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Toxicity of Polybrominated Biphenyls (PBBs) in Domestic and Laboratory Animals

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The composition, environmental fate, and effects of the polybrominated biphenyls (Firemaster BP-6 or FF-1) involved in the accidental contamination of cattle feed in Michigan in 1973 are reviewed. Toxic effects referred to in this report are limited to those occurring in domestic and laboratory animals and include general toxicity, neurobehavioral toxicity, immunotoxicity, reproductive toxicity, mutagenicity and carcinogenicity. The absorption, distribution, biotransformation and elimination of these polybrominated biphenyls are discussed along with the interactions with other chemicals and drugs.

Introduction

Polybrominated biphenyls (PBBs) have been widely used as flame retardants in industrial and consumer products. In 1973, PBBs accidentally entered the food chain in Michigan, when Firemaster FF-1, a commercial flame retardant, was inadvertently substituted for magnesium oxide used as a supplement in the formulation of cattle feed (*1*). Ten to twenty bags, 50 lb each, of PBBs were mixed into feeds that were widely distributed to Michigan farmers, and consumed by a large number of farm animals. Three feed preparations containing different concentrations of PBBs appeared initially to be involved Feed No. 405, 2.4 ppm;† No. 410, 1790 ppm; and No. 407, 4300 ppm (*2*). A concentration as high as 13,500 ppm of polybrominated biphenyls was also cited (*3*). Dairy cattle were the first to show signs of intoxication (decreased milk production, anorexia, alopecia, abnormal growth) which were attributed to the ingestion of PBB-contaminated feed. Subsequently, other farm animals were also found to have been contaminated and adversely affected. For about 9 months following the accident, contaminated dairy and poultry products were consumed by Michigan farmers and residents. In order to minimize further human exposure, at least 29,800 cattle, 5,900 hogs, 1,500 sheep, and 1.5 million chickens were destroyed along with large amounts of animal feed, cheese, butter, dry milk products and eggs (*4*).

Chemical Composition

The Michigan Chemical Corporation produced Firemaster FF-1 by adding an anticaking agent, calcium polysilicate (2%) to a commercial flame retardant known as Firemaster BP-6. Firemaster BP-6 is a mixture of polybrominated biphenyl compounds, containing 2-8 bromine atoms (*5*). Table 1 shows the major components of Firemaster BP-6 analyzed by gas chromatography combined with mass spectrometry. The major constituents of Firemaster BP-6 are 2,4,5,2′,4′,5′-hexabromobi-

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† 1 ppm = 1 mg/kg of feed or diet.

Table 1. Brominated compounds related to Firemaster FF-1 (BP-6).

Reported components	Concentration	Reference
2,2′-Dibromobiphenyl	200 ppm	(9)
Tetrabromobiphenyl(s)	2%	(10)
2,4,5,2′,5′-Pentabromobiphenyl }	10%	(10)
2,4,5,3′,4′-Pentabromobiphenyl }	8%	(11)
	4%	(6)
Hexabromobiphenyl	56%	(6)
2,4,5,2′,4′,5′-Hexabromobiphenyl }		
2,3,4,2′,4′,5′-Hexabromobiphenyl }	63%	(6, 10)
2,4,5,3′,4′,5′-Hexabromobiphenyl }	14%	(10)
Two heptabromobiphenyls	33%	(6)
2,3,4,5,2′,4′,5′-Heptabromobiphenyl	27%	(6)
Octabromobiphenyl }		
2,3,4,5,2′,3′,4′,5′-Octabromobiphenyl }	11%	(10)
Unknowns		
Tetrabromonaphthalene(s)		(12)
Pentabromonaphthalene(s)	150 ppm	(6)
	1 ppm	(8)
Hexabromonaphthalene(s)	70 ppm	(6)
	25 ppm	(8)

phenyl (56%) and 2,3,4,5,2′,4′,5′-heptabromobiphenyl (27%) (*6, 7*). Firemaster BP-6 was also found to be contaminated with low concentrations (0.02% or less) of penta- and hexabrominated naphthalenes (*6, 8*). Firemaster BP-6 was analyzed for the presence of polybrominated dibenzo-*p*-dioxins and polybrominated dibenzofurans because of structural homology to their known very toxic chlorinated derivatives (Fig. 1). If present, the concentrations were less than 0.5 ppm (*6*). Phenoxyphenols and hydroxybiphenyls, which might be intermediates in the formation of brominated dibenzo-*p*-dioxins and brominated dibenzofurans, respectively, were not identified (*8*).

Small amounts of PBB metabolites and decomposition products have also been detected by chromatographic and spectrometric methods (*13-18*). Several of these are listed in Table 2. Brominated dibenzofurans can be formed by thermal degradation of PBBs. Ultraviolet degradation cleaves bromine atoms leading to several debrominated and oxygenated biphenyl products (Table 2).

Fate of PBBs in the Environment

Because of their stability, PBBs may remain in the environment for a long time, raising questions about their transport and degradation in such environmental components as soil, water, plants, and animals.

