ABSTRACT

POLYBROMINATED BIPHENYLS IN RAW MILK AND PROCESSED DAIRY PRODUCTS

by

Teiko Murata

The present study was aimed at the investigation of the distribution of PBBs in dairy products and the effect of processing on the residue levels. Milk containing less than 0.3 ppm of physiologically incorporated PBBs from each of four dairy herds was separated by physical methods into skim milk and cream as well as being further processed into pasteurized whole milk, pasteurized skim milk, pasteurized cream, butter, cheddar cheese, freeze-dried whole milk, freeze-dried skim milk, and freeze-dried cream. Spray-dried skim milk and whole milk and evaporated whole milk were manufactured individually from milk of two of these herds.

PBBs were extracted using AOAC procedures for chlorinated hydrocarbon pesticides and quantitated with electron capture GLC. The PBB contents of the by-products, buttermilk and cheese whey, and the raw whole milk, skim milk, and cream were also measured. Moisture and lipid contents of all

the manufactured products were also determined.

PBBs were found to be concentrated in the high-fat content products and skim milk, buttermilk, and cheese whey had very low levels of these compounds on a total weight basis. However, when the concentration of the PBB residues in these products was expressed on a fat basis, they had slightly higher levels than that which occurred in the pasteurized whole milk suggesting PBBs may be associated with lipoprotein or may be soluble in the serum of milk.

The variations in levels among the raw and processed dairy products were not significantly different when the PBB levels were expressed on a fat basis.

Condensation was not effective in the removal of PBBs from whole milk while spray-drying appeared to significantly promote losses of PBBs from whole milk (30 - 40%) and skim milk (60 - 69%). The higher losses of residue observed in spray-dried skim milk suggest PBBs are more easily removed from low-fat content products and when the concentration in the original product is low.

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INTRODUCTION

The growing concern over environmental contamination has undoubtably stemmed from the rapid influx of synthetic compounds and their accompanying waste materials. Of these compounds, much attention has been focused on the halogenated hydrocarbons. Organochlorine pesticides and polychlorobiphenyls, with their lipophylic properties, have been found in the fat of virtually all animals. They resist biological degradation and consequently are expected to be present in the ecosystem for an extended period of time. Occurrence of chlorinated hydrocarbon residues are well documented (Abbott et al., 1966; Anderson et al., 1969; Holden et al., 1967; Holmes et al., 1967).

Polychlorinated biphenyls (PCB) have been used industrially in the manufacture of resins, rubbers, plastics, building materials, paper, electrical equipment, hydraulic fluids, etc. (U.S. Dept. of HEW, 1969) and have been mentioned as capable of extending insecticide kill-life (Sullivan et al., 1953; Lichtenstein et al., 1969). They were first identified by Jensen (1966) and since then PCB residues have been detected almost the world over, in fish, birds, and wildlife (Holmes et al., 1967; Reynolds, 1969; Jensen et al., 1969; Risebrough et al., 1968; Peakall et al., 1970; Anderson et al.,

1969; Holden et al., 1967), and in humans (Musial et al., 1974; Savage et al., 1973; Doguchi et al., 1975; Biros et al., 1970).

Human exposure to these contaminants has generally been through their residues in foods. Surveys of foods indicate varying amounts of organochlorine pesticide residues in many commercial foods (U.S. Dept. of HEW, 1969) and food contamination by PCBs has been reported (Kuratsune, 1969; Kolbye, 1972; Fujiwara, 1975).

Polybrominated biphenyls (PBB), currently manufactured by Michigan Chemical Corporation, are members of the halogenated hydrocarbon class of compounds with properties and uses closely related to PCBs. They are not present in the ecosystem in as large amounts as PCBs and would not have been a subject of great concern if the accidental contamination of Michigan cattle and other farm animals with this product had not occurred.

PBBs were mistakenly used in place of magnesium oxide in dairy feeds (Jackson et al., 1974) and this incident has led to their occurrence in milk. The FDA has set the actionable PBBs residue level in milk at 0.3 ppm (fat basis). Although there is no evidence indicating low levels of contaminant chemicals are detrimental to man, it seems prudent to minimize concentration in foods, until more conclusive data firmly establish safe levels.

Since milk is one of the major sources of human foods, it is important to investigate the possibility of eliminating or

reducing any contaminant level in this product. Some studies have shown the effectiveness of removal of halogenated compound residues from milk. Chlorinated hydrocarbon pesticides have been removed from milk by molecular distillation (Bills et al., 1967), by roller-drying of whole milk (Ruzicka et al., 1967), and by spray-drying of whole milk (Li et al., 1970). The distribution of PCBs in dairy products and the effective decrease of their residue concentration by heating skim milk was reported by Platonow et al. (1971), however, information concerning the distribution of polybrominated biphenyls in dairy products and their removal during processing of milk is non-existent. Thus, this project is focused on the possibility of eliminating or reducing the PBB residue levels in the processing of PBB contaminated milk as well as studying their distribution in related dairy products.

REVIEW OF LITERATURE

Polybrominated biphenyls (PBBs) are fairly new chemicals introduced essentially as a fire retardant for the manufacture of safe end-products. They belong to the halogenated hydrocarbon class of chemical compounds and share many chemical properties with polychlorinated biphenyls (PCBs) and organochlorine pesticides.

Uses and characteristics of PBBs are presented in this review. Similarities of the properties of PBBs to PCBs are summarized. The parallelism between reactivity of PCBs and many of the chlorinated hydrocarbon pesticides has been extensively documented. Since there have been no studies on the effect of processing on PBB levels in foods, studies of the effect of processing of dairy products on the levels of PCBs and chlorinated hydrocarbon pesticides are also reviewed.

Polybrominated Biphenyls

Physical and Chemical Characteristics - Uses

PBBs reduce the flammability of thermoplastics. This property, in addition to their low cost, has led to their production on a commercial scale. Michigan Chemical Corporation currently manufactures a product under the name of Fire Master® BP-6 which is a mixture of brominated biphenyls with

approximately 63% of hexabromobiphenyls, 14% of heptabromobiphenyl, 10.5% of pentabromobiphenyl, 2% of tetrabromobiphenyl, and others, unidentified (Kerst, 1974). This product is employed in industries as a plasticizer and fire retardant. Its specific uses are summarized in Table 1.

Fire Master® BP-6 is solid at room temperature with a softening point of 72°C. It shows very low solubility in water, 11 ppb at 25°C, but it is soluble in most organic solvents (Kerst, 1974).

Studies concerning stability of brominated biphenyls indicate Fire Master® BP-6 undergoes reductive dehalogenation when exposed to ultraviolet light under certain conditions (Ruzo et al., 1975). This reaction has also been reported to occur with mono- and di-brominated biphenyls (Bunce et al., 1975) and octabromobiphenyls (Norris et al., 1974). The photoreactivity of this class of compounds has been shown to be greater than that of the chlorinated biphenyls. This increased reactivity is probably attributable to the lower C-Br bond energy (Ruzo et al., 1975; Bunce et al., 1975) which is 71 kcal/mole compared to the C-Cl bond energy of 85.6 kcal/mole (Kerst, 1974).

Residues in the Environment

Reports on the detection of PBB residues in the environment are so far non-existent. However, since these compounds are closely related to PCBs, both in uses and chemical properties, it is expected they may escape into the

Table 1. Specific industrial uses and approximate allocations of Fire Master® BP-6 produced (Kerst, 1974).

Industrial use	App. allocation of total Fire Master® BP-6 produced, %	Examples
Business machines and industrial equipment	48	Typewriter, calculator and microfilm reader housings; business machine housings.
Electrical	35	Radio and TV parts, thermostats, shaver and hand test housings.
Fabricated products	12	Project housings, movie equipment cases.
Transportation	1	Miscellaneous small automotive parts, i.e. electrical wire connectors, switch connectors, speaker grills
Miscellaneous	4	Small parts for electrical applications, motor housings, components for industrial equipment.

environment in similar routes as that of the PCBs.

Residues in Biological Systems

The accidental use of Fire Master® BP-6 in place of magnesium oxide in feed led to the inadvertent contamination of numerous Michigan dairy herds, swine, and poultry during the fall of 1973 and the winter of 1974 (Jackson et al., 1974; Gutenmann et al., 1975; Detering et al., 1975; Prewitt et al., 1975).

Jackson and Halbert (1974) were the first to document a toxic syndrome in a herd from one Michigan dairy farm caused by intake of polybrominated biphenyls contaminated feed. These compounds were identified by a U.S.D.A. laboratory and were further traced to the same manufacturer who supplied magnesium oxide to the feed company. By July, 1974, 92 food producing premises involving cattle, poultry, and swine were identified as contaminated by PBBs and quarantined. A further euthanasia program was planned to eliminate the contaminated animals.

