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COMPARATIVE PATHOLOGIC EFFECTS OF PURIFIED POLYBROMINATED BIPHENYL CONGENERS IN RATS

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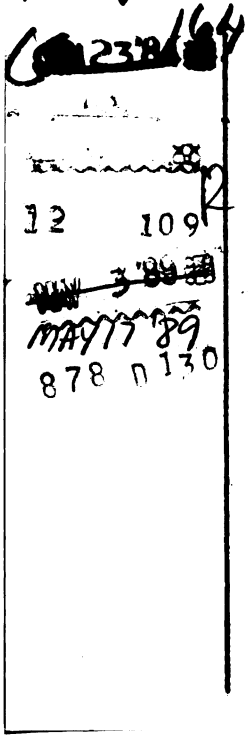

COMPARATIVE PATHOLOGIC EFFECTS OF PURIFIED POLYBROMINATED BIPHENYL CONGENERS IN RATS

By

Budi Tri Akoso

A DISSERTATION

Submitted to
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DEDICATED WITH LOVE TO MY PARENTS

Asih Soepranti and S. Poedjohadioetojo

ABSTRACT

COMPARATIVE PATHOLOGIC EFFECTS OF PURIFIED POLYBROMINATED BIPHENYL CONGENERS IN RATS

By

Budi Tri Akoso

Male rats were assigned into groups of 6 rats each and were fed diets containing 0, 1, 10 or 100 ppm of Firemaster BP-6 (FM BP-6), 2,2',4,4',5,5'-hexabromobiphenyl (-HBB), or 2,3',4,4',5,5'-HBB. In addition, 3 groups of 6 rats were given diets containing 0, 1 or 10 ppm 3,3',4,4',5,5'-HBB. Rats were killed on the 30th day of each experiment.

The objectives were to characterize and compare the pathologic effects of FM BP-6 and 3 purified polybrominated biphenyl (PBB) congeners. The major congener in FM BP-6 is 2,2',4,4',5,5'-HBB (47.8%) and it is strictly a phenobarbital (Pb)-type inducer of hepatic microsomal enzymes. Congener 2,3',4,4',5,5'-HBB induces Pb and 3-methylcholanthrene (MC)-type microsomal enzymes and constitutes approximately 5.5% of FM BP-6. Although 3,3',4,4',5,5'-HBB is not a constituent of FM BP-6, it was chosen because it is strictly an MC-type inducer.

Decreased feed intake and depressed growth rates in rats given diets containing 3,3',4,4',5,5'-HBB were the only

clinical signs observed. Results of urinalyses and hematologic examinations were normal.

Hepatic weights were increased in rats fed diets containing FM BP-6 or any of the 3 congeners. Swollen hepatocytes and cytoplasmic vacuolation were most prominent with 3,3',4,4',5,5'-HBB. Ultrastructurally, FM BP-6 caused proliferation of smooth endoplasmic reticulum (SER), decreased numbers of mitochondria and increased fat droplets. Similar but less severe changes were seen with 2,3',4,4',5,5'-HBB or 2,2',4,4',5,5'-HBB. The latter congener caused the least severe changes. Hepatocytes of rats fed diets containing 10 ppm 3,3',4,4',5,5'-HBB had extensive proliferation and disorganization of rough endoplasmic reticulum (RER), increased fat droplets and some proliferation of SER. Myelin bodies in hepatocytes were seen in rats fed FM BP-6 but were not seen with 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB.

In general, FM BP-6 and each of the 3 congeners decreased concentrations of liver vitamin A, serum triiodothyronine (T_3) and thyroxine (T_4) and increased T_3/T_4 ratios. Changes were most severe with FM BP-6 and 3,3',4,4',5,5'-HBB.

Thyroid follicular cell hyperplasia and hypertrophy with scanty or absent colloid were associated with the dietary administration of FM BP-6 and each of the 3 congeners. Ultrastructurally, increased numbers of dense bodies and colloid droplets and dilated cisternae were most prominent in rats given FM BP-6 or 3,3',4,4',5,5'-HBB.

The results indicated that 3,3',4,4',5,5'-HBB, an MC-type inducer, was the most toxic among the 3 congeners,

whereas 2,2',4,4',5,5'-HBB, a Pb-type inducer of hepatic microsomal enzymes, was the least toxic. Firemaster BP-6, a mixed-type inducer (MC- and Pb-type), was more toxic than either 2,3',4,4',5,5'-HBB (mixed-type inducer) or 2,2',4,4',5,5'-HBB. Apparently, the latter congener, which is strictly a Pb-type inducer and the major congener in the FM BP-6, contributes little to the toxicity of the mixture. Toxicity of FM BP-6 can be mostly attributed to congeners with MC-type induction capability.

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INTRODUCTION

The awareness of the presence of toxicants in the environment has been recorded since ancient centuries when the Rig-Veda of Hindu origin (5000 B.C.) mentioned the use of "soma" plant as an hallucinogen by priests. Meanwhile, the Hebrew pentateuch was concerned about human beings and their relationship with the environment (Auerbach and Gehrs, 1980). Attention to environmental contamination has become more and more intense as the development of chemical technology has met the demand for an increasing need for chemicals. Environmental contaminants such as 1,1,1,-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT), organophosphates, polyhalogenated aromatic hydrocarbons and many others, including the recent Michigan livestock feed contaminant, polybrominated biphenyls (PBB), have drawn some attention to the potential hazards not only to the livestock industry but also to human health as well.

Polybrominated biphenyls were used as a flame retardant for plastic and synthetic fibers and were manufactured by the Michigan Chemical Corporation in the early 1970s under the trade name Firemaster BP-6 (FM BP-6). More than 13 million pounds of FM had been produced until the chemical was banned due to widespread contamination of livestock feed in the late summer of 1973. Up to a thousand pounds of FM were accidentally included in a shipment of "Nutrimaster" (magnesium

oxide), a livestock feed supplement. Severe herd problems associated with food contamination were reported following the accident, but the source of contamination was not identified until April 1974 (Jackson and Halbert, 1974; Kolbye, 1977).

At least 8 months elapsed between the onset of contamination and the detection of the chemical. Over 10,000 Michigan residents were exposed to PBB through consumption of contaminated milk and meat which resulted in bioaccumulation in their bodies (Dunckel, 1975; Kay, 1977). More than 34,000 cattle and 1.5 million chickens were destroyed, as well as tons of milk, eggs, cheese, and other livestock products.

The PBB are potent inducers of liver microsomal drug metabolizing enzymes and are characterized by both phenobarbital (Pb) and 3-methylcholanthrene (MC) type induction (Dent et al., 1976a,b). Firemaster BP-6 has been analyzed by electron capture gas chromatography and consists of a mixture of brominated biphenyls. Purification of the chemical revealed that approximately 30 different congeners are in the PBB mixture. While the PBB mixture is usually quantified based on its major congener, 2,2',4,4',5,5'-hexabromobiphenyl (HBB) (Fries and Marrow, 1975; Willett and Irving, 1976), many scientists contend that other peaks may contribute the most harmful effects. The 2,2',4,4',5,5'-HBB (peak-4), a Pb type inducer, has been demonstrated to be less toxic than the PBB mixture or 2,3',4,4',5,5'-HBB (peak-6) (Akoso and Sleight, 1979; Dharma, 1980). The latter 2 chemicals are both mixed-type inducers. The most toxic effect might be

induced by a strict MC-type inducer, 3,3',4,4',5,5'-HBB (Aust et al., 1981). This chemical is characterized by a structure related to its ability to exist in a planar configuration typical for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) receptor binding.

Continuing anxiety and concern over long-term effects of PBB in livestock and on public health have resulted in the need for research in many aspects related to the toxicity. The first objective of this study was to compare the pathologic features of FM BP-6 with its purified congeners, represented by 2,2',4,4',5,5'-HBB and 2,3',4,4',5,5'-HBB. The second objective was to compare and characterize the pathologic features in rats fed different types of inducers of hepatic drug-metabolizing enzymes, including Pb (2,2',4,4',5,5'-HBB), MC (3,3',4,4',5,5'-HBB) or mixed type (FM BP-6 and 2,3',4,4',5,5'-HBB). The third objective was to determine the magnitude of toxicity due to each purified congener.

While the types of induction of the purified congeners presented in this study have been documented, the comparative study on the degree of tissue alteration in response to each chemical should provide worthwhile information for exploring toxicopathological features of FM BP-6.

