to dieldrin metabolite II, though a new chemical name was given as dieldrin metabolite II'.

Effects of Chlorinated Pesticides in Biological Systems

The use of pesticide chemicals in agricultural and food production may affect the yield and the composition of some crops, or the flavor of some fruits and vegetables. The literature in this aspect has been reviewed by Marth (1965). A recent report showed that treatment with pesticides had no significant effects on the fatty acid, oil content, and protein content in soybean seeds (Johnson and Jullum, 1969). However, DDT apparently affected the photosynthesis of some marine phytoplankton (Wurster, 1968) and the nervous system of brook trout (Anderson and Peterson, 1969).

Significant reports have been published concerning the effects of pesticides, especially DDT and its derivatives, on birds and rats. Many studies pointed out that DDT and its metabolites had an effect on the thickness of egg shell and were responsible for the reduction of population of several species of wild birds (Bitman et al., 1969; Wurster and Wingate, 1968). It has been suggested that DDT

and dieldrin affect the deposition of calcium to eggshell, by inducing some hepatic microsomal enzymes which affect the metabolism of steroid hormones.

Some chlorinated pesticides were found to have an inhibitory effect on enzyme activities, such as that DDT inhibited the ATP-ase in rat brain (Matsumura et al., 1969), and chlordane, aldrin, dieldrin and DDT inhibited ATP-ase functions of rabbit brain (Koch, 1969). Casterline et al. (1969) showed that chlordane and some other pesticides had an effect on the esterase activities in serum and tissues of rats fed variable casein diets.

Long term administration of DDT to mice has caused liver lesions, hypertrophy and hyperplasia. When mice had been receiving small doses of DDT daily over five generations, the later generations showed more serious tumors and leukemia than the earlier generations (Tarjan and Kemény, 1969).

Phillips (1963) reported that DDT affected the utilization of vitamin A and carotene in rat liver. Dieldrin produced a similar effect in rats on a low protein diet (Lee et al., 1964). Tinsley (1969) found that rats receiving DDT with α-protein diet had depressed growth and vitamin A level in the liver. Rats may become more susceptible to the lethal effect of single toxic oral doses of DDT or other chlorinated pesticides on a deficient protein diet (Boyd and Stefec, 1969).

Tinsley (1966) reported that dieldrin accentuated an essential fatty acid stress and the transformation of linoleate to arachidonate in rat livers. On the other hand, when rats were raised on a diet deficient in essential fatty acids, a reduced growth rate of female rats, and an increased level of polyunsaturated fatty acid together with stearate in the liver lipids of rats were observed (Tinsley and Lowry, 1969).

Chlorinated Hydrocarbon Residues in Human Tissues in Relation to Public Health

Because of the wide incidence of some persistent pesticide chemicals in food, it is of interest to examine the residue levels in human tissues. There have been a number of publications providing the results of these surveys carried out in several countries and in various areas of the United States (Hoffman et al., 1964; Dale et al., 1965; Quinby et al., 1965; Brown, 1967; Abbott et al., 1968).

Generally, adipose tissue or body fat was examined. The most commonly found residues were DDT, DDE, dieldrin, lindane, heptachlor epoxide, DDD and BHC. The concentration of dieldrin in body fat is close to 0.2 ppm throughout the world, while DDT and its metabolites are found at levels from 1 ppm to 30 ppm. The general population in the United States carries approximate 9 to 12 ppm of total DDT and its derivatives (Brown, 1967; Abbott et al. 1968).

Casarett et al. (1968) and Radomski et al. (1968) further analyzed the distribution of residues in various human tissues. Adipose fat generally contained the highest levels of residues on a whole tissue basis, whereas bone marrow and liver followed by adipose tissue contained higher concentrations of residues on a fat basis. Brain lipids contained the lowest amounts of residues (Casarett et al., 1968).

Whether the trace amounts of pesticide residues consumed daily over long periods of time will have an adverse effect on human health has developed great interest, especially in recent years.

Early in 1956, Hayes and associates performed some experiments with human volunteers who were given daily doses of DDT at levels up to 200 times more than ingested by the general population. It was reported that the storage of DDT in adipose fat was proportional to the total intake, but an equilibrium was achieved in about one year. No illness was observed among the volunteers during the feeding period. Although from this study and other surveys on the general population in different years it appeared that an equilibrium between the intake and storage of DDT was possible, Quaife et al. (1967) questioned the validity of those experiments in the methods of sampling the general population and pointed out that the results of analyses by the spectrophotometric method were not comparable to those by gas-liquid chromatography.

A series of studies of dieldrin in relation to human health have been carried out by Hunter and his co-workers in England (Hunter et al., 1967; Hunter and Robinson, 1967, 1968; Hunter et al., 1969). They reported that the health of occupationally exposed workmen and the volunteers was not affected by body burdens of dieldrin 8 to 60 times greater than the general population. They also showed that there was a correlation between concentration of dieldrin in the adipose tissue and blood, while both were proportional to the daily dosage and total intake, and suggested that the concentration in either tissue might be predicted from the concentration found in the other. This correlation for the concentration of dieldrin in different kinds of samples from the same person was also demonstrated by Hayes and Curley (1968) by the analyses of plasma, fat and urine samples of workers exposed to aldrin, dieldrin and endrin.

The storage of dieldrin in human subjects during the surgical stress or in periods of complete fasting was examined by Hunter and Robinson (1968). They found that there was no increase in the concentration of dieldrin in the whole blood in the above cited conditions and since the concentrations in adipose tissue and blood were related, it was apparent that the total amount of dieldrin in adipose fat would not increase with starvation.

As additional information on studies of human volunteers ingesting dieldrin for two years, Hunter and associates (1969) pointed out that a steady state of dieldrin

concentration in the human body had been achieved. The average biological half-life was about 369 days. When the feeding period had ceased, the concentration of dieldrin in blood decreased gradually.

Although several studies pointed out that the present exposure level for the general population should not have any hazard to health, there is still a lack of investigations concerning the effect of pesticide residues on enzyme activities, hormone functions, and genetic systems in human subjects. It is known that chlorinated hydrocarbons do produce significant histopathological symptoms in liver of animals on a high dosage over a long period of time (Durham, 1963). From limited statistical analyses, Deichmann and Radomski (1968) showed that there was a correlation between the high residues of DDT in the human body and the frequency of human death from various disorders, including liver cancers.

Extraction and Determination of Chlorinated Hydrocarbon Pesticide Residues in Milk

When DDT was first determined in milk more than twenty years ago, a colorimetric method or a total organochlorine estimation method was applied (Wilson et al., 1946; Carter, 1947). Since 1960, the analytical methods have been improved dramatically. Some of the recent reviews of modern methods for pesticide analysis have been prepared by Lisk (1966), Williams and Cook (1967), and Thornburg and Beckman (1969). Official general procedures have been compiled in

"Pesticide Analytical Manual," by Food and Drug Administration (FDA, 1968).

The analysis of chlorinated hydrocarbon residues in milk usually consists of three steps: the extraction of these substances from samples, removal of co-extracted interferring materials, and the determination of residues in the purified extract (Moffitt, 1963; Henderson, 1965; Kroger, 1966; FDA, 1968). The first two steps may also be combined as a single step (Langlois et al., 1964a; Stemp and Liska, 1965; Crosby and Archer, 1966).

Since these chlorinated pesticides are very hydrophobic in nature, the extraction is commonly accomplished by employing hydrocarbon solvents. It is also because of the hydrophobic properties that they are readily deposited in the fatty portion of biological materials, such as the adipose tissue of animals and the butterfat of milk. In the case of milk, it is known that butterfat is enclosed within the fat globule membrane, which is composed of a lipoprotein complex, together with other constituents (King, 1955). In order to recover the residues quantitatively from milk, the globule membrane should be disrupted to release the butterfat. Various reagents and a mechanical churning process can be applied to break up the membrane of fat globules in milk. Reagents suitable for these purposes are: (a) strong acid, such as sulfuric acid used in Babcock fat determination, (b) ethyl alcohol, as used in the Mojonnier method to extract fat from milk, (c) surface active agents, such as Triton

X-100, and (d) alkaline solution, to hydrolyze the membrane constituents as well as the lipid material and subsequently to make the residues available for solvent extraction.

The first three techniques all release the hydrophobic pesticides with a relatively large portion of butterfat, which, as well as other edible fat, oil or butter samples, will be "cleaned up" in the second step. Two sub-steps may be involved to accomplish this purpose: solvent partition and column absorption procedures. Usually, the residues are isolated from the fat by partitioning of acetonitrile (Mills, 1961; modified by Furman and Fehringer, 1967), dimethyl formamide (deFaubert Maunder et al., 1964), or dimethyl sulfoxide (Haenni et al., 1962; Eidelman, 1962, 1963, 1967) with either hexane or petroleum ether. Chlorinated hydrocarbons are more soluble in acetonitrile (or dimethyl formamide, and dimethyl sulfoxide) than in petroleum ether (or hexane), whereas butterfat has a reverse solubility. Consequently, the acetonitrile layer contains the pesticide residues with only small amounts of fat materials. This extract is then cleaned up on an absorption column, such as Florisil (Mills, 1961; Wood, 1966), alumina (deFaubert Maunder et al., 1964), silica gel (Kadoum, 1968), carbon-celite (Moats, 1964) magnesium oxide (FDA, 1968), Florisil-celite (Shell Chemical Company, 1964), and silicic acid-celite (Samuel, 1966) using various eluting solvents or the combined solvents including petroleum ether, ethyl ether, hexane, methyl chloride, acetonitrile, and dimethyl

sulfoxide. Some pigments and traces of lipids will be absorbed on the column, while the chlorinated hydrocarbon insecticides are eluted in one or more fractions.

Other procedures are available to replace the solvent partition step: for example, saponification (O'Donnel et al., 1954; Crosby and Archer, 1966), cold-bath precipitation (McKinley and Savary, 1962; McCully and McKinley, 1964), sweep co-distillation (Storherr et al., 1967; Malone and Burke, 1969), molecular distillation (Bills and Sloan, 1967), deactivated Florisil column absorption (Giuffrida et al., 1966), and celite absorption techniques (Singh and Lanthier, 1968). The extracts from these procedures are also subjected to the additional cleanup step using an absorption column as mentioned above.

Procedures are also available to clean up butterfat or vegetable oil samples in one step, such as column chromatography on carbon-celite or partially deactivated Florisil columns (Moats, 1964; Moats and Kotula, 1966), and channel layer chromatography (Matherne and Bathalter, 1966; Hetherington and Parouchais, 1970).

Even simpler methods are reported to combine the extraction and cleanup in a single step (Langlois et al., 1964a; Stemp and Liska, 1965; Crosby and Archer, 1966). These techniques will be described later.

In all cases, the final step of analysis is the determination of residue content in the purified extract, qualitatively or quantitatively or both, by one or more of

the following methods: fly bioassay, total organic chloride estimation (Henderson, 1963), paper chromatography (Mills, 1959; Moffitt, et al., 1961), colorimetry for dieldrin (Cueto, 1960) or for DDT (Blinn and Gunther, 1963), thin-layer chromatography (TLC) (Kovacs, 1963; Abbott and Thomson, 1965; Moats, 1966), reversed phase paper chromatography (Wang and Chou, 1968), flame-spectrophotometry (Herrmann and Gutsche, 1969), and gas-liquid chromatography (GLC) with either electron capture detector (Burke and Giuffrida, 1964; Burke, 1965) or microcoulometric titrating system (Bosin, 1963; Burchfield and Wheeler, 1966; Giuffrida and Ives, 1969). Residues can be further identified by infrared (Blinn, 1965) or mass spectroscopy (Mumma and Kaniner, 1965). The most widely used methods probably are electron capture GLC, and TLC.

Excellent reviews or volumes for the analysis of pesticide residues by GLC and TLC have been offered by Gunther (1964), Westlake and Gunther (1967), Zweig (1967), Gudzinowicz (1967), Wise (1967), and McCully (1969).

The literature dealing with methodology and modifications of techniques is massive, only those methods that seem pertinent to the extraction procedure will be described briefly in this review. It should be kept in mind that each extraction step reported may be followed by different clean-up procedures. The final determinations are usually carried out by GLC. The accuracy or the recovery of the residues by

each method may be affected by the cleanup step or GLC performance in addition to the extraction technique.

Acid Hydrolysis

Langlois et al. (1963) used the Babcock method to extract butterfat from milk, and then analyzed the residue concentrations in this butterfat. Recoveries for added DDT were about 90%, but for dieldrin and lindane were only 70-75% and 63-67%, respectively. Endrin was lost in this process.

Stanley and LeFavoure (1965) digested animal tissue with a perchloric-acetic acid mixture to destroy the cellular structure and liberate the pesticide-containing fat, which was further cleaned up and analyzed. This method was reported to be suitable for DDT, DDD, lindane, and heptachlor, but not for heptachlor epoxide, aldrin, dieldrin and endrin.

Alkaline Hydrolysis

Schafer et al. (1963) described a procedure to determine DDT and DDE in milk by alcoholic KOH hydrolysis. Milk fat as well as the fat globule membrane was decomposed and the stable residues were extracted by hexane. In this process, DDT was converted to DDE. Crosby and Archer (1966) and Richardson et al. (1967) reported that this method could be applied to the detection of heptachlor, heptachlor epoxide, dieldrin and endrin as well as DDT+DDE. These workers

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used a rather mild hydrolysis; i.e., heating the mixture of 10 ml milk and 20 ml of 25% alcoholic KOH solution at 65-80°C for 15 min. Shell Chemical Company (1964) and Food and Drug Administration (FDA) (1968) recommended a procedure by which the alkaline hydrolysis was carried out on a steam bath and refluxed for 60 or 30 min under an air condenser for the alkaline stable residues, such as aldrin, dieldrin, and endrin (Shell Chemical Company, 1964), or for the oily materials which were not removed by Florisil and magnesia columns (FDA, 1968).

Surfactant Reaction

Lampert (1964) suggested the recovery of milk fat from whole milk by using a surface active agent-Triton X-100 (alkyl-phenoxy polyethoxyethanol) with the aid of urea, sodium tetraphosphate, isopropyl alcohol and water. The mixture of milk and reagents was heated in a water bath at 80-100°C for 10 min. He reported that added DDT, DDE, DDD, aldrin, dieldrin, heptachlor epoxide, lindane, and toxaphene were recovered at levels comparable to the ether extraction techniques.

Kroger and Patton (1967) reported that the results from two methods, one was referred to the modified Roese-Gottlieb method involving the use of ethanol and ethers; one was the simplified detergent method using Triton X-100, sodium tetraphosphate and water, were not significantly different from each other on a fat basis for the analysis of

dieldrin and heptachlor epoxide in naturally contaminated milk. However, Moubry et al. (1967) found that the simplified detergent method generally extracted 5-10% less of DDT, DDE, dieldrin and BHC than the extraction by the Mills method (similar to the modified Roese-Gottlieb method) on a whole milk basis.

For omental fat samples, Li et al. (1968) reported that Triton X-100 was essentially ineffective in aiding the extraction of various chlorinated residues by petroleum ether, while other surfactants, such as Span 80 and crude lecithin, did improve the extraction efficiency for dieldrin and heptachlor epoxide as compared to the extraction with only petroleum ether.

Extraction Involving the Use of Polar Solvent

Ethyl alcohol has been one of the most widely used reagents in the extraction of organochlorine pesticide residues from fatty foods. The official method prepared by the Food and Drug Administration (FDA, 1968; available since 1962) describes a procedure employing the use of alcohol and potassium oxalate to break up the fat globule membrane and to denature proteins. The released fat, containing pesticide residues, is then extracted by ethyl ether and petroleum ether. This technique is essentially adapted from the Mills method (Mills, 1961), which was a modified Mojonnier fat extraction method.

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Shell Chemical Company (1964) recommended the use of a mixture of hexane and ethyl ether for the extraction following the alcohol disruption process.

Other solvent systems for the extraction of chlorinated hydrocarbon pesticide residues from milk have been suggested by Giuffrida et al. (1966) and deFaubert Maunder et al. (1964) using acetone-petroleum ether and acetone-hexane, respectively. Recoveries of various pesticides were reported generally satisfactory.

