Distribution and Kinetics of PBB Residues in Cattle

by G. F. Fries,* G. S. Marrow,* and R. M. Cook†

Cows fed a constant amount of polybrominated biphenyl (PBB) reached a steady-state concentration in milk fat within 30 days. This concentration was approximately four times the concentration in the total diet. When feeding of PBB was stopped, the concentration in milk was adequately described as a sum of two first-order elimination rates. Biological half-life in environmentally contaminated cows, studied for 6 months about a year after contamination, was 60 days. The stage of lactation affected the rate of elimination, and in some cases concentrations increased shortly after calving. Residues were distributed in body tissues proportionally to concentration of fat in the tissues. Liver and brain were exceptions. Concentration in liver fat was generally higher than other tissues and possibly related to the treatment of some cows with phenobarbital. Residues in brain fat were significantly lower than all other tissues. The ratio of the concentrations in milk fat to concentration of residues in the blood of calves and fat of fetal tissues to the concentration in the corresponding tissue in the dams was 0.36:1. It was estimated that people consuming milk from the most highly contaminated Michigan cows could have received PBB doses as great as 10 g from this source alone.

Introduction

The polybrominated biphenyls (PBB) are nonpolar and chemically unreactive. These two characteristics are the major determinants of the kinetics and distribution of PBB residues in the animal. Residues of PBB are distributed in close association with the fat of tissues, have a long biological half-life, and are mainly excreted in fat-containing products, i.e., milk and eggs. Thus, as expected, the kinetics and distribution of PBB residues are qualitatively similar to the kinetics and distribution of chemically related compounds such as polychlorinated biphenyls (PCB) and chlorinated hydrocarbon insecticides.

We studied the distribution and kinetics of PBB in controlled feeding studies with diary cattle (*1*) and in highly exposed dairy cattle involved in the Michigan feed contamination incident (*2, 3*). Our studies are briefly summarized in this paper. All discussions are confined to the major hexabromobiphenyl component of a commercial PBB (FireMaster FF-1, Michigan Chemical Co., St. Louis, Michigan) unless otherwise noted.

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Distribution

Tissue Levels

Distribution of PBB residues among tissues are determined in 41 dairy cattle (*3*). Of these, 32 were cows that had been exposed to feed contaminated with 0.4% PBB (*4*). The estimated PBB intake was about 400 g over 15 days. The other 9 animals were calves born to these exposed cows. The calves' PBB exposure was from both placental transfer and consumption of contaminated milk. The tissues examined were perirenal fat, omental fat, subcutaneous fat, skeletal muscle, cardiac muscle, kidney, liver, lung, and brain.

The animals were divided into four groups for summarization: group I contained 6 cows killed 9 months after exposure; group II contained 12 cows that became moribund 9 to 12 months after exposure; group III contained 14 cows killed 15 to 20 months after exposure and after they had been used in residue elimination studies (*2*); and group IV contained 9 calves killed at 2-4 months of age.

The geometric mean concentration of PBB in the fat of the tissues of the four groups are shown in Figure 1. All values are expressed as concentration in fat because the variation among tissues was greatly reduced when the values were expressed on
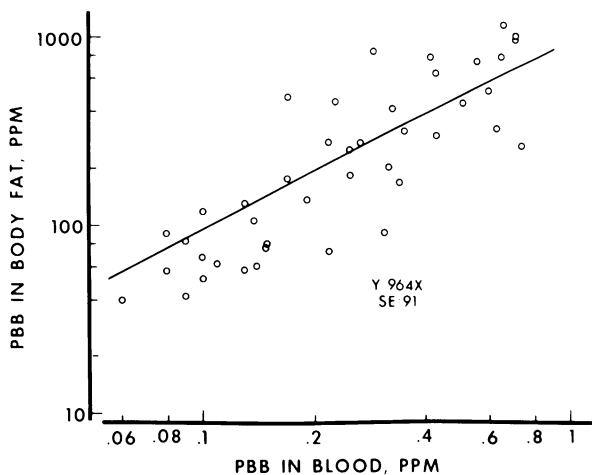
FIGURE 1. Concentration of polybrominated biphenyl in the fat of tissues from environmentally contaminated cows and calves. Group I contained 6 cows 9 months after exposure; group II contained 12 moribund cows 10–12 mo. after exposure; group III contained 14 cows 15–20 mo. after exposure; group IV contained 9 calves from these cows at 2–4 mo. of age.

