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Effects of PBBs on Cattle. IV. Distribution and Clearance of Components of FireMaster BP-6

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Sixty dairy animals were utilized in seven experiments to determine aspects of the distribution and clearance of FireMaster BP-6. Experimental protocols of various studies provided daily exposures from 0.25 to 25,000 mg, exposures for 1 to 202 days, and total study periods from 10 to 1100 days. Necropsy of 28 animals provided information on residue concentrations in 35 tissues, and the excretion in milk was determined in 15 animals. These studies showed that the major brominated biphenyls of this commercial mixture were absorbed from the gastrointestinal tract and appeared in the blood plasma within 4 hr. With continued exposure to the residue plasma concentrations reached a steady state by 15 days. Free PBB was not detectable in urine. During PBB feeding feces was the major route of excretion, representing approximately 50% of the amount fed to animals not displaying signs of toxicosis. Following withdrawal of PBB, fecal concentrations declined to 1 to 2% of concentrations during dosing, yet, feces remained the major excretory route in nonlactating animals. In contrast, in post-exposure lactating animals milk fat became an important excretory route removing three-times the quantity of residue cleared in feces. Following parturition, concentrations of PBB in milk fat declined approximately twofold in 6 days. Thereafter, the residue concentration in milk fat was approximately 0.4 that in depot fats. PBB had a predilection for lipid tissues with similar concentrations in various depot fats. Concentrations of the residue were notably low in tissues of the nervous system despite the high content of lipid material. Liver contained residue concentrations that were disproportionately high when compared to the lipid content of the organ. Calves born to PBB-exposed cows had similar distribution of residues in body tissues although concentrations were less than those of the dam.

Introduction

An understanding of the distribution and clearance of xenobiotic residues in food producing animals is critical for evaluation of possible subacute toxic effects on exposed animals and subsequent transmission of the residue to man. This information is vital for the interpretation of residue concentrations in samples of body tissues or excretions used to regulate the exclusion of animals and/or food from marketing channels.

Development of most of the information on distribution and clearance of polybrominated biphenyls (PBB) was necessitated by the impact of inadvertent mixing of FireMaster, a commercial flame retardant, into cattle feed (*1*). This accident resulted in exposures to animals that have varied greatly in amount and duration. A variety of nonspecific toxic responses were reported by cattle accidentally exposed to high concentrations of the flame retardant (*2*). Claims that many of the same toxic symptoms have occurred in cattle exposed to very low levels of PBB have been made but could not be verified by a field study (*3*). An understanding of the distribution and clearance of FireMaster at acutely toxic levels and below was necessary to understand toxic responses and to aid the establishment of regulatory tolerance levels in food producing animals. Like commercial preparations of polychlorinated biphenyls (PCB), the PBB in FireMaster consists of a mixture of halogenated biphenyls (*4*). By analogy to PCB, each of these specific brominated biphenyls may behave differently *in vivo*. Studies with PCB have shown that the number of chlorines generally affects absorption (*5*), excretion (*6*), and toxicity (*7*). However, the behavior and toxicity of some chlorinated biphenyls is influenced by chlorine position on the biphenyl in addition to the halogen content (*8*). An understand-

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ing of the kinetics of each of the brominated biphenyls in FireMaster was needed.

This paper reports an overview of several experiments conducted over a three year period designed to elucidate mechanisms of clearance and toxicity of polybrominated biphenyls in dairy cows and calves.

Methods and Materials

Animals

Sixty dairy animals have been involved in seven studies to determine the distribution, clearance, and health effects of FireMaster BP-6. The protocol for these experiments with cows, heifers, and calves are in Table 1. Detailed descriptions of methods used for experiments: 1, 2, 3 (*9*); 4 (*10*); and 7 (*11*) have been reported.

Experiments 1, 2, and 3 were designed to carefully monitor the short-term distribution and clearance of FireMaster. In these studies close interval sampling of blood and excreta, complete collections of excreta, inert plus radiolabeled tracers, and necropsies were employed to determine the fate of the residue. Experiment 4 provided information on residue distribution when from 0 to 25 g was given for 60 days to pregnant heifers. Samples of blood, body fat, and excreta were collected frequently throughout the dosing period and currently extends beyond the second parturition for many of these animals. In order to determine the distribution of PBB in 35 tissues, animals from each group were allotted for necropsy immediately following the

Table 1. Protocol for seven experiments on the distribution and effects of PBB on dairy cattle.