LITERATURE REVIEW

Chemical and Physical Properties

Polybrominated biphenyl (Firemaster BP-6) is insoluble in water, highly soluble in organic solvents, and is classified as a lipophilic substance. The PBB mixture has a melting point of 72 C and will decompose at 300 to 400 C. The vapor pressure is relatively low and under ultraviolet radiation, PBB are degraded readily (Kay, 1977).

The potential hazard from PBB-contaminated soil is low. Plant uptake of PBB was not a problem on the farms where contaminated livestock were located. The PBB were not taken up by orchard grass cuttings or carrot tops (Jacobs et al., 1976). However, the persistence in soil may become a source of environmental contamination (Dunckel, 1975; Kolbye, 1977; Fine, 1976).

As indicated above, PBB are complex mixtures of bromobiphenyl compounds. The reported percentages include: tetra- (2.0), penta- (10.6), hexa- (62.8) and heptabromobiphenyl (13.2) (Michigan Chemical Corporation, 1974). In addition, Hass et al. (1978) reported the presence of 150 ppm pentabromonaphthalene and 70 ppm hexabromonaphthalene. However, these contaminants were not considered to influence PBB toxicity to any great extent (Goldstein et al., 1978).

There are 14 distinct congeners which are differentiated by gas chromatographic elution and are identified by their numerical order of appearance in the chromatographic profile. Peaks 1 and 2 are penta-, 3 to 7 are hexa-, 8 and 9 are hepta-, 10 to 12 are octa-, 13 is nona-, and 14 is decabromobiphenyl (Moore and Aust, 1978; Aust et al., 1971). Among the congeners, 10 have been identified structurally. These include peaks 1 to 9 and peak 12. The structural characteristics and the percentage by weight of individual congeners are listed in sequence: 2,2',4,5,5'-penta- (4.5%); 2,3',4,4',5-penta- (4.2%); 2,2',3,4',5',6-hexa- (1.4%); 2,2',4,4',5,5'-hexa- (47.8%); 2,2',3,4,4',5'-hexa- (12.0%); 2,3',4,4',5,5'-hexa- (5.5%); 2,3,3',4,4',5-hexa- (5.0%); 2,2',3,4,4',5,5'-hepta- (15.1%); 2,2',3,3',4,4',5-hepta- (1.1%); and 2,2',3,3',4,4',5,5'-octabromobiphenyl (1.1%) (Aust et al., 1981). The chemical and physical properties of PBB are similar to polychlorinated biphenyl (PCB), with the main structural difference being the attachment of bromine atoms rather than chlorine in the biphenyl ring. Bromine is more labile than chlorine and therefore PBB may be less stable in the environment than PCB (Hutzinger et al., 1976).

Metabolism

Among the major congeners, 2,2',4,5,5'-penta- (peak 1) and 2,2',3,4',5',6-hexabromobiphenyl (peak 3) are the 2 congeners metabolized rapidly (Dannan et al., 1978b). According to Moore et al. (1980), PBB metabolism is facilitated when the number of para-substitutions decreases, the number

of orthos increases, and the total number of substitutions decreases. The ideally brominated biphenyl with a structure which facilitates metabolism is 2,2'-dibromobiphenyl. This chemical is metabolized at a rapid rate (Dannan et al., 1978b). Similar evidence has been reported for 2,2'-dichlorobiphenyl (Greb et al., 1975; Hesse and Wolf, 1977). Unlike peak 1 and peak 3, all other PBB congeners have at least 2 para-substitutions; hence, metabolism is not likely to occur.

Biochemical Pharmacology

Polybrominated biphenyls are potent inducers of liver microsomal drug-metabolizing enzymes. The induction is classified as a mixed type, in which the microsomal enzymes have induction properties similar to both Pb and MC (Dent et al., 1976a,b). One of the major properties of hepatic microsomal drug metabolizing enzymes is to make lipid soluble compounds more polar and thus water soluble, thereby making them more susceptible to urinary or bile excretion (Milburn et al., 1967). Reactions such as oxidation, reduction, hydrolysis, and conjugation are involved in the metabolism of xenobiotics (Kappas and Alvares, 1975; Gillette, 1966).

The liver microsomal drug-metabolizing enzymes metabolize many drugs and other xenobiotics as well as some endogenous compounds, such as steroids, fatty acids, bile acids and heme (Conney, 1967; Kuntzman, 1969). This complex of enzymes consists of many types of enzymes, primarily mixed function oxidases (MFO) and cytochrome P_{450} (Gillette et al., 1972). This system catalyzes the consumption of one molecule of

oxygen per molecule of substrate. One oxygen atom is complexed with the product, while the other oxygen atom is incorporated into water (Mason, 1957). Cytochrome P_{450} is the substrate and oxygen binding site of the MFO system (Omura et al., 1965; Imai and Sato, 1966; Schenkman et al., 1967). Its name originates from the property of exhibiting an absorption maximum at 450 nm when the reduced form of cytochrome is complexed with CO (Omura and Sato, 1964).

Among the congeners, 2,2',4,4',5,5'-hexa- (peak 4), 2,2',3,4,4',5,5'-hepta- (peak 8) and 2,2',3,3',4,4',5,5'-octabromobiphenyl (peak 12) are known to be Pb-type inducers (Moore et al., 1978b; Moore et al., 1979; Besaw et al., 1978). Goldstein et al. (1977) described a chlorinated analog represented by 2,2',4,4',5,5'-hexachlorobiphenyl (HCB) to also be a Pb-type inducer. While there is no strict MC-type inducer in FM BP-6, 2,3',4,4',5-pentabromobiphenyl (peak 2), 2,2',3,4,4',5-HBB (peak 5), 2,3',4,4',5,5'-HBB (peak 6) and 2,3,3',4,4',5-HBB (peak 7) are mixed type inducers (Aust et al., 1981; Robertson et al., 1981). Moore et al. (1980) described peak 2 as an MC-type and a weak Pb-type inducer. A strict MC-type inducer, 3,3',4,4',5,5'-HBB, has been purified from a mixture obtained from RFR Corporation (Aust et al., 1981). This congener is not found in FM BP-6 but is suspected as the most toxic PBB congener. Structurally, the congener is characterized by para- and meta-substitutions with a planar configuration. The meta-substitution and planar configuration are required for binding to a cytoplasmic receptor associated with aryl

hydrocarbon hydroxylase (AHH) induction. This characterization has been documented in the chlorinated analog, 3,3',4,4',5,5'-hexachlorobiphenyl (HCB) (Poland and Glover, 1977).

Kinetics

Absorption

After gastrointestinal absorption, PBB are circulated throughout the body, appearing in the plasma within 4 hours and reaching a steady state by 15 days. The PBB will then be distributed to various tissues with the largest amount to the fat of liver, muscle, and kidney (Fries et al., 1978; Kolbye, 1977). Approximately 2 to 6 hours after intraruminal administration in cattle, PBB were detected in plasma with peak concentrations occurring at 24 to 48 hours (Willett and Irving, 1975). Matthews et al. (1978) postulated that absorption of polyhalogenated hydrocarbon is less with increasing halogenation. Similarly, PBB with less bromine atoms are more readily absorbed (Fries et al., 1976).

Retention

As a lipophilic substance, the distribution and retention of PBB are associated closely with the fat available in tissue. With the exception of certain organs, especially liver and brain, the PBB residue in tissue is directly proportional to the fat content of an organ (Gutenmann and Lisk, 1975; Willett and Irving, 1975, 1976). Fries (1978) and Fries et al. (1978) also reported that there was no difference in the

concentration of PBB in the fat of tissues, including perirenal, omental and subcutaneous adipose tissue, skeletal muscle, cardiac muscle and kidney of cattle originally exposed to PBB contaminated feed in Michigan. However, brain, a lipid-rich tissue, usually has the lowest level of PBB residue, either due to the effectiveness of the blood-brain barrier or the inability of PBB to accumulate in the types of lipid in nervous tissue, such as phospho-, glyco- and sulfolipids (Willett and Durst, 1978). Norris et al. (1974) reported that decabromodiphenyl oxide or octabromobiphenyl were not accumulated in the kidney, skeletal muscle or testis.

Excretion

The PBB are excreted through the milk, eggs, feces, and urine. While feces are the most important route of PBB excretion in male or nonlactating animals, milk or eggs are the most effective route for excretion of lipophilic compounds such as PBB in lactating mammals or laying birds.

The tissue residues of PBB are higher in the male than in the female Japanese quail (Babish et al., 1975) or laying hens (Fries et al., 1973). Fries (1978) estimated about 50% of the daily intake of PBB is excreted through the eggs.