Onley (1964) reported that pesticide residues could be extracted directly from the fluid milk instead of butterfat. According to his procedure, milk was blended with anhydrous sodium sulfate and a mixture of solvents consisting of acetonitrile, ethyl ether, dioxane and acetone. The extract was chilled in ice, filtered, and diluted with water in the presence of petroleum ether. The petroleum ether solution was further cleaned up on an activated Florisil column, where dioxane was included in the second eluting mixture.

The residues in animal tissues are usually extracted by blending the sample with anhydrous sodium sulfate and an organic solvent or mixture of solvents. Taylor et al. (1964) used acetone for this purpose. An acetone-acetonitrile system was used by Saschenbrecker and Ecobichon (1967). Li et al. (1968) found that chloroform-methanol extracted more lindane and DDT from animal fat than the extraction

with petroleum ether, but this was not true for heptachlor epoxide. The use of Span 80 with petroleum ether provided a better extraction for heptachlor epoxide, a slightly better extraction for DDT, and a similar recovery for lindane as compared to the extraction by chloroform-methanol.

Onley and Bertuzzi (1966) developed a method using a mixture of acetone, methyl cellosolve, and formamide to extract the pesticide residues, and to coagulate the fatty materials with calcium stearate. Wood (1969) reported that animal tissues, such as liver and eggs, could be mixed with celite and dimethyl sulphoxide, and then the mixture was packed into a column. Chlorinated residues were eluted from the column with dimethyl sulphoxide.

One Step Extraction and Cleanup

Langlois and associates (1964a) ground dairy products with Florisil to produce a free-flowing powder and which was added to the top of a partially deactivated Florisil column. Organochlorine pesticide residues were eluted with a solvent mixture of 20% methylene chloride in petroleum ether. Recoveries of added DDT, lindane, heptachlor, dieldrin, and endrin were 90-96%.

Stemp and Liska (1965) modified the above procedure and reported that milk was mixed with methylene chloride-petroleum ether solvent and Florisil, and the liquid was then decanted through a Florisil column. The slurrying technique was repeated several times and the residues were

obtained in the elutant from the column. They further reported that an aliquot of milk could be directly pipetted onto the top of a Florisil column, and pesticides were eluted with the solvent mixture as above. Good recoveries were observed for chlorinated hydrocarbon residues.

The hexane extract from the alkaline hydrolysis procedure was sometimes suitable for electron capture GLC analysis without cleanup (Crosby and Archer, 1966; Richardson et al., 1967), especially when the residue levels were sufficiently high. This procedure may be considered as an one-step method.

The principle of these rapid methods also involves the disruption of protein and fat globule membrane structures in milk by chemical or physical forces, or both forces, and the extraction of pesticide residues was performed at the same time.

Recently, Lawrence and Burke (1969) presented a comparison of various extraction and cleanup procedures used in ten methods for the analysis of chlorinated hydrocarbon residues in milk. All the final determinations were carried out by electron capture GLC with the same conditions.

In general, they found that Lampert's method (Lampert, 1964, modified by California State Department of Agriculture) showed the lowest recoveries for the added DDD and the naturally incorporated heptachlor epoxide in milk. Moubry et al. (1967) method was better, but was still lower in recoveries. The method of deFaubert Maunder et al.

(1964) also showed lower values for both residues. It was pointed out by Lawrence and Burke that the weakness of this method appeared to be associated with the dimethyl formamide-hexane partitioning technique rather than the extraction procedure. Highest values were obtained by Onley's method (1964). However, Lawrence and Burke questioned the accuracy of the empirical corrections which were used for five variables in Onley's method. Comparable average values, but with varied ranges, were obtained by other methods, which included the method of (a) FDA (1968, using either single extraction or triple extraction techniques), (b) Samuel (1966), (c) Sweep co-distillation (Storherr et al., 1967) following the single extraction of fat by FDA method, (d) Langlois et al. (1964a), (e) deFaubert Maunder et al. (1964), (f) Giuffrida et al. (1966).

Distribution of Residues in Milk

There is no difficulty in realizing that most of the chlorinated hydrocarbon chemicals are found in the lipid portion of milk, since these substances are highly soluble in fat.

Mann et al. (1950) reported that the concentration of DDT was much higher in the cream and butter than in the skim milk and whole milk, on a weight basis, providing all fractions were made from the same source of milk obtained from cows receiving DDT in their ration.

This pattern of distribution was also proved by Langlois et al. (1964b, 1965) for other kinds of chlorinated pesticides. They found that, following separation of pasteurized milk which had DDT and dieldrin added or pasteurized milk from cows fed lindane or endrin, residues were found almost entirely in the cream and butter fractions and the skim milk was essentially free of residues. The amount of residue in each fraction was generally proportional to the fat content in that fraction. Therefore, on a weight basis, butter contained the greatest quantity of residues, followed by cream, whole milk, buttermilk, and skim milk in decreasing order. However, if expressed on a fat basis, butter showed lesser residues of DDT, lindane and dieldrin, but not endrin, than cream and raw milk. Buttermilk contained higher levels of the first three residues, but a lower level of endrin than other fractions. The residue levels in skim milk were reported to be too low to be determined, either on a weight basis or on a fat basis.

Beroza and Bowman (1966) demonstrated that both organochlorine (such as aldrin and lindane) and organophosphate (ethion and imidan) pesticides were essentially recovered in the cream phase, and only a small percentage remained in the aqueous phase following centrifugation of raw whole milk which was spiked with the pesticides. They suggested that most of the organic pesticides were adsorbed onto the cream phase in milk, for both the non-polar compounds (aldrin, ethion, and lindane) and relatively polar

compounds (such as imidan). However, the non-polar compounds were poorly recovered from milk by extraction with hexane and ethyl ether, while the polar compounds were readily extracted by this solvent mixture.

Recently, Hugunin and Bradley (1969) reported that skim milk and buttermilk had higher concentrations of DDT and dieldrin per gram fat than the raw whole milk, but in two cases involving Kelthane, the residue level in buttermilk was lower than that in raw whole milk. A decreasing order of the concentration of dieldrin and Kelthane (one case) was observed from a high density buttermilk pellet, to a buttermilk serum phase, to the butteroil. They drew the conclusion that organochlorine pesticide was associated with phospholipid in the milk fat.

From the same laboratory, the results of Li et al. (1970) showed that skim milk contained higher concentrations of several chlorinated pesticides, but not of dieldrin, than other fractions which included whole milk, butter, cream, and buttermilk, all expressed on a fat basis. The residue levels of lindane, heptachlor epoxide, and in some cases of DDT and its derivatives were higher in buttermilk than those in raw whole milk. However, buttermilk contained the same concentration of chlordane, as well as in some cases of DDT and its analogs, and a lower value of dicofol (Kelthane) as compared to the content in raw whole milk on a fat basis. Moreover, the concentration of dieldrin was essentially not different among various fractions in all cases.

Processing Techniques and Residues in Milk

It has been the concern of many researchers as well as food processors whether the presence of organochlorine pesticide chemicals in milk will affect routine dairy processing, or that some processing techniques will effectively reduce the residue levels in milk and milk products.

The effect of residues on dairy processing has been studied by several workers regarding the effects on some microorganisms used in manufacturing of dairy products. Kim and Harmon (1968) and Kim (1969) reported that no effect of methoxychlor, dieldrin, heptachlor and malathion was observed on the growth and lactic fermentation ability of lactic starter cultures which have been usually used in producing sour cream, yoghurt, cultured buttermilk and cheese. However, Bradley and Li (1968) stated that dieldrin slightly reduced the acid production by a lactic acid producing organism in making Cheddar cheese. Li et al. (1970) again found that most of the pesticides studied, such as dieldrin, heptachlor, and chlordane showed some bacteriostatic or bactericidal action against starter microorganisms in the manufacturing of Cheddar cheese.

Comparatively more studies have been made concerning the effects of processing on the residues in milk. Mann et al. (1950) demonstrated that pasteurization had no significant effect on DDT levels in dairy products. Langlois et al. (1964b, 1965) and Liska (1968) reported that the concentrations of various chlorinated residues remained fairly

constant on a fat basis during the manufacture of butter, cheese, and ice cream throughout the processes of pasteurization, homogenization, churning, fermentation and storage. Processing techniques involving high temperature treatments caused some reduction of certain residues. The manufacture of dry whole milk powder either by spray drying or roller drying significantly decreased the concentrations of all residues except methoxychlor, while condensing affected the levels of dieldrin and heptachlor epoxide, and slightly affected telodrin, methoxychlor, chlordane, endosulfan and endosulfan sulfate, but not DDT, lindane and heptachlor. Sterilization was fairly effective, except for lindane, heptachlor, and total DDT-like substances. However, a change of DDT to DDE and DDD was observed in some instances.

Li et al. (1970) manufactured several products including pasteurized whole milk, 30% cream, butter, cheese, and spray-dried, condensed or sterilized condensed whole milk from the milk of cows fed insecticides. They concluded that, in general, most of the pesticides studied were very stable under various processing conditions. However, reduced concentrations of dieldrin, lindane, and chlordane were found in spray-dried products, of dicofol in sterilized condensed whole milk, and of DDE in stored products. Dieldrin and toxaphene showed a slightly higher concentration during storage.

Molecular distillation at 200°C and under a pressure of 5×10^{-4} torr (1 torr = 1 mm Hg) was found very efficient

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in removal of added lindane, heptachlor, heptachlor epoxide, aldrin, DDT, DDD and DDE in butteroil as reported by Bills and Sloan (1967).

Kiermeier et al. (1967) pointed out that freeze-drying of milk at a reduced pressure of 1×10^{-3} torr might have some reduction effect on the concentration of chlorinated hydrocarbon residues. However, Kroger (1968) found that freeze-drying under 0.1 mm Hg pressure and mild deodorization treatments were not effective in reducing the amount of dieldrin and heptachlor epoxide in butteroil. On the other hand, he observed that heating at very high temperature (up to 300°C) as well as steam deodorization at 180-195°C and 0.01-0.5 mm Hg significantly reduced the residue levels. The high temperature treated butteroils were found unacceptable for further food use, whereas the steam deodorized product showed no deleterious side effects, except for the loss of odor and color. Commercial steam distillation-vacuum processing was also fairly effective for reduction of lindane, little effective on dieldrin and DDT and not effective on heptachlor in milk (Ledford et al., 1968).

Ultraviolet light treatment of milk and butteroil had some effects on reduction of chlorinated residues, but also rendered the milk unacceptable in flavor at the same time (Li and Bradley, 1969).

The possible effect of manufacture and storage of Monterey and Cheddar cheese on the content of DDT, DDE and DDD was investigated by Montoure and Muldoon (1968). Their

results showed that DDT was not detected in samples of whey at dipping, but higher levels of DDE were found in this phase, and there was a definite change in the ratio of DDE: DDD. Distribution was similar for DDE, DDD and DDT in samples from all other phases, including raw milk, pasteurized milk, whey from pressing, and cheese after pressing.

Kim (1969) and Kim and Harmon (1970) reported that lactic cultures did not modify nor degrade the residues of DDT, lindane and aldrin in milk. However, Ledford and Chen (1969) found that some isolated cheese microorganisms degraded DDT and DDE to DDD in a media of tryptone and yeast extract, and the aerobic growth of geotrichum species caused almost complete disappearance of DDT and DDE. Some other isolated organisms showed no effect.

For comparison, it is worthwhile to mention that a significant amount of residues can be removed from fruits and vegetables by washing and peeling, although the following cooking or canning process has no further effect (Lamb et al., 1968; Elkins et al., 1968; Farrow et al., 1968). Chlorinated residues in crude vegetable oils are removed very efficiently by deodorization, a process usually included in commercial processing of vegetable oils for human consumption (Duggan, 1968; Smith et al., 1968).

EXPERIMENTAL

Materials

Chemicals

The major chemicals used in this research and the sources of these chemicals are listed as follows:

Dieldrin (99% HEOD, chemical name and structure are shown in the Appendix) was purchased from Applied Science Laboratories, Inc., Ann Arbor, Michigan. Ethyl alcohol was 200 proof and was from Commercial Solvents Corporation, Terre Haute, Indiana. Acetonitrile (CH_3CN), hexane (C_6H_6), petroleum ether, and ethyl ether (CH_3OCH_3) were all of nano-grade and were supplied by Mallinckrodt Chemical Works, St. Louis, Missouri. Acetone was spectrophotometric grade and was also from Mallinckrodt Chemical Works. Distilled water was used throughout the experiments unless otherwise specified. Florisil, 60-100 mesh, was a product of the Floridin Company, packaged by Fisher Scientific Company, Fair Lawn, New Jersey. Celite 545 was manufactured by Johns Manville, and packaged by Fisher Scientific Company.

All the minor chemicals used were either of reagent grade or were specified in the text.

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The milk used in this research was from three sources: (a) commercial pasteurized homogenized vitamin D milk from a local market, (b) fresh raw whole milk from Holstein cows, Michigan State University Dairy, which was fed with regular rations containing corn silage, alfalfa hay and grain, (c) naturally contaminated raw whole milk from Holstein cows fed dieldrin in their rations; also from Michigan State University Dairy. Each cow received an initial dose of 2 g dieldrin (in capsules) followed by a 60 mg dose daily. The feeding periods and the collection dates of milk samples are indicated in Table 1.

Methods

The methods employed in the present studies for the determination of dieldrin in milk as well as milk fractions consisted of three steps: (a) extraction of dieldrin from samples, (b) cleanup of the extract by removal of co-extracted fat and other substances, (c) determination of dieldrin by electron capture gas chromatography.

Extraction of Dieldrin from Milk

Commercial pasteurized homogenized milk was used for the studies on the comparison of extraction methods. Dieldrin was dissolved in acetone at appropriate concentration. An aliquot of this solution was added to the milk samples and the mixture was allowed to equilibrate for

Table 1.--Source of naturally contaminated milk: a record of dieldrin doses, feeding periods of dairy cows, and the collection dates of milk samples.[a]

Table 1.--Source of naturally contaminated milk: a record of dieldrin doses, feeding periods of dairy cows, and the collection dates of milk samples.[a]

Holstein Cow No.	Age (yrs)	Lactation Period Start	Doses of Dieldrin mg/cow/day[b]	Dieldrin Feeding Period		Milk Collection Date		
				Start	End	1	2[c]	3[c]
304	5	9/27/68	60	11/27/68	1/7/68	12/11/68 and 12/12/68	1/21/69	3/26/69 through 4/7/69
799	4	6/20/68	60	12/10/68	1/24/69	--	1/21/69	3/26/69 through 4/7/69

[a]Milk was obtained from the Michigan State University Dairy herd. The feeding management data and other information were provided by Kim Wilson, Department of Dairy.

[b]Dosage was based on 0.1 mg/kg body weight/day.

[c]The second and third sample collections consisted of pooled milk from both cows.

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45 min at room temperature with intermittent stirring. An equal volume of acetone was added to the control milk samples and these were analyzed in the same manner as the contaminated samples. Four methods were evaluated and described as below.

Food and Drug Administration Method (FDA, 1968; with Slight Modifications)

An aliquot of 100 ml of milk was transferred into a 500-ml separatory funnel. Approximately 1 g of sodium oxalate (Na oxalate) was added to the separatory funnel and the content was mixed well. One-hundred milliliters of ethyl alcohol (ethanol) were added and again the separatory funnel was shaken thoroughly for 2 min. Fifty ml of ether and 50 ml of petroleum ether were added successively to the separatory funnel which was shaken thoroughly for at least 1 1/2 min following each addition. The mixture was allowed to separate well and then the aqueous phase was drawn into a second separatory funnel and reextracted twice with 50-ml portions of a mixture of ethyl ether and petroleum ether (1:1,v/v). The final aqueous layer was discarded and the ether layers were combined in an 1,000-ml separatory funnel containing 600 ml of 2% sodium chloride (NaCl) solution. The content was mixed cautiously, and again the layers were allowed to separate completely before discarding the aqueous phase. The ether extract was rewashed twice with 100-ml portions of water which was

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subsequently discarded each time, and was then passed through a column of anhydrous sodium sulfate (Na_2SO_4) (2.5 cm) using a small amount of petroleum ether to rinse the separatory funnel and the column. All of the ether effluents were collected in a 400-ml beaker and evaporated on a steam bath under a stream of nitrogen. When a large portion of the solvent had been eliminated, the content of the beaker was quantitatively transferred into a tared 30-ml beaker in which the evaporation of solvent was continued until all of the solvents were driven off. The small beaker containing fat and pesticide residue was further dried in an oven at 50°C, and then weighed. A portion or all of this fat was used in cleanup procedures for residue analysis.