this basis rather than as concentration in whole tissue. In other words, the concentration of PBB in tissue parallels the concentration of fat in tissue. There were no significant differences among the concentrations of PBB in perineal fat, omental fat, subcutaneous fat, skeletal muscle, cardiac muscle, and kidney when all of the animals are considered as a group. Some of the differences in concentration among these tissues were statistically significant within individual groups (3). However, the differences were small, inconsistent, and do not appear to have biological or practical importance.

Concentrations of PBB in liver were significantly higher than concentrations in other tissues in groups III and IV but not in groups I and II. The reason for the higher values in groups III and IV is unknown, but most of the higher values of group III were associated with 9 cows that received phenobarbital (2). Phenobarbital is a liver microsomal enzyme inducer, and the increase is possibly associated with enzyme induction.

The concentration of PBB in the fat of lung was significantly lower than the concentration in the fat of all other tissues except brain; where the concentration was significantly lower than in all other tissues. Phospholipids make up a large portion of the lipid in brain and this may be one explanation of the lower residue concentrations in brain.

Our findings on PBB distribution in this study are consistent with the results of studies on the distribution of chlorinated hydrocarbon compounds in cattle (5–7). However, we are not in good agreement with some limited studies on PBB by others

(8, 9). In those limited studies there was a much wider range of concentrations among tissues than in our study. In our study the cows were killed at least 9 months after exposure to PBB; in the other studies the cows were killed within a few days after exposure to PBB. The greater uniformity of the concentrations in our studies suggest that PBB is slow in establishing equilibrium concentrations in the fat of the various body tissues.

Placental Transfer

Placental transfer of PBB was evaluated with two independent sets of observations (3). Fetal samples were obtained from eight animals that were pregnant when killed, and blood samples were obtained from nine calves at birth and compared with blood samples simultaneously obtained from their dams. The average ratio of PBB concentration in fetal or calf tissue to PBB concentration in dam tissue was 0.36:1 for fat and 0.37:1 for blood. We conclude that PBB is readily transferred across placental membranes.

Tissue and Product Relationships

Knowledge of the relationships among the concentration of PBB in tissues and excretory products provides important basic scientific information as well as useful practical information. The relationships of primary interest are those among body fat, blood, milk fat and feces.

Subcutaneous body fat, blood, milk fat and feces were obtained from 12 cows every 4 weeks for 24 weeks (2). Some body fat biopsy samples had too little fat to provide reliable analytical information. Therefore, this discussion will involve the 6 cows from which complete sets of data were obtained.

The relationships between blood and the other samples are presented in Figures 2–4. Blood was arbitrarily denoted the independent variable because it is the common physiological connection among all samples. The relationships were evaluated with the regression model

$$Y = bX$$

where Y is the dependent variable, X is the independent variable, and b is the regression coefficient. This model differs from standard linear regression because the regression line passes through the origin. The model is considered theoretically more appropriate when the error is proportional to the magnitude of the observed values (10).

In addition to the relationships shown (Figs. 2–4), other combinations of PBB concentrations in tissues and products are also closely related. The relationships involving blood, milk fat and feces were
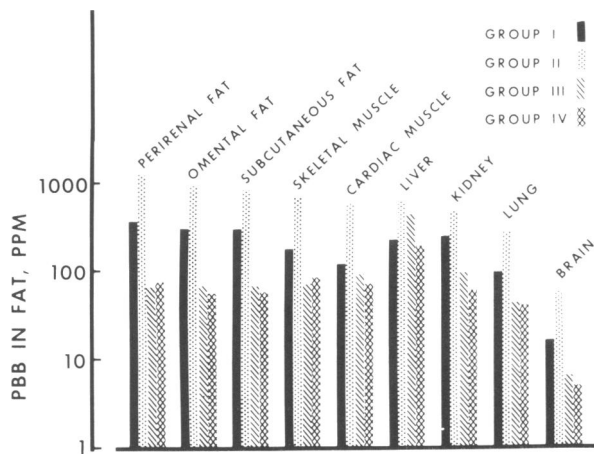
FIGURE 2. Relationship between the concentration of polybrominated biphenyl in blood and subcutaneous body fat biopsy samples. The values are from six cows sampled every 4 weeks for 24 weeks.
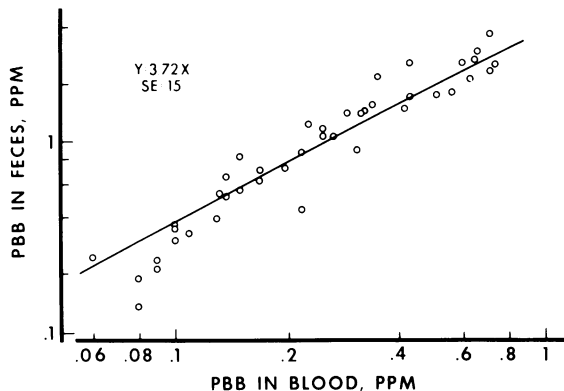