In a lactating animal PBB tends to accumulate in mammary tissue and in milk fat. Hence, milk was a major route of excretion in lactating cows (Gutenmann and Lisk, 1975). Willett and Irving (1976) detected PBB in milk within 13 hours after oral administration. The PBB reached a peak in milk

at about 60 hours. In the meantime, there was approximately 23% elimination of the chemicals through the milk. The PBB level remained higher in milk than in the body fat as long as cows were given PBB (Fries and Marrow, 1975). Upon termination of the oral administration, the steady state level ratio was 0.42 in milk fat to 1 in the body fat (Fries, 1978). This author also found that the concentration of PBB in milk fat concentration had declined by 60 to 70% after 60 days. He proposed that the decline may be influenced by the level of milk production, total amount of body fat, and changes in body fat concentration.

Fecal excretion of PBB has been described in rats (Matthews, 1977), pigs (Ku et al., 1978), cattle (Willett and Irving, 1976) and chickens (Ringer and Polin, 1977). Norris et al. (1974) reported a rapid fecal elimination of octabromobiphenyl in rats. When a single dose of ^{14}C-octabromobiphenyl is used, about 62% of the isotope is detected in the feces within 24 hours and about 73% of the dose is excreted by 16 days after administration. Rozman et al. (1981) studied fecal excretion in Rhesus monkeys given 100 mg/kg BW of ^{14}C,2,2',4,4',5,5'-HBB. They concluded that the excretion of the chemical through the feces is due to both biliary and intestinal elimination. They also found that mineral oil stimulated fecal excretion after a month of treatment and had an additive effect with cholestyramine in enhancing biliary HBB excretion.

Urine is a minor route for PBB excretion. Willett and Durst (1978) failed to detect free unconjugated PBB in the

urine of cattle. Kohli and Safe (1976) detected only 1% of a single intraperitoneal dose of PBB in the urine and feces of pigs within 7 days.

Pathology

Clinical Pathology

Red blood cell count (RBC), packed cell volume (PCV), hemoglobin (Hb) and total and differential white blood cell counts (WBC) in rats fed diets containing PBB were not affected (Sleight and Sanger, 1976; Sleight et al., 1978). This is in contrast to the effect of other halogenated aromatic compounds (McConnell and Moore, 1979). Kately (1977) did not observe significant differences among the various hematologic values for exposed and unexposed cattle. Hematologic data obtained from cows from one involved herd were inconclusive (Trapp et al., 1975).

Blood urea nitrogen (BUN) values in rats fed a diet containing PBB were reported as within normal limits (Sleight et al., 1978; Garthoff et al., 1977; McCormack et al., 1978a,b) but increased in cattle (Durst et al., 1978), pigs (Werner and Sleight, 1981) and guinea pigs (Hall, 1980). Meanwhile, Moorhead et al. (1977) observed increased levels of aspartate aminotransferase (AST), lactic dehydrogenase (LDH), blood urea nitrogen (BUN) and bilirubin in cows fed 25 g PBB/day.

Kasza (1977) found a decreased total of hematopoietic cells and an increased M/E ratio in Beagles fed 4 mg/kg/day of PBB for 61 days. The bone marrow had foci of necrosis and proliferation of reticuloendothelial cells.

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 decreased at 10 ppm of 3,3',4,4',5,5'-HBB (Thompson et al., 1981). The latter workers emphasized that the decreases were mainly in the high density lipoprotein fraction. However, Howard et al. (1980) did not find changes in serum cholesterol values of pigs fed diets containing up to 200 ppm of PBB.

Clinical Signs

There were no clinical signs of toxicosis in rats fed up to 100 ppm of PBB for 30 or 60 days. The weight gains and feed efficiency decreased when rats were fed up to 500 ppm PBB (Sleight and Sanger, 1976). The body weight of mice fed diets containing up to 200 ppm PBB for 2 weeks was not affected (Cagen et al., 1977). Feed consumption and feed efficiency were not affected when rats were fed up to 1000 ppm octabromobiphenyl (OBB) for 4 weeks (Lee et al., 1975b) or up to 1 mg/kg/day of OBB for 180 days (Norris et al., 1974). Decreases in body weight due to dietary treatment with PBB have been reported in Rhesus monkeys (Allen et al., 1978) and pigs (Ku et al., 1978).

The signs of PBB toxicosis in cattle in the field were variable and somewhat inconsistent. The variability may be due to the lack of previous information concerning the health of the herds, the dose and duration of PBB exposure (Durst et al., 1977). In a herd of 400 dairy cattle exposed to high levels of PBB contaminated feed, the clinical signs were

anorexia, reduced milk production and increased frequency of urination and lacrimation (Jackson and Halbert, 1974). Lameness, shrunken udders, abnormal growth of hooves and loss of hair were also reported. In a field observation of dairy cattle contaminated with PBB there was a marked decrease in feed consumption and an increase in reproductive disorders (Prewitt et al., 1975). Pregnant heifers given 25 g PBB/day had signs of depression, dehydration, diarrhea, emaciation and abortion (Moorhead et al., 1977).

Gross Lesions

Hepatomegaly was the most pronounced and consistent finding resulting from PBB toxicosis mainly in laboratory animals. The evidence has been reported in rats (Sleight and Sanger, 1976; Akoso, 1977), guinea pigs (Sleight and Sanger, 1976), mice (Corbett et al., 1975; Cagen et al., 1977), Japanese quail (Babish et al., 1975) and cockerels (Dharma, 1980).

Thyroid enlargement has been reported in rats fed a diet containing 10 ppm of PBB or higher for 60 days (Sleight et al., 1978). The PBB also exaggerated thyroid enlargement in rats fed a basal iodine deficient diet for 30 days (Akoso, 1977). Thyroid enlargement has also 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 cockerels fed 45 ppm PBB (Ringer, 1978).

In a herd of cattle that had been fed PBB contaminated feed, the gross lesions were reported as hematomas, abscesses of peritoneal and thoracic cavities, adhesions of the rumen to the ribs, liver enlargement or abscesses, necrotic metritis, and suppurative bronchopneumonia (Jackson and Halbert, 1974). Moorhead et al. (1977) reported that pregnant heifers fed experimental diets containing 25 g PBB/day had lesions including subcutaneous emphysema and hemorrhage, thymus atrophy, enlarged kidneys, thickened gallbladder, inspissated bile, abomasal edema, and mucoid enteritis. Ku et al. (1978) also found an increase in relative weights of liver, heart, kidney, and adrenal of pigs fed a diet containing 20 or 200 ppm PBB for 16 weeks.

Concerning the effects of PBB on reproduction, Harris et al. (1978b) reported no effect on fetal mortality, length of fetuses or the weight of fetuses when PBB was administered orally to pregnant rats in doses up to 10 mg/day from day 7 through day 15 of gestation. However, Corbett et al. (1975) stated that PBB is weakly teratogenic. These investigators observed that 1000 ppm PBB given to pregnant mice caused exencephaly or cleft palate. Mink appear to be sensitive to PBB. Dietary treatment of 0.1 to 2.5 ppm caused a decrease in litter size, kit weight at birth and kit survival (Aulerich and Ringer, 1979). Reproductive effects of PBB in birds have been studied by Ringer and Polin (1978). They reported that dietary feeding of 45 ppm PBB caused decreased egg production, hatchability and viability of offspring.

After cessation of PBB treatment, the egg production returned to normal.

Histopathology

Fatty metamorphosis and amyloidosis were among the hepatic lesions of PBB contaminated cattle described by Jackson and Halbert (1974), as well as renal lesions including nephrosis and interstitial nephritis. Moorhead et al. (1978), in experimentally exposed cows, reported hepatic glycogen depletion and early centrilobular fatty metamorphosis. The kidneys had an extreme dilatation of collecting ducts and convoluted tubules with evidence of cloudy swelling and hydropic degeneration. There were hyperplasia and cystic dilatation in the mucous glands of the lamina propria of the gallbladder. The hair follicles of the eyelid had an accumulation of keratin.

Rats fed a diet containing 1 ppm PBB or higher had cytoplasmic vacuolation with the degree of lesions related to the dose and duration of treatment. 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. (1977) observed enlarged and vacuolated hepatocytes as well as some 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 evidence of steatosis, megalohepatocytes, necrosis and interstitial fibrosis in the liver.

Sleight et al. (1978) reported mild follicular epithelial hyperplasia with absent or poorly staining colloid of the thyroid gland in rats fed diets containing 100 ppm PBB. These authors also reported a squamous metaplasia of bronchiolar epithelium of rats fed diets with excessive iodine and containing 100 ppm PBB.