Several works concerning PBB residues have shown that chemical is excreted into the milk of cows (Gutenmann et al., 1975; Fries et al., 1975; Willet et al., 1975; Detering et al., 1975). Appearance of PBB in milk, plasma and feces were observed by Willet and Irving (1975) after intraruminal doses of the product were given to cows. In the experimental cow and sheep feeding with PBB at levels of 50 ppm, Gutenmann

et al. (1975) found the half-life for PBB in milk was about 10.5 days which would give, after 105 days, a total daily excretion of less than 0.01 mg/day of this compound. They also reported the distribution of PBB residues in the tissues with highest concentrations of PBBs being in fatty tissues. The content of PBBs in blood, milk, and body fat of cows contaminated 7 to 9 months previously showed that body fat and milk fat contained 600 and 300 times, respectively, of that which the blood contained (Detering et al., 1975).

Fries and Marrow (1975) studied the excretion of Fire Master® BP-6, PCB, and DDE into the milk of cows. They found the rate of disappearance of PBB in milk was 58 days, with mode of excretion (decay rate and steady state excretion levels) being similar to those for PCBs and chlorinated hydrocarbons.

A study conducted by Fries et al. (1973) concerning polybrominated biphenyls and PCBs in hens and cows, had previously shown that residues of PBBs in animal systems behave similarly to the residues of PCBs with a comparable degree of halogenation.

Toxicity of PBBs

The varying degree of toxicity of polybrominated biphenyls in animals is directly correlated to the concentration of these chemicals at which the animals are exposed. The oral lethal dose for Japanese quail exceeds 1.0 g/kg (Strik, 1973), for rats the value found is in the range of

21.5 g/kg (Michigan Chemical Corp.), and the acute dermal LD_{50} for rabbits is between 2.15 - 10.0 g/kg (Michigan Chemical Corp.).

Liver lesions were the most common symptoms of acute and chronic toxic effects seen in the Michigan cattle (Jackson et al., 1974). These were seen when the level of PBBs in the body fat were approximately 200 ppm. Hematomas and abscesses in the peritoneal and thoracic cavities of these cows were also observed.

From field observations of PBB contaminated cattle, Prewitt et al. (1975) reported that animals which had over 20 ppm in the milk fat, at parturition, had unrelaxed pelvic ligaments and the calves were stillborn or died shortly after birth. Metrites and retained placentas as well as liver and kidney adhesions were also common. Examinations of dead calves showed PBBs were transferred through the placenta (Detering et al., 1975). PBB concentration in the body fat of these calves was in the range of 50 to 400 ppm.

Strik (1973) reported hexabromobiphenyl fed to Japanese quail and chicken induced porphyria, especially in the liver. Hepatic microsomal enzymes of Japanese quail were induced by dietary PBB at 10, 20, and 100 ppm (Babish et al., 1975). Hatchability was affected by the diet which contained 100 ppm PBBs and 40% of these embryos died in the first day or two of development. However, when the feed contained 10 and 20 ppm of PBBs no effect was observed.

Farber and Baker (1974) found that hexabromobiphenyl

was a more potent microsomal enzyme inducer than PCB (Arochlor^{R} 1254) at 5 ppm level in rats.

PBBs were also found in the blood of farmers exposed to the contaminated feed (Chem. Eng. News, 1975), but these farmers appeared to be healthy.

Polychlorinated Biphenyls - Chlorinated Hydrocarbons

Occurrence and Reactivity

The world wide occurrence of PCBs in the marine and aquatic environments is fully documented. Studies indicate that sources of these chemicals are diffuse and generally associated with waste disposal materials (Carnes et al., 1972; Schmidt et al., 1971; Fujiwara, 1975; Martell, 1975).

Toxicological studies with PCBs have shown they have similar mode of action to that of chlorinated hydrocarbons. They promote abnormal changes in the liver and induce many microsomal enzymes (Turner et al., 1974; Holub et al., 1975; Villeneuve et al., 1971; Grant et al., 1971). PCBs with higher chlorine content appear to have greater effect in enzyme activity (Rhee et al., 1973; Iverson et al., 1975) and are more resistant to biological degradation than the low chlorinated biphenyls (Greb et al., 1975; Yoshimura et al., 1975; Jan et al., 1975).

A number of studies have shown that humans contain low

levels of halogenated compounds. Biros et al. (1970) found polychlorinated biphenyls and DDE in human adipose tissue. Comparable results were obtained in Japan, in the work conducted by Fujiwara (1975). The adipose tissues contained PCBs, DDT, DDE, and several BHC isomers. He pointed out that the PCB levels were similar to the levels found in Germany but higher than those in the United States.

Analyses of human blood showed varying concentration of PCBs, BHC, DDT, and DDE residues (Doguchi et al., 1975; Fujiwara, 1975). The levels of PCB were assumed to be associated to the degree at which the sampled people were exposed since the residue concentrations were proportionally higher in more severely contaminated areas.

Savage et al. (1973) detected PCBs in human milk in rural Colorado, although the amounts were not appreciable. However, in Japan the PCB concentrations found in human milk were relatively high and it was estimated that babies would ingest between 4.8 - 5.3 µg/kg body weight of PCBs per day (Fujiwara, 1975). Musial et al. (1974) reported that milk from people of provinces of Canada contained PCBs as well as DDT, and DDE.

These evidences, in addition to several documented works of the occurrence of organochlorine pesticides in human tissues (Brown, 1967; Abbott et al., 1968; Dyment et al., 1971) indicate the ultimate deposition of these chemicals in man.

The sources of human contamination has generally been associated with ingestion of food containing halogenated

compounds (Kolbye, 1972; Kuratsune, 1969). Reported results of surveys of food items conducted in the United States (Duggan et al., 1967; Duggan and Lipscomb, 1969; Lipscomb, 1968) and in Japan showed that low levels of PCBs and several organochlorine pesticides are present in most of the commercial food. Marth and Ellickson (1959) have reported that 25 to 62% of market milk supplies contained varying amounts of chlorinated hydrocarbon insecticides. Cummings (1966) has shown that the organochlorine pesticides dieldrin, heptachlor epoxide, lindane, and DDT were present in dairy products from market basket studies, although the levels were very low.

Milk Contamination

Several studies have documented the transfer of chlorinated hydrocarbon pesticides from the feed into the milk of dairy cows. It has also been shown that spray treatment of cows with these pesticides led to their appearance in the milk by entering into cows through the skin.

Marth et al. (1959) had suggested that insecticide residues in milk are primarily a result of spraying dairy barns and cattle and of ingestion of treated forages by dairy cattle.

The major concern about chlorinated hydrocarbon pesticides is that once the cows are exposed to these chemicals the residues will be present in the milk for a long period of time as a result of storage in the fat body tissues and

gradual translocation into milk from these tissues.

Similar behavior of polychlorinated biphenyl residues has been pointed out by Fries et al. (1972). They reported that the rates of decline in milk concentrations of PCB and DDE residues after environmental exposure were identical. In the long-term studies of PCB residues retention and excretion by cows, Fries et al. (1973) found that the rate of decline in milk fat of PCB concentration was 50% within 15 days after removal of the chemical from the diet. By comparison with DDE from previous data (Fries et al., 1969) they showed the similarity of behavior of DDE and PCB in the accumulation and excretion in cows. They also observed that the decrease, in concentration in body fat, paralleled the decrease in concentration in milk fat.

Although retention and excretion of polychlorinated biphenyls into milk has been well documented, the precise sources of contamination are not known. Some studies have indicated contamination of cows might occur through ingestion of feed containing these compounds. Savage et al. (1973) reported the presence of PCB residues in silage stored in pits and upright silos although they were at very low levels. Similar results were obtained by Skrentny et al. (1971) from silage stored in upright silos. They stated that the PCBs had leached into the silage from the sealants used to coat the silo walls.

Furr et al. (1974) reported that waste paper has been incorporated into farm animal feeds to serve as a substitute

form of cellulose in the ration. In their experimental feeding the cows with waste paper, they observed PCBs were transferred from the paper to the milk. Moreover, there was an evident increase in the concentration with the increase in the length of time of feeding.

Stanovick et al. (1973) pointed out that foodstuffs might get PCB contamination from paperboard which contains recycled waste paper with appreciable amounts of carbonless "carbon" paper. They suggested PCBs would migrate from the paperboard into the food.

Effect of Processing on Residues

Studies concerning the possibility of removing halogenated compounds from foodstuffs upon processing have stemmed from the possible chronic effects of continued assimilation and accumulation of these compounds in the body fat.

Several studies concerning the effect of residues on dairy processing have been conducted. Kim (1969) reported that no effect was observed on the growth of lactic starter cultures in milk containing different organochlorine pesticides. On the other hand, Bradley and Li (1968) observed that dieldrin slightly reduced the production of acid by a lactic culture organism in making cheddar cheese. Pasteurization had very little effect on the DDT residues in dairy products (Mann et al., 1950). Langlois et al (1964, 1965)

manufactured several dairy products and reported that the concentrations of the pesticides studied remained fairly constant. However, they observed significant losses of DDT and lindane during the spray-drying of whole milk and loss of lindane only during the roller-drying. They also observed lower insecticide content in butter and cheese than the raw milk on a fat basis.

