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thesis entitled

ACUTE PATHOLOGIC EFFECTS OF POLYBROMINATED

BIPHENYLS (PBB) IN THE GUINEA PIG AND GOLDEN HAMSTER

presented by

James Edward Collins

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ACUTE PATHOLOGIC EFFECTS OF POLYBROMINATED BIPHENYLS (PBB) IN THE GUINEA PIG AND GOLDEN HAMSTER

By

James Edward Collins

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Pathology

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ABSTRACT

ACUTE PATHOLOGIC EFFECTS OF POLYBROMINATED
BIPHENYLS (PBB) IN THE GUINEA PIG AND GOLDEN HAMSTER

By

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Groups of 6 guinea pigs or hamsters were given oral doses of Firemaster BP-6, a mixture of PBB, in corn oil. Single doses of 0, 100, 200, 400 or 800 mg/kg body weight were given to guinea pigs. Hamsters were given 4 doses of 0, 100, 200, 400 or 800 mg/kg singly at 3 hour intervals. Body weights were measured and survivors were killed 14 days after treatment. Guinea pigs given 400 or 800 mg/kg died and had severe body weight loss. No compound related deaths occurred in hamsters given PBB but all had reduced weight gain. Thymuses were atrophied in both species. Absolute liver weights were increased in hamsters but not in guinea pigs. Livers of hamsters had diffuse hepatocellular hypertrophy and hepatocyte necrosis, whereas guinea pig livers had mild centrilobular fatty change. Variations in organ pathology or in biochemical findings did not explain differences in species susceptibility to the lethal effects of PBB.

Dedicated with love to my wife,

Barb, and my son, Brian.

ACKNOWLEDGEMENTS

I wish to express my appreciation to Dr. Stuart Sleight, my major professor, for his support during my course of study.

I also wish to thank Drs. Allan Trapp, Stuart Levin, and Howard Stowe, members of my guidance committee, for their helpful suggestions.

Special thanks to my friend and fellow student, Dr. Richard Jensen, who was always willing to help and provide constructive criticism.

I also wish to thank the many technicians who contributed to the project.

My deepest appreciation to my wife, Barb, and my son, Brian, for their support, love and understanding throughout my studies.

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INTRODUCTION

Polybrominated biphenyls (PBB) are members of a class of compounds, the polyhalogenated aromatic hydrocarbons, typified by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). TCDD occurs as a contaminant during the synthesis of trichlorophenols and the germicide hexachlorophene (Kimbrough, 1974). It has been involved in several accidental environmental contaminations, the most recent being an accident in Seveso, Italy in 1976, that disseminated approximately 2 kg of TCDD over an urban area (Firestone, 1978).

These environmental accidents have led to many investigations into the toxic effects of TCDD in laboratory animals. The guinea pig is the most sensitive to the lethal effects with an oral LD_{50} of 2 µg TCDD/kg (Gupta et al., 1973) whereas the hamster is the least sensitive with an oral LD_{50} of 1157 µg TCDD/kg (Olson et al., 1980).

The reasons for the species variation to the lethal effects of polyhalogenated aromatic hydrocarbons are unknown. No interspecies correlation exists between liver damage and TCDD toxicity. Sufficient exposure, however, does produce thymic atrophy and loss of body weight in all species studied.

A cytoplasmic receptor which binds TCDD has been identified (Poland et al., 1980). It is postulated that this receptor translocates to the nucleus in a fashion similar to steroid hormones. Once in the nucleus it initiates the synthesis of 3-methylcholanthrene (MC)-type of microsomal drug metabolizing enzymes. Poland and

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Glover (1980) have shown that 3,3',4,4',5,5'-hexabromobiphenyl (HBB) also competitively binds to this receptor and stimulates MC-type microsomal drug metabolizing enzymes.

Although there is a correlation between the ability to induce MC-type of drug metabolizing enzymes and toxicity, the biochemical basis of toxicity is unknown. Work by Gasiewicz et al. (1982) has shown variations in receptor number and affinity among species. The guinea pig, which has been found to be sensitive to TCDD-induced thymic atrophy, has the highest number of thymic receptors. The hamster is less sensitive and has a lower number of receptors. Also, hepatic receptors in the guinea pig had the highest affinity for TCDD whereas hamster hepatic receptors had lower affinity.

The marked interspecies variation in toxicity to TCDD led to the examination of PBB, a related polyhalogenated aromatic hydrocarbon, in two species that differ in their sensitivity, the hamster and guinea pig. This preliminary investigation emphasized gross, histologic and ultrastructural pathology and clinical features.

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LITERATURE REVIEW

Chemical and Physical Properties

The polybrominated biphenyl (PBB) mixture used in this experiment was manufactured under the trade name of "Firemaster BP-6" (FM) by the Michigan Chemical Corporation. At room temperature it is a nonpolar, white, odorless solid that begins to melt at 72 C and decomposes at 300 to 400 C. The vapor pressure is low and, unlike polychlorinated biphenyls, ultraviolet radiation will readily degrade PBB to lesser brominated biphenyls (Kay et al., 1977).

The commercial mixture of PBB (FM) contains about 12 major components (congeners) (Aust et al., 1982). An identification system that correlates molecular structure with retention time in a gas chromatography column has been developed (Figure 1) (Moore and Aust, 1978, Aust et al., 1982). The percentage by weight of individual congeners has been reported with the major component being 2,2',4,4', 5,5'-HBB (47.8%) (Aust et al., 1982). Firemaster FF-1 (BP-6 mixed with 2% calcium silicate) was found to be contaminated with trace quantities of hexabromonaphthalene, pentabromonaphthalene, and tetrabromonaphthalene (Hass et al., 1978).

Metabolism

The metabolism of PBB is facilitated when the number of para-substitutions decreases, the number of ortho-substitutions increases, and the total number of substitutions decreases (Moore et al., 1980).

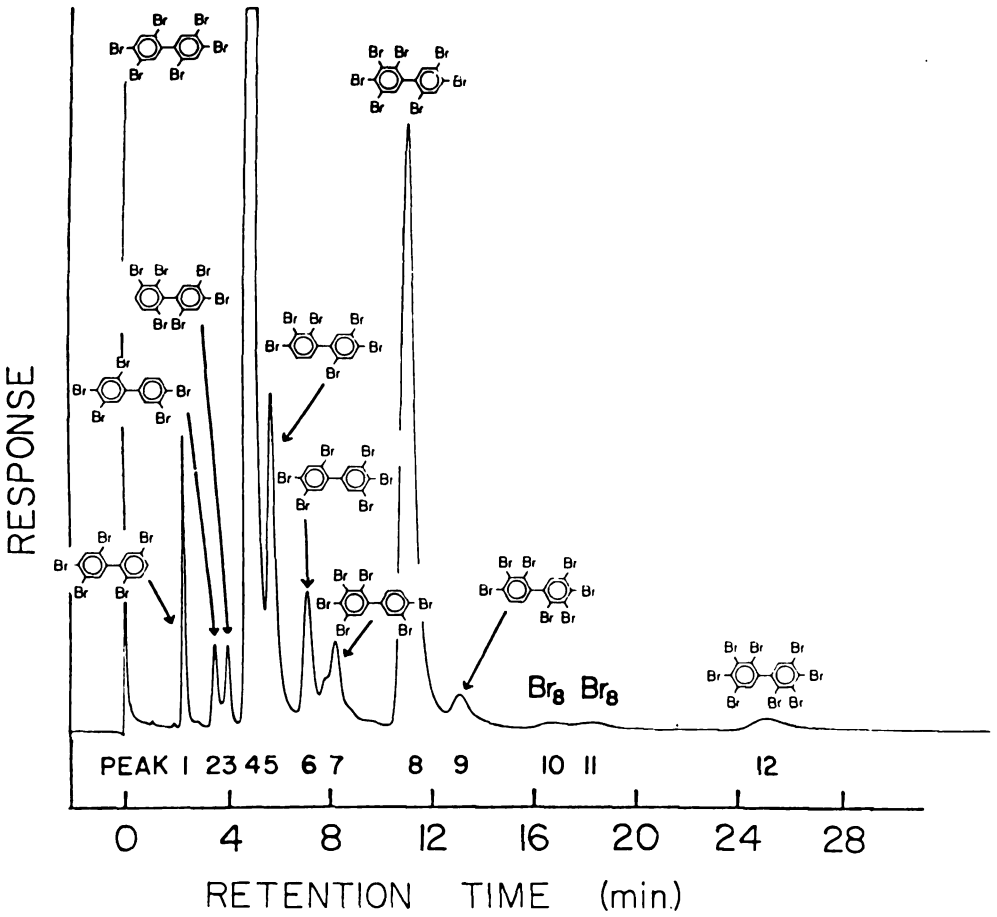

Figure 1. Gas chromatogram of Firemaster BP-6.

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It follows, then, that 2,2'-dibromobiphenyl would be most rapidly metabolized and this has been demonstrated (Dannan et al., 1978). Among the major congeners in FM, 2,2',4,5,5'-penta and 2,2',3,4',5',6-HBB are the congeners most rapidly metabolized (Dannan et al., 1978). The remainder of the PBB congeners in FM have at least two para-substitutions and consequently metabolism is slow or does not occur.

Pharmacology

Microsomal drug metabolizing enzyme systems are involved in oxidation, reduction, hydrolysis and conjugation reactions used for the metabolism of many drugs and foreign compounds (xenobiotics) (Kuntzman, 1969). These reactions make lipid soluble compounds more polar, i.e., more soluble, enabling them to be excreted in the bile and urine (Milburn, et al., 1967). Drug-metabolizing systems are composed of many different enzymes. Of primary importance are a group of mixed function oxidases consisting of NADPH-cytochrome P450 reductase and the terminal oxidase called cytochrome P450 (Gillette et al., 1972).