			PBB exposure			
Experiment	Animals	Status[a]	Daily amount, mg	Duration, days	Total dose to body weight ratio, mg/kg BW	Post exposure study, days
1[b]	1 Cow	P L	3000	1	4.59	1100[e]
	1 Cow	P L N	3000	1	5.95	10
2[b]	1 Calf	N	0.54	44	0.78	—
	1 Calf	N	[d]	95		44
3[c]	1 Calf	N	25,000	9	5000	—
4[c]	2 Heifers	P N	0	60	0	—
	1 Heifer	P L N	0	60	0	170
	3 Heifers	P L	0	60	0	640[e]
	2 Heifers	P N	0.25	60	0.0375	—
	1 Heifer	P L N	0.25	60	0.0375	170
	3 Heifers	P L	0.25	60	0.0375	640[e]
	2 Heifers	P N	250	60	37.5	—
	1 Heifer	P L N	250	60	37.5	170
	3 Heifers	P L	250	60	37.5	640[e]
	6 Heifers	P N	25,000	32-60[f]	≃ 2650	—
5	3 Calves		[g]	160		475[e]
	1 Calf	N	[g]	160		10
	3 Calves		[h]	160		475[e]
	1 Calf	N	[h]	160		10
	3 Calves		[i]	160		475[e]
	1 Calf	N	[i]	160		10
6[c]	1 Heifer	P L	250	180	90.0	500[e]
	5 Heifers	P	0	202	0	200[e]
	4 Heifers	P	250	202	100	200[e]
7[c]	2 Cows	N	25,000	25	1470	35
	2 Cows	N	0	25	0	35

[a] During course of experiment animals were: P = Pregnant; L = Lactating N = Necropsied.
[b] Lot 158RP of FireMaster BP-6 was used.
[c] Lot 6244A of FireMaster BP-6 was used.
[d] Calf from experiment 1.
[e] Under continued observation—days as of 10-1-1977.
[f] All animals moribund between days 33 and 66.
[g] Calves from experiment 4, 0.0 mg/day heifers.
[h] Calves from experiment 4, 0.25 mg/day heifers.
[i] Calves from experiment 4, 250 mg/day heifers.

dosing period and 10 days postpartum. All moribund animals were also necropsied. In experiment 5, the residue concentrations in calves from experiment 4 have been studied.

Experiments 6 and 7 were designed as toxicity studies; however, additional information on residue dynamics was obtained from blood, body fat, and excreta samples.

Doses of FireMaster were administered to animals in gelatin capsules with balling guns in order to insure complete consumption of the residue and minimize cross-contamination. Separate equipment was used for handling and management of experimental groups in order to further reduce chances of cross contamination.

Analyses

The procedures used for the analysis of PBB in this laboratory have previously been reported in considerable detail (*9*, *12*). In experiments 1, 2, and 3 only the major hexabromobiphenyl (peak 3, Fig. 1) was quantitated. The position of the bromines on this biphenyl have been identified as 2,2',4,4',5,5' (*4*, *13*). In experiments 4 through 7 all six peaks (Fig. 1) were quantitated, although chromatographic conditions were optimized for peak 3. Unfortunately peak 1 could not be accurately quantitated in all samples: therefore, all data for this peak has been omitted from this paper. Peaks 1 and 2 have been identified as pentabromobiphenyls, peaks 4 and 5 as hexabromobiphenyls, and peak 6 as heptabromobiphenyl (*4*). Hereafter the components of the mixture (Fig. 1) will be referred to as: peak 2, $PtBB_2$; peak 3, HBB_3; peak 4, HBB_4; peak 5, HBB_5; and peak 6, $HpBB_6$.

Results and Discussion

The two lots of FireMaster BP-6 used in our studies, 158-RP and 6244A, differed slightly in the relative concentration of the brominated biphenyls (*9*). These differences were minor and probably have no significant effect on interpretation of the kinetics of PBB. In the studies in which lot 158-RP was used only HBB_3 was quantitated. Since HBB_3 represented greater than 60% of lot 6244A, primary emphasis has been placed on determining the concentration and kinetics of this component of FireMaster in tissues and excretions of cattle.