The Michigan soils have been contaminated with PBBs from the manure of contaminated animals and from the disposal of feed, milk, carcasses, dust cleanings, etc. Once PBBs have been introduced into the soil, they appear to have little tendency to translocate. A study on the ability of rainfall to

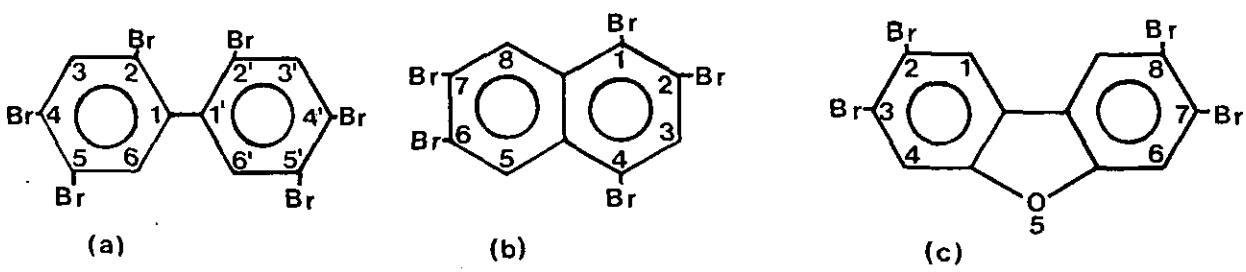

FIGURE 1. Representative polybrominated (a) biphenyl, (b) naphthalene, (c) dibenzofuran.

Table 2. Metabolites and decomposition products of polybrominated biphenyls.

Compound	Occurrence	Reference
Metabolites		
6-Hydroxy-2,4,5,2′,4′,5′-hexabromobiphenyl	Metabolite in dog feces	(*13*)
Pentabromobiphenylol	Metabolite in pig urine and/or feces	(*14*)
Several mono- to pentabromobiphenyls that are not present in BP-6	Metabolites present in fish	(*15*)
Thermal decomposition products		
1,3,4,7,8-Pentabromodibenzofuran 1,3,4,6,7-Pentabromodibenzofuran	Decomposition products of 6-hydroxy-2,4,5,2′,4′,5′-hexabromobiphenyl in gas chromatograph at 230-260°C	(*13*)
2,3,7,8-Tetrabromodibenzofuran Pentabromodibenzofuran	Decomposition products of Firemaster FF-1 by open tube pyrolysis at 380-400°C	(*16*)
Ultraviolet decomposition products		
2,4,5,3′,4′-Pentabromobiphenyl 2,4,5,2′,4′-Pentabromobiphenyl Tetrabromobiphenyl(s) Methoxylated product Dimethoxytetrabromobiphenyl	Decomposition products of Firemaster BP-6 at 2537 or 3000 A	(*17, 18*)

carry PBBs through the soil involved a laboratory simulation which analyzed only the major component of Firemaster, hexabromobiphenyl. This study indicated that hexabromobiphenyl was retained in the top soil and did not leach further (*19*). Although laboratory studies indicated that PBBs can be degraded by ultraviolet light (*20*), no significant degradation occurred in the field even after a year, and, what little degradation did occur, resulted in products with a much greater affinity for soil than that of parent compounds (*21*). Therefore, degradation by ultraviolet light is only a minor decontamination process for PBBs (*22*), affecting the small amount of PBBs on the soil surface. PBBs also appear to be fairly resistant to microbial degradation in soil (*23*).

Because of the apparent persistence of PBBs in soil, several studies have been done to determine whether forage (orchard grass, etc.) and root crops (onions, radishes, carrots, etc.) might take up these contaminants and reintroduce PBBs into the food chain (*21, 23, 24*). Both laboratory and field studies indicate that there was no uptake of PBBs into the plant foliage (*21, 23*), but that trace amounts of PBBs were associated with the root surfaces of edible root crops (*24*). Recontamination of animals from plants grown on contaminated soil is not likely to occur. No degradation of PBBs by plants has been recorded.

Water sampling in the Pine River downstream from the Michigan Chemical Corporation showed detectable levels (0.01-0.07 μg/l.) of PBBs in 1974 (*25*). River sediments also contained detectable levels (100-6200 μg/kg) of PBBs. No degradation of PBBs in water has been reported, although the possibility of photodegradation at the water-air interface exists. With the exception of the heptabromobiphenyls, the PBBs in water are readily taken up by fish and accumulated in their tissues (*15*). As an example of the efficiency of this bioaccumulation, fish living in water containing less than 0.1 μg PBB/liter of water concentrated these contaminants in their bodies 10,000-fold within two weeks (*25*). Fish are capable of metabolizing PBBs, but not to a degree sufficient for any significant degradation (*15*). Ducks living near contaminated waters also accumulate PBBs in their adipose tissue (*25*). Follow-up surveys since the termination of Firemaster BP-6 production show no significant decline of PBB levels in river sediments and fish and duck tissues. Thus, PBBs are likely to remain an environmental problem for a long time.

Absorption, Distribution, Biotransformation and Elimination of PBBs

An understanding of the true biological behavior of PBBs in the form of Firemaster BP-6 is complicated by the nature of the commercial product which is a mixture of compounds whose biological activity differs according to the position of bromine in the molecule and to a lesser degree by the number of bromines in the molecule (*26-29*).

A number of experiments have been performed in cows, pigs, rats, and birds on the absorption, biotransformation, tissue distribution, and excretion of the Firemaster BP-6 and several of its major components. These studies indicate that PBBs are rapidly and almost completely absorbed (absorption apparently decreases with increasing bromine con-

tent) from the gastrointestinal tract and are initially distributed widely throughout the body tissues with major concentrations in liver and fat (*1, 5, 29-44*). Based on the available data, the PBBs in Firemaster BP-6 are apparently metabolized to give hydroxylated degradation products by metabolic routes that are similar to those for the structurally related polychlorinated biphenyls (*14, 45-48*).

Pharmacokinetic studies in rats have revealed that the individual PBBs in Firemaster BP-6 are eliminated from the body at different rates (*26-29*). As a result of this differential rate of elimination, those components that are more slowly removed seem to become more concentrated relative to the other PBBs (*28*). The rates of metabolism appear to be determined primarily by the location of the bromine in the biphenyl molecule and secondarily by the bromine content or the availability of two adjacent unsubstituted sites in the biphenyl molecule (*26-29*). Similar relationships have been reported for the PCBs (*48, 49*). The major technical hexabromobiphenyl component, 2,4,5,2′,4′,5′-hexabromobiphenyl, appears to be the most persistent component in the species studied (*26, 27, 43*).