All of the polyhalogenated hydrocarbons are potent inducers of hepatic microsomal drug-metabolizing enzymes. This includes the halogenated biphenyls, naphthalenes, dibenzofurans and dibenzo-p-dioxins. The type of induction can be divided into two classes. Those which induce cytochrome P450 as phenobarbital does are called phenobarbital-type inducers. The other class is the 3-methylcholanthrene-type which induce a distinct form of cytochrome P450 called cytochrome P448 or $P450_1$ (Haugen et al., 1976). Some compounds, such as the PBB, have properties of both and are called mixed-type inducers.

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The type of microsomal enzyme induction produced by individual congeners in FM is given in Table 1 and has been reported (Moore et al., 1978; Moore et al., 1979; Robertson et al., 1981; Aust et al., 1982).

No strict MC-type of inducer is in FM but Aust et al. (1981) has purified 3,3',4,4',5,5'-HBB, which is strictly an MC-type of inducer, from a mixture obtained from RFR Corporation (Hope, RI). This compound is the most toxic PBB congener that has been tested. The most toxic congener in FM is only about 1% as toxic as 3,3',4,4', 5,5'-HBB (Aust et al., 1982).

The mechanism of MC-type induction has been studied by Poland and Glover (1980). They identified a cytoplasmic receptor involved in the induction of aryl hydrocarbon hydroxylase (AH) by 3-MC and 2,3, 7,8-tetrachlorodibenzo-p-dioxin (TCDD). This receptor may translocate to the nucleus in a manner similar to that described for steriod hormones where it initiates gene expression.

Table 1. Type of liver microsomal drug-metabolizing enzyme induction by polybrominated biphenyl congeners in Firemaster BP-6.*

Phenobarbital-Type	Mixed-Type
2,2',4,4',5, 5'-hexabromobiphenyl	2,3',4,4',5-pentabromobiphenyl
2,2',3,4,4',5,5'-heptabromobiphenyl	2,2',3,4,4',5-hexabromobiphenyl
2,2',3,3',4,4',5,5'-octabromobiphenyl	2,3',4,4',5,5'-hexabromobiphenyl
	2,3,3',4,4',5-hexabromobiphenyl

*No strict 3-methylcholanthrene-type of inducers are in Firemaster BP-6.

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Compounds that induce MC-type of hepatic drug-metabolizing enzymes have the ability to bind to this receptor. This relationship has been found for dibenzo-p-dioxins, dibenzofurans, and for 3,3',4,4', 5,5'-HBB (Poland et al., 1980). There is a high correlation between ability to bind to this receptor and toxicity; but, the biochemical basis for toxicity is unknown.

Kinetics

Since PBB are lipophilic compounds they are distributed to tissues with high amounts of lipid. Thus, adipose tissue, liver and kidney have the highest concentrations of PBB. An exception to this is the brain which, although lipid rich, generally has one of the lowest levels of PBB. This may be due to the different types of lipids found in the brain, i.e. glycolipids and phospholipids or due to the effectiveness of the blood brain barrier (Willett et al., 1978). Ability to penetrate the brain has been shown to be affected by position and number of bromine atoms (Domino et al., 1980). Bromine substitutions also affect transport across biological membrane barriers because as the number of bromine atoms increases there is more resistance to crossing (Fries et al., 1976).

PBB are excreted through milk, eggs, feces, and urine. In the lactating animal milk is a major route of excretion (Gutenmann and Fisk, 1975) and PBB were detected in milk within 13 hours after oral administration (Willett and Irving, 1976).

In laying birds, the egg is a major route of excretion. Fries (1978) estimated about 50% of the daily intake of PBB is excreted through the egg.

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Feces are the major route of elimination of PBB in nonlactating and nonegglaying animals and fecal excretion has been studied in rats (Matthews, et al., 1977), pigs (Ku et al., 1978), cattle (Willett and Irving, 1976), and chickens (Ringer et al., 1977). When a single dose of octabromobiphenyl was administered to rats about 62% of the isotope was detected in the feces within 24 hours and about 73% of the dose was excreted by 16 days after administration. Rozman et al. (1981) studied fecal excretion in Rhesus monkeys given 100 mg/kg body weight of ^{14}C, 2,2',4,4',5,5'-HBB. They concluded that excretion of the chemical through the feces is due to both biliary and intestinal elimination.

Urine is a minor route of excretion of PBB. Willett et al. (1978) did not detect free unconjugated PBB in the urine of cattle. Pigs given a single intraperitoneal dose of PBB excreted only 1% of the administered dosage in the urine (Kohli et al., 1976).

Pathotoxicology

Prior to the 1973 accidental contamination of the Michigan environment with PBB, little work had been done on the toxicity of these chemicals. Since that time, however, many reports of ill effects in exposed people and animals (Jackson et al., 1974; Prewitt et al., 1975; Stross et al., 1981) and toxic effects in laboratory animals (Allen et al., 1978; Aulerich et al., 1979; Gupta et al., 1981; Render et al., 1982) have been reported. Some of the toxic effects in laboratory animals are summarized below.

Clinical Pathology

PBB fed in the diet at 0, 1, 10, 100 or 500 ppm for 30 or 60 days did not affect red blood cell count (RBC), packed cell volume (PCV),

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hemoglobin (HGB) and total white blood cell counts (WBC) in rats (Sleight et al., 1976; Sleight et al., 1978). Gupta et al. (1981), however, reported a decrease at 30 and 45 days but not at 60 days in PCV, HGB, and RBC in rats given 22 oral doses of 30 mg Firemaster FF-1 per kg body weight. Hematologic effects were also seen with other polyhalogenated aromatic hydrocarbons such as the chlorodibenzo-p-dioxins which cause anemia, leukopenia, and thrombocytopenia in guinea pigs and nonhuman primates (McConnell et al., 1979). Gamma glutamyl transpeptidase levels were elevated in female rats given FM and 2,2',4,4',5,5'-HBB (Gupta et al., 1981). BUN levels were increased and sorbitol dehydrogenase levels were unchanged in guinea pigs (Hall, 1980). Serum cholesterol values were increased in rats fed 100 ppm of PBB (Akoso et al., 1977) or 2,2',4,4',5,5'-HBB but were decreased by 10 ppm of 3,3',4,4',5,5'-HBB (Thompson et al., 1981). In humans exposed to high levels of PBB, elevation in serum triglyceride values was the most common finding (Stross et al., 1981).

Clinical Signs

Rats fed up to 100 ppm of PBB for 30 or 60 days had no clinical signs of toxicosis (Sleight and Sanger, 1976). Also, rats given 22 oral doses of 30 mg FM/kg body weight over a 30 day period had no clinical signs of toxicosis (Gupta et al., 1981). In both of these studies, there was a decrease in weight gain and feed efficiency. Decreases in body weight have been reported with other animal species given PBB (Allen et al., 1978; Howard et al., 1980) or given other polyhalogenated aromatic hydrocarbons (McConnell et al., 1978).

Sleight and Sanger (1976) performed a pilot study on PBB toxicosis in guinea pigs. When fed 500 ppm in the diet guinea pigs refused food,

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had severe weight loss and died within 15 days. At a dietary level of 100 ppm, anorexia occurred and 4 of 6 died by day 30. At 1 and 10 ppm there were no clinical signs of toxicosis.

Gross Pathology

PBB toxicosis resulted in hepatomegaly in rats (Sleight and Sanger, 1976; Akoso, 1981; Render et al., 1982) and mice (Corbett et al., 1978). Effects on liver size and weight were inconsistent in the guinea pig. Liver weight was not significantly or consistently increased by feeding 1 or 10 ppm PBB in the diet. Liver-to-body weight ratios were increased in guinea pigs fed 100 ppm or 500 ppm but this was associated with severe body weight loss (Sleight and Sanger, 1976).

Thyroid enlargement has been reported in piglets born from sows fed a diet containing 100 ppm PBB during the last half of gestation (Werner and Sleight, 1981), and in rats fed a diet containing 10 ppm or higher for 60 days (Sleight et al., 1978).

A variety of gross lesions was reported in cattle that had been fed PBB-contaminated feed. These included hematomas, abscesses, liver enlargement, metritis and bronchopneumonia (Jackson and Halbert, 1974).

Histopathology

Rats fed diets containing 10 or 100 ppm FM, 3,3',4,4',5,5'-HBB, or 2,2',4,4',5,5'-HBB had enlarged hepatocytes that contained lipid vacuoles. The degree of vacuolization was dose-related and most prominent in the centrilobular to midzonal area and most severe in rats given 3,3',4,4',5,5'-HBB (Render et al., 1982). Bile duct hyperplasia and portal fibrosis were observed in rats fed iodine-deficient diets containing 100 ppm PBB for 60 days (Akoso, 1977). Kimbrough et al.

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(1977) observed enlarged and vacuolated hepatocytes and neoplastic nodules in the liver of rats given a single oral dose of 1 g PBB/kg body weight. Kimbrough et al. (1980) also reported megalohepatocytes, necrosis, and interstitial fibrosis in the liver. Similar hepatic alterations in rats given FM and 2,2',4,4',5,5'-HBB were observed by Gupta et al. (1981) and lesions produced by FM were more pronounced than those produced by 2,2',4,4',5,5'-HBB.

In a preliminary study of PBB toxicosis in guinea pigs there was marked centrilobular fatty change in animals that died after being fed 100 and 500 ppm PBB. Two guinea pigs fed 10 ppm had swollen hepatocytes with many large vacuoles. Livers from guinea pigs fed 1 or 10 ppm PBB were not enlarged and were histologically normal (Sleight and Sanger, 1976).