Electron Microscopy

Ultrastructural features of rats fed a diet containing PBB included an increase of smooth endoplasmic reticulum, enlarged hepatic mitochondria and the presence of myelin bodies in the cytoplasm of hepatocytes (Sleight and Sanger, 1976). Corbett et al. (1978) observed decreased rough endoplasmic reticulum, increased smooth endoplasmic reticulum, degeneration of mitochondria, and increased numbers of lysosomes in the liver of mice fed a diet containing 1000 ppm PBB for 14 days.

PBB induced similar and dose-dependent lesions in the thyroid when compared to PCB. Kasza et al. (1978) observed an accumulation of colloid droplets and lysosomal bodies within the cytoplasm of follicular cells of the thyroid in rats fed diets containing PBB or PCB. There were vacuolation of the mitochondria and disruption of the cristae. The lesions were observed at daily dose levels as low as 5 ppm, and similar but more severe changes were seen at 50 or 500 ppm.

Immunity

The effect of PBB on immunity has been studied by a number of investigators. The PBB altered the immune system in rats and mice (Luster et al., 1978), sows and their offspring (Howard et al., 1980), guinea pigs (Vos and Van Genderen, 1973), dogs (Kasza, 1977), chickens (Ringer, 1978; Vos and Van Genderen, 1973), and man (Bekesi et al., 1978). However, other researchers did not find immunosuppressive effects (Kateley and Bazzell, 1978; Kauffman et al., 1978). Fraker and Aust (1979) proposed that PBB have a deleterious effect on B-cells and T-helper cells of mice. Luster et al. (1978) also reported depression in cell-mediated immunity in both rats and mice due to oral administration of FM FF-1. Howard et al. (1980) found a decreased response to mitogen stimulation of peripheral blood lymphocytes from sows fed diets containing 100 or 200 ppm PBB for 12 weeks. They further indicated that mitogen responses of lymphocytes from the piglets were normal at birth but were decreased after 4 weeks of age.

MATERIALS AND METHODS

Experimental Design

Seventy-eight young male Sprague-Dawley rats[a] initially weighing 148 ± 16 g were used. All rats were in good general condition at the start of the experiment. The experimental design is illustrated in Table 1. The rats were assigned into 2 groups. The first group comprised 10 subgroups of 6 rats each and they were fed either 0, 1, 10 or 100 ppm of FM BP-6, 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB. The second group comprised 3 subgroups of 6 rats each and were fed 0, 1 or 10 ppm of 3,3',4,4',5,5'-HBB.

The rats were housed 3 to a cage in metal wire-top plastic cages. The cages were cleaned and the bedding was changed once a week. Room temperature was maintained between 21 and 27 C with relative humidity of 45 to 55%. Lights were controlled automatically to allow 10 to 12 hours of darkness.

Chemicals

The chemicals used in this study were FM BP-6, two of its purified congeners and 3,3',4,4',5,5'-HBB. Congeners

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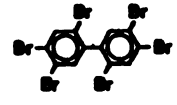
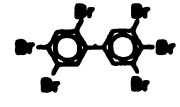
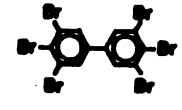
[a]Spartan Research Animals, Haslett, MI.

Table 1. Experimental design of dietary treatment of rats fed FM BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-HBB or 3,3',4,4',5,5'-HBB for 30 days

Group	Modification of Dietary Treatment	Chemical Structure	Chemical Concentration in Feed (ppm)	No. of Rats
I	Control	---	0	6
	Firemaster BP-6		1	6
			10	6
			100	6
	2,2',4,4',5,5'-HBB		1	6
			10	6
			100	6
	2,3',4,4',5,5'-HBB		1	6
			10	6
			100	6
II	Control	---	0	6
	3,3',4,4',5,5'-HBB		1	6
			10	6

2,2',4,4',5,5'-HBB and 2,3',4,4',5,5'-HBB were purified from FM BP-6 by a combination of crystallization and chromatography on neutral alumina in hexane and chromatography on Lipidex 5000 in acetone, heptane and methanol. The 3,3',4,4',5,5' was purchased from the RFR Corporation, Hope, Rhode Island, and purified by repeated alumina chromatography (Aust et al., 1981). The purification of these congeners was done in the Toxicology Laboratory, Department of Biochemistry, Michigan State University.

Feeding Practices

During 2 days of acclimation the rats were fed a regular commercial pelleted diet[b] and tap water *ad libitum*. The rats were then adapted to the finely ground commercial diet for another 2 days before dietary treatment. The first 10 subgroups of rats were given a ground feed diet containing 0, 1, 10 or 100 ppm (mg chemical/kg feed) of FM BP-6,[c] 2,2',4,4',5,5'-HBB[d] or 2,3',4,4',5,5'-HBB[d] while the last 3 subgroups were fed diets containing 0, 1 or 10 ppm of 3,3',4,4',5,5'-HBB.[d] Mazola^{R} corn oil[e] was used as the vehicle for each chemical to assure proper mixing with the

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[b]Purina Rat Chow, Ralston Purina Co., Checkerboard Square, St. Louis, MO.

[c]Firemaster BP-6, Michigan Chemical Co., St. Louis, MI.

[d]Dr. S. D. Aust, Department of Biochemistry, Michigan State University, East Lansing, MI.

[e]CPC International, Inc., Englewood Cliffs, NY.

feed. The amount of corn oil added to the feed was calculated so as to get the same concentration of oil in every dietary treatment, including the control. The feed was fed in porcelain containers with stainless steel tops. Drinking water was available *ad libitum*, in inverted bottles with rubber stoppers and stainless steel sipper tubes. The water was changed twice a week.

Clinical signs, feed consumption and body weights were recorded every other day. Precautions were appropriately employed to prevent any possible contamination among the different feed diets or of the people working with the rats. Each batch of feed was separated and labeled.

Laboratory Investigation Procedure

On the 30th day of the experiment the rats were killed after feed was withheld overnight. The final body weights were recorded prior to necropsy and the rats were killed with ether anesthesia or carbon dioxide.

Blood Samples

The blood samples were obtained from the heart while the rat was anesthetized. Blood samples for hematologic examination were collected into a tube with ethylenediaminetetraacetic acid (EDTA) as the anticoagulant, and direct blood smears were made immediately. Blood without anticoagulant was collected in tubes, and the serum was removed after coagulation and centrifugation. The serum was placed in tubes and stored at -4 C for further chemical analyses. The Hb

concentration was determined by the standard cyanmethemoglobin[f] method, and readings were made with a spectrophotometer.[g] The PCV was measured in microhematocrit tubes,[h] centrifuging for 5 minutes at 3,000 g and reading with a microhematocrit reader.[i] Red blood cells and WBC were counted by using an electronic counter.[j] The blood smears were stained with Wright's stain[k] and examined for the differential leukocyte count.

Blood Chemistry

The amounts of BUN, serum alkaline phosphatase (SAP) and AST were determined by using Eni-Gemsaec reagents.[l]

Thyroid Hormone Analysis

Serum samples for thyroid hormone were analyzed in the Clinical Endocrinology Laboratory, Animal Health Diagnostic Laboratory, Michigan State University. Serum concentrations of triiodothyronine (T_3) and thyroxine (T_4) were determined by radioimmunoassay methods as described by Chopra et al. (1971, 1972).

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[f]Hycel, Inc., Houston, TX.

[g]Perkin-Elmer Coleman 4, Coleman Instruments Division, Oak Brook, IL.

[h]Capillary tubes, Scientific Products, Evanston, IL.

[i]International Micro-Capillary Reader, International Equipment Co., Boston, MA.

[j]Coulter Electronics, Inc., Hialeah, FL.

[k]Hemateck Automatic Stainer.

[l]Smith Kline Instrument, Inc., Sunnyvale, CA.

Serum Electrophoresis

Values for serum protein and LDH-isoenzymes were determined by electrophoretic analysis. Serum for protein determination was applied to cellulose acetate[m] plates, and the serum proteins were separated by electrophoresis at 180 V for 15 minutes. The plates were stained for 6 minutes in Ponceau stain.[n] Following dehydration in methanol for 2 minutes, the plates were cleared in a 25% acetic acid in methanol solution for 5 to 10 minutes. The plates were then dried in an oven at 50 to 60 C approximately 4 to 5 minutes and scanned in a densitometer.[o]

Serum for LDH isoenzyme analysis was applied to cellulose acetate plates,[p] and the LDH isoenzymes were separated by electrophoresis at 300 V for 10 minutes. In the meantime, a substrate plate was prepared by pipetting 1 ml of the LDH substrate onto a wetted acetate plate. The plates were scanned in a densitometer with a 570 nm filter.