Mojonnier Method (Modified by Walstra and De Graaf, 1962)

This method was essentially the same as the FDA method; minor differences involved the use of a small quantity of concentrated ammonium hydroxide (NH_4OH) solution and larger proportions of reagents in the extraction. The original design involved the use of a Mojonnier bottle for the fat extraction from 10 ml of milk. In order to obtain an adequate amount of fat for residue analysis, a separatory funnel and an aliquot of 50 ml of milk were used in the present study. The quantities of other reagents were also increased proportionally. Outlines of these procedures are given as follows: 50 ml of milk were mixed with 0.75 g of NaCl and 6.25 ml of concentrated NH_4OH solution. Fifty

ml of ethanol, 125 ml of ethyl ether and 125 ml of petroleum ether were used for the first extraction, and 25 ml of ethanol, 75 ml of ethyl ether and 75 ml petroleum ether were used in the second extraction. The combined ether extract was evaporated on a steam bath and the resulting fat was weighed.

Surfactant Method

The method reported by Kroger and Patton (1967) was used in this study. The reagent was prepared by dissolving 70 g of sodium tetraphosphate and 30 g of Triton X-100 (octyl phenoxy polyethoxyethanol, Sigma Chemical Co., St. Louis, Missouri) in a total of 1,000 ml of water. To 130 ml of milk in a 250-ml volumetric flask, were added 80 ml of the above reagent. The flask was heated in boiling water bath for 15 min with intermittent swirling every 4-5 min. More reagent was added to bring the liberated fat into the neck of the flask, and the fat was pipetted into a centrifuge tube and spun for 1 min in a clinical centrifuge. The clear fat obtained was weighed and saved for further uses.

Alkaline Hydrolysis Method

Two procedures were used: one was the method reported by Richardson et al. (1967), referred to as the mild alkaline hydrolysis; one was the technique recommended by Shell Chemical Company (1964), referred to as strong alkaline hydrolysis in this study.

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Mild alkaline hydrolysis.--Alcoholic alkaline solution was prepared by dissolving 25 g of potassium hydroxide (KOH) in 15 ml of water and the volume was brought to 100 ml with ethanol. Twenty ml of this solution were added to an aliquot of 10 ml of milk in a flask, which was then heated in a water bath at 65°C for 15 min. The content of this flask was quantitatively transferred into a separatory funnel using a total volume of 20 ml of water to rinse the flask. Ten ml of hexane were added to the separatory funnel which was thoroughly shaken for 4 min. The aqueous layer was discarded after separation and the solvent extract was evaporated to appropriate volume on a steam bath under nitrogen.

Strong alkaline hydrolysis.--Fifty ml of milk were hydrolyzed with 12.5 g of KOH pellets and 25 ml of ethanol by heating on a steam bath and refluxing under an air condenser for one hour. The mixture was then transferred into a separatory funnel using 25 ml water, and the pesticide residues in the aqueous hydrolyzate solution were extracted three times with 40 ml of hexane each time. The extracts were combined and reduced to a suitable volume as described earlier.

Both the mild and strong alkaline hydrolysis procedures were also used in the determination of residues in lipid-depleted milk and skim milk fractions.

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Separation of Milk Fractions by Differential Solvent Extraction Methods

Pasteurized homogenized milk containing added dieldrin was used in this study. The first fraction was extracted by non-polar organic solvents including ethyl ether and this fraction was referred to as the ether-extractable fraction of fluid milk. The residual portion was then extracted with ethanol and non-polar organic solvents and the resulting fraction was designated as the second fraction or the alcoholic-ether-extractable fraction.

Three methods employing different non-polar organic solvent systems were compared and described as follows:

(a) One hundred ml of milk were mixed well with 100 ml portion of a mixture of hexane and ethyl ether (1:1, v/v) in a suitable separatory funnel. Then the mixture was transferred into a centrifuge bottle and centrifuged at 2,000 x G for 15 min. The solvent layer was removed with a pipette and the remaining content was transferred into the original separatory funnel followed by extraction with the same solvent mixture for two more times. The solvent extracts were then combined, measured, concentrated and the resulting lipid was weighed. The residual portion was mixed with 1 g of Na oxalate and 100 ml of ethanol and shaken well prior to extraction with the same solvent mixture by the same technique as described above. The combined extract was further washed with 100 ml of 5% NaCl solution, and passed through a column of anhydrous Na_2SO_4, evaporated

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and the fat weighed. This solvent system was used by Beroza and Bowman (1966).

(b) Petroleum ether was used for the extraction of the first fraction. The basic technique was essentially the same as in method (a), except that 100 ml, 50 ml and 50 ml of petroleum ether were used successively for the extraction; 1.5 g of Na oxalate was added before the second extraction, and that all the extracts were washed with 600 ml of 2% NaCl solution. The amount of each reagent used was mainly according to the FDA technique (FDA, 1968), which was used in the next method.

(c) The first fraction was extracted by the use of ethyl ether-petroleum ether, and the second fraction was extracted by using Na oxalate, ethanol, ethyl ether and petroleum ether. The techniques involved have been described earlier as the FDA method. A diagram showing the fractionations is given in Figure 1.

Cleanup Procedures for the Residue Extract

All of the extracts containing lipids were cleaned up by acetonitrile-petroleum ether partition and Florisil-celite absorption column techniques prior to the final analysis by electron capture GLC. The hexane extract following the alkaline hydrolysis was cleaned up directly on a Florisil-celite column.

Figure 1

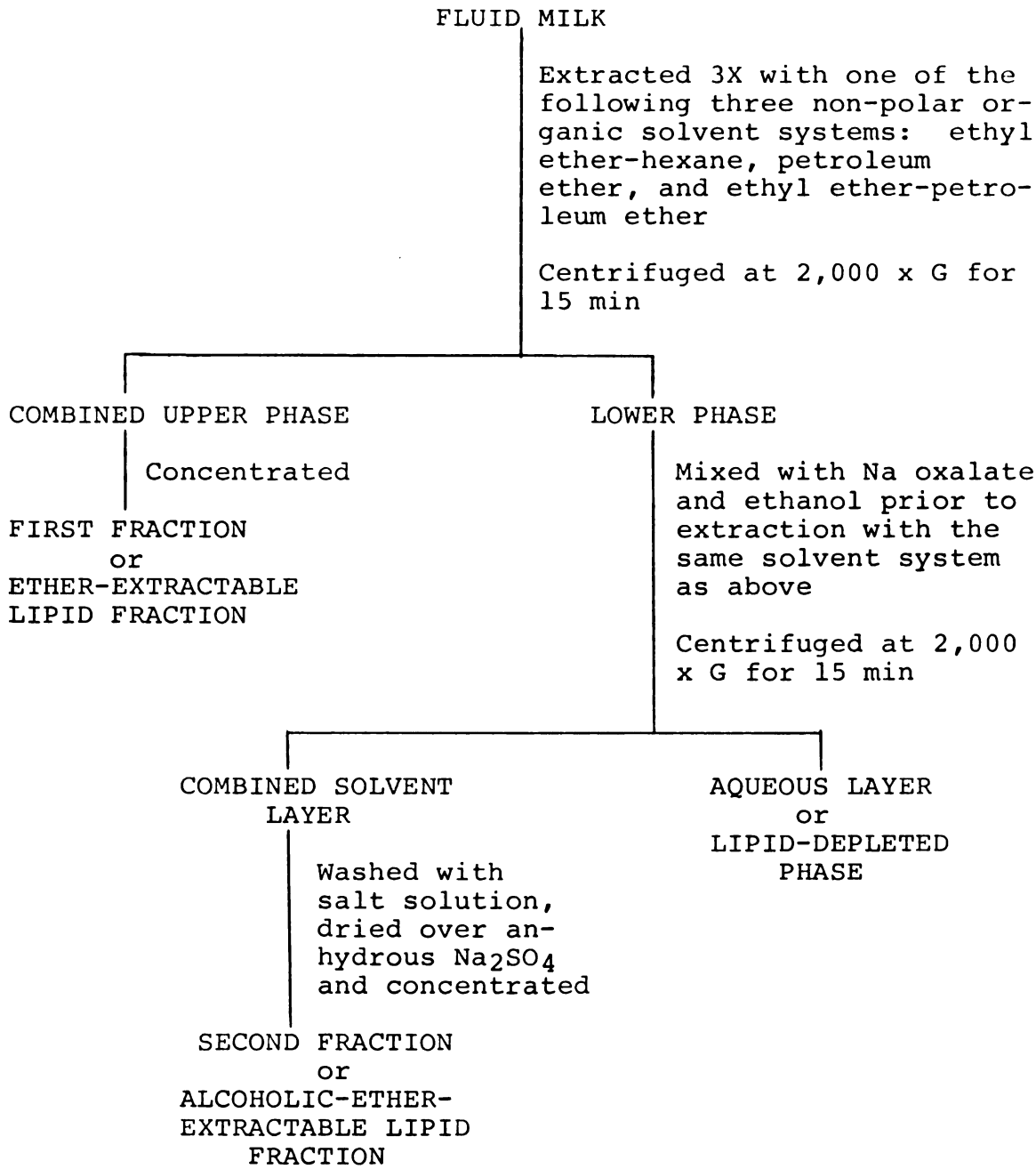

Figure 1.--Scheme for the separation of milk fractions by differential solvent extraction.

Acetonitrile Partition

This technique was reported by the FDA (1968). Up to 3 g of extracted fat were dissolved in petroleum ether and transferred into a 125-ml separatory funnel using a total volume of 15 ml of petroleum ether. Thirty ml of acetonitrile saturated with petroleum ether were added to the separatory funnel, and the contents thoroughly mixed for one min. When the layers were well separated, the lower phase (acetonitrile) was transferred into a 1,000-ml separatory funnel containing 650 ml of 2% NaCl solution and 100 ml of petroleum ether. The acetonitrile partition step was repeated three more times and all of lower phase was transferred into the same 1,000-ml separatory funnel, while the final petroleum ether layer with relatively large portions of lipids was discarded. The large funnel was then gently mixed for diluting the acetonitrile with aqueous salt solution and allowing the lipophilic pesticide residue to be redistributed into the petroleum ether layer. The aqueous acetonitrile portion was drawn into another 1,000-ml separatory funnel and was extracted again with 100 ml of petroleum ether by shaking the funnel vigorously for 15 sec. The aqueous phase was discarded. The petroleum ether extracts were combined in the original separatory funnel, washed twice with 100 ml of 2% NaCl solution, passed through an anhydrous Na_2SO_4 column (2.5 cm) and concentrated on a steam bath to ca. 20 ml.

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Florisil-Celite Column Cleanup
(Shell Chemical Co., 1964)

The Florisil-celite absorbent mixture (5:1, w/w) was deactivated with 10% water by mixing the content on a wrist action shaker for 16 hours. A chromatographic column of 20 mm x 400 mm was packed with a layer of glass wool, 2.5 cm of anhydrous Na_2SO_4, 10 g (4.2 cm) of the Florisil-celite mixture, and again a 2.5 cm layer of anhydrous Na_2SO_4 and a thin layer of glass wool. This column was prewashed with about 50 ml of hexane. The concentrated residue extract, either in hexane or in petroleum ether, was transferred onto the column, and eluted into a 400-ml beaker at a flow rate of 2-3 ml per min. Hexane was used to elute pesticide residues from the column until a total of 300 ml of effluent was received, which was then evaporated to an appropriate concentration on a steam bath under a stream of nitrogen gas. Usually the final solutions containing 0.02 to 0.2 ppm of dieldrin were suitable for GLC analysis.

Determination of Dieldrin by Electron Capture Gas Chromatography

A F & M Hewlett-Packard Model 5750B research gas chromatograph (Hewlett-Parkard Analytical Instruments, Palo Alto, California) equipped with a tritium electron capture detector was used in the later part of this research. The operating conditions were: 4 ft x 1/4 in o. d. coiled glass column prepacked with 3.8% SE-30 on 80/100 mesh Diatoport S; injection port temperature 240°C; column oven

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200°C; detector 210°C; chart speed 0.25 or 0.5 in./min.; flow rate of carrier gas, helium, 45-50 ml/min; and purge gas of 90% argon-10% methane mixture, 50 ml/min. The sample size was 1-4 µl, usually 2 µl.

Standard solutions of dieldrin ranging from 0.005 to 0.5 ppm were prepared in hexane. Usually no less than four standards (different concentrations) were injected into the GLC before and after the sample injections on each operation day. A standard curve of dieldrin concentration versus peak height on the chromatogram was made, and the quantity of residue in the final sample extract was determined from this standard curve. Gaul (1966) has reported that the method of using peak height for calculating gas chromatographic peaks showed no significant difference from other methods including disc integration, triangulation, peak height x width at half height, and R_t x peak height in the calculated results for dieldrin. A new standard curve was constructed on each operation day because the slope of the standard curve varied from day to day. A typical GLC chromatogram for determination of dieldrin in milk is given in the Appendix.

In the early stage of this research, a Beckman GC-4 gas chromatograph was used. It had an electronic wire as the source of electrons in the detector. A variety of columns have been used on this GC, including: glass or stainless steel column, 4 or 6 ft long, packed with QF-1 and OV-17 or DC 11, or DC 200 on Gas Chromo Q 60-80

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mesh or on Chromosorb W 60/80 mesh. The column oven temperature was 150°C to 220°C varied with the column used. It would be very tedious and superfluous in presenting the specific conditions used in each individual determination, since the standard solutions were always injected between samples during the period with the same operation conditions.

Separation of Milk Fractions by Physical Methods

Three kinds of milk samples were used: (a) naturally contaminated milk from cows fed dieldrin, (b) raw whole milk containing added dieldrin, and (c) control raw whole milk. All milk was from the Michigan State University dairy herd. Milk was collected in stainless steel milk cans at the afternoon milking and brought back to the laboratory immediately for separation. For the preparation of milk containing added dieldrin, an appropriate quantity of dieldrin solution (1 mg/ml in acetone) was added to the milk and the mixture was allowed to equilibrate by intermittent stirring in a water bath at 37°C for 45 min.

Preparation of Major Milk Fractions

The method employed was essentially that of Brunner (1965) and Swope (1968). Milk was first separated into skim milk and cream with a DeLaval disc-type separator (Model 9). The resulting cream was diluted with three volumes of warm

(39-40°C) 0.25 M sucrose solution (for the naturally contaminated milk sample 1 and 2) or deionized distilled water (39-40°C) (for other samples) and reseparated as before. This process was repeated three times. A suitable amount of the original skim milk was saved for later analysis while the remainder and the washings were discarded. Washed cream was stored in a large Erlenmeyer flask (3,000 or 4,000 ml) and cooled overnight at 4°C. On the following morning, it was allowed to warm up to 12°C and then churned on an Eberbach rotatory shaker at room temperature for about one hour or until the separation of butterfat and liquid phase was well observed. The buttermilk (liquid phase) was decanted into another flask for further treatment and the butter was melted in the original flask or other suitable container at 45°C. Butteroil and butter serum fractions were thus obtained. A scheme of the separation procedure is presented in Figure 2.