Excretion of PBBs is primarily via the bile into feces. Additional excretory routes for these chemicals are via lipophilic secretions such as hair and skin oil. Milk and eggs are the major routes in lactating mammals and egg-laying birds, respectively (*43*). Transplacental transfer of PBBs has been demonstrated in rats and cows and detrimental effects in young suckling on PBB-contaminated mothers have been reported (*5, 29, 37, 40-42*).

A number of metabolic studies of PBBs have been performed in cows. In one of the largest experiments to date, sixty dairy cows at nearly all stages of development and productive life were given daily doses of 0.25 mg to 25 g of Firemaster BP-6 for 1 to 202 days and were followed experimentally from 10 days to 3 years (*29*). It was found that the major (hexabromobiphenyls) and minor (pentabromobiphenyls, heptabrombiphenyls) brominated biphenyls of the commercial mixture were absorbed from the gastrointestinal tract and appeared in the blood plasma within 4 hr. With continued exposure, a steady state for the chemical in the plasma was reached after 15 days. PBBs had a predilection for high lipid-containing tissues of the cows and similar concentrations were found in the various fat depots. However, PBB concentrations were low in the nervous system despite its high lipid content and disproportionately high in liver considering the relatively low lipid content of that organ. In cows not showing signs of toxicity, the major route of elimination, representing 50% of the amount of PBBs fed, was the feces. Nonmetabolized PBBs were not detectable in the cows' urine, although this route of excretion may account for minor or metabolized brominated biphenyls. Following withdrawal of PBBs, the fecal concentration declined to 1–2% of fecal levels during dosing, but feces remained the major elimination route in non-lactating animals. Milk fat was the major route of excretion in lactating animals, accounting for three times the quantity of PBB residue eliminated in feces. The half-time of PBB excretion via the maternal milk was 28 days. PBB concentrations in milk declined approximately twofold in six days following birth of young. Following parturition, the PBB residues remained at a level of about 0.4% of that in the depot fat. Calves born to PBB-exposed cows had distributions of PBB residues in body tissue similar to those in the mothers but the concentrations were lower (*29*). This study and others (*1, 30, 36, 37*) have demonstrated that growth, fat deposition, fat metabolism, lactation, pregnancy and level of exposure can modulate the biotransformation of PBBs and that simple kinetic models are inadequate to describe the long-term behavior of PBBs in cows.

To the limited extent that they have been studied, the patterns of metabolism and tissue distribution in the pig, guinea pig, and rat are comparable to that in the cow (*5, 26-28, 33, 40, 47, 49*). However, in the rat, unlike the cow, the heptabromobiphenyl component was retained in significant proportions 42 days after administration (*26*). In hens, elimination of PBBs through the egg is more important than elimination through the excreta or storage in body fat (*34, 38*). The level of PBBs in eggs has been reported as about 1.5 times the dietary levels (*34*). After 63 days of dietary exposure to PBBs, the concentration of these chemicals in body fat of chickens was about four times that in the diet (*34*). The PBB residues in female egg-laying quail were generally lower than in males of the same species (*39*).

The kinetics of several individual components of Firemaster BP-6 have also been studied. When the major component, 2,4,5,2′,4′,5′-hexabromobiphenyl, was fed to male rats at a dose of 1 mg/kg, as single or multiple doses, 90% was absorbed from the gastrointestinal tract. This compound was distributed throughout the body with predominant initial localization in muscle, fat, and skin. The chemical was extremely resistant to metabolism. It was estimated on the basis of the short-term metabolic studies that only 9.5% of the total dose would ever be excreted in the feces. These findings suggested that male and nonnursing females and non-egg-laying birds would be unable to effectively clear

their tissues of ingested PBBs in their lifetimes (*43*). There is evidence that another PBB component, heptabromobiphenyl, disappears more quickly from milk (*36*) and eggs (*38*) than the hexabromobiphenyl described above.

Finally, metabolic studies of "octabromobiphenyl" (OBB), which consists of a mixture of hepta-, octa-, nona- and decabromobiphenyl averaging approximately eight bromine atoms per molecule, provide an insight into the disposition of the octabromobiphenyl component of Firemaster BP-6 that represents about 11% of the commercial mixture. This mixture, when fed to rats at 1000 ppm for 4 weeks, produced bromine concentrations in the fat that were approximately 600 times greater than the bromine levels in the fat of nontreated animals. Eighteen weeks after OBB was removed from the diet, the fat bromine residues had continued to increase and were 800 times greater than the bromine levels in the fat of control animals, implying that these compounds are retained in the tissues over a prolonged period. However, no comparisons with hexabromobiphenyl retention were possible from these studies (*44*).

Enzyme Induction by PBBs

Basic biochemical studies in laboratory rodents have shown that Firemaster BP-6 is a potent inducer of a group of liver (*50-56*) and kidney (*57*) microsomal enzymes, identical to those induced by phenobarbital and those induced by 3-methylcholanthrene (*51, 52*). Phenobarbital-type enzyme inducers increase the concentrations of cytochrome P-450, NADPH-cytochrome c reductase, and a wide range of microsomal enzymes whereas 3-methylcholanthrene-type inducers increase the concentration of cytochrome P_1-450 and stimulate a more specific group of enzyme activites not including the reductase (*58*). While phenobarbital-like microsomal enzyme stimulators also enhance bile flow and the biliary excretion of drugs, 3-methylcholanthrene-like inducers do not produce such effects (*59, 60*).