Collection of Tissues

Necropsy was performed soon after the animal was killed. The trachea was exposed, and the lungs were infused with 1 to

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[m]Titan III, Helena Laboratories, Beaumont, TX.

[n]Helena Laboratories, Beaumont, TX.

[o]Quick Scan and Quick Quant II, Helena Laboratories, Beaumont, TX.

[p]Titan III L, Helena Laboratories, Beaumont, TX.

2 ml buffered formalin which was injected intratracheally so as to obtain better fixation of the lungs. All tissues were examined grossly. The brain, liver, kidneys, thymus, and spleen were weighed with a top-loading balance.[q] The thyroid gland was weighed on an analytical balance[r] immediately after being removed. Urine was drawn from the bladder by direct puncture at the time of necropsy.

Tissues for histological examination were preserved in 10% neutral buffered formalin. Tissues collected included trachea, lungs, heart, spleen, liver, kidneys, stomach, intestines, skeletal muscle, thyroid, pituitary gland, adrenal gland, salivary gland, eye, skin, bone, urinary bladder, thymus, pancreas, testes, and brain. Bone was decalcified[s] prior to the histological processing.

Small pieces of liver and thyroid gland were sliced into approximately 2 mm blocks, fixed in Karnovsky's fixative and stored at 4 C prior to further preparation for ultrastructural examination.

Fat, liver, kidney, and thymus for chemical and liver vitamin A analyses were wrapped with aluminum foil, labeled and saved at -70 C for later analysis.

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[q]Mettler Series P, Model 163 (readability 0.001 g), Mettler Instrument Corp., Hightstown, NY.

[r]Model H-15 (readability 0.0001 g), Mettler Instrument Corp., Hightstown, NY.

[s]RDO, Du Pa Ge Kinetic Lab, Inc., Downers Grove, IL.

Urinalysis

The urine specific gravity was determined by using a refractometer.[t] The concentration of urobilinogen, blood, bilirubin, ketones, glucose and protein was estimated,[u] and the pH was determined.

Chemical Analysis

Tissue samples included liver, fat, kidneys, and thymus to be analyzed for FM BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-HBB or 3,3',4,4',5,5'-HBB concentration according to the chemical added to the diet. Pooled samples from 3 rats each weighed approximately 0.5 g. The samples were washed with petroleum ether and ground together with washed ignited sand.[v] The mixture was dehydrated by adding 10 to 20 g of granular anhydrous sodium sulfate.[v] Fifteen milliliters of distilled hexane[w] was added and the mixture was brought to a boil over an 80 C water bath. The content was filtered into a 100 ml volumetric flask. The addition of hexane and filtration were repeated 3 times. Hexane was added to bring the volume up to 100 ml. Two aliquots of 20 ml each were separated and each was condensed to approximately 0.5 ml by evaporation.[x] The first aliquot was dried in a preweighed aluminum pan by

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[t]Golden Refractometer, American Optical Co., Buffalo, NY.

[u]Multistix, Ames Co., Division Miles Lab., Inc., Elkhart, IN.

[v]Mallinckrodt, Inc., Paris, KY.

[w]J. T. Baker Chemical Co., Phillipsburg, NY.

[x]N-Evap, Model III, Meyer Organomation Assoc., Inc., Shrewsbury, MA.

evaporation and then weighed again to record the lipid weight.

Acetone-prewashed columns[y] measuring 200 mm x 7 mm ID were filled with 1.6 g of activated magnesium silicate.[z] The tapered end was plugged with a small amount of glass wool to hold the magnesium silicate. A small amount of granular anhydrous sodium sulfate was added to the top, the content of the column washed with 5 ml of glass-distilled hexane and the washing discharged. The previously condensed sample was transferred into the column and repeatedly rinsed with hexane. The eluate was condensed to 0.5 ml and then brought up to 2 ml with the addition of iso-octane.[aa]

Two microliters of the sample eluant was injected into the gas chromatograph.[bb] The gas chromatograph was equipped with an electron capture detector and operated with an injector temperature of 280 C. The column temperature was 250 C and the detector temperature was 310 C. The carrier was gaseous nitrogen at a flow rate of 30 ml/min. The result was compared to a standard sample containing 0.05 or 0.1 µg of FM BP-6, 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB/ml.

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[y]Chromaflex, 200 mm x 7 mm ID.

[z]Florisil, 60-100 Mesh, Fisher Scientific Co., Cleveland, OH.

[aa]Burdick and Jackson Laboratories, Inc., Muskegon, MI.

[bb]GC Model 3700, Varian Instrument Division, Palo Alto, CA.

Normal calf liver tissue was used to control the accuracy of the extraction procedure. The result was expressed as ppm of chemical on a fat basis.

The above procedure was modified for 3,3',4,4',5,5'-HBB analysis by using distilled toluene[cc] for the extraction solution instead of hexane.

Microsomal Enzyme Assays

A portion of liver was placed in cold 1.15% potassium chloride containing 0.2% nicotinamide. The liver was homogenized, and the homogenate was centrifuged at 10,000 x g for 20 minutes. The supernatant was recentrifuged at 105,000 x g for 90 minutes. The microsomes were washed with a 0.3 sucrose containing 0.1 M sodium pyrophosphate to remove ribosomes and absorbed proteins and then stored at -20 C in 0.05 M Tris-HCl, pH 7.5, with 50% glycerol and 0.01% butylated hydroxytoluene (Welton and Aust, 1974). The amounts of benzo(α)pyrene hydroxylase and aminopyrine demethylase were quantitated as described by Moore et al. (1978b). Microsomal isolation and assays were done in the Toxicology Laboratory, Department of Biochemistry, Michigan State University.

Vitamin A Analysis

Dried liver weight was determined by the following procedure. One gram liver samples were placed in an aluminum pan and dried in an oven at 56 C. The dried liver was weighed after 48 hours in the oven.

[cc]J. T. Baker Chemical Co., Phillipsburg, NY.

Samples for liver vitamin A analyses were extracted with the same procedure as for chemical analyses. The determination of vitamin A was done in the Clinical Nutrition Laboratory, Animal Health Diagnostic Laboratory, Michigan State University. The vitamin A was quantitated by a modification of the high pressure liquid chromatography procedure described by Dennison and Kirk (1977).

Histologic Preparation

The 10% buffered formalin-fixed tissues were trimmed, processed in an Autotechnicon[dd] and embedded in paraffin. The tissues were then sectioned with a microtome at 6 μ and stained with hematoxylin and eosin or other selected special stain including oil red O, Best's carmine, periodic acid-Schiff (PAS) and Ziehl-Nielsen as described by Luna (1968).

Transmission Electron Microscopy

Karnovsky's-fixed liver and thyroid for electron microscopy were cut into approximately 0.5 to 1.0 mm^3 blocks and were then washed into Zetterqvist's solution (Pease, 1964) at pH 7.4. The tissues were then postfixed in 1% osmium tetroxide in Zetterqvist's fixative. The tissues were transferred to propylene oxide after dehydration with graded alcohols and subsequently embedded into a mixture of Epon and Araldite.

The embedded tissue was cut with a glass knife on an ultramicrotome.[ee] For tissue-lesion orientation, a 1 μ-semithin

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[dd] Histomatic, Model 166, Fisher Scientific Co., Pittsburgh, PA.

[ee] LKB Ultratome III^R, Instrument Group 8800, Sweden.

section was stained with toluidine blue and observed under light microscopy. Thin sections, approximately 900 Å thick, were made and stained with uranyl acetate and lead citrate. The sections were observed by using an electron microscope.[ff]

Statistical Evaluation

The data were analyzed by analysis of variance. The significant differences were determined by Student's t-Bonferroni test (Gill, 1978).

[ff] CEM 952, Carl Zeiss, Germany.

RESULTS

Clinical Signs

There were no clinical signs of toxicosis observed in rats fed diets containing up to 100 ppm of FM BP-6, 2,2',4,4',5,5'-HBB, or 2,3',4,4',5,5'-HBB for 30 days. The animal behavior was not changed, and there was no mortality. The daily feed intake was increased in rats fed diets containing 1 ppm 3,3',4,4',5,5'-HBB but decreased at 10 ppm (Table 2). Firemaster BP-6, 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB did not alter the daily feed intake.