Li and Bradley (1969) observed that ultraviolet light treatment of milk and butteroil reduced the levels of chlorinated hydrocarbon pesticide residues, but the treatment rendered the milk unacceptable for consumption.

Reduction of organochlorine pesticide residues upon freeze drying of milk at a system pressure of 1×10^{-3} torr was suggested by Kiermeier et al. (1967). However, freeze drying at a pressure of 0.1 mm Hg and mild deodorization were not effective on the reduction of the dieldrin and heptachlor epoxide levels in butteroil (Kroger, 1968), but heating at very high temperature (300°C) and steam deodorization at 180 - 195°C and 0.01 - 0.5 mm Hg significantly reduced the amount of the residues. Ledford et al. (1968) found that commercial steam distillation-vacuum processing was also fairly effective on reduction of lindane, but little or no effect was observed for dieldrin, DDT and heptachlor in milk.

Li et al. (1970) reported that manufacture of 30% cream, butter, cheese, spray-dried whole milk, condensed whole milk, and pasteurization and sterilization in general did not affect the amounts of pesticide residues in these products.

On the other hand, spray drying reduced dieldrin, lindane, and chlordane, and sterilization of condensed milk significantly removed dicofol while storage of cheddar cheese for six months slightly reduced dieldrin content. In addition, they observed that skim milk, buttermilk and whey contained higher concentrations of these pesticides than the whole milk on a fat basis.

Bills and Sloan (1967) found that molecular distillation at high temperature (200°C) and under reduced pressure (5 x 10^{-4} torr) was very efficient in removal of lindane, heptachlor epoxide, heptachlor, aldrin, DDT, DDD, and DDE from butteroil.

A possible degradation of DDT and DDE by cheese microorganisms was studied by Ledford and Chen (1969). Their results show that aerobic growth of geotrichum species caused almost complete disappearance of the DDT and DDE residues.

Platonow and Funnell (1971) studied the effect of processing on the levels of metabolically incorporated polychlorinated biphenyls. They pointed out that the manufacturing processes did not significantly alter the residue levels. However, heating skim milk at 70°C and holding for 10 minutes reduced the amount of PCB by approximately 67%. They also observed that most PCB was present in the cream and whole milk although the actual amounts were not always proportional to the fat content. This fact was indicated by the higher concentration found in whey per fat unit than any other sample.

Losses of 15 to 25% of several organochlorine pesticides from milk during roller drying were reported by Ruzicka et al. (1967). Residues in skimmed milk were negligible.

Stemp and Liska (1966) found that during the processing of concentrated, dried, and sterilized dairy products 40-50% of the telodrin residues were destroyed in evaporated milk and 10-20% in the dried whole milk. However, methoxychlor was more stable under the milk-processing treatments. They also observed a higher concentration of telodrin in the cream on a fat basis when compared to raw whole milk while methoxychlor remained fairly constant.

Comparable effects were obtained by Liska (1968) during heat treatment of milk containing chlorinated insecticide residues. Spray drying destroyed more than 80% of the lindane residues and heptachlor was destroyed more easily than heptachlor epoxide. He concluded that the amount of residue destruction varied with the treatment used and nature of the insecticide residue.

The effect of storage of Monterrey and cheddar cheese on the chlorinated hydrocarbon residue level was found to be not effective by Montoure and Muldoon (1967). However, they observed a change in the residue distribution during manufacturing of the cheeses. DDT was not detected in whey samples at dipping while high levels of DDE and TDE were evident. In all other phases the distribution of the residues was quite similar.

EXPERIMENTAL PROCEDURE

In order to investigate the relationship between the polybrominated biphenyls (PBB) residue levels in milk and the processing of the milk, environmentally contaminated milk with less than the actionable level (below 0.3 ppm) of PBB was processed into a variety of dairy products.

Many Michigan dairy farms had their herds accidentally contaminated through feedings with feed containing PBB. The Michigan Department of Agriculture has been monitoring these farms and the milk from them is being analyzed periodically. From these monitoring data, herds with low levels of PBB were identified. Milk was purchased from four of these herds to be processed into dairy products. It was collected at the farms in stainless steel milk cans and brought back to the laboratory (iced) and kept refrigerated until processing.

In June, 1975, the Michigan Department of Agriculture data showed PBB levels for the milk collected as follows (Van Patten, 1975):

Herd	Collection Date	PBB Level, ppm (fat basis)
1	June 26	0.223
2	July 6	0.138
3	July 21	Trace
4	July 23	0.132

Separation of Milk Fractions by Physical Methods

Milk was separated into skim milk and cream with a Westfalia disc-type separator, model LWA 205, at approximately 6000 rpm. The separator was first warmed up with hot water (60°C) and fed with milk at temperatures of 60-62.8°C. The resulting cream was measured volumetrically and the yield of skim milk was estimated by the difference between the whole milk and cream.

Duplicate quantitative portions of each fraction and whole milk were taken after thorough mixing and stored in glass bottles at -23°C for further analysis.

Pasteurization

Immediately following separation, the whole milk and skim milk were pasteurized at 62.8°C (145°F) for 30 minutes in a Kusel culture cabinet and the cream was pasteurized at 71.1°C (160°F) for 30 minutes, according to the "Milk Ordinance and Code" of the U.S. Dept. of HEW (1953).

A measured portion of the pasteurized cream was stored at 2.5°C in a glass beaker, covered with wrapping film, and kept for at least 12 hours for further butter processing.

Approximately one-quarter of the cream, whole milk, and skim milk was stored in freeze-drier trays at -23°C and the remaining portions were kept refrigerated for further

processing, after two aliquots were taken for lipid and residue analysis.

Butter

The separated cream fraction was allowed to reach 5°C and then churned in a Kitchen-Aid, model K5-A, at approximately 240 rpm for 30 minutes or until separation of the buttermilk from butterfat occurred.

Following collection and yield measurement of the buttermilk, the butterfat was washed once with cold tap water, wrapped in a double layer of cheese cloth and gentle pressure was applied manually to remove the excess of water. The butter was wrapped in a plastic wrapping film and kept frozen at -23°C.

Cheese

A stirred curd cheddar type cheese was made from pasteurized whole milk following the procedure outlined by Kosikowski (1966). Lactic culture and rennet extract were obtained from the Dairy Plant of Michigan State University. A stainless steel container with dimensions of 24 cm x 30 cm x 6 cm and equipped with a spicket was used as a cheese vat and heated with a water bath.

Twenty grams of lactic starter culture (1% of Streptococcus lactis and Streptococcus cremoris secondary culture)

were added to approximately 2000 g of warm (31.1°C), whole pasteurized milk. This was stirred well and left for 15-30 minutes at 31.1°C (88°F), with continuous agitation. After the ripening period, 0.4 ml of single-strength rennet extract diluted to 10 ml with distilled water were added, stirred and left for 45 minutes at 31.1°C (88°F), or until the appropriate strength of the curd for cutting was obtained. The curd was cut into small cubes with approximately 6.5 mm (1/4 in) per side, healed, and then with continuous agitation of the curd the temperature was slowly raised to 38.8 - 39.4°C (102 - 103°F). After the temperature of 38.8 - 39.4°C was reached, the curd was cooked for approximately 45 minutes more or until an acidity of 0.13 - 0.14% of lactic acid was attained. The whey was then drained to the level of the curd.

The acidity was determined by titration of 10 ml of whey with 0.1N NaOH using phenolphthalein as an indicator.

The whey was completely drained after approximately 30 minutes and cooking was allowed to continue with agitation of the curd every 5 - 10 minutes until the percent lactic acid was 0.23 - 0.25%. During this period, an aluminum foil cover was used to keep a homogeneous temperature through the entire curd. After the recommended acid value was reached, 5 g of coarse cheese salt (2.5% of the finished product) were spread over the curd and stirred for 10 minutes. The stirred curd was placed in a circular metal mold with a proper sized cheese cloth fixed at the bottom and covered with the cheese cloth followed by an adapted wood rim. Pressures of

24.8 torr (0.48lb/sq in) and 38.3 torr (0.74 lb/sq in) were applied consecutively for a one hour period each and then the cheese was dipped into hot water (60°C) for approximately 3 minutes, placed back in the hoop, and soaked liberally with the hot water. By this procedure the curd was softened sufficiently so that it could be efficiently molded. A final pressure of 43.9 torr (0.85 lb/sq in) was applied over the cheese and left overnight.

A fraction of the cheese whey at the time it was completely drained was taken for analysis. The cheese was weighed and the yield of cheese whey was estimated by the difference of the whole milk and cheese. It was cut into two halves and wrapped in plastic wrapping film. One half was allowed to age at 2.5°C for approximately 2 months and the other half was stored at -23°C.