Commercial mixtures of polychlorinated biphenyls have produced a similar mixed type of induction (*61*) which has been attributed to the different inducing capabilities of individual components of the mixture (*62*). Thus far, two components have been identified. They are 2,4,5,2′,4′,5′-hexabromobiphenyl and 2,3,4,5,2′,4′,5′-heptabromobiphenyl, which together comprise 83% of the commercial mixture (*54*). Constituents of Firemaster BP-6 that are responsible solely for the 3-methylcholanthrene-type enzyme induction are unknown (*54-56*). Since the toxicity of several polyhalogenated biphenyls appears to be correlated with an ability to induce enzymes like those induced by 3-methylcholanthrene (*63, 64*), identification of such components might help to better characterize the toxicity and mechanism of action of PBBs in the commercial mixture. Indirect evidence for the presence of such components comes from a study in mice which revealed that the liver enzymes induced within 24 hr of exposure to Firemaster BP-6, were equivalent to those induced by phenobarbital, whereas those present 96 hr later were the same as the enzymes stimulated by 3-methylcholanthrene (*51*), implying a sequential metabolism of components with phenobarbital and 3-methylcholanthrene-like inducing properties.

In tests of the potency of the enzyme-inducing capability of Firemaster BP-6, the commercial mixture produced minimal enzyme induction at a dose level of 0.3 mg/kg/day and maximum induction at 30 mg/kg/day (*54*). Mixed induction was produced via both the transplacental route and the milk (*65, 66*). Lactating rats exposed to a dietary level of 1 ppm of Firemaster BP-6 showed no significant enzyme induction, whereas several enzymes were induced in nursing young at this dose level (*67, 68*).

Chemical Interaction of PBBs with Other Chemicals and Drugs

The ability of PBBs to induce a wide range of microsomal enzymes in liver and kidney involved in activation and deactivation of foreign compounds implies that PBBs have a high capability for altering the biologic activity of a variety of chemicals and drugs (*69, 70*). Several experimental studies support this assumption. It has been shown that mice ingesting PBBs in the feed for two weeks (as Firemaster BP-6, in concentrations of 1, 20, 25 or 100 ppm) developed an increased susceptibility to the renal and hepatic lesions induced by single intraperitoneal injections of the chlorinated hydrocarbon solvents, chloroform and carbon tetrachloride (*71*). As little as 0.025 ml/kg of chloroform produced abnormalities in liver and kidney function. While a dose of 0.005 ml/kg of carbon tetrachloride caused similar changes in the livers of these mice, 0.125 ml/kg of the solvent was required to produce kidney changes. Kidney changes were also observed in the PBB-treated mice when they were given 1.0 ml/kg trichloroethylene or 0.15 ml/kg or 1,1,2-trichloroethane. Since PBB administration stimulates microsomal enzyme systems in liver and kidney that appear to be necessary for the generation of toxic metabolites from these chlorinated hydrocarbon solvents, it can be assumed that the mechanism of PBB-induced potentiation of solvent

toxicity is stimulation of a particular enzyme or enzyme pathway resulting in the generation of greater amounts of toxic metabolite in PBB pretreated mice. These results imply that long-term exposure to PBBs might also sensitize humans or animals to the toxic action of chemicals requiring biotransformation (*71*).

There is also limited evidence that enzyme induction by PBBs may decrease the biological activity of some chemicals. Female rats maintained for approximately one year on a diet containing 50 ppm PBBs, as Firemaster BP-6, and 300 ppm N-2-fluorenylacetamide (2-FAA), a potent carcinogen, lived longer and developed 50% fewer tumors of the ear duct and mammary glands than did animals fed only 2-FAA in the diet. The PBB supplement also appeared to prolong the time to appearance of these tumors (*72*). Since the chemical 2-FAA requires metabolic change to the active carcinogen, it is possible that the enzymes induced by PBBs may have converted the 2-FAA mainly into inactive metabolites and thus reduced the amount of carcinogen bound to DNA (*73*, *74*). Further studies are needed to verify this hypothesis.

The results of a recent experiment imply that PBBs may also affect the activity of chemicals by influencing their excretion without affecting their biotransformation (*58*). In this study, dietary exposure of mice to Firemaster BP-6, enhanced the disappearance from plasma of two drugs, indocyanine green and ouabain, which do not require biotransformation for their activity. At 10 and 15 min following the intravenous injection of 40 mg/kg indocyanine green, the plasma levels of the drug were lower in animals that had been fed PBBs in the diet at levels of 100, 150, and 200 ppm than in those controls receiving no PBBs. The plasma concentration of ouabain given intravenously at 0.1 mg/kg was also much lower after 60 min in mice fed 100 and 200 ppm PBB than in controls. PBB-fed mice showed an enhanced uptake of both drugs by the liver, and an increase in liver weight. These effects appeared to be similar to those observed after phenobarbital pretreatment at 75 mg/kg/day for 4 days. Since indocyanine and ouabain are not metabolized prior to excretion into the bile, their enhanced disappearance from plasma cannot be explained by an altered rate of biotransformation. As was the case with phenobarbital pretreatment, however, the increased rate of disappearance from plasma was associated with an increased liver mass. Additional factors, not yet explored for PBBs, but important to the phenobarbital effect, are increases in hepatic blood flows and bile flow (*58*).