Body Weight

All rats gained weight throughout the experimental study. The weight gains in all rats given up to 100 ppm of Firemaster BP-6, 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB as well as 1 ppm 3,3',4,4',5,5'-HBB were normal. However, 10 ppm 3,3',4,4',5,5'-HBB significantly decreased the weight gains beginning from day 20 ($p<0.025$), as illustrated in Figure 1, and the weight gains continued to decline up to the end of the experiment ($p<0.01$).

Table 2. Initial body weight, weight gains and feed consumption in rats fed 3,3',4,4',5,5'-HBB for 30 days

Chemical Concentration in Feed (ppm)	Initial Weight (g)	Weight Gain (g)	Daily Feed Intake (g)
0 (control)	177 ± 1.04	188 ± 0.23	23.5 ± 0.21
1	179 ± 6.05	198 ± 9.98	24.7 ± 0.21[b]
10	175 ± 5.66	154 ± 8.58[a]	21.3 ± 0.28[c]

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

[a,b,c]Significantly different from control value ($p<0.025$, $p<0.005$, $p<0.0005$, respectively).

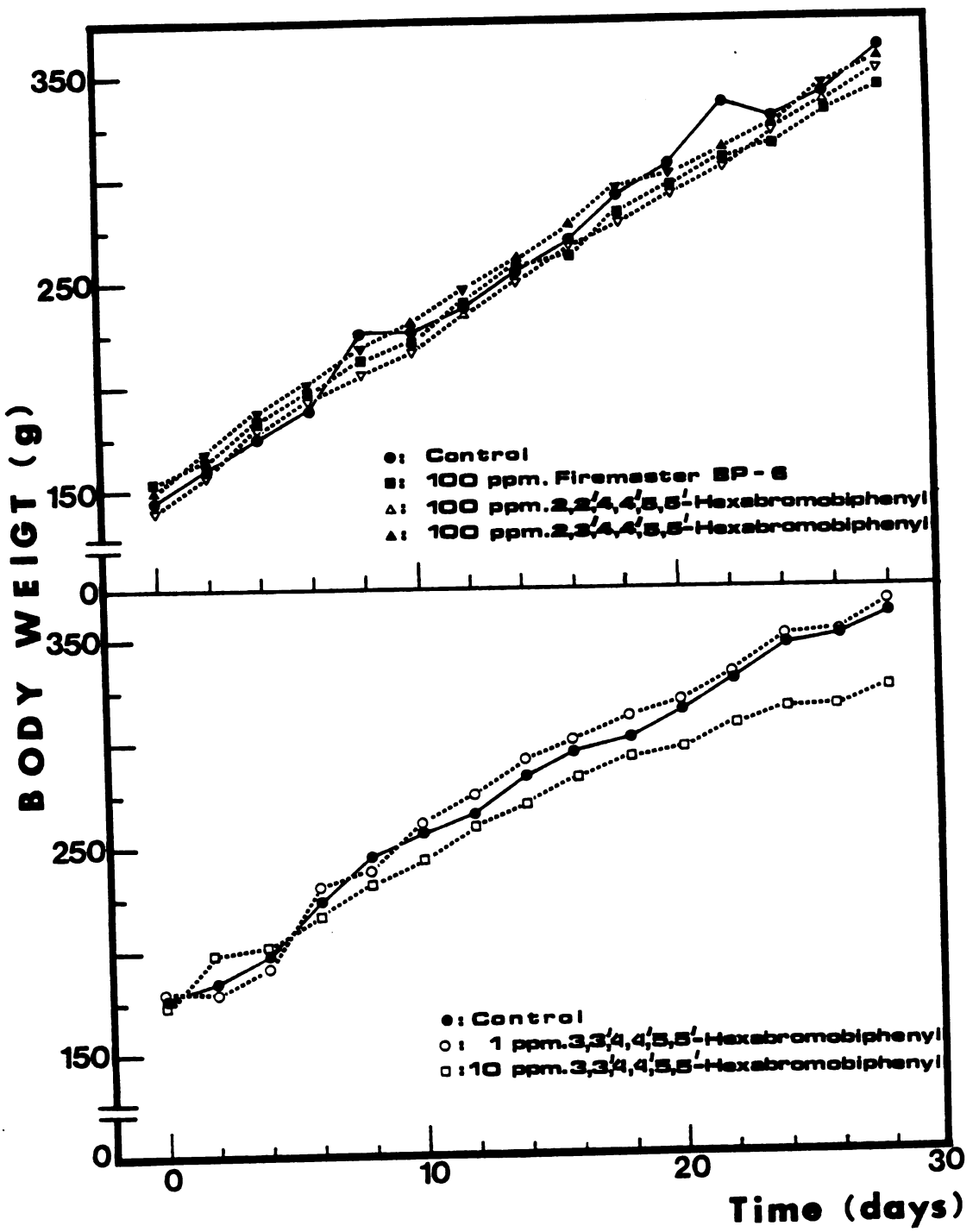

Figure 1. Means of body weight of rats fed diets containing Firemaster BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-HBB or 3,3',4,4',5,5'-HBB for 30 days.

Laboratory Investigations

Hematology

Red blood cell, Hb, and WBC values for all rats were within normal range. Values for PCV and differential leukocyte count were not significantly affected by FM BP-6, 2,2',4,4'5,5'-HBB, 2,3,4,4',5,5'-HBB or 3,3',4,4',5,5'-HBB. Morphological appearance of RBCs, WBCs and platelets was normal.

Urinalysis

Results of urinalysis were inconclusive. The inconsistent results obtained may be due to the lack of urine available in the urinary bladder. Many rats had empty or nearly empty urinary bladders by the time necropsy was performed. However, interpretation of the available data revealed that protein content and specific gravity tended to increase in treated rats, especially at the high dose of each of the chemicals.

Blood Chemistry

Serum alkaline phosphatase was not significantly affected. Firemaster BP-6 increased AST at 100 ppm ($p<0.05$), while 2,2',4,4',5,5'-HBB increased AST at 1 ppm ($p<0.0005$) but not at 10 or 100 ppm. The effects of treatment on BUN concentrations were inconclusive. The 3,3',4,4',5,5'-HBB did not significantly alter BUN, AST or SAP. The results of tests for BUN, AST and SAP are listed in Appendix Table A-1.

Serum Electrophoresis

Protein Fractions

The total serum proteins in all rats were within normal values, and there were no indications of dose-dependent responses. There was an increase in the gamma globulin fraction in rats fed 100 ppm 2,2',4,4',5,5'-HBB, but there were no alterations in other protein fractions. In general, there were no significant effects on albumin, alpha globulin and albumin/globulin ratio. The serum protein fraction determinations are listed in Appendix Table A-2.

Lactic Dehydrogenase Isoenzymes

Dietary treatment with 2,2',4,4'5,5'-HBB decreased LDH-1 significantly at 1 ($p<0.01$), 10 ($p<0.005$) or 100 ($p<0.005$) ppm, but there was no effect on total LDH or on the other fractions (LDH-2 to LDH-5). The results of tests for serum lactic dehydrogenase isoenzymes are given in Appendix Table A-3.

Organ Weights

Data were calculated for organ weight to body weight ratios for spleen, thymus, liver, thyroid and brain. Results are given in Table 3. In general, all the changes in the organ weights were dose dependent. The absolute organ weights and final body weights are listed in Appendix Table A-4.

Spleen

Neither Firemaster BP-6 nor 2,3',4,4',5,5'-HBB affected the spleen weight. However, in rats fed diets containing

Table 3. Organ weight to body weight ratios in rats fed HBB or 3,3',4,4',5,5'-HBB for 30 days

Group	Modification of Dietary Treatment	Chemical Concentration in Feed (ppm)	Spleen (g/100 g BW)
I	Control	0	0.24±0.03
	Firemaster BP-6	1	0.25±0.02
		10	0.23±0.01
		100	0.22±0.03
	2,2',4,4',5,5'-hexabromobiphenyl	1	a
		10	a
		100	a
	2,3',4,4',5,5'-hexabromobiphenyl	1	0.24±0.02
		10	0.26±0.03
		100	0.24±0.02
II	Control	0	0.26±0.03
	3,3',4,4',5,5'-hexabromobiphenyl	1	0.25±0.03[b]
		10	0.23±0.01[b]

Data are expressed as mean ± SD.

[a]Not done.