Freeze-Drying

Portions of the pasteurized cream (900 - 1100 g), skim milk (1000 - 1300 g), and whole milk (900 - 1300 g) were placed in freeze-drier trays, giving a depth of approximately 6.5 mm (1/4 in). These were covered securely with plastic wrapping film, and frozen at -23°C overnight. The frozen samples were freeze-dried in a Virtis REPP freeze-drier, model FFD42 WS, for 24-30 hours with a system pressure of 5 μ (5×10^{-3} torr). The temperature of the platin was 65.6 - 71.1°C (150 - 160°F) giving a final product temperature

of 48.8 - 51.6°C (120 - 125°F).

The freeze-dried samples were removed from the trays, and stored in tightly sealed plastic bags at -23°C.

Spray-Drying and Condensation

Spray-drying and condensation processes were done for milk from Herds 1 and 2. The pasteurized whole milk and skim milk were forewarmed at 48.8°C (120°F) and dried in a Swenson spray-drier, model 175505. Air outlet temperatures ranged from 76.6 to 98.8°C (170 - 210°F) with the following specific conditions being used:

Herd 1	Skim milk	Lot 1	87.8 - 93.3°C
		Lot 2	76.6°C
	Whole milk		87.8°C
Herd 2	Skim milk	Lot 1	87.8 - 93.3°C
		Lot 2	87.8 - 98.8°C
	Whole milk	Lot 1	87.8 - 93.3°C
		Lot 2	85.0 - 90.5°C

For the condensation process, the pasteurized whole milk was heated to 54.5°C (130°F) and then condensed in a research model Rogers vacuum pan under vacuum of 635 torr (25 in of Hg) to approximately 35% total solids for the milk of Herd 2 and 60% total solids for Herd 1. The evaporated milk of Herd 1 was further diluted to give approximately 30% total solids.

Duplicate aliquots of the evaporated milk were taken and

stored in tightly sealed glass bottles at -23°C. The spray-dried samples were placed in plastic bags and kept frozen at -23°C until needed.

Chemical and Residue Analysis

All chemical and residue analyses were carried out in duplicate. The chemicals used were ACS reagent grade and the solvents were redistilled from glass. All glassware was acetone-rinsed followed by petroleum ether rinsing before being used. Standard solutions were prepared in petroleum ether using the commercial Fire Master® BP-6, hexabromobiphenyl (Lot No. 5143, Michigan Chemical Corporation, Chicago, Illinois).

Moisture Analysis

Moisture analysis was carried out in a Hotpack vacuum oven, model 633, at 100°C and a vacuum of 660 - 711 torr (26 - 28 in of Hg) to a constant weight. The percentage of total solids was expressed as the dry weight of the sample.

Lipid Analysis

Total lipid content of the samples was determined by two methods. One of these methods, the soxhlet method (official AOAC method) using petroleum ether was applied for

low moisture content samples after they were previously dried in a Precision Thelco air oven, model 18, at 70°C for 30 - 60 minutes. This method was also used for the dehydrated samples except for the spray-dried samples. The samples were extracted for 5 - 6 hours, the extract was first evaporated in a steam bath and then dried to a constant weight in a Lab line vacuum oven, model 3615, at 70°C and a vacuum of 660 - 711 torr (26 - 28 in Hg).

The second method involved the extraction of the crude fat for the high moisture content samples and spray-dried samples (dissolved in 50 ml of hot (60°C) distilled water) and utilized a petroleum ether-ethyl ether solvent mixture following the extraction procedure outlined in the Pesticide Analytical Manual (PAM) of the Food and Drug Administration, Section 211.13h (1971). The lipid fraction was extracted three times with petroleum ether-ethyl ether (1:1). Ethyl alcohol was proportionally adjusted to the volume of the samples, and potassium oxalate, approximately 1 g was used. The extracts were combined and washed with 500 ml of distilled water containing 30 ml of saturated sodium chloride solution, the water layer was discarded, and then washed once with 200 ml of 1% sodium sulfate solution. No centrifugation nor further drying of the extract by passing through a column of anhydrous sodium sulfate was involved. From this extract a 10 ml aliquot was taken and dried in a Labline vacuum oven at 70°C and vacuum of 660 - 711 torr (26 - 28 in Hg) for fat determination.

Residue Analysis

Extraction of the lipid fraction for residue analysis was done as previously described. Specific sample weights for the PAM method were as follows: evaporated milk, 13.0 - 27.0 g, diluted 1:2 with distilled water; spray-dried samples, 4.0 - 7.0 g; whole milk, 33.0 - 58 g; skim milk, 56.0 - 78.0 g; buttermilk, 65.0 - 83.0 g; and cheese whey, 65.0 - 78.0 g.

For the Soxhlet method the sample weights were: cream, 2.0 - 5.0 g; butter, 2.0 - 2.5 g; cheese, 1.5 - 5.5 g; freeze-dried whole milk, 3.5 - 4.5 g; freeze-dried skim milk, 4.5 - 6.0 g; and freeze-dried cream, 2.0 - 2.5 g.

The clean up procedures used are described in the Pesticide Analytical Manual, Section 211.14a, petroleum ether-acetonitrile partitioning, and Section 211.14d, florisil column. Modifications in these procedures were as follows: acetonitrile extract was washed with 400 ml of 1% sodium sulfate solution containing 100 ml of petroleum ether, shaking vigorously for one minute. The aqueous acetonitrile layer was discarded and the petroleum ether was collected in an erlenmeyer flask containing anhydrous sodium sulfate. The extract was concentrated to approximately 10 ml using a Kuderna Danish concentrator and then placed on a 23 cm (8 in) long Florisil column and eluted with 300 ml of 6% ethyl-ether-petroleum ether. This eluate was concentrated to approximately 10 ml as previously described and evaporated

to dryness using a N-Evap evaporator, model 111, under a stream of dried air. Before GLC analysis, exactly 1 ml of petroleum ether was added to each sample.

GLC Analysis

Gas chromatograph analyses were carried out using a Tracor GLC, model 560, equipped with a 63 Ni electron capture detector. The GLC was interfaced to a Digital PDP-8-Pamila GC data system. The column for GLC was a pyrex column, 1.83 m (6 feet) long x 4.0 mm i.d., packed with 3% OV-1 on chromosorb W 80/100 mesh H.P. The carrier gas was nitrogen with a flow rate of 40 ml/min. Temperatures at the injection port, column, and EC detector were 270°C, 240°C, and 300°C, respectively. Standards were injected at the beginning of each run, after every 5 - 6 samples and at the end of the run. Quantitations were based on the peak area of the standards (hexabromobiphenyl peak used) and concentrations were expressed on the wet weight, dry weight, and weight of the fat components.

In order to determine the percentage of recovery of the PBB residues by the utilized method, different levels of PBB (Fire Master® BP-6) were added to raw whole milk at concentrations of 0.06, 0.02, and 0.006 ppm based on the total weight. The samples were extracted and cleaned up in duplicate at each level according to the Food and Drug Administration method with the same modifications as previously described for the samples.

The recovered fractions and percentage of recovery are

shown in Table 2. The values obtained are slightly low in comparison to the percent recoveries obtained by Gutenmann et al. (1975).

The presence of the PBB residues was confirmed by ultraviolet and mass spectrometric analysis. Ultraviolet degradation test was applied to raw whole milk and freeze-dried whole milk of Herd 1, raw cream of Herd 3, pasteurized skim milk of Herd 2, and spray-dried whole milk of Herd 4. These samples and a PBB (Fire Master® BP-6) standard solution were exposed to ultraviolet light for 0, 15, and 30 minutes in a Rayonet photochemical reactor, model 1162, equipped with short wave (254 NM) UV lamps. Gas chromatographic analyses were carried out immediately after exposure with the same conditions as that for the samples. Mass spectrometric analysis was run on a pool of all of the extracted samples. A GC-MS-CPU system was used which consisted of a Beckman GC-65 gas chromatograph interfaced to a DuPont 21-490 Mass Spectrometer which was in turn interfaced to a Digital PDP-12 computer. The mass spectra was obtained at an ionizing voltage of 70 eV with a source temperature of 210°C.

Analysis of the Data

The PBB concentrations based on solids, total weight and on fat were analyzed for variance. Duncan's multiple range test (1957) was used to sort out significant differences

Table 2. Recoveries of PBB (Fire Master® BP-6) from milk[a] at different added levels by the Food and Drug Administration method.

PBB concentration in milk, ppm, wet weight basis		Percent recovery of PBB, ppm, wet weight basis
Added	Recovered	
0.006	0.00265[b]	44.17
0.02	0.01314	65.70
0.06	0.04295	71.58

[a] Raw whole milk, average fat content 4.1%.

[b] Values are average of two determinations, except one for the 0.02 ppm added level.

revealed by the analysis of variance.

The PBB residue contents of spray-dried products and condensed whole milk were analyzed by the t statistic (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

In order to investigate the effect of processing on the polybrominated biphenyl levels and their distribution in dairy products, milk from four dairy herds containing low levels of these compounds (<0.3 ppm) was processed into cream, skim milk, butter, and cheddar cheese. The effects of pasteurization, freeze-drying, condensation, and spray-drying processes were also studied.