The minor brominated biphenyls of the mixture, having varying halogen content and/or position, provide valuable insight into the kinetics of PBB in cattle. Therefore, quantitation of these components was made, although GLC conditions were optimized for HBB_3. The minor components were not

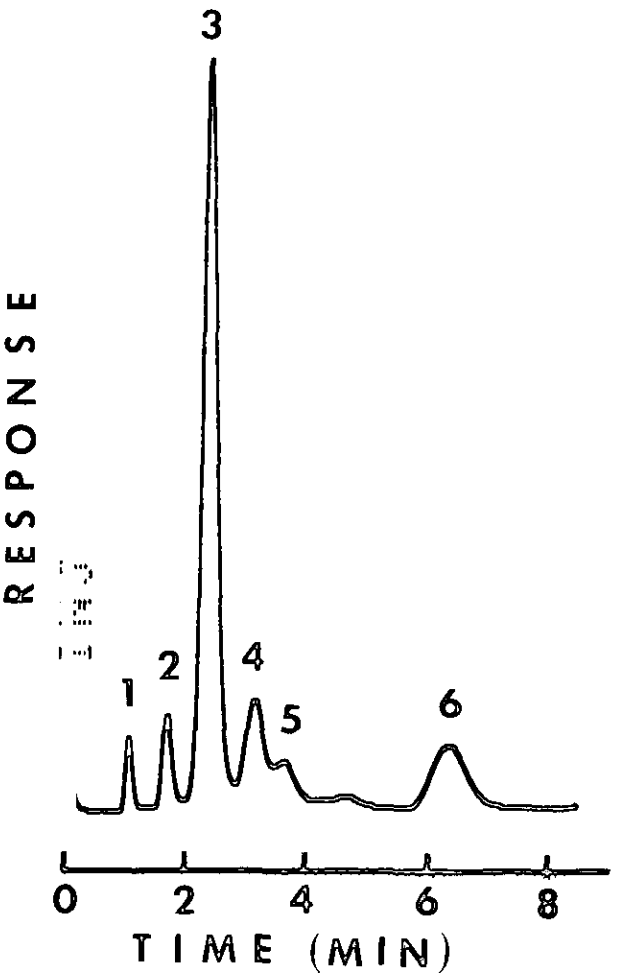

FIGURE 1. Gas-liquid chromatographic separation of the major brominated biphenyls in FireMaster BP-6. Conditions for the H. P. 5736 with ^{63}Ni detectors were: 0.5 m column of 5% OV-17 on Gas-Chrom Q, with column temperature at 270°C and argon: methane (95:5) as carrier and purge. Halogen content of peaks 1 and 2 (penta), 3 through 5 (hexa), and 6 (hepta) have been reported (*1*).

quantitated as parts per million of that compound because they comprised minor quantities of a commercial compound which still has not been fully identified. Changes *in vivo* of inert, nonhalogenated biphenyl or other biphenyl components could cause profound errors in reported concentrations of these minor components. In order to minimize these errors, yet quantitate the behavior of the minor components, samples from experimental animals were

quantitated against a standard of FireMaster BP-6 (lot 6244A) in which the concentration of each component was assigned the value of 1. Thus absolute concentration could not be determined, but the relative concentration of each component could.

PBB Concentrations in Feces

When FireMaster BP-6 was fed to dairy animals, feces was clearly the most important excretory pathway during exposure. The rate of passage of unconjugated PBB through the gastrointestinal (GI) tract was much the same as the flow of alimentary contents. The appearance and decline of HBB_3 in feces of animals given single 3-g doses of FireMaster were significantly correlated with passage of a chromic oxide (Cr_2O_3) marker (*9*). Presence of both PBB and Cr_2O_3 were detected in feces approximately 12 hr after administration, reached peak concentrations between 20 and 36 hr after dosing, then declined to about 2% of peak concentration during the subsequent 48 hr. Approximately half of each single dose administered was accountable in feces by day 8 (*9*).