Thymuses in mice had a dose-related decrease in cortical thickness (Fraker and Aust, 1980). Fraker suggested that PBB might affect lymphocytopoiesis. Luster et al. (1978) suggested that the immunotoxic effect may be manifested via an elevation in the concentration of plasma glucocorticoids. However, glucocorticoid levels were only midly increased in mice fed 100 ppm PBB (Fraker and Aust, 1980), and were not elevated in animals exposed to other polyhalogenated aromatic hydrocarbons (Vos et al., 1977). Rats fed up to 100 ppm FM or 2,2',4,4',5,5'-HBB had histologically normal thymuses (Render et al., 1982). In the same study, rats fed dietary levels of 10 or 100 ppm of 3,3'4,4',5,5'-HBB for 10 days had decreased cortical thickness and loss of demarcation between the cortex and medulla. Thymus weights of rats given daily oral doses of 30 mg FM/kg for a total of 22 doses in 30 days were reduced by 15 days (Gupta et al., 1981). Gupta suggested

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that thymus weights may be considered nonspecific because many toxic compounds or "stressful events" may induce side effects.

Other polyhalogenated aromatic hydrocarbons produce thymic atrophy. TCDD consistently produces involution of the thymus in all animal species. McConnell et al. (1979) stated that the lesion is in part produced by necrosis.

In general, histologic evaluation indicates that the thymus and liver of animals are the major target organs of PBB toxicity.

Electron Microscopy (EM)

Ultrastructural hepatic lesions were produced when rats were fed diets containing up to 100 ppm of 2,2',4,4',5,5'-HBB or FM BP-6 for 9 days. For 2,2',4,4',5,5'-HBB and FM at 10 and 100 ppm the changes consisted mainly of increased smooth endoplasmic reticulum (SER) and lipid vacuolation. Additional alterations seen with 3,3', 4,4',5,5'-HBB included disorganization of rough endoplasmic reticulum (RER), myelin body formation and bile ductule hyperplasia (Render et al., 1982). Gupta et al. (1981) reported similar electron microscopic changes in rats given FM or 2,2',4,4',5,5'-HBB and the EM alterations were most severe in rats given FM. Corbett et al. (1978) observed decreased rough endoplasmic reticulum, degeneration of mitochondria, and increased lysosomes in the liver of mice fed a diet containing 1000 ppm PBB for 14 days.

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MATERIALS AND METHODS

Young male guinea pigs (Hartley cross) weighing between 300 and 350 grams and young male hamsters (outbred Golden Syrian) weighing between 70 and 88 grams were used. Using a random number table, animals were numbered and assigned to polycarbonate cages, one per cage. All animals had access to water and commercial pelleted diets (guinea pigs -- Guinea Pig Chow #5025, Ralston Purina Company, St. Louis, MO; hamsters--Wayne Lab-Blox, Allied Mills, Inc., Chicago, IL) ad libitum, except feed was removed 12 hours prior to killing. Heat-treated hardwood bedding (Northeastern Products Corp., Warrensburg, NY) was changed every day for 3 days following gavage and thereafter as needed. Room temperature was 23 C and lights were adjusted for 12 light hours per day.

Firemaster BP-6 (FM) (Lot 6224A) was used. FM was manufactured by Michigan Chemical Company, St. Louis, Michigan, which has since merged with Velsicol Chemical Corporation, Chicago, Illinois (Dunkel, 1975). FM is composed of a mixture of polybrominated biphenyls and the chemical composition has been reported (Hass et al., 1978).

FM was ground to a powder with a mortar and pestle, weighed on an analytical balance (Mettler Type H15 Analytical Balance, Mettler Instrument Company, Highstown, NJ) and dissolved in corn oil (Mazola) with heat (100 C). The concentration of FM was adjusted so that 5 ml corn oil/kg body weight was given. Dosages of 0, 100, 200, 400 or

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800 mg FM/kg body weight required solutions of 0, 2, 4, 8 or 16% respectively. On days 11 through 13, two guinea pigs and two hamsters were given 75 mg of crystalline phenobarbital (Pb) (Sigma Chemical Company, St. Louis, MO) per kg body weight dissolved in water. Two additional guinea pigs and hamsters were given 20 mg of 3-methylcholanthrene (MC) (Sigma Chemical Company, St. Louis, MO) per kg body weight by gavage. Pb- and MC-treated animals served as positive controls for liver microsomal enzyme assays.

After a one week acclimation period the guinea pigs and hamsters were given FM by gavage as outlined in Tables 2 and 3. Animals were observed daily for clinical signs of toxicosis. They were weighed at the beginning and every other day during the study. In addition, survivors were weighed at the time feed was removed and just before killing. Body and organ weights were measured and histologic changes were evaluated in all animals. Fourteen days after treatment survivors were weighed, anesthetized with ether (Mallinckrodt Inc., St. Louis, MO), bled by cardiac puncture and killed by decapitation. Weights of the brain, kidneys, liver, spleen, testicles, thymus, and adrenal glands were recorded. In addition to these tissues, specimens of colon, duodenum, gall bladder, heart, lung, lymph node, pancreas, intercostal skeletal muscle, eyelid skin, spleen, sternal bone marrow, stomach, thyroids, and urinary bladder were fixed in 10% neutral buffered formalin. Tissues were embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin. Sections of liver were also stained with oil red O for lipid identification (Luna, 1968). Samples of liver were collected, wrapped in aluminum foil and stored at -20 C until analyzed for PBB.

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Table 2. Treatment schedule for hamsters. On day 0, hamsters in groups 1-5 were given corn oil or Firemaster BP-6 in corn oil by gavage at 3 hour intervals. Treatment groups 6 and 7 were given a single dosage of phenobarbital or 3-methylcholanthrene by gavage on days 11-13.

Treatment Group	Test Compound	Dosage mg/kg	Number of Dosages	Number per Group
1	vehicle only	0	4	6
2	Firemaster BP-6	100	4	5*
3	Firemaster BP-6	200	4	5*
4	Firemaster BP-6	400	4	6
5	Firemaster BP-6	800	4	6
6	phenobarbital	75	3	2
7	3-methylcholanthrene	20	3	2

*Hamsters that developed enteritis within 2 days of gavage were removed from the study.

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Table 3. Treatment schedule for guinea pigs. On day 0, hamsters in groups 1-5 were given a single dosage of corn oil or Firemaster BP-6 in corn oil by gavage. Treatment groups 6 and 7 were given a single dosage of phenobarbital or 3-methylcholanthrene by gavage on days 11-13.

Treatment Group	Test Compound	Dosage mg/kg	Number of Dosages	Number per Group
1	vehicle only	0	1	6
2	Firemaster BP-6	100	1	6
3	Firemaster BP-6	200	1	6
4	Firemaster BP-6	400	1	6
5	Firemaster BP-6	800	1	6
6	phenobarbital	75	3	2
7	3-methylcholanthrene	20	3	2

For electron microscopy, portions of liver from three surviving animals per group were fixed in Karnovsky's fixative (Pease, 1964) at pH 7.4 and then post fixed in 1% osmium tetroxide in Zetterqvist's fixative. Tissues were dehydrated in alcohol (50, 70, 90, and 100%) and were transferred to propylene oxide. A mixture of epon and araldite was used for embedding.

One micron sections of liver from one representative animal per control and FM-treated group were stained with toluidine blue (Luna, 1968) and examined with the light microscope. Ultrathin sections (900 A^{o}) of selected areas were stained with uranyl acetate and lead citrate and viewed with an electron microscope.

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Clinical chemistry examinations were performed on blood serum obtained from surviving animals at the time of necropsy. Sorbitol dehydrogenase (SDH), gamma glutamyl transpeptidase (GGTP), triglyceride, and total cholesterol levels were determined using the Eni-Gemasaec reagents (Electronucleonics, Inc., Fairfield, NJ).

Portions of liver from control and FM-treated guinea pigs, two per group, were collected and immersed separately in cold 1.15% KCl containing 0.2% nicotinamide. Portions of liver from hamsters in the control and FM-treated groups were divided into two containers of the same solution with portions of three livers per group in each container. Liver samples from Pb- and MC-treated guinea pigs and hamsters were collected and pooled in their respective containers. Procedures for isolation, washing, and storing of microsomes were previously described (Pederson et al., 1970; Welton et al., 1974). Aminopyrine demethylation (AP) was assayed as described by Moore et al. (1978) and ethoxyresorufin-o-deethylase (EROD) was assayed according to the method of Burke and Mayer (1974). Cytochrome P450 and the cytochrome P450 CO-difference spectra absorption maxima were measured according to the methods of Omura and Sato (1964).

Standard procedures for extraction and analysis for PBB in tissues were followed (Thompson, 1977). Analysis was with a gas chromatograph (Varian 3700). Column temperature was 250 C, detector temperature was 310 C, and nitrogen was the carrier gas at a flow rate of 30 ml/minute. Standards were used. Lipid concentrations were determined in 20-ml aliquots of each extracted sample. Solvent was evaporated and the sample was vacuum-dried in a preweighed aluminum foil pan. After drying, the pan and the remaining lipid were weighed and the percentage of lipid in the original sample was calculated.

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Data were analyzed statistically by using the one-way analysis of variance. Differences between group means were analyzed by using the Student-Newman-Keuls' test. Differences from control values were considered significant at the level of $p<0.05$.

RESULTS

Clinical Signs

Hamsters

Hamsters treated with up to 3200 mg FM/kg body weight had no clinical signs of toxicosis. Two hamsters treated with 400 and 800 mg/kg respectively, developed proliferative ileitis within 2 days of treatment and were removed from the study.

Guinea Pigs

Guinea pigs given 0 or 100 mg FM/kg did not have clinical signs of toxicosis. Two given 200 mg/kg and all given 400 or 800 mg/kg died by day 14. The time of death was dose-related with animals given 800 mg/kg dying on days 8-11. Guinea pigs given 400 mg/kg died on days 10-13.

Clinical signs of toxicosis developed by day 5 in guinea pigs given 800 mg/kg and all that died had similar clinical signs including rough hair coat, decreased activity, wetting of the ventral intermandibular and cervical fur with saliva, and severe body weight loss.

Body Weight

Hamsters

Hamsters given FM did not gain weight or had significant weight loss compared to control animals (Table 4). There was no significant difference in weight loss between treatment groups.

Table 4. Initial body weight, final body weight, and weight gain (loss) in hamsters given oral doses of Firemaster BP-6.