FIGURE 3. Relationship between the concentration of polybrominated biphenyl in blood and milk fat. The values are from six cows sampled every 4 weeks for 24 weeks.
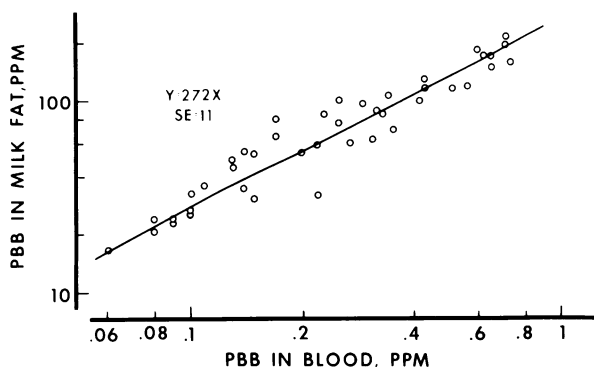

FIGURE 4. Relationships between the concentration of polybrominated biphenyl in blood and feces. The values are from six cows sampled every 4 weeks for 24 weeks.

particularly close. Relationships involving subcutaneous body fat biopsies were more variable. However, when the relationship involving milk fat and body fat was examined at the time lactating cows were killed, the agreement was much better (Fig. 5) and in line with the variation of other sample combinations. The greater variation with body fat biopsy samples suggest that these samples can lead to unreliable conclusions, particularly if the animals are thin and the samples only contain a small amount of fat (2).
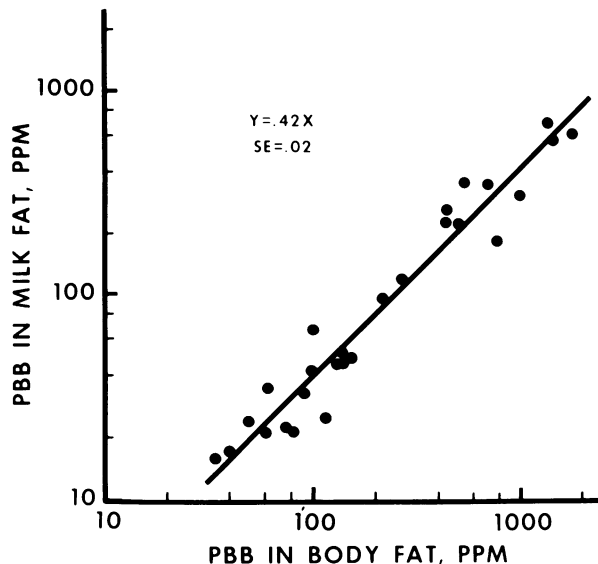

FIGURE 5. Relationship between the concentration of polybrominated biphenyl in milk fat and subcutaneous body fat obtained when the cows were killed.

Our observations suggest the body burden of PBB can be reliably estimated by sampling the most accessible tissue or product. Blood or milk samples are usually much easier to obtain than body fat biopsies. Blood suffers a disadvantage at lower body burdens because the concentration is much lower than in milk or body fat. Thus, detectable levels could be present in milk or body fat but the levels in blood would be below the limit of analytical sensitivity.