Finally, a further example illustrates the range of biological effects that can result from the interactions of PBBs with other chemicals in the body. It has been shown that a dose of 200 mg/kg of PBBs in the form of Firemaster BP-6 increased iron transport in the rat intestine by 50-60% as determined in everted duodenal gut sacs (*75*). The effect of PBB on iron transport was biphasic; iron transport was maximal two days after treatment and returned to normal levels two days later, but increased significantly to about 20% more than in controls at 7 and up to 18 days after treatment. This effect on iron absorption could be elicited by a dose as small as 2.5 mg/kg PBB without any overt signs of intoxication or weight loss. This experimental finding is corroborated by an increased serum iron content in cattle accidentally exposed to PBBs on farms in Michigan. Whether increased absorption is the sole mechanism for the increases in serum iron, however, is unknown, and further studies on the effect of chronic exposure to PBBs on iron absorption and total body iron are necessary (*75*).

General Toxicity of PBBs

Until the Michigan accident, very little was known about the toxicity of PBBs. Toxicological assessments of Firemaster FF-1 have been complicated because this commercial preparation contains a mixture of PBBs as well as traces of impurities (brominated naphthalenes) which have their own toxic properties. Adverse effects may also vary with the species exposed, the age of the animal, and the duration and dose of exposure. Gupta and Moore (*76*) showed that PBBs are more toxic when given in small repeated doses versus a single acute dose. In most acute and chronic feeding studies, signs of PBB toxicity include weight loss or a decreased weight gain, and an increase in liver size (*76-85*). Weight loss is not necessarily accompanied by decreased food intake (*76*, *81*) suggesting that PBBs may cause poor feed utilization.

Several studies on PBB toxicity in rats found that concentrations as low as 50 ppm (Firemaster BP-6) in the diet of male rats for 10 weeks produced an enlarged liver (*77*). Female rats fed 100 ppm Firemaster BP-6 for 90 days, showed retarded weight gain and altered activity of several kidney enzymes, but no loss of kidney function (*78*). Rats given 30, 100, 300 and 1000 mg/kg/day (5 days/week, 22 total doses) of Firemaster FF-1 by stomach tube were observed for 180 days after the beginning of treatment (*76*). All the animals exposed to dose rates greater than 30 mg/kg/day died within 73 days, with the exception of 62% of the males at 100/mg/kg/day which survived 180 days until sacrifice. All surviving rats demonstrated decreased body

weight gain (accompanied by significant decreases of food consumption), anemia, and enlarged livers. Histopathologic studies showed differences between the toxicities of PBBs to surviving male and female rats. Liver, kidney, prostate and thyroid glands were primarily affected in male rats, whereas only the liver was altered in female rats. The animals which became moribund or died early had marked atrophy of the thymus and spleen (*76*).

Mice fed 1000 ppm of Firemaster BP-6 in the diet for 4, 8, 11 and 14 days showed progressive signs of intoxication, body weight loss, and enlargement of the liver (*79*). Electron microscopy of liver tissue revealed increases in smooth endoplasmic reticulum and lysosomes, mitochondrial degeneration and decreases in glycogen.

Chickens fed Firemaster FF-1 in the diet at levels ranging from 50 to 250 ppm demonstrated the following adverse effects which were accentuated by increasing dose and/or length of exposure: decreased body weight due to decreased feed intake, decreased comb, testes, spleen, and bursa weights, increased liver and thyroid weights, hydropericardium and ascites (*80*). In another study, chicks fed 75 or 150 ppm of Firemaster FF-1 in the diet for 9 weeks showed cardiovascular and hematological effects which included decreased heart rate, cardiac output, packed cell volume, and hemoglobin concentration. This anemic condition was thought to be due to both decreased erythropoietin production essential for red blood cell formation by the kidney and to a direct action on the bone marrow (*80*).

Nonhuman primates experimentally exposed to Firemaster FF-1 also showed anemia which may have been due to decreased erythropoietin production (*81*). Seven female rhesus monkeys fed 0.3 ppm in the diet of Firemaster FF-1 for approximately 2 years showed reduced body weights for 6 months despite unmodified food intake, and prolonged menstrual cycles and decreased progesterone levels (*81*). Three monkeys fed 1.5 ppm for 38 weeks had a moderate weight loss, moderate fatty infiltration of the liver and decreases in serum cholesterol, while two monkeys on a diet containing 25 ppm for 14 weeks developed hyperplastic gastroenteritis (*82*). This hyperplastic gastroenteritis and the accompanying ulcerations were the most severe and acute lesions that were observed in PBB-exposed monkeys. Additional signs seen at 25 and 300 ppm PBB included enlarged livers, dry scaly skin, hair loss, generalized subcutaneous edema and marked edema of the eyelids (*81*).

Dairy heifers fed 250 mg/day of Firemaster BP-6 for 202 days, showed no clinical evidence of hepatic, renal, or intestinal toxicity (*83*). But dairy heifers fed 25 g/day of Firemaster BP-6 for 33-60 days showed weight loss, dehydration, atrophy of the thymus, and liver and kidney enlargement (*84, 85*). Histopathological and microscopic changes were most pronounced in the kidneys, gall bladder, and eyelids. In calves, a dose rate of 100 mg/kg/day of Firemaster FF-1 in the diet for 2-12 consecutive weeks produced histopathological lesions in the kidney, liver, skin, and testes (35).

In order to evaluate the comparative toxicity of PCBs and PBBs, adult male rats were fed 0, 5, 50, 500 ppm Aroclor 1254 (PCBs) or Firemaster BP-6 (PBBs), in the diet, for 2, 3 or 5 weeks. Firemaster BP-6 tended to cause equivalent adverse effects at lower doses, or in shorter time periods or had a greater effect at the same dose level than Aroclor 1254. The effects which were more pronounced with Firemaster BP-6 were an enlarged liver, an increase in liver cholesterol and plasma cholesterol and a decrease in liver RNA synthesis. Firemaster BP-6 was also found to be about three times more potent than Aroclor 1254 in inducing microsomal enzyme activities (*86*).