The concentration of HBB_3 in feces of heifers fed 250 mg/day of BP-6 for 60 days reached steady state by day 10. Following withdrawal of the PBB the concentration of HBB_3 declined, and in 10 days had decreased to approximately 1% of the concentration present during exposure.

Following the initial decline, the residue concentration in feces remained stable until parturition. Total collections of feces were not made during this period so daily excretion can only be estimated. However, assuming 20 kg of fecal output daily, these animals were clearing only 0.7 mg of unconjugated HBB_3 daily.

In experiments in which heifers were given 250 mg of FireMaster for 202 days, concentrations of HBB_3 in feces during exposure were virtually identical to animals given the same daily dose for 60 days. Presently, direct relationships between the amount of PBB fed and fecal concentration cannot be made. The animals given 25 g daily displayed toxic effects which included diarrhea, anorexia, and dehydration (*12*). Concentrations of PBB in samples of feces from these animals sometimes were greater than 40,000 ppm. In contrast, PBB concentrations in feces from heifers receiving 0.25 mg daily were often at or below the sensitivity limit of our analytical procedures (*14*).

Few studies with PBB have reported fecal excretion. Detering et al. (*15*) and Cook et al. (*16*) studied cows that had been environmentally contaminated (*2*) 7–9 months prior to study. The authors estimated exposures of between 200 and 400 g of PBB over a 2-week period. Concentration of PBB in feces at the time of their study averaged 0.22 ppm with a daily excretion of 10 mg. Animals in our studies which received similar daily doses of PBB became moribund during the dosing period, and thus post-exposure clearance was not determined. Animals that received 250 mg daily had PBB concentration ratios in body fat to feces of about 750:1, which correspond with ratios reported for these environmentally exposed animals (*15*); however, the feces to plasma ratio of 0.7:1 in our studies does not correspond with reported ratios of 4.2:1 (*16*).

Free (unconjugated) residues of PBB have not been detected in urine of cows in our experiments. This route of excretion must not be overlooked as a possible route of clearance of minor or metabolized brominated biphenyls. For that matter, hydroxylated, conjugated, or otherwise bound PBB may escape detection in feces. During our initial study a ^{14}C-labeled tetrachlorobiphenyl was used as a tracer compound and administered with the FireMaster BP-6 (*9*). Total radioactivity accumulated in urine accounted for 24.4% of the ^{14}C dose, none of which was ether-extractable.

Although a large proportion of ^{14}C from the PCB with four halogens was recovered, presumably in conjugated form, this route still may be of little importance in PBB exposed cows. Most of the major biphenyls in FireMaster contain five or more halogens, thus reducing the chances of metabolism and excretion (*5*). Further, if HBB_3 were efficiently conjugated and excreted it would not be likely that tissue PBB concentrations would persist as have been observed in our studies.

Whereas feces was the most important excretory route in nonlactating animals, milk became the major route in lactating animals. Animals that previously received 250 mg/day for 60 days had a clearance of PBB via milk that was three times that in feces. This clearance in milk was only about 2 mg/day.

When FireMaster was fed to lactating cows, HBB_3 was detected in milk 13 hr post-exposure (*9*), and milk concentrations were stable by 20 days (*19*). Upon withdrawal of the PBB source, Fries and Marrow (*19*) reported a 71% decline in the concentration of HBB_3 in milk fat within 15 days, and thereafter the decline had a half-life of 58 days.

Animals in our studies exposed to PBB prior to parturition exhibited similar initial declines in milk residue concentrations postpartum. Perhaps accumulations of PBB in the mammary tissue may have contributed to these initially high concentrations. Thereafter, the reported 58 day half-life does not agree with our data. During peak lactation, when our experimental animals were metabolizing

body fat, concentrations of HBB_3 increased in milk fat and again declined when animals were gaining weight. Whereas the estimate of a 58 day half-life may be applicable for animals not changing weight, a more complex model is necessary to describe excretion of PBB in milk throughout a complete lactation.