Total Dosage (mg/kg)	Initial Weight (g)	Final Weight (g)	Weight Gain (Loss) (g)
0	80 ± 1.96	92 ± 1.28	12 ± 1.14
400	80 ± 1.79	75 ± 4.40	-5 ± 4.28[a]
800	82 ± 1.58	82 ± 1.66	0 ± 2.94[a]
1600	78 ± 2.09	75 ± 4.93	-3 ± 3.99[a]
3200	79 ± 1.76	71 ± 3.32	-8 ± 2.38[a]

Data are expressed as mean ± SE, (n=6, except n=5 for hamsters given 400 or 800 mg FM/kg).

[a] Significantly different from control ($p<0.05$).

Guinea Pigs

Guinea pigs treated with 200, 400 or 800 mg FM/kg had severe dose-related body weight loss (Figure 2). Guinea pigs given 400 or 800 mg/kg began losing weight immediately after treatment and at necropsy both groups had lost an average of 58% of their original mean body weights (Table 5). Although guinea pigs given 100 mg/kg had reduced weight gain, it was not significantly different from control values.

Organ Weights

Hamsters

Livers of hamsters given FM had a significant increase in absolute weights and in liver-to-body weight ratios (Table 6 and 1A). Absolute

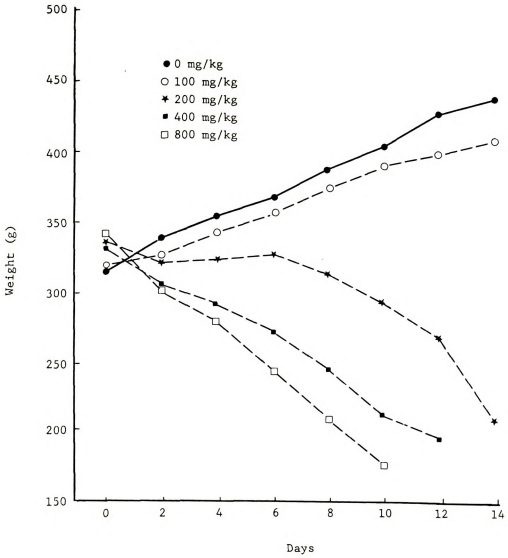

Figure 2. Body weight of guinea pigs that were given a single oral dosage of 0, 100, 200, 400 or 800 mg/kg Firemaster BP-6. There were 6 animals per dose group. All animals given 400 and 800 mg/kg and two animals given 200 mg/kg died.

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Table 5. Initial body weight, final body weight and weight gain (loss) of guinea pigs given a single oral dosage of Firemaster BP-6.

Total Dosage (mg/kg)	Initial Weight (g)	Final Weight (Necropsy) (g)	Weight Gain (loss) (g)
0	316 ± 9.20	426 ± 13.67	110 ± 7.07
100	318 ± 10.82	401 ± 7.02	83 ± 12.68
200	336 ± 9.28	245 ± 31.69	- 91 ± 27.47[a,b]
400	332 ± 16.41	193 ± 11.35	-139 ± 7.69[a,b]
800	337 ± 9.11	195 ± 8.65	-142 ± 5.62[a,b]

Data are expressed as mean ± SE (n=6).

[a] Significantly different from control ($p<0.05$).

[b] All guinea pigs given 400 or 800 mg FM/kg body weight and two given 200 mg/kg died and were necropsied before the end of the study (day 14).

Table 6. Organ weights of hamsters given oral doses of Firemaster BP-6.

Dosage (mg/kg)	Liver (g)	Thymus (mg)	Adrenals (mg)	Kidneys (g)	Testicles (g)	Brain (mg)	Spleen (mg)
0	2.86 ± 0.13	58.8 ± 4.35	22.6 ± 1.65	0.71 ± 0.02	3.13 ± 0.07	974 ± 8.83	845 ± 3.33
400	5.61 ± 0.26[a]	13.3 ± 4.79[a]	20.2 ± 1.18	0.75 ± 0.02	2.69 ± 0.26	943 ± 12.92	607 ± 5.32[a]
800	5.90 ± 0.16[a]	16.7 ± 3.64[a]	20.9 ± 1.21	0.77 ± 0.02	3.13 ± 0.07	970 ± 7.85	682 ± 2.61[a]
1600	5.91 ± 0.19[a]	11.4 ± 2.59[a]	21.6 ± 1.18	0.72 ± 0.03	2.19 ± 0.47	957 ± 18.29	637 ± 5.70[a]
3200	6.38 ± 0.28[a]	6.8 ± 1.32[a]	21.1 ± 0.51	0.70 ± 0.04	2.58 ± 0.25	948 ± 16.32	543 ± 5.04[a]

Data are expressed as the mean ± SE (n=6) except n=5 for groups given 400 or 800 mg FM/kg.

[a]Significantly different from control ($p<0.05$).

and relative thymus weights of hamsters given FM were significantly decreased compared to control values. There was no significant difference in liver or thymus weights between hamsters in FM-treated groups. Spleen weights were decreased on an absolute basis but spleen-to-body weight ratios were not significantly affected suggesting that the reduced size was due to weight loss (Table 2A). Absolute organ weights of adrenals, brain, kidney and testicle were not different from control values.

Guinea Pigs

Absolute liver weights and liver-to-brain weight ratios were not significantly different from control values (Tables 7 and 3A). Guinea pigs given 200, 400 or 800 mg/kg had a significant increase in liver-to-body weight ratios due to severe body weight loss. Dose-related decreases were seen in absolute thymic weights, thymus-to-brain weight ratios and thymus-to-body weight ratios. There was a significant increase in adrenal-to-brain and adrenal-to-body weight ratios for guinea pigs given 400 or 800 mg/kg (Tables 3A and 4A). Absolute adrenal weights were increased for guinea pigs given 400 mg/kg but were not increased at 800 mg/kg. Kidney, testicle and spleen weights and organ-to-body weight ratios either were not affected or were altered by severe body weight loss.

Clinical Chemistry

Hamsters

Serum triglyceride values in hamsters were elevated when compared to control values and groups treated with 800 or 3200 mg/kg had significantly elevated values. Cholesterol levels were significantly

Table 7. Organ weights of guinea pigs given single oral doses of Firemaster BP-6

Dosage (mg/kg)	Liver (g)	Thymus (g)	Adrenals (g)	Kidneys (g)	Testicles (g)	Brain (g)	Spleen (g)

Table 7. Organ weights of guinea pigs given single oral doses of Firemaster BP-6

Dosage (mg/kg)	Liver (g)	Thymus (g)	Adrenals (g)	Kidneys (g)	Testicles (g)	Brain (g)	Spleen (g)
0	17.76 ± 0.83	0.650 ± 0.02	0.179 ± 0.01	3.14 ± 0.15	1.23 ± 0.06	3.75 ± 0.09	0.560 + 0.04
100	19.39 ± 0.81	0.514[b] ± 0.02	0.152 ± 0.01	2.90 ± 0.08	1.08 ± 0.05	3.77 ± 0.06	0.595 ± 0.06
200[c]	14.24 ± 1.58	0.189[b] ± 0.05	0.227 ± 0.03	2.90 ± 0.08	0.60[b] ± 0.08	3.64 ± 0.05	0.333[b] ± 0.07
400[c]	14.45 ± 0.34	0.103[b] ± 0.02	0.261[b] ± 0.02	3.11 ± 0.10	0.58[b] ± 0.07	3.67 ± 0.10	0.213[b] ± 0.02
800[c]	14.42 ± 0.84	ND[a]	0.242 ± 0.03	2.99 ± 0.13	0.66[b] ± 0.06	3.52 ± 0.03	0.211[b] ± 0.01

Data are expressed as the mean ± SE (n=6).

[a]Not Done.

[b]Significantly different from control ($p<0.05$).

[c]All guinea pigs given 400 or 800 mg FM/kg body weight and two given 200 mg/kg died before the end of the study.

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elevated in all FM-treated groups. SDH levels were increased in all groups and were significantly elevated in hamsters given 3200 mg/kg. GGTP levels were within the normal reference range (Table 8).

Guinea Pigs

Serum analyses were performed on survivors from those given 0, 100 and 200 mg/kg. Levels of SDH were within the normal reference range. Serum cholesterol and triglyceride levels were elevated in guinea pigs given FM and in the group given 200 mg/kg the elevation was significant. GGTP levels were significantly decreased (Table 9).

Necropsy Findings

Hamsters

Gross lesions consisted of hepatomegaly in all FM-treated groups and two hamsters given 3200 mg/kg had pale livers with a pronounced lobular pattern.

Guinea Pigs

Control animals or guinea pigs given 100 mg/kg did not have significant gross lesions. Guinea pigs given 200, 400, or 800 mg/kg had severe muscle wasting, loss of body fat, and thymic atrophy. Other gross lesions were seen only in those guinea pigs that died. In these animals there was hemorrhage, necrosis, and excess mucus on the mucosal surface of the fundic and pyloric regions of the stomach. Adrenal glands had subcapsular and medullary congestion and hemorrhage.

Table 8. S
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Total Dosage (mg/kg)	
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Table 8. Serum chemistry values for hamsters given oral doses of Firemaster BP-6.

Total Dosage (mg/kg)	SDH (IU/L)	GGTP (IU/L)	Triglyceride (mg/dl)	Cholesterol (mg/dl)
0	19.2 ± 1.74	4.2 ± 0.81	106.0 ± 9.71	102.0 ± 5.08
400	91.2 ± 15.29	3.2 ± 0.97	167.8 ± 21.89	151.5 ± 6.06[a]
800	76.6 ± 11.36	4.8 ± 1.66	232.4 ± 36.25[a]	145.8 ± 8.16[a]
1600	75.7 ± 8.40	2.2 ± 0.54	160.8 ± 39.99	147.0 ± 13.78[a]
3200	149.3 ± 55.96[a]	6.5 ± 1.85	229.3 ± 26.93[a]	156.5 ± 11.21[a]

Data are expressed as mean ± SE (n=5, except n=4 for hamsters given 3200 mg FM/kg).