During the entire separation process, each yield of the resulting fractions was weighed, except for skim milk which was estimated by the difference between whole milk and cream. A quantitative portion of each fraction was taken for lipid and dieldrin analysis before that fraction was further separated. The storage conditions and further treatments of various fractions were as follows:

Whole milk, skim milk, cream, and washed cream were stored in Nalgene bottles and kept frozen at -20°C until needed. Butter and butteroil were stored in glass bottles

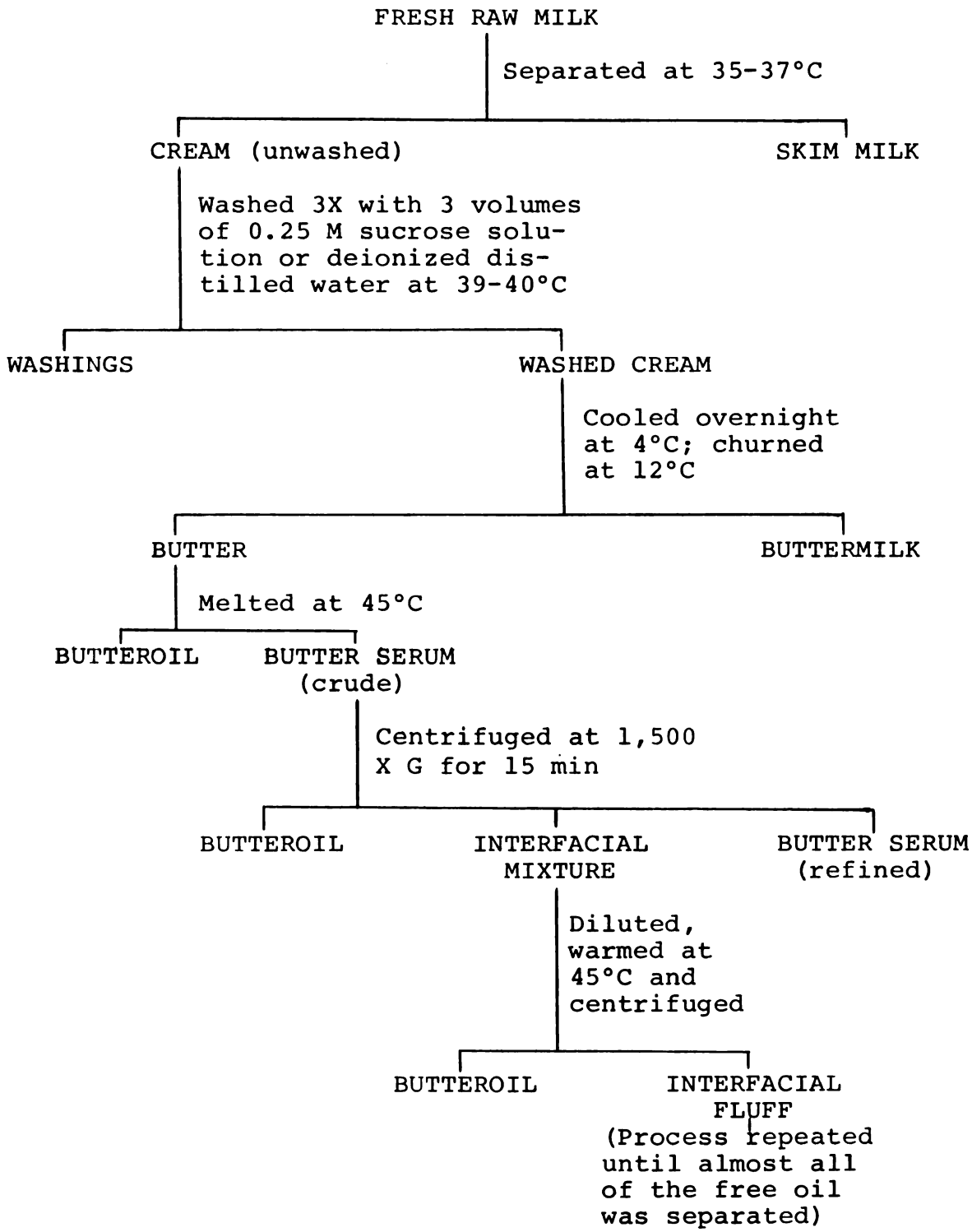

Figure 2.--Scheme for the separation of major milk fractions by physical methods (adapted from Brunner, 1965).

with 0.01% BHT (butylated hydroxy toluene) added and were kept frozen until needed.

Butter serum for the first two preparations of naturally contaminated milk fractions, the crude butter serum containing fairly large amounts of butteroil was collected and analyzed; for other preparations, this butteroil was further separated from butter serum by centrifugation at 1,500 X G for 15 min. However, there was still no clear-cut differentiation between the oil and the serum phases. After removal of oil (as much as possible), the pure butter serum phase in the lower portion of the layers was pipetted out. The middle layer which consisted of butteroil, interfacial fluff and some butter serum was thus left in the centrifuge bottle. This mixture was diluted with an equal volume of water, stirred, warmed at 45°C and centrifuged as described previously. This washing and reseparation process (Figure 2) was adapted from Chien and Richardson (1967). The pure oil as well as the pure aqueous phase was removed and the process was repeated until almost all of the butteroil phase was separated from the interfacial fluff. The butteroil collected in these later steps were all combined to the original butteroil fraction and stored. Only the original pure butter serum, designated as refined butter serum, was saved and stored in Nalgene bottles at -20°C.

Buttermilk was preserved by the addition of 0.01% BHT as well as penicillin G (46 mg/1,000 ml) and streptomycin sulfate (75 mg/1,000 ml) as suggested by Quirk (1965).

Preliminary tests showed no interference peak on the GLC chromatogram as a result of the use of these chemicals. A small portion of this buttermilk was stored in Nalgene bottle at -20°C for later analysis while the remainder was stored at 4°C until needed for fat globule membrane pellet collections.

Preparation of Fat Globule Membrane Pellet Fractions

The procedures involved were primarily those of Swope (1968) with slight modifications as shown in Figure 3. Buttermilk was filtered through four layers of cheesecloth and centrifuged at 2,000 X G for 20 min to eliminate the unchurned fat globules. The liquid was designated as refined buttermilk, and this was subsequently centrifuged at 12,500 X G for 14 min at 4°C to yield a 7,500 S membrane pellet. The resulting cream-like flotation layer was carefully removed from the centrifuge tube with a spatula and a small piece of Kimwipe and discarded. The supernatant was pipetted into a second centrifuge tube (care was taken to avoid disturbance of this very loosely-packed 7,500 S pellet) until approximately 1 cm depth of the liquid including the pellet was left in the bottom of the centrifuge tube (50 ml capacity). Deionized distilled water was added to this tube, the contents were stirred, and recentrifuged at the same speed. This process was repeated once more to remove the fine particles which might be trapped in the pellet, and the washings were discarded. The original supernatant was

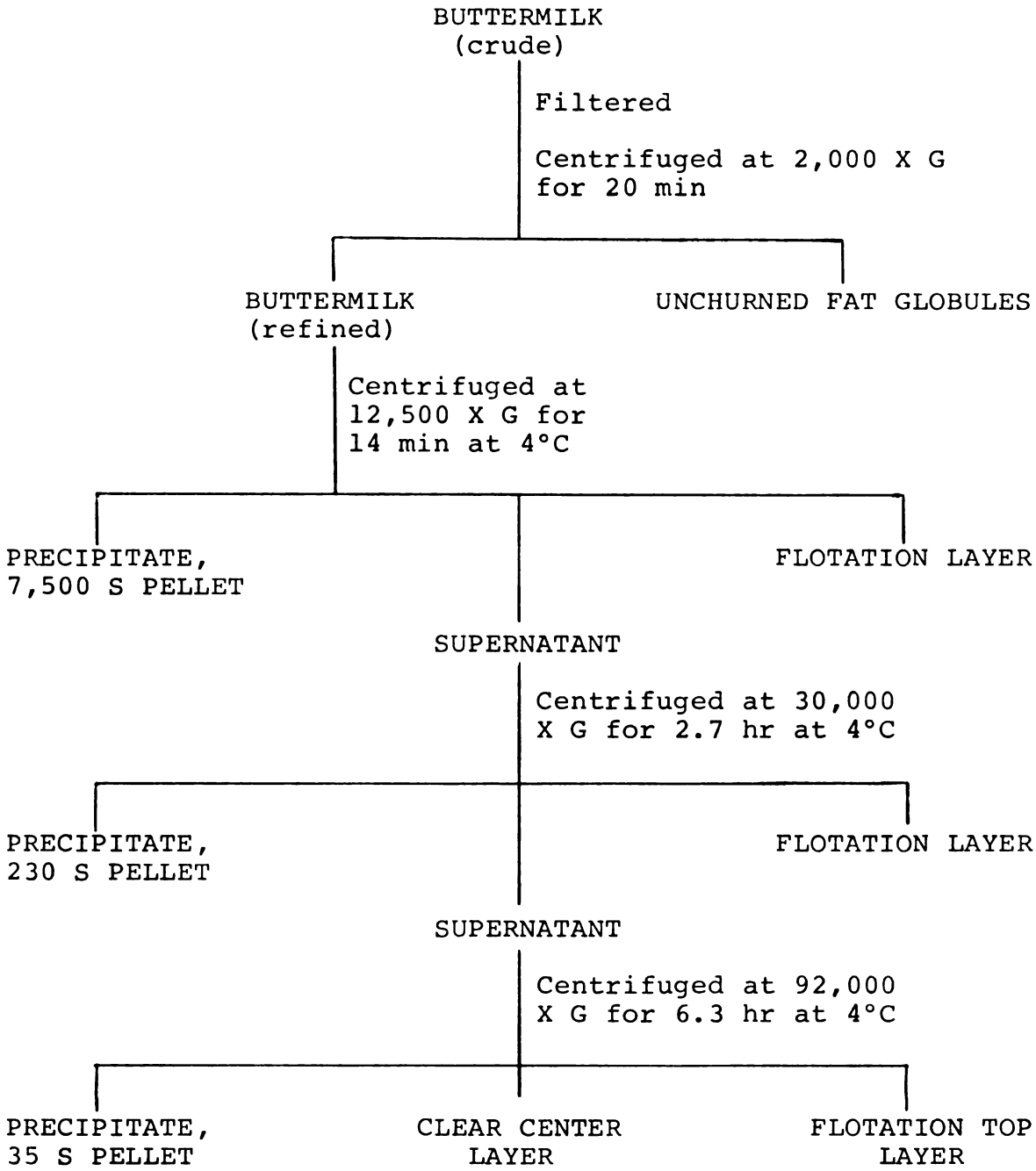

Figure 3.--Scheme for the separation of fat globule membrane fractions (adapted from Swope, 1968).

further centrifuged at 30,000 X G for 2.7 hr to yield a 230 S pellet. Again the flotation layer was removed, and the pellet was washed twice by the same technique as described for the 7,500 S fraction.

An International Model HR-1 high-speed refrigerated centrifuge (International Equipment Company, Needham Heights, Massachusetts) was employed for the collection of these two membrane fractions. The No. 856 rotor used in this centrifuge had dimensions as follows: r_a (radius from the center of rotation to air-liquid meniscus) = 5.5 cm; r_b (radius from the center of rotation to bottom of liquid) = 10.6 cm. The Svedberg units (S) of the resulting pellets were calculated according to the following equation:

$$S = \frac{10^{13} \ln (r_b/r_a)}{W^2 T}$$

where T = separation time in seconds, and

$$W = \text{angular velocity in rad/sec} = \frac{2 \pi (\text{rpm})}{60}.$$

Therefore, the operating speed and time for collection of 7,500 S and 230 S pellets were computed as 10,000 rpm for 13.3 min (ca. 14 min) and 16,500 rpm for 2.7 hr, respectively. A Beckman Model L-2 preparative ultracentrifuge (Beckman Instruments Inc., Spinco Division, Lincolnwood, Illinois) was used for collection of the 35 S pellet. The r_a and r_b values for the type-30 rotor used in this centrifuge were 5.0 and 10.5 cm, respectively. The operating

speed and time for 35 S pellet were 28,000 rpm (92,000 X G) and 6.3 hr, respectively. No washing process was involved for this final precipitate. For the control milk sample, the 35 S pellet was collected directly from the refined buttermilk. The flotation top layer and the final supernatant of this sample and of the milk samples containing added dieldrin were collected for further analysis, whereas the supernatant and top layer of other samples were discarded. Each pellet was removed from the centrifuge tube with a spatula and a minimal amount of water into a suitable container and stored at -20°C until used.

Analysis of the Physically Separated Milk Fractions

Extraction of Lipids and Dieldrin

The major fractions were thawed and extracted according to the procedures described on page 41 (FDA method). Modifications for different fractions were as follows: skim milk, 150-300 ml, reagents used were proportionally increased; cream, diluted with approximately six volumes of water prior to the extraction step; butter, 5 g were dissolved in 25 ml ether mixture (ethyl ether: petroleum ether, 1:1) and 50 ml of 50% ethanol were used in extraction; butteroil, 5 g were dissolved in ethers, washed with salt solution and water, dried and weighed, no further extraction was involved. For buttermilk and butter serum, since poor separations between ether layer and aqueous layer were

observed, a technique was then adopted, which used 3.5 volumes of ethanol, one volume of ethyl ether and 2.5 volumes of petroleum ether in the first extraction and one volume of petroleum ether in each of the second and third extraction. The residual phase was diluted with 2.5 volumes of water (of the sample original volume), and was again extracted with ethyl ether and petroleum ether as the original FDA procedure.

The membrane materials were dried under a hood on a warm steam bath (for the first preparation of naturally contaminated milk) or freeze-dried in a Stokes freeze-dryer at 100 μ Hg pressure for 24 hr with the heating plate temperature at 13-16°C, finally dried in an oven at 50°C to remove the traces of moisture which might be in the freeze-dried samples. The dried solids were weighed and used for the lipid and dieldrin determinations.

The extraction procedures for free lipids and bound lipids were adapted from Cerbulis (1967). Free lipids of the membrane material were extracted with petroleum ether (80 ml per gram of solids) by continuous stirring for 3 hr and the extract was removed by centrifugation. This process was repeated three times, and the extracts were combined, evaporated to dryness on a steam bath with a stream of nitrogen and the resulting lipids weighed. Bound lipids were next extracted three times from these samples with chloroform-methanol (2:1, v/v) by continuous stirring for 3 hr using 30 ml of solvent per gram of dry solids. The extracts were combined, washed twice with 0.2 volume of 0.2%

NaCl solution, dried and the resulting lipids weighed. Since chloroform caused some interference on the GLC chromatograms, later extractions of bound lipids used ethanol, ethyl ether, and petroleum ether following the procedure as described earlier for buttermilk. The free-lipid-extracted membrane materials were rehydrated with ten volumes of water prior to the extraction for bound lipids. A preliminary test showed that this revised lipid extraction technique provided a comparable result as the extraction by a solvent mixture of chloroform-methanol (2:1, v/v) reported by Folch et al. (1957).

Determination of Dieldrin

Dieldrin contents of the extracted lipid fractions were determined by electron capture GLC using the same cleanup techniques and GLC conditions as described previously.

Phosphorus Determination

Lipid phosphorus was determined essentially according to the procedure of Morrison (1964). The extracted lipids were redissolved in a mixture of chloroform and methanol (2:1, v/v) to a concentration of approximately 30 mg lipids per ml of solution. Aliquots of the solution containing 2-50 µg phosphorus were placed in 40-ml test tubes accurately calibrated at the 12.5 ml and 25 ml level. Solvent was then removed with a stream of nitrogen on a steam bath. One and one-tenth ml of sulfuric acid (95%, sp. gr. 1.84) were

added to each tube and this was then heated in a multi-block tube heater (Research Specialty) at 220-230°C until charring was completed; usually for 12-15 min. Hydrogen peroxide (30% H_2O_2, w/v) was added dropwisely and the tube was heated for 1 min after each addition. This process was repeated until a clear solution was obtained. The tube was further boiled for 3 min to drive off the excess H_2O_2. After the tube was cooled completely, deionized distilled water was added to bring the volume to 12.5 ml. Five-tenths ml of sodium sulfite (16% solution of Na_2SO_3 anhydrous), 5 ml of ammonium paramolybdate (2% solution of $(NH_4)_6Mo_7O_{24} \cdot 4 H_2O$) and 0.5 ml of ascorbic acid (10%) were added successively. Each tube was well shaken and then heated in boiling water for 1 min. After cooling, deionized water was added to bring the volume to 25 ml. The absorbance of the solution was read at 822 nm with a Beckman DU-2 spectrophotometer. Duplicate blanks and standard solutions were carried out at the same time with samples. The phosphorus concentrations in sample solutions were read on the standard curves which were prepared individually for each set of digestion and determination. Phospholipid contents were estimated by multiplying the values of phosphorus by 25 (Swope, 1968). The averages of duplicate determinations are reported.

RESULTS AND DISCUSSION

Comparison of the Extraction Efficiency of Lipid and Dieldrin in Milk

Four methods were compared. The results are shown in Table 2. The lipid extraction efficiency by the FDA method was comparable to the Mojonnier method. This was no surprise since the FDA method was essentially a modification of the Mojonnier method. The ether extracts obtained in the FDA method were further washed with a 2% NaCl solution and water to remove some impurities which might affect the baseline on GLC chromatograms for residue analysis. This washing process might also account for the slightly lower lipid recovery by the FDA method. However, this quantity was not significant. The total recoveries of added dieldrin by these two methods showed no significant difference, although the method of Mojonnier seemed to give a slightly higher value. Usually, as described in the "Pesticide Analytical Manual" (FDA, 1968), a portion of the extracted fat was weighed and analyzed for the residue content, and subsequently the results were expressed on a fat basis. For the above two methods, the concentrations of dieldrin in the extracted lipids were also comparable with each other.