PBB Concentrations in Tissues

Understanding the transfer of PBB to and from plasma is critical to an evaluation of distribution, clearance, and potential target organ toxicity. Following administration of FireMaster to dairy cattle, the major brominated biphenyls were readily absorbed from the GI tract and were detectable in plasma within 2–4 hr. In single-dose experiments, the concentration in blood plasma was maximal 24 hr after exposure. When multiple doses were administered, plasma concentrations were at equilibrium by 15 days. The concentration of HBB_3 was remarkably constant among animals when fed 250 mg PBB daily for 60 or 202 days. Average plasma concentrations of HBB_3 were about 0.13 ppm.

When dosing with FireMaster was terminated, concentrations of HBB_3 in plasma declined approximately 50% in 10 days and 66% by day 20. Thereafter, plasma residues do not fit a consistent decline model. To describe plasma changes in PBB concentration among environmentally exposed animals mathematically is difficult to achieve with accuracy. Changes in plasma concentrations of HBB_3 among experimentally exposed animals have indicated that corrections for pregnancy, lactation, and body weight (fat) changes will be necessary.

Table 2 shows mean concentrations of HBB_3 in plasma and major tissue groups of heifers and their calves that were necropsied when moribund, at the end of PBB exposure, or 10 days postpartum. Tissue:plasma residue concentration ratios are also presented. The relative tissue concentrations in various tissues were not unlike those found in necropsied tissues of cows and calves environmentally and experimentally exposed to PBB (*9, 17, 18*). Basically PBB has a strong predilection for fatty tissues with the notable exception of tissues of the nervous system. Whether the latter effect reflects failure of the residue to cross the blood brain barrier or simply reflects the inability of PBB to accumulate in the phospholipids, glycolipids, and sulfolipids of nervous tissues has not been demonstrated.

Most of the tissues of the body will fit nicely into one of the five tissue groupings in Table 2 having similar concentrations of extractable lipids and therefore similar PBB content. Bile, liver, and mammary tissues would not fit these categories, as they contained disproportionately large residue concentrations.

Where residue concentrations were above the limits of analytical sensitivity, the tissue:plasma ratios were remarkably consistent for nervous tissues (spinal cord, pons, and cortex) contractile tissues (smooth, striated, and cardiac) and liquid tissue (allantoic, amniotic, CSF, and synovial). This relationship probably represents an equilibrium between blood and the liquid tissues. However, it is

Table 2. Mean concentrations of hexabromobiphenyl in tissue groups and the ratios of tissue/plasma.

Animals	Exposure, g	Necropsy day		2,2',4,4',5,5'-hexabromobiphenyl in tissue, ppm							
				Plasma	Fats	Nervous	Contractiles	Glands	Liver	Liquids	Bile
6 Heifers	≈ 1125	33-66	Mean	53.3	1836	73.2	57.3	236	267	2.0200	53.3
			T/P Ratio	1.0	102	4.1	3.2	13.1	14.8	0.1	3.0
2 Heifers	15	61	Mean	0.1368	25.3	0.641	0.524	1.246	3.999	0.0198	0.536
			T/P Ratio	1.0	184	4.7	3.8	9.1	29.2	0.1	3.9
1 Heifer	15	230	Mean	0.0461	26.0	0.085	0.179	1.107	3.164	0.0244	0.117
			T/P Ratio	1.0	563	1.8	3.8	24.0	68.6	0.5	2.5
1 Calf	[a]	10	Mean	0.0563	12.03	0.011	0.656	0.632	0.575	0.0112	0.061
			T/P Ratio	1.0	213	4.1	11.6	11.2	10.2	0.2	1.1
2 Heifers	0.015	61	Mean	0.0026	0.163	Tr[c]	0.008	0.015	0.246	Tr	Tr
			T/P Ratio	1.0	62.7	—	3.2	5.6	94.6	—	—
1 Heifer	0.015	230	Mean	Tr	0.123	ND[d]	0.015	0.011	0.063	Tr	Tr
			T/P Ratio	—	—	—	—	—	—	—	—
1 Calf	[b]	10	Mean	Tr	Tr	ND	Tr	Tr	0.030	Tr	ND
			T/P Ratio	—	—	—	—	—	—	—	—

[a] Exposure by placental transport from preceeding dam plus 18.4 mg from dam's milk.
[b] Exposure by placental transport from preceeding dam plus .24 mg from dam's milk.
[c] Trace.
[d] None detected.

unknown whether an equilibrium exists between nervous or contractile tissues and the blood or whether the constancy simply reflects bound residue resulting from the initial exposure.