Neurobehavioral Toxicity of PBBs

Although clinical studies of farmers exposed to PBBs in Michigan have revealed a high incidence of neurologic and behavioral signs and symptoms (*87*), relatively little information is available from experimental studies concerning the effects of PBBs on the nervous system.

Tilson and Cabe (*88*) used a battery of tests to determine whether known amounts of PBBs given to laboratory rats and mice resulted in adverse behavioral and neurological effects. Animals were given doses of FF-1 or 2,4,5,2′,4′,5′-hexabromobiphenyl (HBB). Neurobehavioral toxicity was assessed at the end of a 30-day dosing regimen, and also 30 days after cessation of dosing in order to determine the delayed onset of signs or the duration of any effects noted. These investigations indicated that PBBs given orally to mice and rats reduced body weights, decreased motor activity, depressed motor reflexes, and impaired forelimb grip strength. Such effects were noted in rats receiving a total dose of 100-150 mg/kg of FF-1 during 30 days of dosing. The effects in rats tended to worsen over the 30 days after treatment, but those in mice tended to improve. HBB, the major component of the FF-1 mixture, was less toxic than the FF-1 mixture. Performance on tests sensitive to detecting peripheral neuropathy and emotionality changes was not affected by doses of FF-1 up to 1300 mg/kg. These authors concluded that the neuromuscular weakness and behavioral depres-

sion seen in laboratory animals after repeated exposure to PBBs are relatively nonspecific, and may be due to the effects of PBBs on organ systems other than the nervous system.

In another study (*89*), however, PBB (FF-1) levels were still detectable in the brains of rats 10 months after the last of a series of twenty 1.0 mg/kg doses. In a related study (*90*), rat tissue concentrations of PBBs, measured under conditions of weight loss severe enough to cause the disappearance of body fat, continued to exhibit a marked dose-response relationship with respect to retention of PBBs. PBB concentrations were consistently highest in liver, followed by kidney, brain, and plasma levels. Geller et al. (*89*) used operant conditioning techniques to evaluate whether known doses of PBBs affected the learning or performance of a discrimination task by laboratory rats. Neither the learning nor the performance of this task was affected by administration of doses up to 6 mg/kg/day for 20 days of dosing. However, extra responses (hyperactivity) occurred in the low dose group (1 mg/kg) whereas decreased responses (CNS depression) occurred in the high dose group (6 mg/kg). This latter observation led these authors to speculate that PBBs may produce behavioral changes similar to those of barbiturate-type drugs which can produce a continuum of excitation and depression depending upon the dose administered. Such speculation needs to be followed by further studies.

Immunotoxicity of PBBs

Following the Michigan accident, a large number of studies reported PBB-associated immunological effects in cattle and laboratory animals. Early studies reported that feeding low levels of Firemaster BP-6 to chickens and guinea pigs caused atrophy of various lymphoid organs and depressed antibody responses in the guinea pigs. Cattle inadvertently exposed to PBBs (accumulating up to 30 ppm PBB in body fat) showed no obvious immune alterations (*91*). Feeding Firemaster BP-6 to dogs for 61 days resulted in lymphoid depletion of lymph nodes, particularly in the T-cell zones, and a reduction in IgC containing lymphocytes in popliteal lymph nodes, but only at near toxic doses. In rhesus monkeys, alterations in B- and T-cell functions occurred after feeding PBBs at concentrations of 1.5 ppm for 5 months and 25 ppm for 10 weeks (*93*).

Preliminary studies in rodents exposed to PBBs for 30 days at dose rates ranging from 0.03 to 30.0 mg/kg body weight per day showed decreased immune responsiveness at 3.0 and particularly 30.0 mg/kg/day (*94*). In chronic studies (0.1 to 10.0 mg/kg body weight per day, 5 days per week for 6 months) several immunological responses were depressed in mice and rats given the higher dose rates although there was no significant decrease to bacterial challenge (*95*). The authors concluded that although PBBs could induce immune alterations in rodents, its immunological effects were apparent only at exposure levels near doses that caused other significant harm such as liver lesions and decreased weight gains.

Wilson et al. (*96*) reported depressed antibody responses following PBB exposure in mice (167 ppm in the diet for 3 weeks). After 6 weeks of feeding, however, the antibody response returned to normal indicating immunological recovery. Further studies showed the mean survival time of mice fed a diet containing 167 ppm PBB for 3 weeks was not affected by *Plasmodium berghei* infection (*97*). Fraker and Aust (*98*) reported that antibody responses to sheep red blood cells in mice fed diets containing 1, 10 or 100 ppm PBB for 30 days were 80%, 30% or 12%, respectively, of control values, although cell mediated immunity was not affected by this exposure regimen.

In summary, while PBB exposure in most laboratory animals causes immunosuppression, doses that come near to inducing clinical signs of overt toxicity are required.

Reproductive Toxicity of PBBs

Adverse reproductive effects were first observed in PBB contaminated cattle and have been subsequently reported to occur in a number of species. Effects on the reproductive process and on the offspring of three laboratory (rat, mouse, and monkey) and two commercial (cow and mink) mammals will be discussed.

Beaudoin (*99, 100*) reported that Firemaster BP-6 was fetotoxic and teratogenic in rats. Bi-daily exposure to 5 (and 25) mg/kg/body weight of Firemaster BP-6 for 14 days (starting with the day sperm was identified in the vagina as day 0), resulted in a decreased number of implantation sites and 25 mg/kg gave an increase in resorptions and fetal death (*99*). Doses of 50 and 100 mg/kg produced both effects. Administration of single doses of 400 and 800 mg/kg reduced fetal weight at term and resulted in an increased incidence of cleft palate and diaphragmatic hernia (*100*). Corbett et al. (*101*) also observed an increase in fetal death and resorption and reduced fetal weight at term in pregnant rats exposed to 100 and 1000 ppm Firemaster BP-6, but no increase in the incidence of malformations at these concentrations. No fetotoxic or teratogenic effects were observed in rats given

daily doses of up to 40 mg/kg Firemaster FF-1 from days 7-15 of gestation (*102*).