Table 2.--Comparison of extraction efficiency by different methods of lipid and dieldrin in milk.[a]

Method[b]	% Lipid Extracted	Dieldrin Concentration, ppm		% Recovery of Dieldrin
		Extd. Fat	Whole Milk	
FDA	3.54 ± 0.03	1.30 ± 0.04	0.0459 ± 0.0014	91.87 ± 2.73
Mojonnier	3.59 ± 0.02	1.29 ± 0.04	0.0463 ± 0.0013	92.60 ± 2.50
Surfactant	3.23 ± 0.02	1.27 ± 0.06	0.0410 ± 0.0032	82.00 ± 4.00
Alkaline hydrolysis (mild)	--[c]	--	0.0450 ± 0.0030	90.00 ± 6.00
Alkaline hydrolysis (strong)	--[c]	--	0.0270 ± 0.0023	54.00 ± 4.50

[a]Commercial pasteurized homogenized milk containing 0.05 ppm of added dieldrin.

[b]Average and range of duplicate determinations.

[c]Milk fat was not extracted by this method.

The surfactant method showed a lower lipid recovery as compared to the above two methods. Consequently, the total recovery of dieldrin by this method was also much lower than the FDA or Mojonnier method; approximately 10% less lipid as well as dieldrin were extracted by this technique. However, when the isolated fat was analyzed and the results were expressed on a fat basis, only 2-3% less amounts of dieldrin was observed as compared to that found by the other two methods. Lampert (1964), who first developed the surfactant technique for residue analysis in milk, used urea and isopropyl alcohol in addition to Triton X-100 and sodium tetraphosphate. He showed generally good recoveries on a fat basis for several added insecticides including dieldrin. However, he indicated that no intention was made to give a quantitative fat separation by this technique. He also stated that good but slower fat separation was feasible if not using urea and isopropanol. The procedure used in the present study was that of Kroger and Patton (1967); they and Moubry et al. (1967) applied the simplified technique using Triton X-100, sodium tetraphosphate and water to separate fat for residue analysis. But neither of them have reported the percentage of fat recovered by this technique. Kroger and Patton found that comparable results for heptachlor epoxide and dieldrin in milk were obtained by this technique and by the Roese-Gottlieb method (similar to the FDA method) on a fat basis. Moubry et al. did not report the results on a fat basis, but

indicated that 5-10% less residues were extracted from milk than by the Mills method (Mills, 1961; also similar to the FDA method). From the results of the present study and the reports of earlier workers, it seems that the lower recovery of the total quantity of residue was due to the incomplete fat recovery; whereas the residue concentration in the isolated fat was similar or slightly lower than that in the solvent extracted fat. A report of Lawrence and Burke (1970) suggested that the low recoveries of residues by this surfactant technique probably was due to the low concentrations of residues in the fat released by this technique.

In examining the alkaline hydrolysis method, it is obvious that the mild hydrolysis, as a consequence of the low heat and a shorter hydrolysis time, showed more advantages than the strong hydrolysis with high temperature and longer hydrolysis time. The recovery of added dieldrin as determined by the mild alkaline hydrolysis was comparable to that by the FDA method, whereas the strong alkaline hydrolysis provided a much lower recovery for dieldrin. This was somewhat surprising because of reports that indicated that dieldrin is thermally stable and not attacked by bases (Porter, 1964). No break-down products were observed on the resulting product GLC chromatograms from either hydrolysis procedure. It appeared that the fragments of dieldrin, if there were any from the strong hydrolysis process, might be hydrophilic and lost in the aqueous phase and washings. Some amounts of dieldrin possibly could be lost with the

vapor under the vigorous distillation process of the strong alkaline hydrolysis procedure. Another possibility was that some components in the milk samples might have an effect on the stability of dieldrin with this hydrolysis condition. Neither the FDA (1968) nor the Shell Chemical Company (1964) who recommended the strong alkaline hydrolysis methods has given any information on the recoveries of residues in milk.

The mild alkaline hydrolysis technique had an advantage over the FDA solvent extraction method in eliminating the acetonitrile partition step. However, the Florisil-celite column cleanup step was still needed in this technique to provide a suitable extract for GLC analysis. One disadvantage of this method is that an independent fat determination should be conducted in order to be able to convert the results from a weight basis of whole milk to a fat basis. If a laboratory has personnel to perform fat determinations for all samples routinely, or if the results are not required to be reported on a fat basis, then this mild alkaline hydrolysis would be the method of choice in saving analytical time and chemical reagents.

The control samples of commercial pasteurized homogenized milk were found to contain 0.003-0.005 ppm of dieldrin by all the methods reported as above. No correction for recovery has been made for the purpose of comparing extraction efficiency.

Since it was desired in the present research to determine both the lipid and the dieldrin content in milk

samples, the extraction method of FDA was thus chosen for later experiments. Recoveries of dieldrin at different added levels were also examined before the final decision was made. Both the gross and the net recoveries are given in Table 3. Analysis showed that the blank milk samples contained less than 0.005 ppm of dieldrin, where 0.005 ppm was the limit of the analytical sensitivity under those particular experimental conditions. An attempt, by using TLC techniques ("Changes in methods," 1966) to confirm the results of the blank milk samples was not successful because of the low sensitivity and low selectivity of the TLC method as compared to the electron capture GLC method. It was because of this background concentrations of residue in milk samples that the apparent gross recoveries of dieldrin at low levels were above 100%, whereas the net recoveries of dieldrin were not so high. When the contamination level was 0.05 ppm or above, the presence of this low background residue did not significantly affect the difference between the gross and net recoveries. Generally, the recovery of added dieldrin at various levels was satisfactory. Statistical analysis showed that an average value of 92.65% with a standard deviation of $\pm$ 3.29% for the gross recovery and 91.03% $\pm$ 3.78% for the net recovery were obtained for added dieldrin at levels of 0.050 to 2.000 ppm. Blank samples using water in the place of milk showed 95.0 $\pm$ 1.0% recovery of dieldrin. Therefore, the less than 100% recovery of dieldrin from the milk samples was probably due to losses in

Table 3.--Recoveries of dieldrin from milk[a] at different added levels by the Food and Drug Administration method.

Dieldrin Concentration in Milk, ppm		% Recovery of Dieldrin	
Added	Observed	Gross	Net
None	<0.0050[b]	--	--
0.005	0.0087	174.00	74.00
0.010	0.0132	132.00	82.00
0.050	0.0460	91.90	86.90
0.100	0.0904	90.42	89.20
0.200	0.1862	93.10	90.60
0.400	0.3794	94.86	93.60
0.800	0.7162	89.52	88.89
1.000	0.9878	98.78	98.28
2.000	1.8000	90.00	89.75
Average and standard deviation[c]		92.65±3.29	91.03±3.78
Coefficient variation[c]		3.55%	4.16%

[a]Commercial pasteurized homogenized milk.

[b]Detection limit.

[c]Calculations were based on the dieldrin concentration levels of 0.050 ppm and above.

the multiple steps of transferring, washing, cleaning up and evaporation, and only a small percentage of the loss could be due to the inefficient extraction, if there was any. This FDA method has been subjected to collaborative studies in a number of laboratories, and generally satisfactory results were reported for the analysis of fruits and feed products (Wells, 1967), butterfat (Carr, 1970) and various fatty foods (Johnson, 1965). It was concluded to use this procedure for later experiments in this research.

Distribution of Lipids and Dieldrin in Solvent Separated Milk Fractions

Three solvent systems were compared in this study. The first fraction was extracted with non-polar hydrocarbon solvents and the second fraction was extracted from the residual portion of the first fraction by using both polar and non-polar organic solvents. Results in Table 4 show that only a small percentage of the total lipid was obtained in the first fraction, although method No. 3 had a higher recovery than the recoveries of the other two methods. However, the concentrations of dieldrin in the first fraction (ether-extractable fraction) were much greater than in the second fraction (alcoholic-ether-extractable fraction). The ratio of dieldrin concentration in fraction 1 and 2 ranged from 3:1 to 27:1, varied with the solvent systems used. In considering the total amount of dieldrin in these two fractions, an almost equal recovery of dieldrin was

Table 4.--Distribution of lipids and dieldrin in solvent separated milk fractions.[a]

Method No.[b]	Fraction No.[c]	% Lipid Extracted	Dieldrin Concentration in Extracted Lipid		% Recovery of Dieldrin	% Approximate Recovery of Solvent
			ppm	Ratio		
1	1st	0.106	1,878.00	19	40.60	20
	2nd	2.560	97.50	1	47.60	100
	Total	2.666	168.29		88.20	
2	1st	0.092	2,353.00	27	43.40	20
	2nd	3.170	87.65	1	51.95	100
	Total	3.262	151.77		95.35	
3	1st	0.842	285.50	3	47.35	43
	2nd	2.452	91.80	1	45.00	100
	Total	3.294	141.31		92.35	

[a]Commercial pasteurized homogenized milk fortified with 5 ppm dieldrin on a weight basis.

[b]Solvent systems were hexane-ethyl ether, petroleum ether, and ethyl ether-petroleum ether for method No. 1, 2, and 3, respectively.

[c]Ether-extractable lipid and alcoholic-ether-extractable lipid for the first and second fraction, respectively.

obtained in either fraction for all three solvent systems applied.

Beroza and Bowman (1966) reported that about 10% of the total lipids in milk were extracted with the solvent mixture of hexane and ethyl ether, the same solvents as those used for the first fraction of method No. 1 in the present study, and almost 100% were recovered with solvent system of ethanol and hexane-ethyl ether (same as that used in the second fraction) from fluid milk samples. The present results show a substantially lower recovery of fat with the above solvent system; such as that the first fraction accounted for only 4% of the total extracted lipids, and the sum of the two fractions accounted for only 80% of the total lipids as extracted by method No. 3. The concentration of dieldrin when calculated on the total extracted lipid basis of method No. 1 was higher than the other two methods, however, the total amount of lipid was low, therefore the absolute recovery of dieldrin was not as high as by the other two methods. Lawrence and Burke (1970) also observed that the residue levels tended to be high on a fat basis when only 50-80% of the total fat was extracted from milk by a single solvent extraction technique.

Methods No. 2 and No. 3 gave comparable results in total lipid extraction and total dieldrin extraction, where the slight difference could be due to the analytical variance. All of the above studies apparently suggested that the distributions of residue in the lipid fractions

extracted differently would be very likely to be different among one another. Therefore, it seems to be definitely important to extract all of the lipids in milk, if the subsequent residue analysis is to be based on that isolated lipid fraction.

Another aspect of the present results concerning the incomplete recovery of dieldrin with non-polar organic solvents will be tentatively elucidated in this discussion. The literature has offered excellent information in the importance of milk fat extraction techniques with polar solvents or other suitable agents to break the fat globule membrane. A question was then raised on whether the added pesticides in milk could penetrate the membrane and why the recoveries of these non-polar organochlorine compounds were so low with hydrocarbon organic solvents, if they had not penetrated into the butterfat. Beroza and Bowman (1966) thought that this low recovery was due to the adsorption of these pesticides onto the surface of fat globules rather than penetration into the fat body. A low recovery of these compounds with hexane-ethyl ether was also observed for skim milk in which the pesticides were added, as reported by Beroza and Bowman (1966). They suggested that it was due to the binding forces of non-polar residues to the aqueous phase of milk, although this binding was much weaker than the adsorption on the cream phase in whole milk. However, they did not notice, or did not report the recovery of the solvents used. It was experienced in the present research

that most of the non-polar organic solvents, such as hexane, ethyl ether, or petroleum ether, or the combined mixtures of them, were poorly recovered (20-43% as shown in Table 4) once they were mixed with the fluid milk. In some instances, the recoveries of solvents were even lower than that of dieldrin. Poor separations of the non-polar solvents from the skim milk were also observed. This finding implies that the low recovery of added dieldrin (possibly as well as milk fat) might be due to the low recovery of solvents, or that it may be due to causes similar to those which depressed the complete recovery of added solvents. Unfortunately such causes are not yet thoroughly understood. Milk is a complex system composed of various proteins, lipids and other components in various forms. The added non-polar organic solvents as well as dieldrin could be adsorbed on the hydrophobic groups of the serum proteins and/or the membrane proteins. In connection with this finding, it was then speculated that the function of alcohol for pesticide and fat extraction appeared to be no less important in rupture of the emulsion between the solvents and milk phases than the rupture of fat globule membrane, where the later was indicated as the only essential function of alcohol in the discussion of Moffitt (1963) and Kroger and Patton (1967).

Additional investigation was made to detect the remaining residue, if there was any, in the lipid-depleted phase of whole milk and skim milk. The naturally contaminated milk samples were used in this experiment and the

technique of alkaline hydrolysis was the method of choice because the solvent-extractable residue should have been removed with the lipid fraction. Table 5 shows the results of such analyses. The concentrations of dieldrin in the lipid-depleted fractions of both the milk and skim milk samples were below the detection sensitivity, under these particular experimental conditions. The strong alkaline hydrolysis procedure again provided a much lower recovery of dieldrin in either whole milk or skim milk as compared to recovery by the mild hydrolysis method. Thus, no attempt was made to use this strong hydrolysis method for the determination of residues in the lipid-depleted fractions.

To gain further information concerning the distribution of dieldrin in milk fat, the total extracted lipid was separated into neutral and phospholipid fractions by a batchwise silicic acid technique described by Abramson and Blecher (1965). Isolated milk fat was mixed with silicic acid and packed into a column. The neutral lipids were eluted with ethyl ether and petroleum ether from the column and the phospholipids were subsequently eluted from the column by methanol. The relative quantities of neutral and phospholipids recovered were 99.46 and 0.54%, respectively and the relative quantities of dieldrin recovered with these two lipid fractions were 99.85 and 0.15%, respectively. When a blank solution of petroleum ether containing dieldrin was fractionated in a similar manner, it was observed that 99.65% of the dieldrin was recovered with the effluent of

Table 5.--Distribution of dieldrin in lipid-depleted milk fractions.[a]

Fraction	Dieldrin Concentration on a Weight Basis, ppm	
	Method 1[b]	Method 2[b]
Whole milk	0.0285	0.0035
Lipid-depleted milk[c]	<0.0003[d]	--
Skim milk	0.0032	0.0001[d]
Lipid-depleted skim milk[c]	<0.0001[d]	--

[a]Fresh raw whole milk from cows fed dieldrin.

[b]Method 1 and 2 refer to the mild and strong alkaline hydrolysis techniques respectively.

[c]Lipids were depleted by the FDA extraction procedure.

[d]Detection limit.

ethers and only 0.35% with methanol. Apparently dieldrin had solubility similar to that of neutral fat in ethers, whereas the trace residues eluted out with the phospholipid or methanol might be due to an incomplete ether extraction, or such a small amount of dieldrin was slightly adsorbed on the silicic acid just as were the phospholipids. However, not much significance could be ascribed to either factor, since the absolute quantity of dieldrin recovered in the methanol fraction was near the detection limit, and the small amount of residue observed might be an artifact under that particular set of experimental conditions. Additionally, once the lipids and residue had been extracted from the natural milk system with solvents, the original binding forces between the pesticide residues and the milk components could very well be altered. A second series of experiments were, therefore, designed to separate milk fractions by physical forces. The correlation of dieldrin with lipids in these fractions would provide a better insight of the natural deposition of such an organochlorine pesticide residue in milk.

Distribution of Lipids and Dieldrin in Physically Separated Milk Fractions

Naturally Contaminated Milk

Tables 6, 7, 8, 9a and 10 present the results of lipid and dieldrin determination in naturally contaminated milk fractions. Three collections and preparations were

made in different periods. It is very obvious that the concentrations of dieldrin residue in milk fat were significantly decreased in the later collections when the feeding of pesticide had been discontinued.

Slightly modified procedures were adapted for each of these three preparations. In preparation 1 (Table 6) fractions of whole milk, skim milk, cream, butteroil, buttermilk and butter serum were analyzed for total lipid, dieldrin and phospholipid content. The concentrations of dieldrin in these fractions were generally proportional to the total lipid contents in these fractions, except that butter serum contained slightly lower levels. The high phospholipid content in this fraction might have an influence on the distribution of residue. However, the same speculation can not be applied to buttermilk, which also contained a high level of phospholipids but had dieldrin level compatible with that in other fractions.