[a]Significantly different from control ($p<0.05$).

Table 9.

Total Dosage (mg/kg)	
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Table 9. Serum chemistry values for guinea pigs given Firemaster BP-6.[a]

Total Dosage (mg/kg)	SDH (IU/L)	GGTP (IU/L)	Triglyceride (mg/dl)	Cholesterol (mg/dl)
0	41.3 ± 3.01	13.5 ± 1.61	65.2 ± 8.85	26.7 ± 1.67
100	61.5 ± 14.57	8.3 ± 0.99[b]	147.0 ± 29.20	45.7 ± 4.28
200	49.8 ± 3.28	6.5 ± 1.32[b]	307.3 ± 62.45[b]	145.8 ± 36.35[b]

Data are expressed as mean ± SE (n=6, except n=4 for guinea pigs given 200 mg FM/kg).

[a]There were no survivors in groups given 400 or 800 mg FM/kg and clinical chemistry measurements were not done.

[b]Significantly different from control ($p<0.05$).

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Histopathologic Findings

Hamsters

Histopathologic alterations were limited to liver and thymus and were similar between FM-treated groups. Hepatic lesions consisted of diffuse hepatocellular hypertrophy with an occasional necrotic hepatocyte (Figures 3 through 6). Hepatocyte cytoplasm contained coarse eosinophilic clumps and sometimes, in the midzonal region, fine vacuoles. Hamsters given 3200 mg/kg often had randomly scattered necrotic hepatocytes sometimes accompanied by mixed populations of inflammatory cells (Figure 7).

Hamsters given FM had thymic atrophy which was most severe in those given 1600 or 3200 mg/kg. It was characterized by reduced cortical thickness and exposure of underlying thymic epithelium due to a loss of cortical lymphocytes. The cortico-medullary junction was irregular and necrosis was not evident (Figures 8 and 9).

Guinea Pigs

Livers of guinea pigs given 200, 400 or 800 mg/kg had mild histologic alterations. There was hepatocellular vacuolization that was most prominent in the centrilobular area (Figures 10 and 11). Midzonal and periportal regions contained scattered hepatocytes with single large vacuoles. Vacuoles stained for lipid with oil red O. One guinea pig given 200 mg/kg had diffuse hepatocellular vacuolization. Two guinea pigs given 100 mg/kg had minimal centrilobular hepatocellular vacuolization. Livers of control animals were histologically normal.

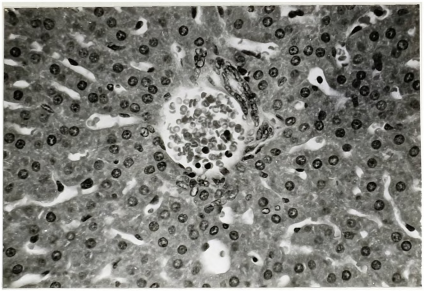

Figure 3. Portal region of liver from a control hamster. (H&E stain, 300X).

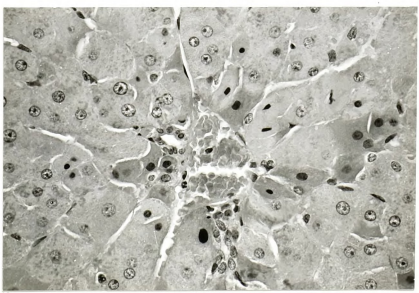

Figure 4. Portal region of liver from a hamster given 3200 mg FM/kg. Notice hepatocellular hypertrophy, decreased sinusoidal space, and degenerated hepatocytes with pyknotic nuclei. (H&E stain, 300X).

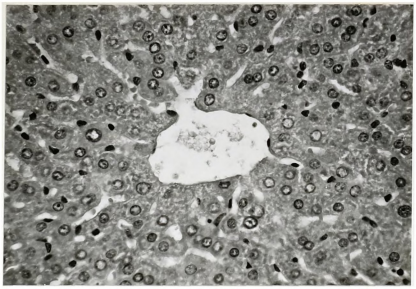

Figure 5. Centilobular region of liver from a control hamster. (H&E stain, 300X).

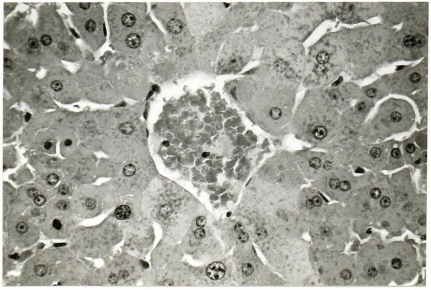

Figure 6. Centrilobular region of liver from a hamster given 3200 mg FM/kg. Notice hepatocyte hypertrophy, decreased sinusoidal space, and coarse clumping of hepatocyte cytoplasm. (H&E stain, 300X).

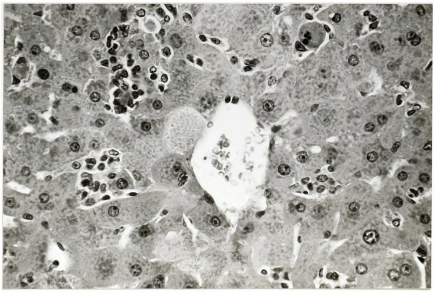

Figure 7. Centrilobular region of liver from a hamster given 3200 mg FM/kg. Notice mixed population of inflammatory cells associated with necrotic hepatocytes. (H&E stain, 300X).

Figure 8. Thymus of control hamster. (H&E stain, 120X).

Figure 9. Thymus of hamster given 3200 mg FM/kg. Notice reduced cortical thickness and irregular corticomedullary border associated with loss of cortical lymphocytes. (H&E stain, 120X).

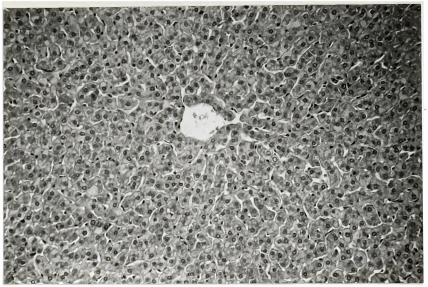

Figure 10. Centrilobular region of liver from a control guinea pig. (H&E stain, 120X).

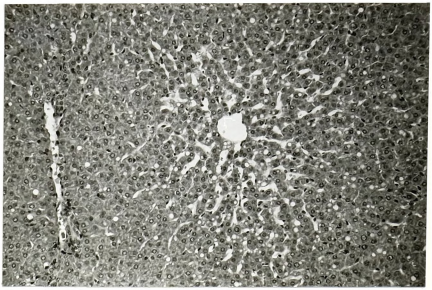

Figure 11. Hepatic lobule of a guinea pig given 200 mg FM/kg. Notice single vacuoles in cytoplasm of centrilobular hepatocytes. (H&E stain, 120X).

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Guinea pigs given 200, 400 or 800 mg/kg had thymic atrophy. Atrophic thymuses had reduced cortical thickness and indistinct separation of cortex from medulla due to a loss of cortical lymphocytes (Figures 12 and 13). Thymic corpuscles were dilated and contained an admixture of neutrophils and keratin (Figure 14). This change was most prominent in the high dosage groups and the cystic corpuscles often displaced much thymic parenchyma. One guinea pig given 400 mg/kg had an increase in the number of cortical macrophages. Necrosis was not evident.

Urinary bladder had dose-related hyperplasia of transitional epithelium (Figures 15 and 16).

Adrenal glands of guinea pigs that died of PBB toxicosis had multifocal areas of hyperemia and hemorrhage in the medulla and cortex.

In the stomach of guinea pigs that died of PBB toxicosis there was multifocal necrosis of the mucosa in the pyloric region. In some guinea pigs, necrosis was restricted to surface epithelium whereas in others, necrosis extended to the muscularis mucosae. Hemorrhage, nuclear debris, coccoid bacterial organisms, and neutrophils were in necrotic areas or free in the lumina. Superficial submucosal connective tissue underlying areas of necrosis was infiltrated by heterophils.

Ultrastructural Findings

Hamsters

Hepatocytes of hamsters given FM had similar ultrastructural changes that were most severe in those given 3200 mg/kg. Smooth endoplasmic reticulum (SER) was increased and sometimes dilated (Figures 17 and 20). A few intracytoplasmic lipid droplets were

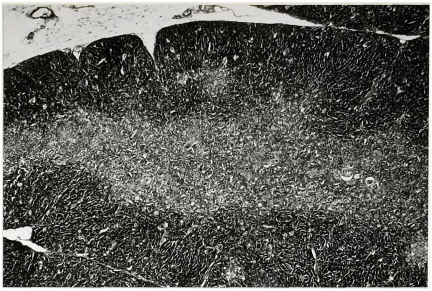

Figure 12. Thymus of control guinea pig. (H&E stain, 48X).

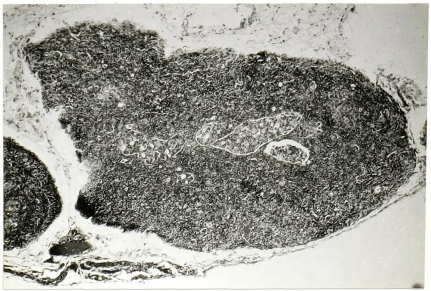

Figure 13. Thymus of guinea pig given 800 mg FM/kg. Notice atrophied thymus with inapparent cortex and dilated thymic corpuscles. (H&E stain, 48X).

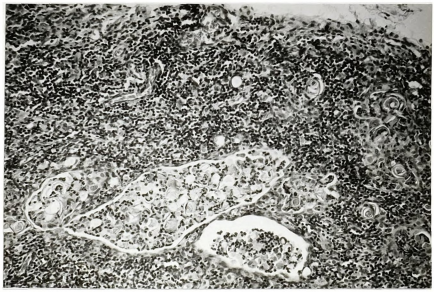

Figure 14. Thymus of guinea pig given 800 mg FM/kg Notice dilated thymic corpuscles which contain squamous debris and heterophils. (H&E stain, 120X).