The grouping of 10 glandular tissues (adrenal, spleen, pancreas, kidney, thymus, thyroid, ovaries, parotid, lymphatic, and lung) (Table 2) was the most heterogenous group. Due to the widely varied function of each of these organs the residue accumulation of each merits further study.

High concentrations of PBB in liver tissue were not surprising in light of the detoxification and excretion functions of the liver. Among the animals directly exposed to the residue, greater efficiency of accumulation of HBB_3 at a very low level was clearly evident, particularly in liver. Ratios of the residue concentration in liver or fat tissue to the total dose consumed by the animals were calculated. Exposures of 25 g and 250 mg/daily resulted in liver:total exposure ratios of approximately 0.25. The ratio was 17 for heifers in the 0.25 mg daily feeding group. While not as pronounced as in liver, the accumulation pattern in fatty tissues was similar, with ratios of 1.7 for the higher exposures and 10 for animals receiving 0.25 mg daily.

Excretion in bile was probably the major source of PBB in feces following withdrawal of the residue. Unfortunately, the low numbers of samples of bile obtainable and unknown daily bile production did not allow quantitation of biliary excretion.

The concentrations of HBB_3 in the various depot fats of the body were quite similar. Within animals receiving the same exposure, the coefficient of variation was approximately 25%. Subcutaneous fats appeared to be the most variable, and concentrations in this pool were particularly affected by body weight changes of animals.

Figure 2 shows the HBB_3 concentrations in subcutaneous fat samples of animals given 0, 0.25, and 250 mg for 60 days or 25 g until moribund. This figure clearly shows the biomagnification or concentration of residues in fatty tissues of animals in contact with very small amounts of PBB. By design, to obtain the toxicological data for this experiment, all animals were housed in the same facility. Despite a meticulously designed protocol to minimize cross-contamination, the animals not experimentally exposed to BP-6 accumulated detectable concentrations in body fat. Based on tissue concentrations observed in animals receiving 0.25 mg daily (a total of 15 mg), we estimate that the experimentally unexposed animals consumed approximately 7 mg. Considering that more than 3 million mg were excreted in the facility by other animals, the experimental protocol appeared very successful.

Animals given 25 g daily lost weight, became moribund, and were necropsied with 33 to 66 days (*10, 14*). Concentrations of HBB_3 in subcutaneous fat from these animals ranged from 1100 to 6000 ppm.

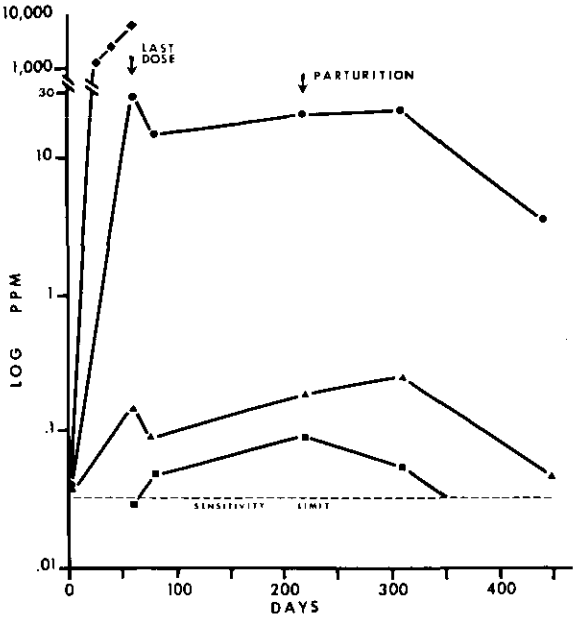

FIGURE 2. Average concentrations of hexabrominated biphenyl (HBB_3) in samples of subcutaneous fat from heifers given daily doses of FireMaster BP-6 for 60 days or until moribund. (■) 0; (▲) 2.5×10^{-1} mg/day; (●) 2.5×10^{2} mg/day; (•) 2.5×10^{3} mg/day.