Distribution of Fractions

The raw whole milk of each herd was first separated into the cream and skim milk fractions by using a conventional separator. The yields and percentage distribution of these fractions are reported in Table 3. The relative distribution of the milk components showed that the skim milk fraction accounted for most of the total weight with yields ranging from 85 to 93%. The percentage distribution of the cream fractions was fairly consistent for the herds except for Herd 1 in which case was lower. Variations in the feeding rate of the milk into the separator during the fractionation process can slightly affect the composition of the cream fraction such that better separation can be achieved

Table 3. Yields, percentage distribution and recovery of milk fractions.

Milk component	Herd 1		Herd 2		Herd 3		Herd 4	
	Weight	%	Weight	%	Weight	%	Weight	%
	g		g		g		g	
Unfractionated milk	39102.84		39117.98		19446.21		19474.56	
Skim milk	36224.85	92.60	34473.89	88.13	16648.37	85.61	16668.33	85.59
Cream	2841.28	7.27	4530.96	11.58	2901.15	14.92	2840.97	14.59
Recovery		99.87		99.71		100.50		100.10

with slower feeding rates resulting in cream fractions with higher lipid content but lower percentage distribution. In this study, yields of 7 to 15% of the cream fraction were obtained.

The yields and percentage distribution of the pasteurized cream fractions used in butter processing are summarized in Table 4. It was observed that the yields of butter were proportional to the butterfat content of the cream. Therefore, a percentage distribution of approximately 73% of the butter from the cream of Herd 1 (which had the highest fat content) was obtained. The yields of butter from Herds 2 and 3 were quite similar being approximately 23%. However, an unexpected higher relative distribution of butter, from Herd 4 (approximately 36%) was observed. After the churning period, the butter fractions were washed with cold tap water and the excess water was removed by wrapping the product in cheese cloth and applying pressure manually. Further moisture determination showed the butter from Herd 4 to have a higher moisture content which contributed to an apparent higher yield. This variation probably resulted from the inadequate removal of excess water.

As can be seen in Table 5, the yield of cheese manufactured from the pasteurized milk was fairly similar for three of the herds, ranging from approximately 10 to 11%, but that the yield from Herd 3 was slightly lower. Numerous factors may influence yields of cheese including casein and fat contents of the milk, quality of starter cultures and

Table 4. Yields, percentage distribution and recovery of cream fractions in butter making procedure.

Cream fraction	Herd							
	1		2		3		4	
	Weight	%	Weight	%	Weight	%	Weight	%
	g		g		g		g	
Unfractionated cream	922.8		948.5		958.1		952.9	
Butter	674.0	73.04	218.6	23.05	221.7	23.14	340.0	35.68
Buttermilk	246.7	26.73	735.1	77.50	734.2	76.63	613.5	64.38
Recovery		99.77		100.50		99.77		100.10

Table 5. Yields and percentage distribution of milk fractions in cheese making procedure.

Milk component	Herd							
	1		2		3		4	
	Weight	%	Weight	%	Weight	%	Weight	%
	g		g		g		g	
Whole milk	2000.00		2000.00		2000.00		2000.00	
Cheese	225.35	11.27	202.15	10.11	174.94	8.75	225.96	11.30
Cheese whey	1774.65	88.73	1797.85	89.89	1825.06	91.25	1774.04	88.70

rennet extracts, and conditions of processing. Care was taken to maintain processing conditions as similarly as possible for the four herds. Therefore, variations seen in the yields might be primarily attributable to the variations in the fat content which in turn affects the casein content (direct correlation).

Moisture and Lipid Analysis

Determinations of moisture and lipid contents were done on the raw milk and processed dairy products of each herd. Their individual and product averages are shown in Tables 6 (moisture content) and 7 (lipid content). In general, the average moisture content of the raw and pasteurized products were quite similar. Variability seen between the raw and pasteurized cream of Herd 1 was due to the techniques involved in the two milk fractionation batches. A second separation was done to obtain samples of raw cream and skim milk. Although the conditions of this fractionation process were maintained as closely as possible to those used in the first batch (which fractions were subsequently pasteurized), the feeding rates of these two batches may have differed. Consequently, variations in fat contents were also found between the raw and pasteurized skim milk of this herd. Differences in the lipid content of individual herds between raw and pasteurized whole milk and cream were primarily due to the difficulty found in homogenizing the thawed samples.

Table 6. Average moisture contents[a], expressed as percent of total weight.

Product	Herd				Product average
	1	2	3	4	
Whole milk (raw)	86.20	86.50	86.70	86.80	86.55
Whole milk (pasteurized)	85.70	87.80	88.60	87.10	87.30
Skim milk (raw)	90.20	91.20	91.00	90.80	90.80
Skim milk (pasteurized)	90.20	91.00	91.00	90.20	90.60
Cream (raw)	55.00	60.30	73.80	60.70	62.45
Cream (pasteurized)	40.00	66.70	73.40	65.70	61.45
Butter	14.00	23.00	30.50	27.40	23.73
Buttermilk	88.20	90.20	89.60	90.00	89.50
Cheese (fresh)	36.60	39.00	38.50	36.70	37.70
Cheese (aged)	34.60	37.60	36.50	35.80	36.13
Cheese whey	92.70	91.70	92.00	91.90	92.08
Freeze dried skim milk	2.30	2.30	2.90	2.30	2.45
Freeze dried whole milk	1.90	1.50	4.30	2.50	2.55
Freeze dried cream	0.50	0.80	0.90	0.90	0.76
Spray dried skim milk[b]	6.60	2.60	-	-	4.60
Spray dried skim milk[c]	3.00	2.10	-	-	2.55
Spray dried whole milk[d]	1.80	0.30	-	-	1.05
Spray dried whole milk[e]	-	1.10	-	-	1.10
Evaporated milk	72.20	64.70	-	-	68.45

[a] Values are average of two determinations.

[b] Air outlet temperature 76.6-93.3°C (170-200°F).

[c] Air outlet temperature 87.8-98.8°C (190-210°F).

[d] Air outlet temperature 85.0-90.5°C (185-195°F).

[e] Air outlet temperature 87.8-93.3°C (190-200°F).

Table 7. Average fat contents[a], expressed as percent of total weight.

Product	Herd				Product average
	1	2	3	4	
Whole milk (raw)	4.40	4.10	4.90	4.50	4.48
Whole milk (pasteurized)	4.56	3.64	2.95	4.55	3.93
Skim milk (raw)	0.77	0.15	0.24	0.15	0.33
Skim milk (pasteurized)	0.15	0.15	0.22	0.16	0.17
Cream (raw)	37.43	31.60	17.20	32.70	29.73
Cream (pasteurized)	55.58	27.20	19.40	27.00	32.29
Butter	83.50	75.77	66.25	69.60	73.78
Buttermilk	0.25	0.63	1.40	0.61	0.72
Cheese (fresh)	30.80	33.77	22.00	28.00	28.64
Cheese (aged)	29.10	32.40	22.10	27.80	27.85
Cheese whey	0.46	1.09	0.43	0.52	0.63
Freeze dried skim milk	0.43	1.14	1.00	0.55	0.78
Freeze dried whole milk	30.90	16.80	24.20	32.50	26.10
Freeze dried cream	91.30	78.50	71.15	78.90	79.96
Spray dried skim milk[b]	0.97	1.98	-	-	1.48
Spray dried skim milk[c]	1.24	2.00	-	-	1.64
Spray dried whole milk[d]	28.15	27.40	-	-	27.78
Spray dried whole milk[e]	-	26.00	-	-	26.00
Evaporated milk	7.80	10.10	-	-	8.95

[a] Values are average of two determinations.

[b] Air outlet temperature 76.6-93.3°C (170-200°F).

[c] Air outlet temperature 87.8-98.8°C (190-210°F).

[d] Air outlet temperature 85.0-90.5°C (185-195°F).

[e] Air outlet temperature 87.8-93.3°C (190-200°F).

Care was taken to homogenize the samples before an adequate aliquot was weighed for further analyses, but variability in the data indicated that this procedure was not always satisfactorily achieved even though the samples seemed visibly homogeneous.

Residue Analysis

Determination of PBB levels in the raw whole milk following the general procedure for chlorinated hydrocarbon pesticides outlined in the Pesticide Analytical Manual showed levels of 0.307, 0.186, 0.093, and 0.214 ppm on a fat basis for Herds 1, 2, 3, and 4, respectively. These values proved to be roughly 1.5 times higher than those obtained by the Michigan Department of Agriculture, although the ratio was not estimated for Herd 3 since levels for this herd were cited as trace amounts.

Distribution of PBB

The total amount of the PBB residues in the raw whole milk and their distribution and recovery in the physically separated milk fractions are summarized in Table 8. The concentration of PBBs was generally very low in the skim milk being approximately 3 to 4.5% of the total amount, while the cream fraction contained most of the residues (39-73%) reflecting the association of PBBs with the lipid

Table 8. Total PBB in milk and its distribution and recovery in the physically separated milk fractions.