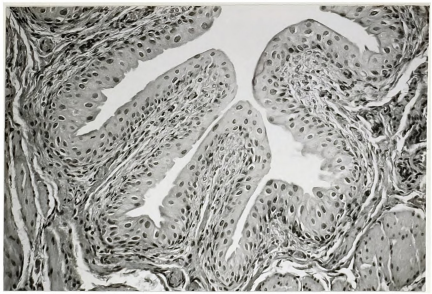

Figure 15. Transitional epithelium of urinary bladder of control guinea pig. Notice epithelium is 2 or 3 cell layers in thickness. (H&E stain, 120X).

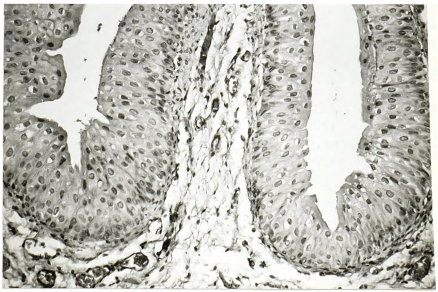

Figure 16. Transitional epithelium of urinary bladder of guinea pig given 200 mg FM/kg. Notice epithelium is 5 or 6 cell layers in thickness. (H&E stain, 120X).

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in hepatocytes (Figure 19). Bile canaliculi were often markedly dilated and had loss or blunting of microvilli (Figures 18 and 19).

Guinea Pigs

Ultrastructural examination of livers of guinea pigs given 0, 100 or 200 mg FM/kg were done. A consistent ultrastructural feature in hepatocytes was intracytoplasmic accumulation of lipid droplets (Figures 21 and 23). Additional changes were highly variable between hepatocytes or could not be adequately (satisfactorily) evaluated because of poor quality specimens. One guinea pig given 200 mg/kg had increased SER, intracytoplasmic lipid droplets, and dilated cisternae of rough and smooth endoplasmic reticulum (Figures 22 and 24).

Hepatic Drug Metabolism

Hamsters

Hamsters given FM had an increase in cytochrome P450 and a spectral shift in the carbon monoxide difference spectra. Ethoxyresorufin-o-deethylase activity was increased but there was little change in aminopyrine demethylase activity (Table 10).

Guinea Pigs

Guinea pigs given FM had an increase in cytochrome P450 and a spectral shift in the carbon monoxide difference spectra. Also, aminopyrine demethylase and ethoxyresorufin-o-deethylase activities were increased (Table 11).

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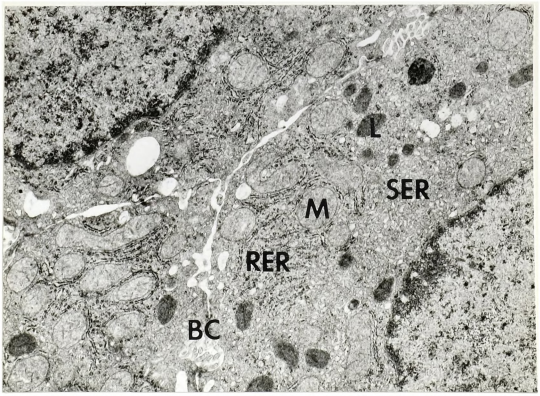

Figure 17. Electron micrograph of hepatocytes of a control hamster. Notice lysosomes (L), mitochondria (M), rough endoplasmic reticulum (RER), some smooth endoplasmic reticulum (SER), and bile canaliculi (BC) with microvilli. (Lead citrate and uranyl acetate stain, 13,600X).

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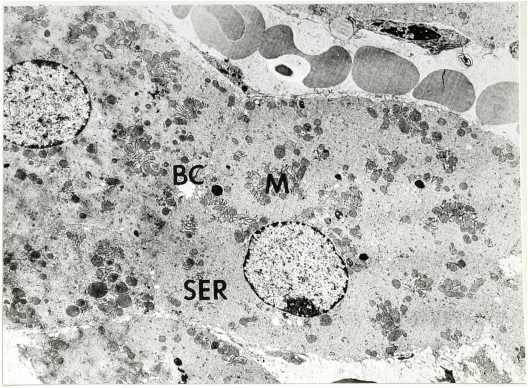

Figure 18. Electron micrograph of hepatocytes of a hamster given 800 mg FM/kg body weight. Notice collections of mitochondria (M) separated by proliferated smooth endoplasmic reticulum (SER) and dilated bile canaliculi (BC) with blunt microvilli. (Lead citrate and uranyl acetate stain, 3,800X).

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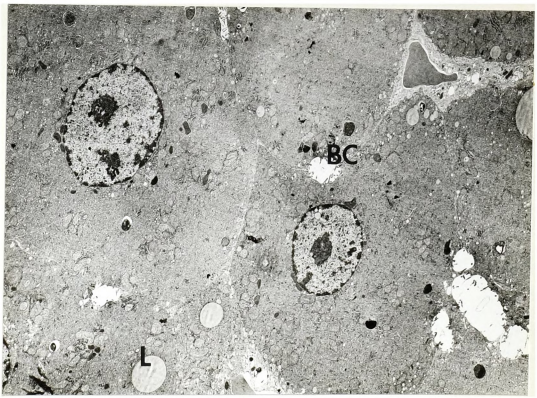

Figure 19. Electron micrograph of hepatocytes of a hamster given 3200 mg FM/kg body weight. Notice intracytoplasmic lipid droplets (L) and markedly dilated bile canaliculi (BC). (Lead citrate and uranyl acetate stain, 3,800X).

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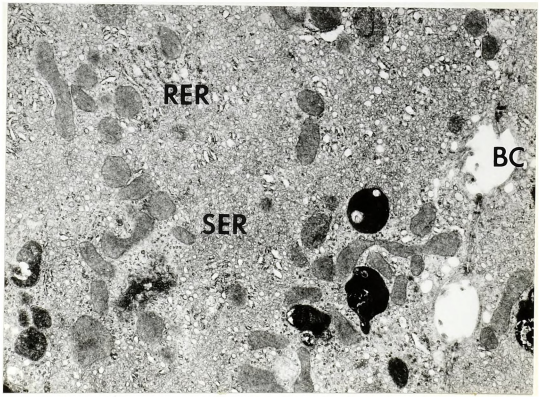

Figure 20. Electron micrograph of hepatocytes of a hamster given 3200 mg FM/kg body weight. Notice smooth endoplasmic reticulum (SER) and rough endoplasmic reticulum (RER) with dilated cisternae and dilated bile canaliculi (BC) with loss of microvilli. (Lead citrate and uranyl acetate stain, 13,600X).

Fig

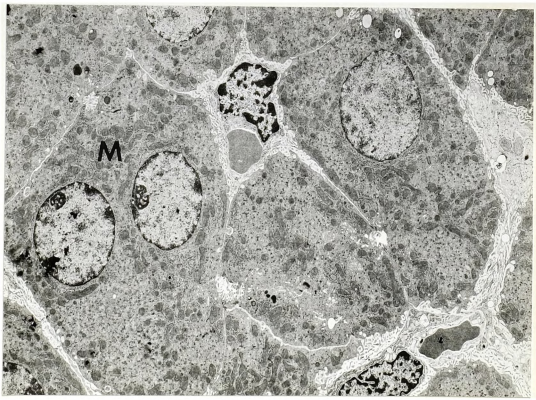

Figure 21. Electron micrograph of hepatocytes of a control guinea pig. Notice abundant mitochondria (M) and glycogen in the cytoplasm. (Lead citrate and uranyl acetate stain, 3,800X).

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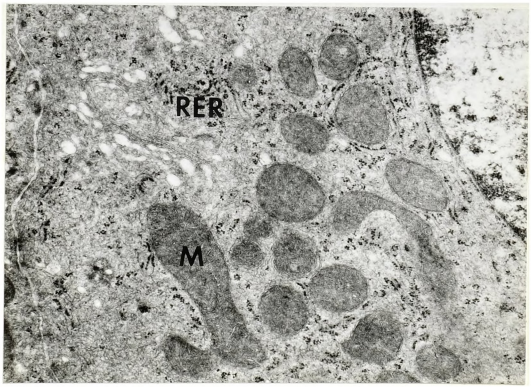

Figure 22. Electron micrograph of hepatocytes of a control guinea pig. Notice mitochondria (M) surrounded by rough endoplasmic reticulum (RER). (Lead citrate and uranyl acetate stain, 24,700X).

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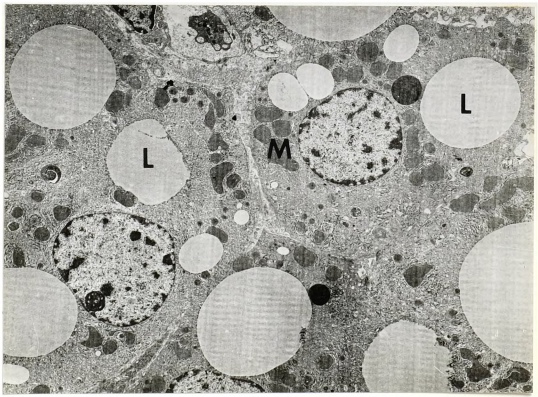

Figure 23. Electron micrograph of hepatocytes of a guinea pig given 200 mg FM/kg body weight. Notice intracytoplasmic lipid droplets (L), proliferated smooth endoplasmic reticulum and few mitochondria (M). (Lead citrate and uranyl acetate stain, 3,800X).