The 0.42:1 ratio of concentration in milk fat to concentration in body fat (Fig. 5) provides the most reliable estimate of this useful measure. The average ratio was 0.37:1 in the six cows followed during lactation in spite of the greater variation with body fat biopsy samples. The average ratio found with four cows with body fat residue levels below 2 ppm was 0.43:1 (1). This suggests that a constant ratio holds over a wide range of concentrations and that milk fat can be used to reliably predict body fat concentrations.

The constant ratios among the concentrations in the tissues and products were obtained from animals no longer consuming PBB. While functional relationships probably exist when animals are consuming PBB, the numerical values will probably be different.

Residue Kinetics

Steady-State Levels

Residue kinetics were studied in four first-lactation cows (2). The cows were fed 10 mg PBB per day for 60 days. Residues in milk were determined during the 60-day feeding period and for 60 days after feeding stopped. The stage of lactation when PBB feeding started averaged 164 days and ranged for 121 to 191 days.

Average concentrations of the major hexabromobiphenyl and heptabromobiphenyl components in milk fat are given in Figure 6. Concentrations of both components reached stable levels after 20 days of feeding and declined rapidly for the first 10 to 15 days after feeding stopped. After 15 days the concentration of hexabromobiphenyl declined much less rapidly while the concentration of heptabromobiphenyl was below the limits of detection.
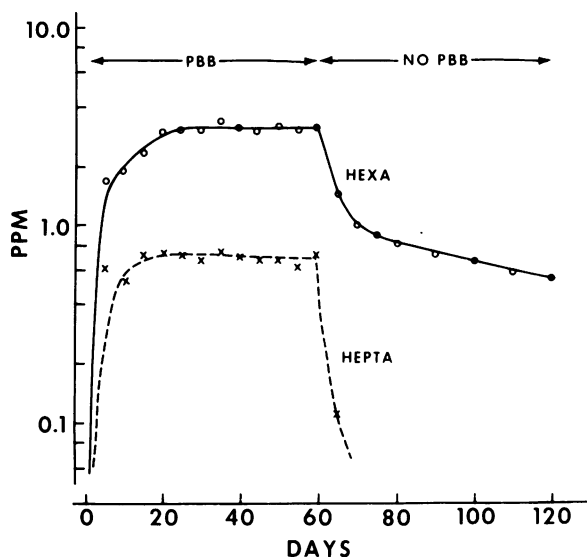

FIGURE 6. Concentration of polybrominated biphenyl in milk fat of cows fed 10 mg/day. Each point is an average of four cows. Calculations of the concentrations of each component was based on the assumption that each was fed at 10 mg/day.

Concentrations of the two components are normalized to equal intakes of each. Differences in the concentrations of hexabromobiphenyl and heptabromobiphenyls (Fig. 6) indicate that, relative to intake, five times more hexabromobiphenyl than heptabromobiphenyl was transferred to milk. We found similar differences in the transfer of the two compounds into eggs of hens with a concomitant higher relative concentration of heptabromobiphenyl in the hen excreta (11). Similarly, the more highly chlorinated polychlorinated biphenyl components are, the less efficiently they are transferred to milk (12) and eggs (13). We have interpreted these observations to mean that less halogenated components of PBB and PCB more readily diffuse across biological membranes than the more halogenated components.

The contribution of heptabromobiphenyl to the total residue is small even when PBB is fed. Not only is there a fivefold difference in relative transfer to milk, but the PBB fed also contains five to six times as much hexabromobiphenyl than heptabromobiphenyl. Thus, the concentration of hexabromobiphenyl in milk was approximately 25 times the concentration of heptabromobiphenyl.

The steady-state concentration (30 to 60 days) of PBB in milk fat was about 3.1 ppm. This accounted for 18% of the daily intake, somewhat lower than is typical of chlorinated hydrocarbon compounds (14). The 3.1 ppm concentration in milk fat was about 4.5 times the concentration in the total diet. In order to provide a margin of safety, total diet concentrations should be less than 0.1 times any regulatory guideline in milk or body fat to prevent excessive residues.