Milk component	Herd							
	1		2		3		4	
	Weight[a]	%	Weight	%	Weight	%	Weight	%
	mg		mg		mg		mg	
Unfractionated milk	0.794		0.485		0.196		0.287	
Skim milk	0.033	4.16	0.021	4.41	0.008	4.23	0.008	2.90
Cream	0.363	45.70	0.355	73.19	0.076	38.77	0.206	71.78
Recovery		49.87		77.60		43.00		74.70

[a] Values are based on the total milk and fraction weights as shown in Table 3.

phase. The recovered PBB fractions ranged from 43 to 78%. The lower recoveries might be attributable to the loss of the residues during the fractionation process since appreciable quantities of milk fat remained in the discs and on the walls of the separator.

The data in Table 9 show the distribution of PBBs in butter and buttermilk during manufacture of butter from pasteurized cream. The percentage recoveries of residues in each fraction as well as total recoveries are also shown. In this study, cream was churned in an institutional mixer to allow separation of the butterfat from the buttermilk. The butter was further washed with cold tap water to reduce residual amounts of buttermilk. The results, again, show that PBB distribution was associated with the lipid content of the fractions. Butter contained approximately 53 to 115% of the total PBB whereas the accumulation of residue in the buttermilk was much lower with a range of 0 to 8%. Recoveries were approximately 115, 55, 62, and 61% for Herds 1, 2, 3 and 4, respectively. Losses of PBB could be due to the losses of butterfat and buttermilk in the churning process and the washing of the butter fraction since appreciable amounts of butter remained on the paddle of the mixer and in the cheese cloth used to remove the excess water from the washed butter.

The distribution and recovery of the PBB contents of the pasteurized whole milk in the cheese and cheese whey fractions are shown in Table 10. Once again, the higher

Table 9. Total PBB in cream and its distribution and recovery in butter making.

Cream component	Herd 1		Herd 2		Herd 3		Herd 4	
	Weight[a]	%	Weight	%	Weight	%	Weight	%
	mg		mg		mg		mg	
Unfractionated cream	0.166		0.053		0.028		0.063	
Butter	0.191	115.06	0.028	52.83	0.015	53.57	0.037	58.73
Buttermilk	0.000	0.00	0.001	1.89	0.002	8.20	0.001	2.06
Recovery		115.06		54.72		61.77		60.79

[a] Values are based on the total cream and fraction weights as shown in Table 4.

Table 10. Total PBB in milk and its distribution and recovery in cheese making.

Milk component	Herd							
	1		2		3		4	
	Weight[a]	%	Weight	%	Weight	%	Weight	%
	mg		mg		mg		mg	
Unfractionated milk	0.037		0.024		0.007		0.029	
Cheese	0.027	72.60	0.013	53.69	0.005	73.85	0.014	48.79
Cheese whey	0.002	4.66	0.010	37.70	0.001	21.54	0.003	9.34
Recovery		77.26		91.39		95.39		58.13

[a] Values are based on the total weight of milk used.

amounts of the residues found in the cheese fraction (49 to 74%) reflected the preferential distribution of PBBs in the lipid portion of the product. Although the average fat content of the cheese whey fractions was less than 1%, the relative amounts of PBB present in these fractions (4.5 to 38%) were somewhat higher than those in the buttermilk milk when compared to the respective lipid contents. Montoure and Muldoon (1967) had observed DDE and TDE to be present in higher amounts in cheese whey from Monterey and cheddar cheese manufacturing while DDT was not detected. The recoveries of the residues in this processing ranged from approximately 58 to 95%. The less than 100% recovery of total PBB from the cheese and whey fractions was probably due to losses of the residues along with the whey and fat in the salting and pressing process of the curd.

It was found in the present study that PBBs are associated with the lipid phase of the products substantiating the similarities of these compounds to polychlorinated biphenyls and chlorinated hydrocarbon pesticides. Through physical separation of milk, Li et al. (1970) and Langlois et al. (1964) showed that organochlorine pesticides were significantly associated with the fat fractions of the cream and butter. Platonow et al. (1971) observed in their study that physiologically incorporated PCBs had a strong predilection for the lipid phase of the processed dairy products and accumulation of DDE and PCB residues in the milk fat have been pointed out by Fries et al. (1973). Additionally, Ang

(1970) indicated that butteroil contained more dieldrin than butter on a weight basis.

Effect of Processing in the PBB Levels

The PBB residue contents of the raw whole milk and the manufactured dairy products under pasteurization, butter and cheese making, and freeze-drying processes are illustrated in Table 11, on the total weight basis and in Table 12, on the total solids basis. Analysis of variance revealed significant differences in the PBB contents of the dairy products on both a total weight and total solids basis. Duncan's multiple range test was used to pinpoint these significant differences among the products and these results are presented in Table 13. Butter contained significantly more PBB than buttermilk at the 5% level of probability on both the solids and total weight basis. Fresh cheese also contained more PBB than cheese whey. In addition, butter and freeze-dried cream had significantly ($p<0.05$) higher PBB levels than pasteurized cream when the residues were expressed on the total weight basis.

The results presented in Table 14 show the PBB contents of the raw milk and dairy products on a fat basis. Analysis of variance showed the PBB levels expressed on a fat basis were not significantly different in these dairy products. Mann et al. (1950) found pasteurization had very little effect on the DDT residues in dairy products. Although the differences found in the present study were not significant,

Table 11. PBB residue[a] in milk and related dairy products (ppm based on total weight).

Product	Herd				Product mean + standard deviation
	1	2	3	4	
Raw whole milk	0.013	0.008	0.005	0.010	0.009 ± 0.004
Pasteurized whole milk	0.012	0.006	0.001	0.007	0.006 ± 0.004
Raw skim milk	0.000	0.003	0.000	0.000	0.000 ± 0.000
Pasteurized skim milk	0.000	0.000	0.000	0.000	0.000 ± 0.000
Raw cream	0.092	0.056	0.017	0.052	0.054 ± 0.031
Pasteurized cream	0.135	0.038	0.019	0.047	0.060 ± 0.052
Butter	0.217	0.088	0.049	0.079	0.108 ± 0.074
Buttermilk	0.001	0.001	0.001	0.001	0.001 ± 0.000
Fresh cheese	0.086	0.047	0.028	0.044	0.051 ± 0.025
Aged cheese	0.054	0.037	0.024	0.060	0.044 ± 0.017
Cheese whey	0.001	0.002	0.001	0.001	0.001 ± 0.001
Freeze dried whole milk	0.106	0.029	0.031	0.052	0.054 ± 0.036
Freeze dried skim milk	0.000	0.001	0.001	0.000	0.001 ± 0.000
Freeze dried cream	0.241	0.132	0.072	0.140	0.146 ± 0.070

[a] Values are average of two determinations; 0.000 values indicate values of less than 0.001 ppm.

Table 12. PBB residue[a] in milk and related dairy products (ppm based on solids).

Product	Herd				Product mean + standard deviation
	1	2	3	4	
Raw whole milk	0.097	0.055	0.034	0.073	0.065 ± 0.027
Pasteurized whole milk	0.083	0.045	0.012	0.050	0.047 ± 0.030
Raw skim milk	0.004	0.003	0.002	0.002	0.003 ± 0.001
Pasteurized skim milk	0.003	0.003	0.002	0.002	0.003 ± 0.001
Raw cream	0.204	0.140	0.066	0.132	0.136 ± 0.051
Pasteurized cream	0.224	0.114	0.072	0.136	0.137 ± 0.064
Butter	0.252	0.114	0.071	0.108	0.136 ± 0.079
Buttermilk	0.006	0.007	0.013	0.009	0.009 ± 0.003
Fresh cheese	0.135	0.082	0.045	0.070	0.082 ± 0.038
Aged cheese	0.082	0.059	0.038	0.094	0.068 ± 0.025
Cheese whey	0.013	0.027	0.009	0.007	0.014 ± 0.009
Freeze dried whole milk	0.108	0.029	0.032	0.053	0.055 ± 0.036
Freeze dried skim milk	0.000	0.001	0.001	0.000	0.001 ± 0.000
Freeze dried cream	0.242	0.133	0.073	0.141	0.147 ± 0.070

[a] Values are average of two determinations; 0.000 values indicate values of less than 0.001 ppm.

Table 13. Summary of the significant differences[a] of the PBB residue contents between products.

PBB content (ppm) based on	Product	Product mean + standard deviation	Statistical significance $p<0.05$
Solid	Butter (A)	0.136 ± 0.079	A > B
	Buttermilk (B)	0.009 ± 0.003	
	Fresh cheese (C)	0.082 ± 0.038	C > D
	Cheese whey (D)	0.014 ± 0.009	
Total weight	Butter (A)	0.108 ± 0.074	A > B
	Buttermilk (B)	0.001 ± 0.000	
	Butter (A)	0.108 ± 0.074	A > E
	Pasteurized cream (E)	0.060 ± 0.052	
	Fresh cheese (C)	0.051 ± 0.025	C > D
	Cheese whey (D)	0.001 ± 0.001	
	Freeze dried cream (F)	0.146 ± 0.070	F > E
	Pasteurized Cream (E)	0.060 ± 0.052	

[a] Duncan's Test (1957).