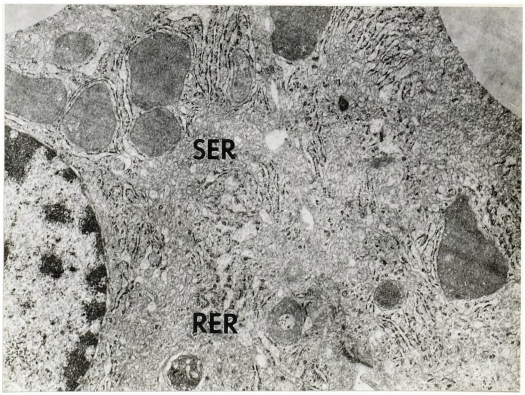

Figure 24. Electron micrograph of a hepatocyte of a guinea pig given 200 mg FM/kg body weight. Notice irregularly dilated cisternae of rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum (SER). (Lead citrate and uranyl acetate stain, 13,600X).

Table 10. Effects of Firemaster BP-6 (FM) on liver cytochrome P450, aminopyrine demethylase, and ethoxyresorufin-o-deethylase in hamsters.

Table 10. Effects of Firemaster BP-6 (FM) on liver cytochrome P450, aminopyrine demethylase, and ethoxyresorufin-o-deethylase in hamsters.

Dosage (mg/kg)	Test Compound	Cytochrome P450 (nmol/mg protein)	Cytochrome P450 λ max (nm)	Aminopyrine Demethylase (nmol/mg protein/min)	Ethoxyresorufin -o-deethylase (nmol/mg protein/min)
0	vehicle	2.28	449.5	32.2	1.12
400	FM	5.03	449.5	33.4	6.74
800	FM	5.49	449.3	33.6	5.84
1600	FM	5.38	449.0	38.4	5.35
3200	FM	5.49	449.0	40.2	4.83
75	Pb[a]	2.85	450.0	31.3	0.96
20	MC[b]	3.26	449.0	25.4	5.10

Data represent the mean of pooled samples, N = 2. Data for Pb and MC represent values from one pooled sample.

[a]Pb, phenobarbital.

[b]MC, 3-methylcholanthrene.

Table 11. Effects of Firemaster PB-6 (FM) on liver cytochrome P450, aminopyrine demethylase, and ethoxyresorufin-o-deethylase in guinea pigs.

Table 11. Effects of Firemaster PB-6 (FM) on liver cytochrome P450, aminopyrine demethylase, and ethoxyresorufin-o-deethylase in guinea pigs.

Dosage (mg/kg)	Test Compound	Cytochrome P450 (nmol/mg protein)	Cytochrome P450 λ max (nm)	Aminopyrine Demethylase (nmol/mg protein/min)	Ethoxyresorufin-O-deethylase (nmol/mg protein/min)
0	vehicle	1.55	449.8	9.5	1.37
100	FM	2.45	449.0	16.0	5.43
200	FM	2.64	449.3	19.1	4.71
75	Pb[b]	2.62	450.0	38.2	1.73
20	MC[c]	2.68	449.0	17.1	6.65

Data for FM represent the mean, N = 2. Data for Pb and MC represent values from pooled samples.

[a]Microsomal enzyme assays were not done on guinea pigs given 400 or 800 mg/kg FM.

[b]Pb, phenobarbital.

[c]MC, 3-methylcholanthrene.

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Table 12.

Dosage (mg/kg)	
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Chemical Analyses

Results of liver analyses for PBB are given in Tables 12 and 13. Values expressed on a fat basis were determined by dividing the chemical concentration in parts per million on a whole weight basis by the fractional amount of lipid in the tissues.

Table 12. Mean concentrations of PBB in the liver of hamsters.

Dosage (mg/kg)	PBB in Liver (ppm)	
	Whole weight basis	Fat basis
0	0.08 ± 0.03	8.07 ± 2.95
400	239.39 ± 75.74	6845.40 ± 1704.68
800	438.54 ± 31.94	9131.00 ± 2273.42
1600	291.04 ± 76.40	9444.40 ± 2578.36
3200	390.87 ± 126.89	8788.37 ± 1613.39

Values are expressed as mean ± (n=5).

Table

Dosage (mg/kg)
0
100
200
400
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Values

Table 13. Mean concentrations of PBB in the liver of guinea pigs.

Dosage (mg/kg)	PBB in Liver (ppm)	
	Whole weight basis	Fat basis
0	0.07 ± 0.29	7.07 ± 1.21
100	0.55 ± 0.17	46.83 ± 13.18
200	72.98 ± 43.62	1825.75 ± 587.00
400	183.30 ± 39.04	4873.00 ± 1040.00
800	471.10 ± 205.00	20049.80 ± 5653.00

Values are expressed as mean ± SE (n=5).

DISCUSSION

The results of this study demonstrate that the guinea pig is much more sensitive to the lethal effects of FM than is the golden hamster. All guinea pigs given 400 or 800 mg FM/kg and two given 200 mg/kg died. Although an exact LD_{50} for guinea pigs could not be calculated, the 14 day LD_{50} would appear to lie between 200 and 400 mg/kg. In contrast, hamsters survived when given up to 3200 mg/kg. This difference in species susceptibility has also been found with TCDD (McConnell and Moore, 1978; Olson et al., 1980) and other toxic compounds (Gak et al., 1976).

Hamsters and guinea pigs each lost body weight when treated with FM but the weight loss was much more severe in guinea pigs. Guinea pigs given 400 or 800 mg/kg had progressive body weight loss that began shortly after gavage (day 2) (Figure 2). At necropsy guinea pigs in these groups had lost approximately 58% of their original body weight. Reasons for body weight loss with polyhalogenated aromatic hydrocarbon toxicity are controversial. Decreased body weight gain unrelated to feed intake has been reported in rats (Gupta et al., 1978) and monkeys (Allen et al., 1978) exposed to FM and in guinea pigs exposed to TCDD (McConnell and Moore, 1978). This suggests that FM causes poor feed utilization. Seefeld et al. (1982), however, demonstrated that reduced food intake alone was sufficient to cause body weight loss in rats treated with TCDD. They suggested that animals

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given toxic compounds frequently spill feed. If the spilled feed is not accounted for, measurements overestimate the amount of food consumed and consequently result in overfeeding of pair-fed controls. Feed consumption was not measured in the present study but consumption was visibly reduced for guinea pigs.

The thymus was a target organ of PBB toxicity in both species. Histologic findings included atrophy of the thymic cortex because of a loss of cortical lymphocytes. The pathogenesis of PBB-induced thymic atrophy is unknown. Luster et al. (1978) suggested that the immunotoxic effect is caused by an elevation in plasma glucocorticoid levels. Glucocorticoid levels, however, were only mildly elevated in mice fed PBB (Fraker and Aust, 1980) and were not elevated in animals exposed to other polyhalogenated aromatic hydrocarbons (Vos et al., 1977). McConnell and Moore (1978) stated that necrosis of cortical lymphocytes was partially responsible for thymic atrophy. Thymuses from guinea pigs and hamsters in the present study had little evidence of necrosis.

PBB caused different toxicologic effects in the livers of the two species. Liver weights of guinea pigs given FM were not affected whereas hamster liver weights were increased. Histologic and ultrastructural changes also varied between species. Livers of guinea pigs had intracytoplasmic lipid droplet accumulation especially in the centrilobular area. Livers of hamsters had diffuse hepatocellular hypertrophy and multifocal necrosis of individual hepatocytes. Hepatocellular hypertrophy of hamsters was due mainly to proliferated SER and to a lesser extent lipid droplet accumulation.

Lipid accumulations in hepatocytes were extensive in guinea pigs but were mild or absent in hamsters. Mechanisms responsible for lipid accumulation are not completely understood. Hinton et al. (1979)

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stated that lipid accumulates after exposure to PBB because of a blockade in the transport of lipids involving the golgi apparatus and sometimes the endoplasmic reticulum. Lipid also accumulates in livers of rats and mice given PBB (Gupta et al., 1981; Render et al., 1982).

Dose-related hyperplasia of the transitional epithelium of the urinary bladder of guinea pigs was found and has been reported with other polyhalogenated aromatic hydrocarbons such as TCDD (McConnell and Moore, 1978). Hyperplasia of transitional epithelium may be associated with increased urinary excretion of PBB or its metabolites. Olson et al. (1980) found decreased urinary excretion of TCDD by the guinea pig when compared to other species. Thus, it is possible that transitional epithelium in the guinea pig is especially sensitive to PBB and other toxic polyhalogenated aromatic hydrocarbons.

Gastric hemorrhage and necrosis were observed only in guinea pigs that died of PBB toxicosis. Since the occurrence and severity of this lesion were not dose-related the changes were most likely associated with debility rather than being caused by a direct toxic effect of PBB.

In addition to proliferation of SER and lipid accumulation, ultrastructural findings in livers of hamsters included dilation of bile canaliculi with loss or atrophy of microvilli (Figure 18 and 19). Ultrastructural changes in bile canaliculi have been reported in mice but were limited to shortening of microvilli (Corbett et al., 1978). Dilation of bile canaliculi may represent cholestasis (Hill, 1966).

Elevations in plasma levels of GGTP indicate hepatobiliary disease in some animals (Cornelius, 1980). Despite the ultrastructural alterations in bile canaliculi of hamsters, GGTP levels were within the normal range. Braun et al., (1977) found no GGTP activity in the liver of

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hamsters. Therefore, this may have been an inappropriate choice for the detection of biliary alterations in this species. GGTP levels were decreased in guinea pigs. This may be an important finding because GGTP is involved in the metabolism of glutathione, a compound which destroys hydrogen peroxide and free radicals and thereby protects cell membranes (Meister et al., 1976). Further investigations on the significance of reduced GGTP levels in guinea pigs may be indicated.

Elevations of SDH, an enzyme which indicates hepatocellular damage, were found in hamsters but not in guinea pigs. This elevation correlated well with the histologic findings of hepatocellular necrosis in the hamster but not in the guinea pig. SDH levels were also unchanged when guinea pigs were given PBB during gestation (Hall, 1980).

Elevations in serum cholesterol and triglyceride were found in both species. Similar elevations have been reported in rats (Akoso et al., 1977) and people (Stross et al., 1981).