Fetal death, resorption, and decreased fetal weight at term were also observed in mice receiving 200 ppm of PBB in the feed on days 4-16 of gestation (*103*). Malformations (exencephaly, cleft palate) were observed at concentrations up to 1000 ppm of BP-6 (*104*). No fetotoxicity or teratogenicity was observed when mice were exposed to the major component of Firemaster BP-6, 2,4,5,2′,4′,5′-hexabromobiphenyl at a dose of 40 mg/kg on days 10-16 of gestation (*105*).

Rhesus monkeys receiving dietary levels of 0.3 ppm of Firemaster FF-1 for 7 months prior to breeding had prolonged menstrual cycles, long periods of implantation bleeding, and decreased progesterone levels (*93*). Infants born to these monkeys were small and gained less weight during the first 16 weeks after birth.

Jackson and Halbert (*106*) first reported that cows exposed to Firemaster FF-1 contaminated feed had a 50% reduction in milk production within one month of exposure probably due to reduced food intake. After the feed was changed their appetite improved, but milk production stabilized at only 60% of normal. Cows that were bred 4-6 weeks before the replacement of feed resumed estrus and had to be rebred in the next 2 months, suggesting early embryonic death and resorption. Cows exposed in the last trimester often had difficult deliveries and delivered larger calves usually 2-4 weeks late. Often these calves were dead or died soon after birth. Metritis occurred in all lactating cows and their milk production was low. Twenty pregnant cows began eating less several weeks prepartum and some died before giving birth. Four of these cows had signs of excess amniotic fluid which resulted in one death and two stillbirths. Mercer et al. (*107*) compared the health status of 16 herds exposed to low levels of PBBs with 15 control herds for the years 1972-1974. Milk production, mastitis and percentage of the herd culled because of sterility and death of the calves did not differ between the PBB-contaminated and control herds. Another study (*108*) compared 46 cows from six exposed herds with 40 cows from five nonexposed herds. There were no differences between the numbers of cows yielding calves, calves born alive, or abortions and stillbirths. No postnatal deaths or malformations were seen for either group. With regard to the maternal cows, there were no differences in milk production or in incidence of mastitis, milk fever, or metritis. Ferris (*109*) suggested that calf mortality varied between 9.7 and 34.9% in Michigan in 1973, and that differences in mortality could not be attributed to PBB exposure; however, the relationship between calf mortality and PBB exposure needs further investigation. A study (*85*) of pregnant cows fed daily doses (corresponding to 0.0375, 37.5 mg/kg body weight) of Firemaster BP-6 for a total of 60 days, showed no effect on milk production, incidence of mastitis, or number of inseminations required for conception. There were no differences in birth weights of the calves or weight gain by 42, 180 and 202 days, respectively, without seeing toxic effects. However, the pregnant cow had difficult labor and yielded one stillbirth, which was normal on necropsy. The administration of a total dose of 2,650 mg/kg over a 32-60 day period to six pregnant cows resulted in three abortions and three dead fetuses (*85*).

Mink fed 1, 2.5, 6.25 or 15.6 ppm of Firemaster FF-1, or 1.5 ppm-contaminated poultry or 12.0 ppm-contaminated beef for up to 10 months (*110*), exhibited increased mortality. The number of young born alive decreased by 25% with the 1 and 2.5 ppm doses, and by 42% with the contaminated poultry. At these doses, kit weights at birth were also reduced, and the percent mortality from birth to 4 weeks was increased. The PBB-contaminated beef and poultry was more toxic than the Firemaster FF-1 contaminated mixture.

Mutagenicity of PBBs

Hexabromobiphenyl (isomeric composition unspecified) was assayed for mutagenicity in bacterial test systems of *Salmonella typhimurium* (strain TA 98, TA 100, TA 1535, and TA 1537), both with and without metabolic activation by liver preparations from rats and hamsters. The hexabromobiphenyl was not found to be mutagenic (Zeiger, 1980, personal communication).

Firemaster FF-1, when given to pregnant rats in corn oil in six repeated doses of 100 mg/kg body weight, did not produce chromosome aberrations in bone marrow cells (*111*). Similarly, the incidence of chromosome breaks in bone marrow cells of male mice did not significantly increase when the animals received single doses of Firemaster FF-1 in dimethyl sulfoxide (50 or 500 mg/kg body weight). However, the number of chromosome gaps did increase after 24 and 48 hours. The genetic significance of gaps (regions in the chromatid structure that cannot be stained by the Feulgen method) is not clear (*112*). Male rats which were given Firemaster BP-6 in food in a concentration of 5, 50 or 500 mg/kg for five weeks showed no increase in the number of chromosome breaks either in bone marrow cells or in spermatogonial cells (*113*).

In both studies (*111*, *112*) some animals were also injected with colchicine (5 mg/kg body weight) and

mitotic and metaphase indices in bone marrow cells were determined. In the study with pregnant rats (*102*), PBBs interacted synergistically with colchicine: bone marrow cells of animals treated with both colchicine and PBBs showed higher metaphase and mitotic indices than untreated animals or animals treated singly either by PBBs or by colchicine. No colchicine synergism was observed in the study with male mice (*112*).