Among animals given 0.25 or 250 mg daily there was a 40% decline in HBB_3 by 20 days post exposure (Fig. 2). Fat samples were not again collected until parturition, when an increase in the residue concentration had occurred. The concentration in subcutaneous fat continued to increase during heavy lactation when the animals were mobilizing depot fats. Biopsies were again collected in late lactation, and significant declines in residue concentrations had occurred. The latter effect probably reflects the redeposition of subcutaneous fat and a reduced body burden of PBB resulting from excretion of the residue in milk.

Distribution of Minor Components of FireMaster

The quantities of hydrocarbon represented by the minor penta-, hexa-, and heptabrominated biphenyls in FireMaster are quite small. In order to understand the significance of each of these components when accumulated in the mammalian system, additional studies will be needed. The positive identification of each biphenyl is especially needed.

Despite very low concentrations of these components and less than ideal conditions for quantitation, a comparison of the relative concentrations of the minor biphenyls has provided insight into the behavior of PBB *in vivo*. The best comparisons of concentrations for the minor peaks were obtained from samples from heifers given 250 mg/day of FireMaster.

During the period when doses were being administered the relative concentration of pentabrominated biphenyl ($PtBB_2$) in feces was decreased by about 45% and elevated in plasma approximately 50%. It was also slightly elevated in bile of the two necropsied animals. At the same time the relative proportion of heptabrominated biphenyl $HpBB_6$ in feces was elevated approximately 15% compared to the FireMaster standard. $HpBB_6$ was proportionately decreased in plasma and bile by 50 and 80%.

Ten days after the last dose was given and the concentration of all of the FireMaster components had declined, the relative concentrations of each had changed. The relative concentration of $PtBB_2$ increased sharply, nearly doubling in feces and plasma; $HpBB_6$ declined by 45 and 90%, respectively. Following initiation of lactation, very high relative concentrations of $PtBB_2$ were detected in milk fat, and the $HpBB_3$ was virtually undetectable.

These relationships indicated that bromine content influenced the absorption of PBB from the gut and subsequent transfer from plasma to depot fat. The pentabrominated compound was apparently more efficiently absorbed during feeding and obtained equilibrium between plasma and tissues at a higher relative concentration than found in the FireMaster that was fed to the animals. The opposite occurred with the heptabrominated biphenyl as a large proportion was apparently excreted in feces during the dosing period. Changes in the accumulation and excretion of the minor hexabrominated biphenyls also occurred; however, these differences cannot be easily explained without positive identification of the bromine position. The position of the halogen on the biphenyl has been shown to influence the distribution and clearance of polychlorinated biphenyls (*5*).

Although these relationships have suggested an effect on halogen content absorption of PBB, the possibility of biotransformation or degradation of one or more components of FireMaster cannot be ruled out. Transformations could greatly alter the relative concentrations of minor components.

General Discussion

These studies have provided pertinent information on the distribution and clearance of the components of FireMaster when dose levels and durations were varied. Animals from nearly all stages of productive life have been utilized. The data from these studies are being used to develop pharmacokinetic models which can be used to predict the behavior of PBB residues in environmentally contaminated livestock. The variety of protocols used for these studies provided data that have clearly demonstrated the simple kinetic models are inadequate to describe the long term behavior of this residual compound in dairy cows. The data from these studies has demonstrated that growth, fat deposition, fat mobilization, lactation, pregnancy, and level of exposure have important effects on the kinetics of PBB in cows. The true behavior of FireMaster *in vivo* is further complicated by the nature of the compound. Since it comprises of a mixture of compounds with differing halogen content and positional isomers, each component has a different biological behavior.

Surprisingly high concentrations of PBB can be accumulated in tissues of cows without apparent signs of toxicity (*10, 14, 20*). Claims that PBB causes toxic responses and death among dairy animals environmentally contaminated with trace levels of the compound must be seriously questioned, as we still have healthy animals on experiment with PBB concentrations up to and exceeding 30 ppm. Along with the information on the distribution of PBB in cattle, we have developed a broad base of information on the clinical (*9, 10, 20, 21*), pathological (*14, 22*), and target organ (*11, 23*) effects of exposed animals. These studies are continuing to develop a data base of residue and clinical information for the lifetime of remaining experimental animals.

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