Liver microsomal drug-metabolizing enzymes in the guinea pig liver had a mixed-type pattern of induction. This is in agreement with findings from studies using other laboratory animal species (Elcombe and Lech, 1978; Render et al., 1982). Ethoxyresorufin-o-deethylase activity and cytochrome P450 levels were increased in the hamster but aminopyrine demethylase activity was unchanged. Since aminopyrine demethylase levels were also not increased in hamsters given Pb, it appears that this enzyme is not an indicator of Pb-type of induction in this species. Additional studies which measure other hepatic microsomal enzymes induced by Pb are required to determine if Pb-type of induction occurs in hamsters. The results clearly demonstrated MC-type induction.

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PBB levels in the livers of guinea pigs were increased in a dose-related fashion. PBB levels in livers of hamsters were increased but there was little difference between FM treated groups. This may in part explain the lack of difference in measured parameters between hamsters given different dosages of FM. Reasons for the lack of difference in liver PBB concentration between groups were unclear. Analysis of PBB concentration in other tissues, i.e. fat, would help confirm and clarify these results.

Guinea pigs and hamsters had similarities and differences in their toxicologic response to FM. Guinea pigs were more sensitive to the lethal effects of FM but the reasons for this species difference could not be explained on the basis of organ pathology or biochemical findings.

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SUMMARY

The purpose of this study was to characterize the toxicopathologic effects of a mixture of polybrominated biphenyls, Firemaster BP-6 (FM), in the guinea pig and hamster. Groups of 6 guinea pigs or hamsters were given oral doses of FM in corn oil by gavage. Single doses of 0, 100, 200, 400 or 800 mg/kg body weight were given to guinea pigs. Hamsters were given 4 doses of 0, 100, 200, 400 or 800 mg/kg body weight singly at 3 hour intervals. This resulted in a total administered dose to hamsters of 0, 400, 800, 1600 or 3200 mg/kg. Body and organ weights were measured and histologic changes were evaluated in all animals. Survivors were killed 14 days after treatment. Blood serum was obtained for measurement of sorbitol dehydrogenase (SDH), gamma glutamyl transpeptidase (GGTP), triglycerides, and cholesterol. Liver was collected for ultrastructural examination, PBB analysis and hepatic drug-metabolizing enzyme assays.

All of the guinea pigs that were given 400 or 800 mg/kg died and had severe body weight loss. There were no compound-related deaths in hamsters but all failed to gain or lost weight when compared to control values. Thymuses were atrophied in both species. Histological findings in the thymus included cortical atrophy due to a loss of lymphocytes. Absolute liver weights were increased in the hamster but not in the guinea pig. Serum triglyceride and cholesterol levels were elevated in both species. SDH levels were elevated in hamsters and

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GGTP levels were decreased in guinea pigs. Livers of hamsters had diffuse hepatocellular hypertrophy and occasional hepatocyte necrosis. Guinea pig livers had mild centrilobular or randomly distributed fatty change. The urinary bladder epithelium was hyperplastic in guinea pigs. Ultrastructural hepatic changes were seen in both species. In the liver of hamsters there were increased SER, occasional intracytoplasmic lipid droplet accumulations and dilated bile canaliculi with atrophy of microvilli. Livers of guinea pigs had intracytoplasmic lipid droplet accumulation. FM caused Pb-type and 3-MC-type of induction of hepatic drug-metabolizing enzymes in the guinea pig whereas only 3-MC-type of induction occurred in the hamster. Variation in organ pathology or biochemical findings did not explain the differences in species susceptibility to the lethal effects of PBB.

APPENDIX

Table 1A. Organ to body weight ratios of hamsters given oral dosages of Firemaster BP-6.

Table 1A. Organ to body weight ratios of hamsters given oral dosages of Firemaster BP-6.

Dosage (mg/kg)	Liver (mg/g)	Thymus (mg/g)	Adrenals (mg/g)	Kidneys (mg/g)	Testicles (mg/g)	Brain (mg/g)	Spleen (mg/g)
0	31.22 ± 1.26	0.64 ± 0.05	0.25 ± 0.02	7.70 ± 0.18	34.23 ± 0.66	10.65 ± 0.14	0.93 ± 0.05
400	75.27[a] ± 5.08	0.14[a] ± 0.05	0.27 ± 0.02	10.09[a] ± 0.46	35.83 ± 3.02	12.69 ± 0.80	0.80 ± 0.05
800	72.15[a] ± 1.17	0.33[a] ± 0.17	0.26 ± 0.01	9.47[a] ± 0.30	38.38 ± 1.31	11.88 ± 0.30	0.84 ± 0.04
1600	79.60[a] ± 3.72	0.21[a] ± 0.05	0.29 ± 0.01	9.70[a] ± 0.33	27.69 ± 5.11	13.00[a] ± 0.91	0.84 ± 0.34
3200	89.58[a] ± 3.33	0.17[a] ± 0.07	0.29 ± 0.01	9.90[a] ± 0.83	35.69 ± 2.27	13.35[a] ± 0.39	0.76 ± 0.05

Data are expressed as the mean ± SE (n=6, except n=5 for hamsters given 400 or 800 mg FM/kg).

[a]Significantly different from control ($p<0.05$).

Table 2A. Organ to brain weight ratios of hamsters given oral dosages of Firemaster BP-6.

Table 2A. Organ to brain weight ratios of hamsters given oral dosages of Firemaster BP-6.

Dosage (mg/kg)	Liver (g/g)	Thymus (mg/g)	Adrenals (mg/g)	Kidneys (g/g)	Testicles (g/g)	Spleen (mg/g)
0	2.94 ± 0.13	60.39 ± 4.61	23.15 ± 1.69	0.72 ± 0.02	3.22 ± 0.07	86.91 ± 3.91
400	5.94[a] ± 0.28	14.18[a] ± 4.11	21.38 ± 1.20	0.80 ± 0.02	2.85 ± 0.26	64.24[a] ± 5.22
800	6.09[a] ± 0.17	17.24[a] ± 3.87	21.63 ± 1.43	0.80 ± 0.01	3.23 ± 0.07	70.29[a] ± 2.43
1600	6.19[a] ± 0.23	11.97[a] ± 2.81	22.50 ± 1.05	0.76 ± 0.03	2.27 ± 0.47	66.44[a] ± 5.71
3200	6.72[a] ± 0.21	7.06[a] ± 1.30	22.25 ± 0.49	0.74 ± 0.05	2.70 ± 0.23	55.97[a] ± 5.15

Data are expressed as the mean ± SE (n= 6, except n=5 for hamsters given 400 or 800 mg FM/kg).

[a]Significantly different from control ($p < 0.05$).

Table 3A. Organ to body weight ratios of guinea pigs given an oral dosage of Firemaster BP-6.

Table 3A. Organ to body weight ratios of guinea pigs given an oral dosage of Firemaster BP-6.

Dosage (mg/kg)	Liver (mg/g)	Thymus (mg/g)	Adrenals (mg/g)	Kidneys (mg/g)	Testicles (mg/g)	Brain (mg/g)	Spleen (mg/g)
0	40.5 ± 1.31	1.49 ± 0.52	0.41 ± 0.02	7.17 ± 0.17	2.80 ± 0.12	8.62 ± 0.32	1.27 ± 0.05
100	47.6 ± 1.36	1.27[b] ± 0.60	0.37 ± 0.01	7.16 ± 0.32	2.66 ± 0.09	9.32 ± 0.33	1.46 ± 0.13
200[c]	59.3[b] ± 7.36	0.75[b] ± 0.10	1.03[b] ± 0.23	12.46 ± 1.71	2.37 ± 0.18	15.50[b] ± 1.88	1.26 ± 0.10
400[c]	75.4[b] ± 3.18	0.54[b] ± 0.07	1.35[b] ± 0.10	16.16 ± 0.63	2.93 ± 0.25	19.12[b] ± 0.86	1.10 ± 0.07
800[c]	74.0[b] ± 2.33	ND[a]	1.24[b] ± 0.12	16.29 ± 0.55	3.35 ± 0.18	18.97[b] ± 0.83	1.10 ± 0.06

Data are expressed as the mean ± SE (n=6).

[a]Not Done.

[b]Significantly different from control ($p<0.05$).

[c]All guinea pigs given 400 or 800 mg FM/kg body weight and two given 200 mg/kg died before the end of the study.

Table 4A. Organ to brain weight ratios of guinea pigs given an oral dosage of Firemaster BP-6.

Table 4A. Organ to brain weight ratios of guinea pigs given an oral dosage of Firemaster BP-6.

Dosage (mg/kg)	Liver (g/g)	Thymus (mg/g)	Adrenals (mg/g)	Kidneys (mg/g)	Testicles (mg/g)	Spleen (mg/g)
0	4.74 ± 0.21	17.40 ± 0.87	47.80 ± 0.27	83.70 ± 4.40	32.80 ± 1.88	14.90 ± 0.98
100	5.16 ± 0.27	13.60 ± 0.63[b]	40.30 ± 0.22	76.80 ± 1.72	28.70 ± 1.55	15.70 ± 1.49
200[c]	3.91 ± 0.43	5.52 ± 1.33[b]	62.20 ± 0.80	79.60 ± 1.76	16.60 ± 2.43[b]	9.13 ± 1.92[b]
400[c]	3.96 ± 0.12	2.76 ± 0.42[b]	71.50 ± 0.67[b]	84.80 ± 2.48	15.60 ± 1.87[b]	5.72 ± 0.38[b]
800[c]	4.10 ± 0.24	ND[a]	69.00 ± 0.69[b]	89.80 ± 3.52	18.70 ± 1.60[b]	5.95 ± 0.38[b]

Data are expressed as the mean ± (n=6).

[a]Not done.

[b]Significantly different from control ($p<0.05$).

[c]All guinea pigs given 400 or 800 mg FM/kg body weight and two given 200 mg/kg died before the end of the study.

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