Residue Elimination

We have previously proposed that a simple model, consisting of two open compartments with an irreversible exit to the exterior from the first compartment (Fig. 7) that adequately describes the residue picture for halogenated hydrocarbon com-
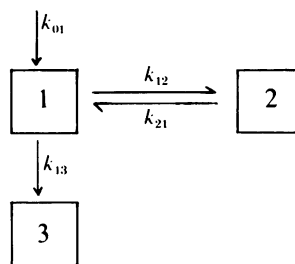

FIGURE 7. Model consisting of two open compartments with an irreversible exit to the exterior from the first compartment.

pounds (14). One may visualize compartment 1 as small with a rapid turnover; i.e. blood, and compartment 2 as large with a slower turnover, i.e., body fat. The third compartment is equivalent to milk and is a sink, not a true compartment. The model does not take into account elimination by

metabolism or enterohepatic recirculation, but the net effect of these routes would be to reduce the value ascribed to input.

We have discussed the major features of the model elsewhere (*14*). The concentration of PBB in milk fat when feeding stops is described by the equation

$$C = C_1 e^{-at} + C_2^{-bt}$$

where C is the concentration in milk fat, C_1 is the initial concentration associated with the first compartment, C_2 is the initial concentration associated with the second compartment, a and b are constants, and t is the time in days.

The shape of the concentration curve (Fig. 6) is consistent with the equation. The first term of the equation has a large rate constant and the value of the term approaches 0 within 10 to 15 days. Thereafter, the curve is described by the second term alone. Thus, the constants of the second term can be readily evaluated by least-squares fitting to the logarithmic form of the second term.

The constants of the first term cannot be accurately evaluated because of the too few points and the rapid change in concentration. In this study the concentration declined 70% within 10 days. The relative magnitude of the initial decline will be greater with a shorter feeding period and smaller with a longer feeding period (*14*).

The constants of the second component of the equation are of the greatest practical importance. The average value of b was 0.012/day. This is equivalent to a half-life of 58 days with a range of 49–85 days.

We have also determined the residue half-lives in 12 environmentally contaminated cows (group III, Fig. 1) over a 24-week period (*2*). The average half-life was 60 days but ranged from 36 to 301 days. Some of these cows did not fit the model particularly well. Some reasons for the poor fit will be discussed below.

Factors Affecting Elimination

The model (Fig. 7) and subsequent calculations of half-lives assume that the various physiological processes of the cow are at steady state. Although the conditions of this assumption can be approximated in some cases, a true physiological steady state never occurs in practice.

Three important factors could affect the half-life; the total amount of fat in the body, changes in the amount of body fat, and the level of milk fat production. Each unit of milk fat will clear the same fraction of the body burden when the amount of

body fat and level of milk fat production are constant because of the constant ratio of the concentrations of PBB in milk and body fats (Fig. 5). Each unit of milk fat will clear a larger fraction of the body burden as the amount of body fat is reduced or as the level of milk fat production is increased. Conversely, each unit will clear a smaller fraction as the amount of body fat is increased or as the level of milk fat production is decreased.

Changes in amount of body fat can have a dramatic effect on the rate of excretion under some conditions. As body fat is lost, the concentration of PBB in the remaining body fat will be increased proportionally. Conversely, there will be a proportional dilution with body fat gain.

These effects were dramatically illustrated in the environmentally contaminated cows we studied (*2*). Those cows had suffered definite ill effects from PBB (*4*) and were in poor condition when studied.

Data from three representative cows studied immediately after calving are shown in Figure 8. Cows 935 and 745 showed dramatic increases in PBB concentration during the first 75 days of lactation when energy deficit and body fat loss are the greatest. However, the concentrating effect of fat loss was not universal as illustrated by cow 16.
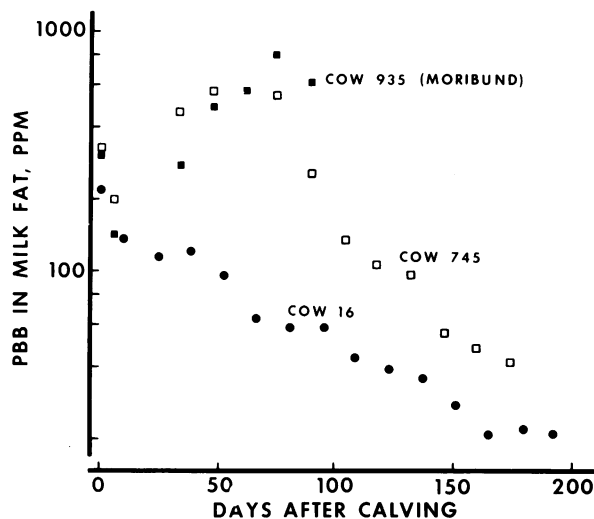

FIGURE 8. Concentration of polybrominated biphenyl in the milk fat of three representative cows after calving.