Table 14. PBB residue[a] in milk and related dairy products (ppm based on total lipid content).

Product	Herd				Product mean + standard deviation
	1	2	3	4	
Raw whole milk	0.307	0.186	0.093	0.214	0.200 ± 0.088
Pasteurized whole milk	0.260	0.151	0.046	0.145	0.150 ± 0.087
Raw skim milk	0.053	0.170	0.067	0.156	0.111 ± 0.060
Pasteurized skim milk	0.234	0.212	0.086	0.113	0.161 ± 0.073
Raw cream	0.246	0.174	0.100	0.159	0.170 ± 0.060
Pasteurized cream	0.243	0.141	0.099	0.173	0.164 ± 0.061
Butter	0.209	0.116	0.075	0.113	0.141 ± 0.081
Buttermilk	0.369	0.105	0.095	0.143	0.178 ± 0.129
Fresh cheese	0.278	0.138	0.126	0.158	0.175 ± 0.070
Aged cheese	0.243	0.118	0.109	0.217	0.172 ± 0.068
Cheese whey	0.211	0.208	0.126	0.109	0.176 ± 0.047
Freeze dried whole milk	0.341	0.170	0.126	0.160	0.199 ± 0.097
Freeze dried skim milk	0.091	0.088	0.094	0.058	0.083 ± 0.017
Freeze dried cream	0.264	0.168	0.101	0.177	0.177 ± 0.067

[a] Values are average of two determinations.

pasteurization seemed to slightly decrease the concentration of PBBs in the whole milk and cream from 0.200 to 0.150 ppm, and from 0.170 to 0.164 ppm, respectively. A slight increase of PBB residues (0.111 to 0.161 ppm) was observed in the skim milk under pasteurization but this variation resulted from the difficulty found in the quantitation of the very low PBBs found in the skim milk variables. It appeared that buttermilk contained more PBBs on a fat basis than butter and pasteurized whole milk. This fact was also observed for the pasteurized skim milk and whey when the levels of PBB were compared to the pasteurized whole milk. Li et al. (1970) pointed out that buttermilk, skim milk, and whey contained higher concentrations, on a fat basis, of the studied chlorinated hydrocarbon pesticides than did the whole milk. These authors suggested that the pesticides were associated with the lipoprotein portion of the products. Attempts to determine the possible causes of the apparent higher quantities of PBBs in these fractions were not made. Ang (1970) found that refined skim milk contained more dieldrin than whole milk on a fat basis. She suggested that dieldrin was slightly soluble in the serum of milk, but she also showed that the levels in the skim milk and whole milk were comparable and that lower levels were found in buttermilk and butter serum.

On the studies of the freeze-drying process, an apparent higher concentration of the PBB residues, on a fat basis, in the freeze-dried whole milk and cream was observed when compared to the levels in the pasteurized whole milk

and cream, respectively. In this aspect, the possibility of occurrence of contamination from the glasswares used in the extraction and clean up procedures of these samples was considered, though it seems to be not very probable since glassware was carefully rinsed with acetone and petroleum ether before each use. On the other hand, gas chromatographic analyses of the freeze-dried samples showed the presence of several unidentified peaks with shorter retention times than the hexabromobiphenyl peak (quantitated peak) at relative concentrations as high as or even higher than the hexabrominated biphenyl peak. Data from the confirmation test by ultraviolet irradiation of freeze-dried whole milk showed the complete disappearance of these extraneous peaks after 30 minutes of ultraviolet light exposure suggesting that they may have been halogenated compounds. Although attempts to identify these compounds by mass spectrometry were not made, they could have been brominated biphenyls with different degrees of bromination formed under the freeze-drying conditions. If this is true, or if other compounds with comparable retention time to the hexabrominated biphenyl compound were present in the milk, the apparent higher levels of PBBS in the freeze-dried whole milk and cream may be justified. It should also be mentioned that quantitation of PBB in the freeze-dried skim milk was found to be extremely difficult due to the presence of interfering peaks with very close retention times to the hexabromobiphenyl peak.

The effect of aging for a period of approximately two

months on the manufactured cheddar cheese showed very little effect on the PBB levels. In fact, the levels found in fresh cheese and aged cheese were comparable. The result of the present study closely agrees with the data of Montoure and Muldoon (1967) on the DDE, TDE, and DDT residue levels in aged Monterey and Cheddar cheese.

In general, the relative concentrations of PBB, on a fat basis, showed that these applied processes were not effective in the removal of the PBB contents. This observation supports the earlier findings of Platonow and Funnell (1971) for PCBs during processing of cream, cottage cheese, and skim milk. They reported these processings did not contribute to significant changes in the polychlorinated biphenyl levels in these products.

Condensation has been shown to be effective in the reduction of telodrin residues in milk (Stemp and Liska, 1966). Pasteurized whole milk of Herds 1 and 2 was evaporated to study the effect of condensation of the PBB levels. Data illustrated in Tables 15 and 16 show the PBB contents on a solids and fat basis, respectively, of the pasteurized whole milk and evaporated whole milk from the individual herds. Results of the statistical analysis are also presented in these tables. The concentration of the residues of the evaporated milk and pasteurized whole milk of Herd 1 were revealed to be significantly different at the 5% level of probability by the t statistic. However, it was observed that the evaporated milk had a higher PBB content than the

Table 15. Effect of condensation on the PBB residue levels[a] of pasteurized whole milk (ppm based on solids)

Herd	Liquid mean + standard deviation	Evaporated mean + standard deviation	t statistic
1	0.0829 ± 0.0022	0.1084 ± 0.0016	-13.257*
2	0.0447 ± 0.0024	0.0426 ± 0.0086	0.333

[a] Values are average of two determinations.

* Significant at the 5% level of probability.

Table 16. Effect of condensation on the PBB residue levels[a] of pasteurized whole milk (ppm based on total lipid content).

Herd	Liquid mean + standard deviation	Evaporated mean + standard deviation	t statistic
1	0.2598 ± 0.0110	0.3870 ± 0.0123	-10.902*
2	0.1509 ± 0.0033	0.1491 ± 0.0273	0.092

[a] Values are average of two determinations.

* Significant at the 5% level of probability.

pasteurized whole milk had, showing an increase in the PBB levels of about 30-40%. Data for Herd 2 showed the residue levels in the evaporated milk to be less than those for pasteurized whole milk but the difference was not significant.

Earlier reports have emphasized that spray-drying was effective in the removal of chlorinated hydrocarbon pesticides from milk. Therefore, in the present study, spray-drying of whole milk and skim milk was done to study the effect of this processing on the PBB residues. Data given in Tables 17 and 18 shows the PBB residue contents based on solids and on total lipid content, respectively, of whole milk and skim milk of Herd 1, before and after spray-drying. Statistical analysis established that the levels in the spray-dried whole milk were significantly lower than the levels in pasteurized whole milk, on a solids basis, showing a reduction of 26.5% of PBBs. Nevertheless, when the residues were expressed on a fat basis, the difference was not significant even though the levels in the spray-dried whole milk were lower. In considering the spray-dried skim milk at air outlet of 87.8 - 93.3°C, the levels in this product were less than the levels in pasteurized skim milk but the difference was not significant.

Tables 19 and 20 show PBB levels on a solids and fat basis, respectively, in the pasteurized and spray-dried whole milk and skim milk of Herd 2. Significant reductions in the PBB residue contents by spray-drying were established at the 5% level of probability by the t statistic. The PBB levels

Table 17. Effect of spray-drying on PBB residue contents[a] of pasteurized whole milk and skim milk of Herd 1 (ppm based on solids).

Product	Liquid mean + standard deviation	Spray-dried mean + standard deviation	t statistic
Whole milk	0.0829 ± 0.0022	0.0622 ± 0.0011[b]	11.902*
Skim milk	0.0034 ± 0.0007	0.0035 ± 0.0003[c]	-1.857
Skim milk	0.0034 ± 0.0007	0.0008 ± 0.0003[d]	4.828

[a] Values are average of two determinations.

[b] Air outlet temperature 87.8°C (190°F).

[c] Air outlet temperature 76.6°C (170°F).

[d] Air outlet temperature 87.8-93.3°C (190-200°F).

* Significant at the 5% level of probability.

Table 18. Effect of spray-drying on PBB residue contents[a] of pasteurized whole milk and skim milk of Herd 1 (ppm based on total lipid).

Product	Liquid mean + standard deviation	Spray-dried mean + standard deviation	t statistic
Whole milk	0.2598 ± 0.0110	0.2174 ± 0.0086[b]	4.294
Skim milk	0.2336 ± 0.0611	0.3371 ± 0.0202[c]	-2.275
Skim milk	0.2336 ± 0.0611	0.0600 ± 0.0208[d]	3.804

[a] Values are average of two determinations.