In the second preparation (Table 7), both unwashed and washed cream were examined, and the buttermilk was further refined by centrifugation to remove any unchurned fat. Washing of cream apparently did not affect the content of residue on a fat basis. An interesting finding was made in the comparison of buttermilk and refined buttermilk. When the unchurned floating fat globules were removed, the total lipid content of buttermilk was reduced, but the relative amount of phospholipid in the total lipid was increased. On the other hand, an inverse distribution pattern of

Table 6.--Distribution of lipids and dieldrin in major fractions of naturally contaminated milk, preparation 1.[a]

Fraction	Lipid (%)	Phospholipid in Total Lipid (%)	Dieldrin Concentration, ppm[b]	
			Extd. Lipid	Fraction
Whole milk	3.48	0.62	11.05±0.50	0.384±0.017
Skim milk	0.66	1.49	11.65±0.75	0.077±0.005
Cream	31.53	0.44	11.45±0.40	3.610±0.126
Butteroil	100.00[c]	0.06	11.90±0.20	11.900±0.200
Buttermilk	0.40	15.79	11.87±0.38	0.047±0.002
Butter serum	12.13	8.38	9.06±1.43	1.098±0.174

[a]Milk from a cow fed dieldrin (see Table 1. Milk Collection No. 1).

[b]Average and range of duplicate determinations.

[c]Assumed value.

Table 7.--Distribution of lipids and dieldrin in major fractions of naturally contaminated milk, preparation 2.[a]

Fraction	Lipid (%)	Phospholipid in Total Lipid (%)	Dieldrin Concentration, ppm[b]	
			Extd. Lipid	Fraction
Whole milk	3.67	0.62	4.64±0.05	0.170±0.002
Skim milk	0.47	2.74	4.67±0.58	0.022±0.003
Cream (unwashed)	25.71	0.28	4.55±0.10	1.170±0.026
Cream (washed)	26.16	0.54	4.39±0.06	1.148±0.016
Butteroil	97.00	0.02	4.80±0.12	4.656±0.116
Butter-milk	4.91	1.56	4.00±0.20	0.196±0.010
Butter-milk (refined)	2.39	3.09	3.20±0.12	0.077±0.003
Butter serum	26.55	3.05	3.65±0.10	0.969±0.027

[a]Milk from cows fed dieldrin (see Table 1. Milk Collection No. 2).

[b]Average and range of duplicate determinations.

dieldrin was observed, i.e., refined buttermilk contained a lower level of residue than the crude buttermilk on a fat basis. It appears that the association of dieldrin with phospholipids was not so significant as it was with the neutral lipids.

The first five major fractions, as shown in Table 7, contained dieldrin at similar levels on a fat basis, whereas lower concentrations of dieldrin were observed in the last three fractions, buttermilk, refined buttermilk and butter serum. These three fractions contained more phospholipids than were in the other fractions (except skim milk) in this preparation, but did not contain as much phospholipids as that in the corresponding fractions of the previous preparation. It seems possible that some other factors may be related to the distribution of residue besides the phospholipid contents in these milk fractions.

In order to understand the possible binding forces of such a pesticide chemical in the fat globule membrane, the harvested membrane pellets were air-dried and successively extracted by petroleum ether and chloroform-methanol into two fractions. Table 8 indicates that relatively greater amounts of the residue were extracted with the free lipids. Although there were still some residues in the bound lipid fraction, the concentrations were much lower. In addition, considerable interference was encountered in the GLC determination of residues in the bound lipid extracts under these particular experimental conditions, and

Table 8.--Distribution of lipids and dieldrin in fat globule membrane pellets of naturally contaminated milk, preparation 1.[a]

Pellet	Free Lipid in Total Lipid[b] (%)	Bound Lipid in Total Lipid[b] (%)	Dieldrin Concentration, ppm		
			Free Lipid	Bound Lipid	Total Lipid
7500 S	12.02	87.98	3.94	0.88	1.13
230 S	45.00	55.00	5.80	0.93	3.12
35 S	30.71	69.29	6.43	0.88	2.58
Total	31.70	68.30	5.87	0.90	2.47

[a]Milk from a cow fed dieldrin (see Table 1. Milk Collection No. 1). Each value is the result of a single determination of pooled pellet fractions.

[b]Free lipids were extracted with petroleum ether from the air-dried pellets, bound lipids were successively extracted with chloroform and methanol (2:1,v/v).

thus the true concentration of dieldrin probably would be lower than observed. At this point, it still could not be concluded that dieldrin was more associated with the free lipids than the bound lipids, since the higher recoveries of dieldrin with the free lipids might be due to their similar solubilities in petroleum ether, and in the original natural state the dieldrin could be deposited in the bound lipid phase as much as in the free lipid phase. Nevertheless, the data reflect that this organochlorine pesticide was more readily extracted with non-polar organic solvent than were the bound lipids, which were composed of relatively high levels of phospholipids. Apparently, dieldrin was not so significantly bound to the phospholipids or the protein moiety in the fat globule membrane system.

The concentrations of dieldrin were generally lower in the membrane pellets than in the refined buttermilk when they were calculated on total lipid basis. This difference might be attributed to the loss of residues during the air-drying process (but this was very unlikely), or to the lower efficiency of extraction for dried materials (not impossible), or more likely was due to the removal of some flotation layers during the multi-centrifugation process, and thus the resulting membrane pellets contained greater amounts of phospholipids than buttermilk. It again appeared that the lower levels of dieldrin in the total lipids were correlated with the high levels of phospholipid content in this fraction. However, the true values of phospholipids

in these samples were not determined since all of the extracted lipids had been used for residue analysis.

A third series of milk samples were collected in much greater quantities. The cream-washing procedure was modified so as to use deionized distilled water instead of a sucrose solution, thus the membrane pellets would be obtained in a water solution and the subsequently dried materials would be the true dry weight of each pellet without the added sucrose. Results of this study are presented in Table 9a and 9b. First, it is to be observed that these milk samples contained much less dieldrin than found in the two previous milk collections, due to the fact that the same dairy cows had received no dieldrin for several months. The decline of residue in milk fat from dairy cows has been well documented in the literature. This observation in the present research only once again confirms those reported by earlier workers.

Results in Table 9a and 9b are the average of analysis of two independent preparations, where each preparation consisted of six consecutive milk collections and individual fractionations. In this study, the butter fraction was included in the analysis and the butter serum was further refined to eliminate residual amounts of butteroil. Only refined buttermilk and butter serum were analyzed because the relatively high contents of phospholipids in these fractions would be of interest in relating to the residue contents. Table 9a, again, shows that dieldrin concentrations were

Table 9a.--Distribution of lipids and dieldrin in major fractions of naturally contaminated milk, preparation 3.[a]

Fraction[b]	Lipid (%)	Phospholipid in Total Lipid (%)	Dieldrin Concentration, ppm[c]	
			Extd. Lipid	Fraction
Whole milk	4.05	0.71	0.83±0.09	0.034±0.004
Skim milk	0.58	1.94	0.89±0.10	0.005±0.001
Cream (unwashed)	53.63	0.37	0.83±0.02	0.445±0.011
Butter	83.00	0.31	0.86±0.15	0.714±0.125
Butteroil	99.20	0.01	0.87±0.09	0.863±0.089
Buttermilk (refined)	1.67	11.84	0.58±0.04	0.010±0.001
Butter serum (refined)	1.92	23.96	0.50±0.07	0.010±0.001

[a]Milk from cows fed dieldrin (see Table 1, Milk Collection No. 3).

[b]Each value is the average of duplicate preparations. Each preparation consists of six individual milk collections and fractionations.

[c]Average and range of duplicate preparations.

Table 9b.--Distribution of lipids and dieldrin in major fractions of naturally contaminated milk, preparation 3.[a]

Fraction	Amount of Fraction of Whole Milk[b] (%)	Amount of Lipid in Fraction[c] (%)	Amount of Dieldrin in Fraction[c] (%)
Whole milk	100.00	100.00	100.00
Skim milk	93.60[d]	13.40	14.49
Cream (unwashed)	6.40	84.74	84.78
Cream (washed)	3.74	--	--
Butter	2.43	49.80	51.60
Butteroil	2.00	48.98	50.76
Buttermilk (refined)	1.31	0.54	0.40
Butter serum (refined)	0.27	0.13	0.08

[a]Milk from cows fed dieldrin (see Table 1, Milk Collection No. 3).

[b]Each value is the average of duplicate preparations. Each preparation is the average of six individual milk collections and fractionations.

[c]Each value is the average of duplicate preparations. Each preparation consists of pooled fractions of six individual milk collections and fractionations.

[d]Estimated by the difference between whole milk and unwashed cream.

comparable within a narrow range in the first five fractions on a fat basis, whereas the concentrations of residue in the refined buttermilk and butter serum were apparently lower than in the other fractions. These two fractions contained significantly less of total lipids than the corresponding fractions from the previous collections and the relative contents of phospholipids were increased. Once again, it shows that the lower dieldrin concentrations in these fractions were correlated with the higher phospholipid contents.

In this series of milk collections and separations, the yield of each fraction was measured before it was further processed. Table 9b indicates the percentage distribution of various fractions in milk, and the lipid and dieldrin accounted for in each fraction. Although skim milk contained very little residue on a weight basis (Table 9a), it accounted for 93.5% of the total milk (by weight); therefore, it still accounted for a significant quantity of dieldrin of whole milk (14.9%). On the other hand, cream accounted for only 6.4% of the total weight of milk, but it accounted for almost 85% of the total lipids of milk, and contained about 85% of the total residue of milk. From this table, it is again observed that the distribution of dieldrin was generally parallel with the total lipid distribution in milk. Losses of fraction weight, and losses of the absolute amounts of lipid and dieldrin could be due to the washing process of cream, although the concentration of

dieldrin on a fat basis in the washed cream was not much different from that in the unwashed cream (Table 8).

A preliminary test showed that freeze-drying had no significant effect on the dieldrin concentrations of whole milk, skim milk, or buttermilk (Table 10). Kroger (1968) also reported that the levels of dieldrin and heptachlor epoxide in butteroil were not affected by freeze-drying. Thus, the membrane materials obtained in this series of preparations were freeze-dried to render it feasible for the extraction of free lipids by petroleum ether; otherwise this solvent could not be quantitatively separated from the water solution of membrane material.

Table 11 illustrates the lipid and dieldrin analyses in these dried membrane pellets. The relative amounts of membrane pellets collected in this study were somewhat different from those reported by Swope (1968). In Swope's results, almost 19% of the total amount of pellets were recovered in the first fraction, 7,500 S pellet; and about 47.5% and 33.5% were recovered in the 230 S and 35 S pellet, respectively; whereas in the present study, the recovery of 7,500 S was only about 2.9% and recoveries of 230 S and 35 S were roughly 43.0 and 54.0%, respectively. This low yield of the 7,500 S pellet in the present research was unfavorable to residue analysis, because the concentrations of dieldrin in this series of milk collections were much lower than the earlier collections, and the total lipid content of this 7,500 S pellet was relatively low as compared to the

Table 10.--Comparison of the lipid and dieldrin contents in fresh and freeze-dried milk fractions.[a]

Fraction	% Lipid Extracted on Fresh Wt. Basis		Dieldrin Concentration in Extd. Lipid, ppm[b]	
	Fresh	Freeze-Dried	Fresh	Freeze-Dried
Whole milk	3.67	3.89	4.62±0.05	4.68±0.29
Skim milk	0.46	0.43	4.67±0.58	4.45±0.14
Cream	26.16	28.99	4.55±0.20	4.38±0.10
Buttermilk	4.91	4.93	4.00±0.20	4.10±0.15

[a]Milk from cows fed dieldrin. Freeze-drying conditions were at 100 μ Hg pressure for 24 hours.

[b]Average and range of duplicate determinations.

Table 11.--Distribution of lipids and dieldrin in freeze-dried fat globule membrane pellets of naturally contaminated milk, preparation 3.[a]

Pellet[b]	Membrane Fraction (%)	% Lipid in Pellet			% Phospholipid in			Dieldrin Concentration, ppm[c]	
		FL[d]	BL	TL	FL	BL	TL	TL	Pellet
7500 S	2.92	9.56	8.62	18.18	--	--	--	--	--
230 S	43.05	26.00	7.65	32.13	12.22	57.33	22.96	0.28±0.02	0.088±0.006
35 S	54.03	39.77	8.38	48.15	14.34	57.70	21.87	0.27±0.03	0.129±0.016
Total	100.00	30.68	7.10	37.78	--	--	--	--	--

[a]Milk from cows fed dieldrin (see Table 1, Milk Collection No. 3).

[b]Each value is the average of duplicate preparations of pellets from pooled buttermilk. Each pooled buttermilk consists of six individual milk collections and separations.

[c]Average and range of duplicate determinations.

[d]FL=Free lipids, extracted by petroleum ether; BL=Bound lipids, extracted by ethanol, ethyl ether and petroleum ether; TL=Total lipids=FL+BL.

other two pellets. Consequently, the absolute amount of dieldrin in this fraction was too little to be determined accurately.

An increasing order of the free lipid contents were observed from 7,500 S, 230 S to 35 S pellets , and the total lipid contents followed a similar distribution pattern which was in agreement with the observation of Swope (1968); although the proportions of total lipid contents in three fractions were slightly different. There were some phospholipids found in the free lipid fraction; it might be due to the effect of freeze-drying, by which some of the lipoprotein complexes were dissociated and the released phospholipids were dispersed into the petroleum ether extract. The total phospholipid contents in the total lipids of these pellets were 22-23% approximately, these values were much greater than refined buttermilk, from which these pellets were collected. This finding confirms the earlier postulation that some neutral fats were removed with the flotation layer during the multi-centrifugation processes. Dieldrin analyses were made using the combined lipids, since the separate recovery of residue in different fat fractions might not reflect the original deposition of such a pesticide chemical in the natural manner in membrane materials. Apparently, the 230 S and 35 S pellet contained comparable concentrations of dieldrin on a fat basis, despite the difference in their free and bound lipid ratios or the total lipid contents. In comparison of the dieldrin concentrations

of these pellets with the refined buttermilk fraction, it was again observed that a lower value was found in the pellets than in the buttermilk, and a higher phospholipid content was associated with this lower level of dieldrin.

Milk Containing Added Dieldrin

Determinations were made on two independent preparations of major milk fractions and their individual and average results are shown in Table 12. In general, the distribution pattern of lipids and added dieldrin were similar to that in the naturally contaminated milk. In this series of experiments, both the crude and refined buttermilk and both unwashed and washed cream were examined. Additionally, a portion of skim milk was also refined by centrifugation at 2,000 X G for 15 min to remove excess cream which was not separated during the first cream separation. The refined skim milk contained much lower lipid contents as compared to the original skim milk, while the phospholipid contents were relatively increased in the refined skim milk; although the direct data of phospholipid contents in the original skim milk were not available for these preparations, they can be assumed not much different from the skim milk with comparable total lipid contents in the previous preparations. The refined skim milk fractions from two preparations contained quite different total lipid contents as well as the relative phospholipid contents. This variation was due to the

Table 12.--Distribution of lipids and dieldrin in major fractions of raw milk containing added dieldrin.[a]

Fraction	Preparation No.[b]	Lipid (%)	Phospholipid in Total Lipid (%)	Dieldrin Concentration, ppm		Amount of Fraction of Whole Milk (%)	Amount of Lipid in Fraction (%)	Amount of Dieldrin in Fraction (%)
				Extd. Lipid	Fraction			
Whole milk	1	3.88	0.57	12.87	0.499	100.00	100.00	100.00
	2	3.85	0.54	13.26	0.510	100.00	100.00	100.00
	A	3.87	0.55	13.07	0.505	100.00	100.00	100.00
Skim milk	1	0.41	--	12.98	0.053	88.99[c]	9.40	9.48
	2	0.61	--	13.40	0.082	91.02[c]	14.37	14.85
	A	0.51	--	13.19	0.067	90.00	11.89	12.17
Skim milk (refined)	1	0.05	14.27	15.02	0.008	88.99[c]	1.22	1.42
	2	0.12	6.37	14.17	0.017	91.02[c]	2.84	3.04
	A	0.09	10.32	14.60	0.013	90.00	2.03	2.23
Cream (unwashed)	1	31.80	0.30	12.20	3.880	11.01	90.24	85.53
	2	37.13	0.36	13.33	4.945	8.98	86.60	87.06
	A	34.47	0.33	12.77	4.413	10.00	88.42	86.30
Cream (Washed)	1	47.54	0.19	12.07	5.738	6.88	84.29	79.05
	2	43.70	0.24	13.33	5.825	5.68	64.67	64.81
	A	45.62	0.22	12.70	5.782	6.28	74.48	71.93
Butter	1	83.29	0.26	12.30	10.245	3.80	81.57	77.96
	2	84.43	0.25	13.28	11.212	2.79	61.18	61.28
	A	83.86	0.26	12.79	10.729	3.30	71.38	69.64

Table 12. (Cont'd).