It should be noted that the concentrating phenomenon after calving has not been consistently observed in healthy well-fed cattle. Brown et al. (*15*) concluded that fresh cows did not have higher DDT residues than other cows in the same herd. We followed some cows from a PCB study (*16*) for as long as 200 days postpartum and the concentration

in milk fat followed an exponential decline remarkably well (Fig. 9). Thus, the increased concentration of residue after calving is probably limited to cows that are in poor condition at calving.

The concentration of PBB in milk fat dropped rapidly for 5 to 10 days in all of the cows that we studied immediately after calving (Fig. 8). Willet and Irving (8) also noted this rapid drop after calving. We suggest that, during the dry period, PBB residues in mammary gland fat are in equilibrium with PBB in the remaining body fat. The PBB in the mammary gland is rapidly transferred to milk as lactation is initiated and after the gland is depleted, the lower transfer rate of PBB from body fat becomes the controlling factor in milk fat concentration. Compounds with larger transfer coefficients do not show this phenomenon after calving as exemplified by DDT (15) and PCB (Fig. 9).
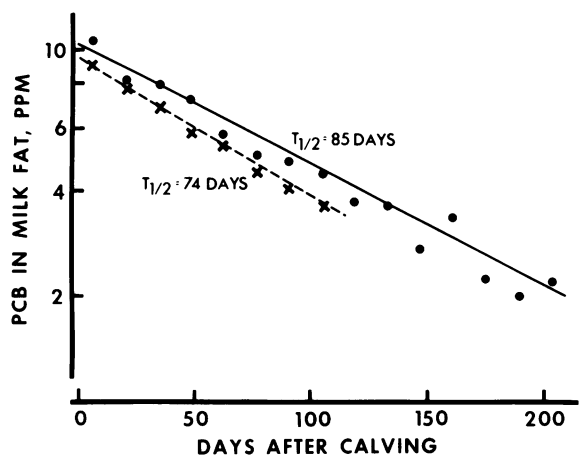

FIGURE 9. Concentration of polychlorinated biphenyl in the milk fat of cows after calving: (●) an average of two cows; (×) an average of those two and an additional four cows.

Caution should be exercised in applying our estimates of half-life despite the good agreement between our two studies. First, the ratio of PBB concentration in milk fat to body fat is wider than any other compound that we have studied and half-life should be directly related to the width of the ratio (14). We found longer half-lives with some compounds having narrower ratios which suggests that the special circumstances of our PBB studies favored shorter half-lives. Second, as lower residue levels are approached, there may be small slow-turnover compartments that make a significant contribution to the milk residue. Third, in the field one may not have an entirely clean environment and the continuing low level intake would give a longer apparent half-life.

Human Exposure

The kinetic data presented here and other independent observations can be used to estimate possible exposure of farm families to PBB in the Michigan incident. The other observations include the time and level of animal exposure, observed residue levels in herds at the time the contamination was identified, and serum levels of exposed people.

Jackson and Halbert (4) provide the best information on the time and level of animal exposure. The cows were fed approximately 15 lb of a concentrate that could have contained as much as 0.4% PBB. Actual analysis of one sample from Halbert was 0.3% PBB (Fries, unpublished). The concentrate was fed for about 2 weeks in late September to early October, 1973. The intake of PBB by these cows could have been in the range of 20–28 g/day. Actual intake may have been somewhat lower because anorexia was experienced. The 0.4% level is assumed for our estimate.

If PBB was transferred from diet to milk fat as efficiently in Halbert's cows as the cows in our study (Fig. 6), milk fat concentration at the end of 15 days exposure could have been as high as 6000 ppm, with an average of 4500 ppm for the 15 days. While the intake of Halbert's cows was probably less than 28 g/day because of anorexia, milk production also dropped, and these two factors could have balanced each other. One would expect a decline to approximately 30% of the maximum exposure value (6000 ppm) within 15 days after exposure ended. This value would have been about 1800 ppm with an average of 2900 ppm for the second 15-day period.