[b] Air outlet temperature 87.8°C (190°F).

[c] Air outlet temperature 76.6°C (170°F).

[d] Air outlet temperature 87.8-93.3°C (190-200°F).

Table 19. Effect of spray-drying on the PBB residue contents[a] of pasteurized whole milk and skim milk of Herd 2 (ppm based on solids).

Product	Liquid mean[b] + standard deviation	Spray-dried mean + standard deviation	t statistic
Whole milk	0.0447 ± 0.0024	0.0268 ± 0.0024[c]	7.458*
Whole milk	0.0447 ± 0.0024	0.0282 ± 0.0006[d]	11.175*
Skim milk	0.0034 ± 0.0001	0.0017 ± 0.0006[d]	3.952
Skim milk	0.0034 ± 0.0001	0.0014 ± 0.0002[e]	12.649*

[a] Values are average of the determinations.

[b] PBB residue content of pasteurized whole milk and pasteurized skim milk.

[c] Air outlet temperature 85-90.5°C (185-195°F).

[d] Air outlet temperature 87.8-93.3°C (190-200°F).

[e] Air outlet temperature 87.8-98.8°C (190-210°F).

* Significant at the 5% level of probability.

Table 20. Effect of spray drying on the PBB residue contents[a] of pasteurized whole milk and skim milk of Herd 2 (ppm based on total lipid).

Product	Liquid mean[b] + standard deviation	Spray-dried mean + standard deviation	t statistic
Whole milk	0.1509 ± 0.0033	0.0956 ± 0.0101[c]	7.360*
Whole milk	0.1509 ± 0.0033	0.1071 ± 0.0018[d]	16.479*
Skim milk	0.2115 ± 0.0013	0.0829 ± 0.0252[d]	7.207*
Skim milk	0.2115 ± 0.0013	0.0662 ± 0.0018[e]	92.546**

[a] Values are average of two determinations.

[b] PBB residue content of original product.

[c] Air outlet temperature 85-90.5°C (185-195°F).

[d] Air outlet temperature 87.8-93.3°C (190-200°F).

[e] Air outlet temperature 87.8 -98.8°C (190-210°F).

* Significant at the 5% level of probability.

** Significant at the 1% level of probability.

in the spray-dried whole milk were reduced by approximately 36% (fat basis) and 40% (solids basis) at air outlet temperatures of 85 - 90.5°C, and 30% (fat basis) and 38% (solids basis) at air outlet temperatures of 87.8 - 93.3°C. Additionally, it was observed that the spray-dried skim milk at air outlet temperatures of 87.8 - 93.3°C showed levels of PBB significantly different than the levels in the pasteurized skim milk, on a fat basis, with reduction of approximately 61% in the residues of the spray-dried product. Nevertheless, when expressed on a solids basis, the difference was not significant. Comparable losses of PBB from the skim milk on a solids and fat basis were obtained when the skim milk was spray-dried at air outlet temperatures of 87.8 - 98.8°C. It was estimated that significant losses of approximately 69% (fat basis) and 67% (solids basis) of PBB occurred during the spray-drying process at this specified condition.

From these studies it was observed that losses of PBB from skim milk were greater than from whole milk, roughly 30 to 40% from whole milk and 60 to 69% from skim milk, suggesting these compounds were more easily removed from low fat content products and/or when the levels were lower. It is possible that some PBB is distributed in the serum phase of milk and that this quantity could be more easily removed than the PBB associated with the lipid phase. Additionally, the greater surface area formed during the spraying of the liquid product and the relatively high temperatures at which the formed particles are exposed could facilitate a more easily volatilization of the PBBs. For the same

reasons, the findings of significant reductions in the PBB levels only in the spray-dried products may be justified and further indicate the data obtained in these studies with PBBs confirms earlier reports with halogenated compounds. Langlois et al. (1964) indicated that significant losses of DDT and lindane occurred during spray-drying treatment of whole milk. Reduction of dieldrin, lindane, and chlordane in the spray-drying of whole milk was observed by Li et al. (1970). Telodrin residues were reduced by approximately 10 - 20% during drying of milk (Stemp and Liska, 1966). Comparable reduction effects were pointed out by Liska (1968) during the spray-drying of milk containing lindane, heptachlor, and heptachlor epoxide.

SUMMARY AND CONCLUSIONS

The effect of processing on the polybrominated biphenyl levels and their relative distribution in dairy products were studied. Milk containing less than 0.3 ppm (fat basis) was separated into skim milk and cream fractions followed by pasteurization of the raw milk and the fractions. These products were further processed into butter, cheddar cheese, and freeze-dried whole milk, skim milk, and cream. Spray-drying of whole milk and skim milk and condensation of whole milk were done for two of the herds.

The relative distribution of milk components showed skim milk accounted for 85 - 93% while cream fraction ranged from 7 to 15%. The yields of butter were proportional to the fat content of the cream used for its manufacture and were approximately 73, 23, 23, and 36% for Herds 1, 2, 3, and 4, respectively. The yields of cheese were also proportional to the lipid content with percentage distribution ranging from approximately 9 to 11%.

Moisture, lipid, and residue analyses were carried out for the raw whole milk and the processed dairy products. Moisture and lipid data were characteristic of the type of dairy product being produced. Recoveries of the total PBB residues during the physical separation of whole milk into skim milk and cream, as well as during butter and cheese manufacture ranged from 43 to 115%. Therefore, some reduction

of PBBs occurred as a result of loss of lipid during these processes. Skim milk contained negligible amounts of the PBB residues (3 - 4%) while cream (39 - 73%), butter (55 - 115%), and cheese (58 - 95%) contained most of the residues. These data indicated the lipid solubility of PBBs as well as the expected accumulation of these compounds in the high-fat content products. From the stand point of minimizing contaminant levels in foods, the physical separation showed promising results, but precise information regarding economical feasibility of using only skimmed milk from PBBs contaminated milk for food consumption should be examined.

Analysis of variance showed no significant difference in the PBB levels (fat basis) in the raw and pasteurized whole milk, cream, and skim milk, butter, buttermilk, cheese, cheese whey, and freeze-dried whole milk, skim milk, and cream. The pasteurized products had slightly lower amounts of the residues indicating pasteurization had little effect on the reduction of PBB levels. Buttermilk, skim milk, and cheese whey contained slightly higher concentrations of the residues than the pasteurized whole milk, on a fat basis, confirming the earlier findings of Li et al. (1970).

Significant differences in the PBB contents were established for the products when expressed on a total weight and solids basis. Duncan's multiple range test was used to sort out the differences among the products. The data showed PBB levels were proportional to the lipid content of the products.

Comparable levels of PBB were found in aged and fresh

cheese indicating aging had very little or no effect on removal of these residues.

Gas chromatographic analysis showed that only the freeze-dried products contained several unidentified peaks at abnormally high concentrations. Exposure of freeze-dried whole milk to ultraviolet light promoted the complete disappearance of these peaks suggesting they may have been brominated biphenyls with different degrees of halogenation.

Whole milk from two of the herds was condensed to produce evaporated milk with 30% solids. Use of the t statistic revealed condensation was ineffective in reducing PBB residues.

A corollary study investigated the possibility of removal of the PBB residues by spray-drying. Pasteurized whole milk and skim milk of Herds 1 and 2 were spray-dried using pilot plant equipment. Significant reductions in the PBB levels in the spray-dried whole milk (ppm based on solids) for both herds were established with losses of approximately 30 to 40% of PBBs. Spray-dried skim milk of Herd 2 contained significantly less PBBs than the pasteurized skim milk, based on the lipid contents with losses ranging from 60 to 69%. When expressed on a solids basis, the significant reduction showed to be approximately 67%.

Significant losses of PBBs only during the spray-drying process indicated these compounds were more easily removed when the surface area of the milk particles was increased and exposed to relatively high temperatures, thus facilitating

greater volatilization of these residues. The findings on the spray-drying studies indicate this method of processing may have promise for the purpose of reducing PBB levels in contaminated milk.

SUGGESTIONS FOR FURTHER RESEARCH

The questions and observations raised in the present study suggest the following additional research:

1 - The relative distribution of the metabolites of DDT (DDE and TDE) have been reported in various phases during the manufacture of cheese. Similarly, the association observed between higher levels of PBBs with cheese whey should be explored further with the purpose of clarifying the possible factors involved in this distribution.

2 - Sufficient quantities of milk should be freeze-dried to facilitate identification of the unknown peaks found in this study. If these compounds are partially debrominated PBBs, the implications of freeze-drying conditions for debromination of polybrominated biphenyls would warrant further investigation. The study should be extended to include a toxicological evaluation of lower brominated biphenyl compounds.

3 - The correlated higher reduction of PBBs in lower fat content products found during spray-drying should be elaborated further on a larger number of samples with varying PBBs and lipid concentrations.

4 - The feasibility of PBB removal from milk by spray-drying should be investigated on a larger scale using commercial conditions.

R E F E R E N C E S

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