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

[c,d,e]Significantly different ($p<0.05$) from FM BP-6, the same dose.

diets containing FM BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-

Thymus (g/100 g BW)	Liver (g/100 g BW)	Thyroid (mg/100 g BW)	Brain (g/100 g BW)
0.23±0.05	4.17±0.18	5.95±0.83	0.49±0.04
0.26±0.04	4.02±0.21[b]	5.46±0.96	0.53±0.04
0.23±0.03[d]	4.84±0.54[b,d,e]	6.16±1.03[b,e]	0.51±0.02
0.20±0.04	7.55±0.46	7.16±0.60	0.53±0.02
0.25±0.05[b]	4.70±0.48[b]	6.92±1.06	0.53±0.02[b]
0.28±0.04[b,e]	5.31±0.49[b,c]	7.06±1.67	0.54±0.04[b]
0.29±0.05	6.53±0.85	6.52±0.89	0.54±0.03[b]
0.26±0.04	4.42±0.68	5.49±0.56	0.54±0.03[b]
0.27±0.05	4.65±0.58[b,c]	5.49±1.20	0.53±0.04[b]
0.26±0.04	6.52±0.57	6.00±0.88	0.55±0.02[b]
0.24±0.02	3.44±0.14	4.27±0.23	0.52±0.02
0.23±0.01[b]	3.97±0.20[b]	5.06±0.37[b]	0.52±0.03
0.14±0.02[b]	5.51±0.43[b]	6.15±0.58[b]	0.57±0.02

2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB, respectively, at

10 ppm 3,3',4,4',5,5'-HBB, the spleen weight to body weight ratio was decreased ($p<0.025$).

Thymus

The thymus weight to body weight ratio was not significantly different from the control when the rats were fed diets containing FM BP-6 or 2,3',4,4',5,5'-HBB for 30 days (Table 3). Feeding rats with diets containing 10 or 100 ppm 2,2',4,4',5,5'-HBB resulted in increased thymus to body weight ratio ($p<0.05$), while 3,3',4,4',5,5'-HBB decreased the ratio at 10 ppm ($p<0.0005$).

Liver

The data on liver weight to body weight ratios are given in Table 3. Dietary exposure of rats to FM BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-HBB or 3,3',4,4',5,5'-HBB for 30 days caused an increase in the liver weight to body weight ratio. Firemaster BP-6 at 1 ppm or 2,3',4,4',5,5'-HBB at 1 or 10 ppm did not significantly affect the liver weight. However, 2,2',4,4',5,5'-HBB increased the liver weight at 1 ($p<0.025$), 10 ($p<0.0005$) or 100 ($p<0.0005$) ppm. At 100 ppm, FM BP-6 and 2,3',4,4',5,5'-HBB significantly increased ($p<0.0005$) the liver to body weight ratio. At the highest dose, FM BP-6 appeared to increase liver weight more than 2,2',4,4',5,5'-HBB ($p<0.025$) or 2,3',4,4',5,5'-HBB ($p<0.005$). The liver weights were dramatically increased when 1 or 10 ppm of 3,3',4,4',5,5'-HBB was added into rat diets ($p<0.0005$) for 30 days.

Thyroid

The absolute or relative weights of thyroid from rats fed 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB were not significantly affected, but FM BP-6 increased the thyroid weight to body weight ratio at 100 ppm. In addition, at 100 ppm the thyroid of rats fed FM BP-6 was larger than in those fed 2,2',4,4',5,5'-HBB ($p<0.025$). A dramatic increase in thyroid weight was recorded in rats fed 3,3',4,4',5,5'-HBB. An increase of thyroid weight to body weight ratio was noticed at the dose level as low as 1 ppm ($p<0.0005$). The data on thyroid weight to body weight ratios are listed in Table 3.

Brain

The brain weight to body weight ratios are given in Table 3. Firemaster BP-6 or 3,3',4,4',5,5'-HBB did not affect the relative brain weight. In contrast, 2,2',4,4,5,5'-HBB or 2,3',4,4',5,5'-HBB increased the brain weight to body weight ratios at 1 ($p<0.05$), 10 ($p<0.05$) or 100 ($p<0.025$) ppm. In addition, the brain weight to body weight ratios of rats fed 100 ppm FM BP-6 were significantly smaller when compared to rats fed 100 ppm 2,3',4,4',5,5'-HBB ($p<0.05$).

Tissue Analyses

Liver Microsomal Enzymes

Results of determinations for benzo(α)pyrene hydroxylation and aminopyrene demethylation are given in Table 4. In general, there was a dose-dependent and chemical-specific induction of liver microsomal enzymes. Aminopyrene demethylation was

Table 4. Liver microsomal enzymes in rats fed diets containing FM BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-HBB or 3,3',4,4',5,5'-HBB for 30 days

Group	Modification of Dietary Treatment	Chemical Concentration in Feed (ppm)	Benzo(α)pyrene Hydroxylation (n mole/mg prot/min)	Aminopyrene Demethylation (n mole/mg prot/min)
I[a]	Control	0	0.8 ± 0.2	5.6 ± 0.8
	Firemaster BP-6	1	2.3 ± 0[c]	12.1 ± 1.1
		10	5.1 ± 0[d]	20.0 ± 2.8[d]
		100	7.0 ± 0.8[d]	17.0 ± 1.5[d]
	2,2',4,4',5,5'-hexabromobiphenyl	1	1.5 ± 0	10.0 ± 0.6[d]
		10	2.2 ± 0[c]	14.7 ± 0.3[d]
		100	1.8 ± 0.5	13.4 ± 0.4[d]
	2,3',4,4',5,5'-hexabromobiphenyl	1	0.9 ± 0.3	7.4 ± 0.2
		10	2.6 ± 0[d]	10.0 ± 0.7
		100	5.6 ± 0.9[c]	13.2 ± 1.1[c]
II[b]	Control	0	2.7 ± 0.3	13.6 ± 1.6
	3,3',4,4',5,5'-hexabromobiphenyl	1	21.2 ± 4.4[d]	14.4 ± 1.0
		10	30.2 ± 3.9[d]	10.1 ± 1.1

[a]Data are expressed as mean ± SD (n=2 pooled samples of 3 rats each).

[b]Data are expressed as mean ± SD (n=6 rats).

[c,d]Significantly different from control value ($p<0.01$, $p<0.005$, respectively).

induced by 2,2',4,4',5,5'-HBB ($p<0.005$) but not by 3,3',4,4',5,5'-HBB. On the other hand, 3,3',4,4',5,5'-HBB administration resulted in dramatic increases in benzo(α)-pyrene hydroxylation ($p<0.005$). Firemaster BP-6 or 2,3',4,4',5,5'-HBB significantly induced aminopyrene demethylation and benzo(α) pyrene hydroxylation especially at the higher doses ($p<0.01$ to <0.005).

Liver Vitamin A

The results of liver vitamin A determinations are given in Table 5. In general, there was a dose-dependent decrease of liver vitamin A content. At 100 ppm the liver vitamin A concentrations of rats fed diets containing FM BP-6 were lower than those treated with 2,2',4,4',5,5'-HBB ($p<0.01$) or 2,3',4,4',5,5'-HBB ($p<0.025$). However, the effects were greater in rats given 2,3',4,4',5,5'-HBB when compared to those given 2,2',4,4',5,5'-HBB ($p<0.01$). As little as 10 ppm 3,3',4,4',5,5'-HBB decreased concentrations of liver vitamin A ($p<0.05$).

Tissue Residue

The concentrations of FM BP-6 and the 3 congeners in fat, liver, kidney and thymus are listed in Table 6. The greatest residues of FM BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-HBB or 3,3',4,4',5,5'-HBB were in the liver. The lowest concentrations in tissues were with 3,3',4,4',5,5'-HBB and the largest concentrations were with 2,2',4,4',5,5'-HBB, with the exception of fat at 100 ppm, in which there was a higher concentration of FM BP-6.