Thereafter the concentration would be expected to decline with a first-order rate constant of 0.012/day (half-life = 58 days). Approximately 230 days elapsed between the time of exposure (Sept. 20, 1973) and the time that a significant number of herds were identified (May 10, 1974). The projected concentration at the time of identification would be 160 ppm.

Two observations support this projection. First, six randomly selected cows from the Halbert herd (group I, Fig. 1) averaged 159 ppm PBB in milk fat (range, 48–351 ppm) when killed in June 1974. Second, the Michigan Department of Agriculture (17) identified 22 herds with residue levels greater than 100 ppm. The discrepancies between the projected values and observed values are not great when one considers the possible introduction of uncontaminated replacements. Thus, the above projection provides a reasonable basis for estimating human exposure to PBB.

The following estimate of exposure of an individual in a farm family consuming its own milk assumed that 1 liter of milk containing 4% fat was consumed per day. The total PBB intake would have been 2.7 g over the 15-day period while the cows were being exposed, 1.7 g during the subsequent 15 days, and 5.4 g during the subsequent 200 days. The estimated total exposure was 9.8 g over the 230-day period. The cumulative intake over time is shown in Figure 10. Note that, if the problem had not been identified, the projected intake over infinite time would have been 10.4 g.
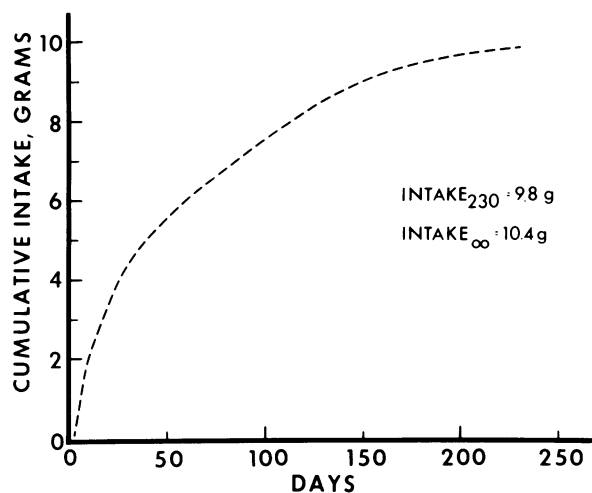

FIGURE 10. Estimated cumulative intake of polybrominated biphenyl by an individual drinking one liter of milk per day from cows that ate 28 g of polybrominated biphenyl per day for 15 days.

The PBB levels in serum determined by the Michigan Department of Health provide a means of checking the reasonableness of our estimate of the higher exposures to farm families. It has been reported (18) that healthy men contain 16% fat (range 4 to 27%). Thus, a 70 kg man would contain about 11.2 kg fat. If there was no metabolism or excretion of a 9.8 g intake, the average fat level would be 875 ppm. In limited observations (19), the ratio of fat PBB to serum PBB was 232 to 1. Thus, one might predict serum PBB levels as high as 3.7 ppm. The highest serum value found by the Michigan Department of Health (20) was 3.8 ppm with a number of individuals in the range of 2 to 3 ppm. This is good agreement considering the assumptions that were made.

It is reasonable to conclude that the most highly exposed people consumed from 5 to 15 g PBB over a 230 day period. This estimate has only considered intake from milk. In a few cases it is known that individuals consumed contaminated meat and/or

eggs. The number of cases where all factors coincided to provide maximum intake were probably small. However, if a cow as used in our example as slaughtered for home consumption within 45 days of initial exposure, the projected intake from meat would have exceeded the projected intake from milk (Fig. 10).

The exposure of an individual in the general population would have a pattern over time as projected for the farm family. However, the exposure level would have been many orders of magnitude less because of dilution in the normal marketing channels. The pattern of exposure over time (Fig. 10) does suggest that prevention of accidents is the only practical means of minimizing exposure. Even when efficient methods are available and used, identification will not be soon enough to prevent a major portion of the potential exposure from a "single-shot" accident.

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