Fraction	Preparation No.[b]	Lipid (%)	Phospholipid in Total Lipid (%)	Dieldrin Concentration, ppm		Amount of Fraction of Whole Milk (%)	Amount of Lipid in Fraction (%)	Amount of Dieldrin in Fraction (%)
				Extd. Lipid	Fraction			
Butteroil	1	98.00	0.08	12.56	12.310	3.19	80.57	78.63
	2	100.00	0.05	13.39	13.390	2.25	58.44	59.02
	A	99.00	0.06	12.98	12.850	2.72	69.51	68.82
Buttermilk	1	4.55	0.68	7.40	0.336	3.01	3.53	2.03
	2	3.11	0.95	9.43	0.294	2.89	2.34	1.66
	A	3.83	0.82	8.42	0.315	2.95	2.93	1.84
Buttermilk (refined)	1	0.87	10.29	6.81	0.060	3.01	0.68	0.36
	2	2.97	2.35	9.20	0.273	2.89	0.23	1.54
	A	1.92	6.32	8.01	0.166	2.95	1.45	0.95
Butter serum (refined)	1	1.22	21.08	6.86	0.084	0.44	0.14	0.07
	2	1.15	24.46	6.92	0.079	0.32	0.09	0.05
	A	1.18	22.62	6.89	0.082	0.38	0.12	0.06

[a]Dieldrin was added to milk at 0.5 ppm level on a fresh weight basis.

[b]Each preparation is an individual milk collection and fractionation. A is the average value of two preparations.

[c]Estimated by the difference between whole milk and unwashed cream.

techniques involved in the first cream separation as well as in the refining separation.

Slightly higher levels of dieldrin were measured in the refined skim milk than in the original skim milk on a fat basis. Speculations on this aspect will be included later in this discussion. In comparison of the total recovery of lipid or dieldrin in washed and unwashed cream, an obvious loss was observed to be due to the cream washing process. But the losses in this series of preparations were not as great as encountered in the previous series of preparations.

Comparable to the study of naturally incorporated dieldrin, the added dieldrin was also recovered essentially (85-87%) in cream phase after separation. Beroza and Bowman (1966) reported a similar observation on recovery of other non-polar chlorinated pesticides. Although the original skim milk accounted for about 10-15% of the total lipids as well as dieldrin of the total contents in milk, the refined skim milk contributed much less of these substances to the whole milk system (1-3%). Therefore, the absolute quantity of dieldrin deposited in any fraction of milk varies significantly with the amount of lipid in that fraction. Comparisons can not be made between two corresponding fractions with different lipid contents.

Relatively lower concentrations of dieldrin were found in buttermilk and butter serum than in other fractions, and refined buttermilk contained less amounts of residue

than the crude buttermilk on a fat basis. These findings were quite similar to those made with the naturally contaminated milk reported earlier.

Analyses of the membrane pellets are given in Table 13. The relative amounts of pellets were slightly different from the previous preparation. The total lipid contents of these pellets were comparable to the previous findings, while the phospholipid levels were somewhat higher. In considering the levels of dieldrin on a fat basis, no conclusion can be drawn for any definite distribution pattern for this pesticide among different pellets. Nevertheless, a lower value of dieldrin concentration was again found in these pellets as compared to the refined buttermilk fraction from which they were collected.

The final supernatant of the membrane collection was also analyzed. It was observed that the final flotation layer was very loosely formed, and it tended to redisperse into the center clear layer with a slight disturbance, such as when the centrifuge tube caps were removed. Care was taken to avoid this disturbance to the floating materials as much as possible and an aliquot of the top layer was pipetted out with a syringe. A significant amount of the center layer supernatant was included in this fraction. The clear center layer collected likewise contained some of the floating material, which was almost unavoidable. Analyses (Table 13) show that the top layer contained definitely higher concentration of residue than that in the center

Table 13.--Distribution of lipids and dieldrin in freeze-dried fat globule membrane materials of raw milk containing added dieldrin.[a]

Pellet[b]	Membrane Fraction (%)	Lipid (%)	Phospholipid in Total Lipid (%)	Dieldrin Concentration, ppm[c]	
				Extd. Lipid	Pellet
7500 S	4.20	17.50	36.79	4.71±0.23	0.824±0.040
230 S	60.72	29.76	33.47	4.93±0.27	1.467±0.080
35 S	35.08	51.35	30.20	3.24±0.16	1.663±0.082
Total	100.00	36.82	31.91	3.96±0.20	1.458±0.075

Supernatant	Dry Matter (%)	Lipid in Dry Matter (%)	Phospholipid in Total Lipid (%)	Dieldrin Concentration, ppm[d]		
				Extd. Lipid	Dry Matter	Solution
Final center layer	0.107	7.22	14.71	3.37	0.243	0.0003
Final top layer	0.163	8.07	14.96	5.12	0.413	0.0007

[a]Dieldrin was added to milk at 0.5 ppm level on a fresh weight basis.

[b]Pellets were obtained from pooled buttermilk of two independent preparations.

[c]Average and range of duplicate determinations.

[d]Each value was from one determination.

layer; although the residue levels in both solutions were substantially low.

Control Milk

Raw whole milk was fractionated in the same manner as the contaminated milk. The purpose of this series of studies was to provide a background information of residue levels in various fractions, and if reliable, to examine the distribution of dieldrin at this low level in milk fractions. In addition to the major fractions, pooled membrane pellets obtained form one process of centrifugation of refined buttermilk at the highest speed (same speed as for 35 S pellet), a clear center layer of supernatant, and the flotation layer were collected and analyzed. Most of these fractions showed practically no peaks in the GLC chromatogram when their extracts were prepared at concentration levels comparable to those milk samples containing added dieldrin. Therefore, no adjustment of residue concentrations was made for the fractions from previous preparations of milk containing added dieldrin. When these extracts of the control samples were concentrated by 10-fold, trace amounts of dieldrin were measured. Results are given in Table 14. It appeared that fractions containing little lipids such as the refined fractions of skim milk, buttermilk and butter serum, and the membrane pellets, contained also higher values of residue on a fat basis. However, little reliability can be given to these results due to the fact that more

Table 14.--Distribution of lipids and dieldrin in various fractions of control milk.

Fraction	Lipid (%)	Phospholipid in Total Lipid (%)	Dieldrin Concentration, ppm[a]	
			Extd. Lipid	Fraction
Whole milk	3.80	0.06	0.063±0.021	0.0024±0.0008
Skim milk (refined)	0.05	14.96	0.195±0.030	0.0001±0.00002
Cream (unwashed)	30.78	0.27	0.053±0.012	0.0163±0.0037
Cream (washed)	46.54	0.24	0.055±0.010	0.0256±0.0047
Butter	83.26	0.21	0.060±0.015	0.0500±0.0125
Butteroil	98.80	0.01	0.059±0.010	0.0583±0.0099
Buttermilk (refined)	2.07	5.22	0.230±0.160	0.0048±0.0033
Butter serum (refined)	2.03	17.15	0.183±0.082	0.0037±0.0017
Membrane pellets[b]	37.75[c]	26.79	0.220±0.039	0.083±0.0147[c]
Clear supernatant[b]	8.07[c]	12.17	--	--
Flotation layer[b]	45.74[c]	2.12	--	--

[a]Average and range of duplicate determinations (apparent values).

[b]Freeze-dried samples.

[c]On a dry matter basis.

interference were observed on the chromatograms of these fractions as compared to the chromatograms of other fractions. Furthermore, the concentrations of dieldrin on a fraction weight basis were very low in various fractions, especially in these low-fat fractions; and the concentration levels as shown in Table 14 probably were the limit of detection sensitivity and accuracy for dieldrin in each particular fraction under the present experimental conditions.

The total lipid and phospholipid contents of the supernatant and flotation layer were also given, although no attempt was made to determine the residue concentrations in these fractions of the control milk. Data showed that the flotation layer contained greater amounts of lipid than the pellets and consequently much lower phospholipid content than the pellets. This observation supports an earlier postulation that a significant amount of neutral fat was removed with the flotation layer during centrifugation, and also it justifies the findings that membrane pellet fractions contained higher levels of phospholipids than the refined buttermilk.

General Discussion

The relative concentrations of dieldrin in the total lipids of various fractions as reported in Tables 6, 7, 8, 9a, 11, 12, and 13 are compiled in Table 15 for a ready comparison. Although the observed ratios of dieldrin levels in various fractions were varied from one preparation to

Table 15.--Comparison of the distribution of dieldrin in various milk fractions.

Fraction	Approximate Ratio of Dieldrin Concentration (fat basis)				
	Naturally Contaminated Milk			Dieldrin Added Milk	
	(1)	(2)	(3)	(1)	(2)
Whole milk	1.00	1.00	1.00	1.00	1.00
Skim milk	1.05	1.01	1.07	1.01	1.01
Skim milk (refined)	--	--	--	1.17	1.07
Cream (unwashed)	1.05	0.98	1.00	0.95	1.01
Cream (washed)	--	0.95	--	0.94	1.01
Butter	--	--	1.01	0.96	1.00
Butteroil	1.08	1.03	1.07	0.98	1.01
Buttermilk	1.07	0.86	--	0.57	0.71
Buttermilk (refined)	--	0.69	0.70	0.53	0.69
Butter serum	0.82	0.79	--	--	--
Butter serum (refined)	--	--	0.60	0.53	0.52
Membrane pellets (pooled)	0.22	--	0.33	0.30[a]	
Final clear supernatant	--	--	--	0.26[a]	
Final top layer	--	--	--	0.39[a]	

[a]Membrane pellets were collected from pooled buttermilk of two preparations.

another, similarities were observed in that buttermilk and butter serum contained generally lower amounts of dieldrin than the fractions of whole milk, skim milk, cream, washed cream, butter and butteroil on a fat basis, while refined buttermilk, refined butter serum and membrane pellets contained even lesser amounts of residue. As pointed out earlier, most of these low-residue fractions contained higher phospholipid contents. Privett et al. (1968), and Swope (1968) have provided excellent information of the high phospholipid contents in the plastic skim milk (equivalent to the buttermilk fraction in the present study) and serum (equivalent to butter serum), and membrane pellets, respectively. Thus an inverse relationship of the concentrations of dieldrin and phospholipid apparently exist.

In a few instances in the present research, the phospholipid contents of buttermilk and butter serum were only slightly greater than in the whole milk, but their dieldrin levels were still low. Therefore, other factors which are associated with the distribution of dieldrin in milk cannot be neglected. The buttermilk fraction was obtained by churning the washed cream at 12°C to room temperature (ca. 24°C), and the butter serum was collected from the melting of butter at 45°C. For these reasons, it is very likely that some high-melting fats were separated into these fractions while the low-melting fats were gathered as butter and butteroil. Studies on the high-melting glycerides in fat globule membrane have been reviewed by Brunner

(1965). In this aspect, the low concentrations of dieldrin in the membrane fractions (buttermilk, butter serum and membrane pellets) may also be related with the high levels of high-melting glycerides in these fractions; in other words, this non-polar chlorinated pesticide residue appeared to be oriented into the low-melting glycerides more readily than into the high-melting glycerides. Additionally, the variance of glyceride species, phospholipid compositions, and the fatty acid moieties among these fractions may also influence the distribution pattern of dieldrin.

A number of reports have emphasized the importance of rupture of the fat globule membrane in residue analysis (Moffitt, 1963; Kroger and Patton, 1967), based on the postulation that chlorinated pesticide residues are mostly deposited in the fat globules of milk. By physical separation of milk, Langlois et al. (1964b) and Li et al. (1970) proved that those physiologically incorporated pesticides were significantly associated with the high-fat fractions, such as cream and butter. The present research confirms these findings, and further indicates that butteroil contained higher concentration of dieldrin than the butter on a weight basis. Additionally, the relative amounts of various fractions collected, and the relative amounts of lipid and dieldrin contained in these fractions are presented. If butteroil may account for most of the lipid in milk, it would also account for most of the deposition of residue in milk; although the present separation technique

did not provide a quantitative recovery of milk fat in the butteroil fraction. So far there has been little or no doubt that most of the naturally incorporated chlorinated residues as well as butteroil are within the fat globules in milk.

On the studies of fortified milk, Beroza and Bowman (1966) reported that above 80% of the total quantity of added pesticides were recovered in the cream phase after separation. They thought that these chemicals were adsorbed onto the membrane of the fat globules rather than having penetrated into the fat body. Langlois et al. (1964b) found that the added DDT and dieldrin were essentially recovered in butter as well as cream. One could still think that these compounds were adsorbed on the serum membrane of the butter phase. However, in the present research, evidence has been given that added dieldrin was mostly found in the butteroil fraction; similar to the distribution of the naturally incorporated dieldrin. Questions have again been raised at this point whether the added pesticide can penetrate through the membrane, or that the recovery of residue in the butter and butteroil phases was only a consequence of re-orientation of this substance during the separation processes; and if the later speculation is true, then would it be also true for the naturally incorporated pesticides? Although the answers are not yet available, nevertheless all the studies showed that a striking similarity is present between the distribution patterns of dieldrin in the milk

system with the different sources of contamination, and that dieldrin is preferentially deposited into butteroil or neutral fat regardless of whether it is the original distribution or is an altered orientation during separation.

Hugunin and Bradley (1969) and Li et al. (1970) reported that in some instances the concentrations of pesticides were higher in the skim milk, buttermilk and high density membrane pellets, in some other instances these high concentrations were observed only for skim milk but not for buttermilk, and in other instances, this difference was not observed at all for the above fractions (such as for dieldrin, reported by Li et al.) as compared to the raw whole milk on a fat basis. They thought that the higher residue levels observed in skim milk and buttermilk were due to the association of pesticides with the phospholipid or lipoprotein portions in these fractions. However, they did not report the fat contents of their milk products or fractions. From the experience in the present research, the fat content of a fraction may vary significantly with the separation techniques involved, especially for the low-fat containing fractions, such as skim milk and buttermilk. This variation may contribute significant differences in the residue concentrations for the low-fat fractions on a fat basis. The variations of the distribution of the same pesticide in corresponding fractions among the data of Hugunin and Bradley and Li et al. might be due to the variance in the lipid contents.

For the same reason, direct comparison cannot be made of their results with the present study.

The skim milk fractions obtained in the present research generally contained rather high amounts of total lipids but relatively low amounts of phospholipids as compared to the commercial skim milk. Analyses showed that these skim milk contained similar concentrations of dieldrin as the whole milk or cream on a fat basis. When excess cream was further removed from the original skim milk, the total lipid content of the resulting refined skim milk was decreased significantly, whereas the relative phospholipid content was increased. Slightly higher residue concentrations on a fat basis were observed in refined skim milk fractions. One possible factor relating to the distribution of dieldrin might be the high amounts of protein in the refined skim milk rather than the high phospholipid contents in the total lipids of refined skim milk; because the protein to lipid ratio in the refined skim milk is significantly high (for the skim milk containing 0.1% fat, this ratio is about 36:1; Bell and Whittier, 1965). It is possible that some dieldrin is distributed in the serum phase of milk and this quantity does not affect the overall concentration of dieldrin in the skim milk which has a relatively high fat content; but this small amount of residue does have a significant effect when the fat content is extremely low as in the refined skim milk. Although some reports showed that the solubility of dieldrin in water is

negligible, some other reports have indicated measurable solubilities at 0.25, 0.54, and 1.0 ppm levels at 25, 35 and 45°C, respectively (Gunther et al., 1968). Park and Bruce (1968) also reported that the maximum solubility of dieldrin in water was 0.186 ppm. The solubilities of non-polar pesticides in protein solutions are even higher. Moss and Hathway (1964) found that dieldrin was soluble at levels as high as 144-210 ppm in the blood serum of rabbits. Similarly, it is rational to postulate that the serum of milk may contribute to a non-negligible quantity of dieldrin, and this quantity is significantly important in the refined skim milk. Consequently, when the residue was extracted by solvent and reported on a fat basis, the resulted concentration would seem higher in the lipid of refined skim milk than in the original skim milk.