Carcinogenicity of PBBs

Several studies have shown that the liver is one of the target organs for polybrominated biphenyls (*1, 114*). Some liver lesions produced are persistent and have the potential to develop into tumors as indicated by Kimbrough et al. (*115*). In this study, Sherman strain COBS rats given a single dose of 1000 mg Firemaster FF-1 by stomach tube in peanut oil developed liver lesions described as "hyperplastic" or "neoplastic" nodules. The neoplastic nodule is a manifestation of the cancer causing process in the liver. It is induced by a variety of carcinogenic agents but not by noncarcinogenic chemicals (*116*). The neoplastic nodules occurred more frequently in females although the levels of PBB in the liver were higher in the male rats. Similar atypical liver nodules are observed in rats after six months when the animals were exposed to multiple doses of PBBs (*76*). The study by Kimbrough et al. (*115*) was evaluated by a working group of the International Agency for Research on Cancer in Lyon, France (*5*), and it was found inadequate for the assessment of carcinogenicity. The number of animals (20 of each sex) was considered too small, and the duration of observations (14 months) too short.

In 1980 (*117*) Kimbrough and her collaborators completed three additional studies. The lot of Firemaster FF-1 and the method of administration was the same as that used in the 1978 study. In one group of 65 female Sherman strain COBS rats, 2-month-old animals were given a single dose of 1000 mg/kg body weight, and in a group of 30 females each animal obtained 12 repeated doses of 100 mg/kg body weight, one dose every 3 weeks. After 26 months, the incidence of liver cell cancer (hepatocellular carcinoma) was 41.4% in the single-dose group, and 64.3% in the multiple-dose group. No liver cancers were observed in the corresponding control groups. A third group of 16 female rats 4 months old was given a single dose of 200 mg/kg also by stomach tube. The incidence of neoplastic nodules 18-22 months later was 31.2%, but no liver cancers were observed.

Similar findings have been observed recently in studies on the carcinogencity of Firemaster FF-1 conducted within the National Toxicology Program (draft NTP Technical Report).* Fischer 344/N (CDF) rats of both sexes were given 125 doses (by mouth) of Firemaster FF-1 over a 6-month period (0.1, 0.3, 1.0, and 10.0 mg/kg/day, 5 days per week). After 6 months of treatment with Firemaster FF-1, some animals of each group were kept for observation during their lifetime. Twenty-three months after the end of exposure, different types of liver tumors were noticed in all male rats which received doses at a rate higher than 0.1 mg/kg/day and in all female rats at a dose rate above 1.0 mg/kg/day. Under the conditions of this bioassay, polybrominated biphenyl mixture (Firemaster FF-1) was considered to be carcinogenic for both Fischer 344 rats and $B_6C_6F_1$ mice of both sexes, inducing neoplastic nodules, hepatocellular carcinomas and cholangiocarcinomas in rats and hepatocellular carcinomas in mice.

In interpreting these data, it should be kept in mind that Firemaster FF-1 is a mixture of PBBs containing mainly compounds with six and seven bromine atoms. In addition it contains penta- and hexabrominated naphthalene impurities in concentrations of about 100 ppm and 2% by weight of an anticaking agent, calcium polysilicate. Furthermore, different strains of rats were used in the Kimbrough et al. studies (*117*) and in the Gupta et al. study (*118*). Lots of Firemaster FF-1 were different as well.

Conclusions

Although the hexa-(2,4,5,2′,4′,5′-) and hepta-(2,3,4,5,2′,4′,5′-) bromobiphenyl compounds constitute the major components of Firemaster FF-1 (BP-6), other minor components and impurities have also been detected, which may have their own toxic properties.

PBBs have contaminated the soil and water in Michigan, but there appears to be little uptake of PBBs by plants from soil. Degradation by sunlight although observable under laboratory conditions is not considered a likely mechanism for PBB degradation, nor is microbial action very effective.

Studies in various animal species indicate that PBBs are rapidly absorbed from the gastrointestinal tract and are widely distributed throughout the tissues with major concentration occurring in liver

*Comments and questions about the DRAFT National Toxicology Program Technical Report on the Toxicology and Carcinogenesis Bioassay of Polybrominated Biphenyl Mixture (Firemaster FF-1) should be directed to Dr. B. Gupta, National Toxicology Program, P. O. Box 12233, Research Triangle Park, North Carolina 27709 (919-541-3233).

and adipose tissue. In general, PBBs are resistant to metabolism and are slowly and incompletely eliminated from the body, via the bile into the feces. Thus, elimination of larger doses will not be completed in the lifetime of most animals except in those instances where egg-laying and milk production facilitate elimination by these routes. Some metabolism of PBBs can occur and hydroxylated derivatives have been identified in the urine of dogs and pigs and in pig feces. PBBs are potent mixed inducers of liver mixed-function oxidase enzymes, and thus provide a high potential for modifying the toxicity of other chemicals and drugs.

In acute and chronic feeding studies, the initial signs of general PBB toxicity include weight loss and increased liver size, which may be accompanied by a broad spectrum of other biological effects depending upon the species studied. Studies on specific target organs suggest that the effects on the nervous system such as neuromuscular weakness and behavioral depression may be the result of nonspecific toxicity. Immunosuppression has also been observed in most laboratory animals exposed to PBBs, but the doses required are close to those inducing clinical signs of overt intoxication.

Adverse reproductive effects were observed in cattle following the original accident and have been observed subsequently in rats, mice, monkeys, cows and mink as well as other species. In general, very early in pregnancy PBB intake can lead to resorption of the fetuses, while administration of PBBs later in pregnancy can give rise to offsprings with lower birth weight and some malformations. PBBs can readily enter the fetus by crossing the placenta and can also be transferred to newborn via breast milk.

Mutagenicity studies on PBBs to date have been negative but inadequate. Exposure to high doses of PBBs has been shown to cause liver cancer in rats and mice.

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