Earlier reports indicating that skim milk contained higher levels of residues on a fat basis (Hugunin and Bradley, 1969; Li et al., 1970) was probably due to a similar reason: extremely low fat content of their skim milk. Actually, the amounts of residues in skim milk are quite low on a weight basis, so that Langlois et al. (1964b) could not detect these substances directly from the skim milk by the one-step method they used (see Literature Review). Therefore they could not report any level of residues on a fat basis for their skim milk, while their whole milk and other fractions contained significant amounts of residues on a fat basis.

In considering the residue concentrations found in buttermilk, butter serum or their refined fractions and fat globule membrane pellets, tentative explanations postulated here are quite different from those for skim milk and refined skim milk. Firstly, the solubility of the protein moiety in buttermilk from thrice-washed cream is different from those in skim milk (Swope, 1968). The solubilities of pesticides in these two systems of protein solutions or suspensions may be different. Secondly, the protein to lipid ratios in these buttermilk and butter serum (approximately 0.72:1.00 to 0.84:1.00, and 0.18:1.00 to 0.20:1.00, respectively, reported by Swope and Brunner, 1968) are much lower than the ratios in skim milk. Thus, the influence of the lipid moiety on the distribution of residue would be greater in a buttermilk or butter serum system than in skim milk; similarly, the protein moiety would be more responsible on the deposition of pesticide in skim milk than in buttermilk. Thirdly, it has been noted that the lipid moiety of buttermilk was very difficult to be completely extracted with the solvent mixture of ethanol, ethyl ether and petroleum ether as used for whole milk, while the lipid in skim milk was readily extracted with these solvents. A revised procedure using higher proportions of ethanol and petroleum ether was found to be necessary in order to denature the lipoprotein complex of buttermilk or butter serum and to recover a greater quantity of lipid. If only a portion of the lipid is extracted, the subsequent analysis of residue

based on that portion of lipid would have a higher concentration of pesticide, as discussed earlier.

For the above reasons, it is apparently suggested that the factors influencing the distribution of dieldrin in skim milk and buttermilk are different. The present results demonstrated that the lower levels of residue in buttermilk and butter serum were probably related to the high levels of phospholipid content or high-melting glyceride content or both in these fractions. Whereas the protein moiety seemed to have played an important role in contributing the residue concentrations when expressed on a fat basis in the extremely low fat fraction, such as refined skim milk.

The variations of the residue levels in buttermilk among the data of earlier workers (Hugunin and Bradley, 1969; Li et al., 1970; Langlois et al., 1964b) and between their data and the present results might be due to the differences in the separation techniques applied, the fat contents of buttermilk fractions, and the amounts of total lipid extracted from the buttermilk. One difference in the separation techniques used was that they used pasteurized unwashed cream to make buttermilk (Li et al., 1970; Langlois et al., 1964b) while a raw and thrice-washed cream was used in the present study. The composition of those buttermilk fractions might be quite differently, and thus influenced the distribution of residues differently.

SUMMARY AND CONCLUSIONS

1. Various methods were compared for the extraction of dieldrin in milk. A method of the Food and Drug Administration (FDA) and the Mojonnier method provided a comparable extraction efficiency for lipid as well as for pesticide. The surfactant method recovered less fat from milk, and slightly lower levels of residue in the isolated fat. A mild alkaline hydrolysis technique was comparable with the FDA method for residue analysis, but needed an independent determination of fat content in order to express the residue concentrations to be on a fat basis. A strong alkaline hydrolysis technique provided lowest recovery of dieldrin in milk.
2. Differential solvent extraction of milk showed that the first fraction, that extracted with non-polar hydrocarbon solvents, contained much higher concentrations of dieldrin than the second fraction, which was subsequently extracted with ethanol and non-polar solvents. It appeared that dieldrin was more readily extracted with hydrocarbon solvents than were the milk lipids. However, the first fat fraction only accounted for about one-half of the total quantity of dieldrin

in milk. This incomplete recovery of dieldrin might be due to the low recovery of solvents used for this extraction or due to the same factor that depressed the recovery of solvents from milk. The lipid-depleted portions essentially contained no residue.

3. Milk containing physiologically incorporated or added dieldrin was separated into various fractions by physical methods. Generally, the distribution pattern of this pesticide was quite similar in the milk system between the two sources of contamination. This observation has led to a proposal that the results of experiments involving dieldrin in milk from either source might be the same.

4. The relative amounts of dieldrin in various fractions closely resemble the relative amounts of total lipid in these fractions and both of them are significantly varied with the separation techniques used. Unwashed cream accounted for approximately 85-87% of the total amount of pesticide in milk, and skim milk accounted for about 10-15%, while refined skim milk accounted for only 1-3% of the total residue in milk. Relative recoveries of dieldrin in other fractions were: washed cream, 65-80%; butter, 52-78%; butteroil, 51-78%; buttermilk, 0.4-2.0%; and butter serum, 0.05-0.08%.

5. Comparable levels of dieldrin on a fat basis were found in whole milk, skim milk, cream, washed cream, butter and butteroil. Lower levels of residue were observed

in buttermilk and butter serum, and even lower values were found in the refined buttermilk and butter serum, and in membrane pellets; although no consistent distribution pattern was observed among the three pellet fractions of 7,500 S, 230 S and 35 S. An inverse correlation of the residue levels with the levels of phospholipid or high-melting glyceride apparently exists. The slightly higher concentrations of dieldrin on a fat basis observed in refined skim milk might be due to the slight solubility of this pesticide in the serum of milk. From another point of view, the residue concentrations on a fat basis as well as on a weight basis for low-fat fractions are significantly related to the total fat content of these fractions.

6. The recovery of added dieldrin in butteroil might be due to the re-orientation during separation processes. Similarly, the low concentrations of dieldrin in buttermilk or butter serum may not necessarily prove that such a substance is less preferentially deposited in these fractions in the natural original milk system. Nevertheless, it showed that dieldrin has a tendency to be distributed more favorably with the neutral lipids or free fats in milk.

7. Control milk was also fractionated and analyzed. However, the quantities of residue in these samples were too low to be determined accurately. Although skim milk, buttermilk and butter serum appeared to contain

higher levels of residue on a fat basis as compared to other fractions, they contained extremely low concentrations of residue on a fraction weight basis. In addition, they exhibited more interference on the GLC chromatogram. Therefore, no firm conclusions on the distribution of dieldrin can be drawn from the analysis of control milk fractions.

PROPOSALS FOR FURTHER RESEARCH

1. Determination of dieldrin metabolite in milk.

The metabolites of DDT, such as DDE and DDD, have been found in milk, and their relative distribution in various phases during the manufacture of cheese have been reported. However, no study has shown the presence of a metabolite of dieldrin in milk. Reports concerning the identification or isolation of some dieldrin metabolites were from samples of urine, or feces of rats, rabbits or sheep. It would be desirable to determine the possible metabolites in a milk system, and furthermore to determine their distribution in various fractions (which resembles various milk products); because some metabolites are more toxic while others are less toxic than the parent compound. Some metabolites as reported are relatively hydrophilic; the excretion of these substances in the urine is favorable to the health aspect, while the possible excretion of them in the aqueous phase of milk would be unfavorable. Therefore, studies should also include the analysis of any possible hydrophilic metabolites and a different procedure may be necessary for the extraction and determination of these

substances. The use of radioactive labeled pesticide chemicals would have advantages in these studies.

2. Effect of enzyme systems on the pesticide residues in milk, and vice versa.

Milk is known to contain a number of enzymes, such as lipase, esterase, phosphatase, xanthine oxidase, and lactoperoxidase. Studies have shown that some liver enzymes have some effects on DDT and dieldrin; similarly the milk enzymes may also affect more or less on pesticide residues, and consequently they (enzymes) may influence the final quality and quantity of residues in the finished milk products processed and stored differently.

Some pesticides are reported to have an effect on natural enzyme activities in birds, rats and rabbits. The effect could be either an enhancing or inhibitory mechanism. In any case, it would be of value to know whether pesticide chemicals may influence the milk enzyme systems, because these may have a great effect on the quality of milk and milk products.

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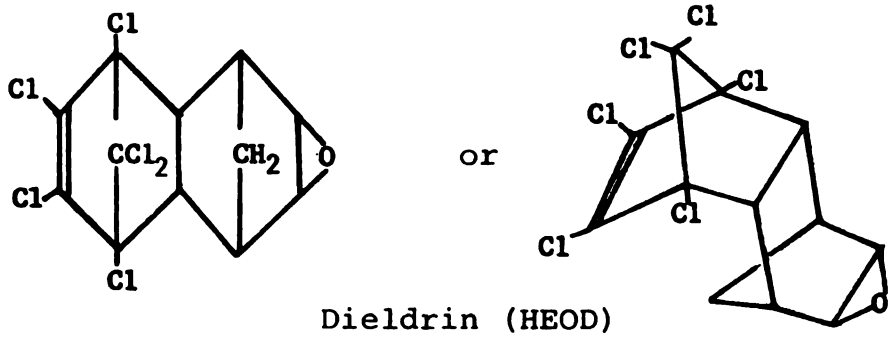
APPENDIX

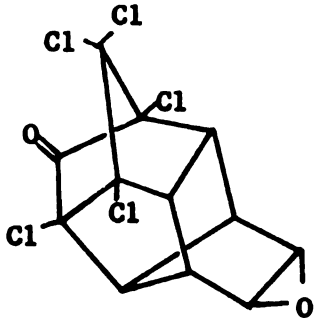
CHEMICAL NAMES OF SOME IMPORTANT CHLORINATED PESTICIDES AND THEIR DERIVATIVES

Pesticide	Chemical Name
Aldrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8-dimethano-naphthalene
BHC	1,2,3,4,5,6-hexachloro-cyclohexane, mixed isomer (Benzene hexachloride)
Chlordane	1,2,4,5,6,7,8,8a-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindan
DDD(TDE)	1,1-dichloro-2,2-bis (p-chlorophenyl) ethane
DDE	1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene
DDT	1,1,1-trichloro-2,2-bis (p-chloro-phenyl) ethane
Dieldrin (HEOD)	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphalene (Technical dieldrin contains not less than 85% of the above ingredient)
Dieldrin Metabolite I	1,1,2,3a,7a-pentachloro-5,6-epoxydeca-hydro-2,4,7-metheno-3H-cyclopenta [a] pentalen-3-one
Dieldrin Metabolite I'	1,9,10,10,11-exo-pentachloro-4,5-epoxy-pentacyclo-(7,3,0,$0^{2.6}$,$0^{3.8}$, $0.^{7.11}$)dodecan-1,2-one
Dieldrin Metabolite II	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-4a(or 5)-hydroxy-exo,1,4-endo-5,8-dimethano-naphthalene

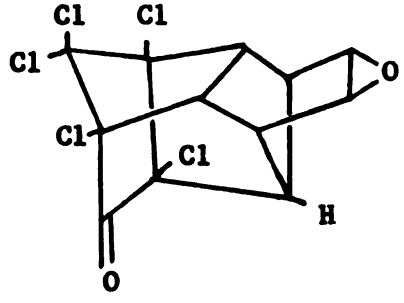
Dieldrin Metabolite II'	9(syn-epoxy)hydroxy-1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-5,8-exo-dimethanonaphthalene
Dieldrin Metabolite III	1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-dihydroxy-exo-1,4-endo-5,8-dimethanonaphthalene
Endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-endo-5,8-dimethanonaphthalene
Heptachlor Epoxide	1,4,5,6,7,8,8a-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan
Lindane	1,2,3,4,5,6-hexachloro-cyclohexane, gamma isomer



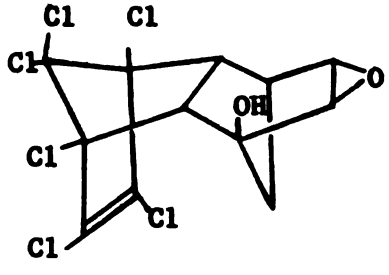
Dieldrin (HEOD)



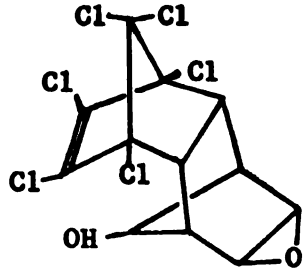
Metabolite I



Metabolite I'



Metabolite II



Metabolite II'

Metabolite III has the epoxide ring of dieldrin replaced by two -OH groups.

Figure 4.--Chemical structures of dieldrin and its major metabolites (see text for references).

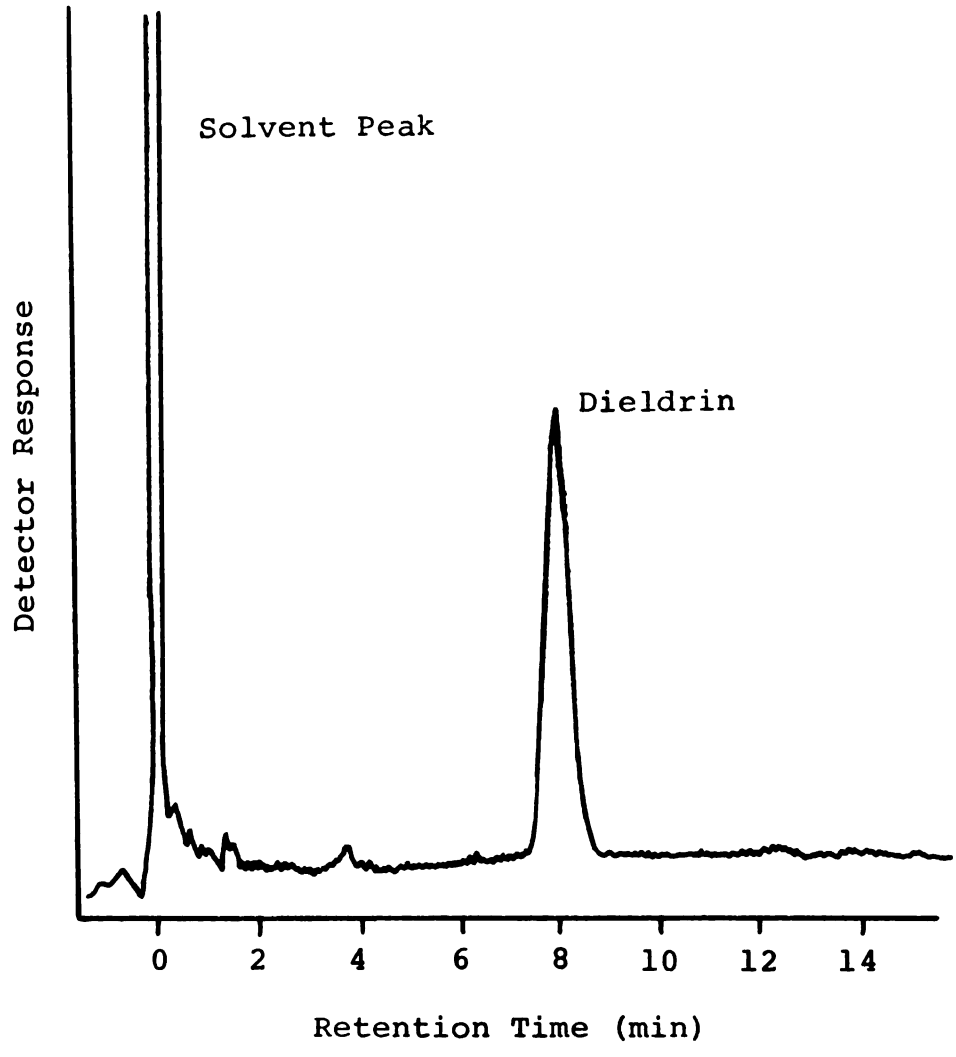


Figure 5.--A typical gas-liquid chromatogram for determination of dieldrin in milk. (GLC conditions: Hewlett-Packard model 5750B gas chromatograph; electron capture detector; 3.8% SE-30 on 80/100 mesh Diatoport S column.)