Table 5. Liver vitamin A concentration in rats fed diets containing FM BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-HBB or 3,3',4,4',5,5'-HBB for 30 days

Group	Modification of Dietary Treatment	Chemical Concentration in Feed (ppm)	Vitamin A Wet-Liver Basis µg/g	Vitamin A Wet-Liver Basis mg/liver	Vitamin A Dry-Liver Basis µg/g
I	Control	0	103.6±10.8	1.55±0.19	349.47±10.79
	Firemaster BP-6	1	106.6±11.3	1.44±0.01	345.81±31.82
		10	85.4±10.1[a,c,d]	1.41±0.05[a,c,d]	265.93±19.13[a,c,d]
		100	29.6± 2.8[a,c,d]	0.73±0.74[a,c,d]	85.79± 5.55[a,c,d]
	2,2',4,4',5,5'-hexabromobiphenyl	1	98.4± 4.5	1.61±0.25	350.27±29.32[a]
		10	81.0± 6.3[a,b,d]	1.39±0.09[b,d]	248.59±14.40[a,b,d]
		100	70.5± 5.5[a,b,d]	1.54±0.19[b,d]	234.00± 3.99[a,b,d]
	2,3',4,4',5,5'-hexabromobiphenyl	1	112.2± 8.7	1.68±0.47	390.37±74.47
		10	86.2± 7.1[a,b,c]	1.35±0.07[a,b,c]	277.60±14.44[a,b,c]
		100	47.6± 6.6[a,b,c]	1.03±0.07[a,b,c]	147.75±13.73[a,b,c]
II	Control	0	119.9±30.1	1.46±0.37	372.38±72.15
	3,3',4,4',5,5'-hexabromobiphenyl	1	67.6±11.5[a]	0.96±0.15[a]	221.75±32.09[a]
		10	49.3±13.0[a]	0.83±0.19[a]	147.94±43.06[a]

Data are expressed as mean ± SD (n=2 pooled samples of 3 rats each).

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

[b,c,d]Significantly different ($p<0.05$) from FM BP-6, 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB, respectively, at the same dose.

Table 6. The chemical concentrations in the tissues of rats fed diets containing different levels of FM BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-HBB or 3,3',4,4',5,5'-HBB for 30 days

Group	Modification of Dietary Treatment	Chemical Concentration in Feed (ppm)	Chemical Concentrations in Tissues (ppm) Fat	Liver	Kidney	Thymus
I	Control	0	0.06	1.47	1.46	1.10
	Firemaster BP-6	1	7.81	22.34	16.04	21.35
		10	61.63	310.44	147.01	89.97
		100	1534.93	2507.04	988.58	1044.36
	Control	0	0.03	0.89	0.73	0.68
	2,2',4,4',5,5'-hexabromobiphenyl	1	15.99	68.57	38.70	28.80
		10	148.65	692.75	372.51	242.73
		100	992.32	6061.83	3818.05	3840.62
	Control	0	0.02	0	0	3.22
	2,3',4,4',5,5'-hexabromobiphenyl	1	9.10	23.54	13.85	12.21
		10	69.07	241.91	144.85	210.95
		100	648.16	4340.37	1103.96	1638.71
II	Control	0	0	0	0	0
	3,3',4,4',5,5'-hexabromobiphenyl	1	0.57	125.15	0	0
		10	6.87	447.80	15.42	20.42

Data are expressed as means of 2 pooled samples from 3 rats each. Results for controls are based on analysis for either Firemaster BP-6 or the 3 congeners.

Serum Thyroid Hormone

Concentrations of serum T_3, T_4 and their ratios are listed in Table 7. The serum samples for T_3 and T_4 determinations in rats fed FM BP-6, 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB were only analyzed in rats given 100 ppm.

Serum T_3 concentrations were decreased by 100 ppm FM BP-6 but were increased by 2,2',4,4',5,5'-HBB. The serum T_3 concentrations in rats fed diets containing PBB or 2,3',4,4',5,5'-HBB were lower than in the rats fed 2,2',4,4',5,5'-HBB ($p<0.05$). The rats fed diets containing 2,3',4,4',5,5'-HBB had lower levels of serum T_3 at 10 ppm as compared with the control ($p<0.025$).

Serum T_4 concentrations were decreased by FM BP-6 ($p<0.01$), but the reduction by 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB was not significant. In addition, the T_4 concentrations in rats fed FM BP-6 were significantly lower than in rats fed 2,2',4,4',5,5'-HBB ($p<0.025$). Reduction in T_4 concentrations were also detected in rats fed 10 ppm 3,3',4,4',5,5'-HBB ($p<0.0005$).

There was an increase in the serum T_3/T_4 ratio in rats fed FM BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-HBB or 3,3',4,4',5,5'-HBB. Firemaster BP-6 increased the T_3/T_4 ratio more than 2,2',4,4',5,5'-HBB ($p<0.005$).

Pathology

Gross Lesions

Gross lesions were observed mainly in the liver and thyroid gland. There were no noticeable differences in the

Table 7. The serum T_3, T_4 and T_3/T_4 ratio of rats fed diets containing FM BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-HBB or 3,3',4,4',5,5'-HBB for 30 days

Group	Modification of Dietary Treatment	Chemical Concentration in Feed (ppm)	T_3 (ng/ml)	T_4 (ng/ml)	T_3/T_4 Ratio (X100)
I[a]	Control	0	0.99	44.2	2.2
	Firemaster BP-6	100	0.81[c,e,f]	11.4[c,e]	7.1[c,e]
	2,2',4,4',5,5'-hexabromobiphenyl	100	1.48[c,d]	36.8[d]	4.0[c,d]
	2,3',4,4',5,5'-hexabromobiphenyl	100	1.06[d]	23.1	4.6[c]
II[b]	Control	0	1.43	56.55	2.5
	3,3',4,4',5,5'-hexabromobiphenyl	1	1.49	54.58	2.7
		10	1.22[c]	21.13[c]	5.8[c]

[a]Data are expressed as a mean of 2 pooled samples of 3 rats each.

[b]Data are expressed as a mean of 6 rats.

[c]Significantly different from control value ($p<0.025$).

[d,e,f]Significantly different ($p<0.05$) from FM BP-6, 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB, respectively, at the same dose.

gross lesions produced by the 4 different chemicals. However, there were definite dose-dependent lesions.

Thyroid. The thyroids of rats fed control diets or diets containing 1 ppm of FM BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-HBB or 3,3',4,4',5,5'-HBB for 30 days were relatively normal. At a level of 10 ppm or higher the thyroid appeared to be darker than normal.

Liver. Livers from the control rats and rats fed 1 ppm of each of the treatment were normal grossly. Addition of 10 ppm of any of the chemicals to the diets caused an enlarged liver with slight yellowish discoloration. At 100 ppm the enlargement was more pronounced, mottling was apparent, and the surfaces were more convex.

Incidental findings. Some of the rats had multifocal areas of hyperemia in the lungs and in some instances they were accompanied by small areas of emphysema. The changes were unrelated to the dose or the chemical added to the diets and were seen in some controls. There were no other incidental findings in the other organs examined grossly.

Histopathology

Liver. The control rats had normal histologic features characterized by hepatic cords radiating from the central veins toward the portal triads (Figure 2). The hepatocytes were relatively large and polyhedral with compact cytoplasm, rounded nuclei and prominent nucleoli.

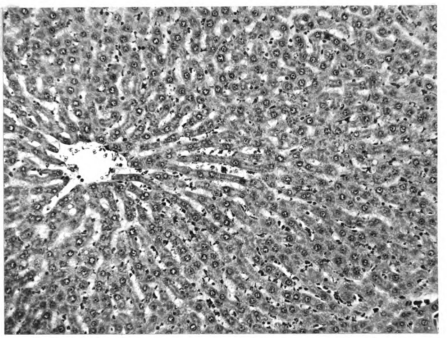

Figure 2. Photomicrograph of a section of liver from a control rat to illustrate a normal lobular pattern with hepatic cords radiating from the central vein towards the portal triads. Notice compact cytoplasm and prominent sinusoids. (H&E stain, 160X)

After 30 days of dietary treatment with 1 ppm of each of the chemicals, there was mild cytoplasmic vacuolation. Vacuoles were generally small and evenly distributed, and the lobular structure was not affected. However, 3,3',4,4',5,5'-HBB produced more numerous and slightly larger vacuoles than the other 3 chemicals.

Rats fed 10 ppm of FM BP-6, 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB had swollen hepatocytes with small to moderately sized vacuoles in the cytoplasm. Oil red O stain revealed lipid substances in the cytoplasm. Lesions produced by these 3 chemicals were similar in type as well as in degree of severity. Rats given 10 ppm of 3,3',4,4',5,5'-HBB had marked disruption of lobular structure. Some of the central veins were no longer identifiable due to extensive swelling of hepatocytes and cytoplasmic vacuolation. The vacuoles were mostly large and were located in the midzonal or central areas of the lobules (Figure 3). Pyknotic nuclei were numerous. Occasionally, there were multifocal areas of necrosis with accumulation of lymphocytes and macrophages (Figure 4).

Rats fed diets containing 100 ppm of each of the chemicals had more prominent lesions than seen at 10 ppm. In rats fed 100 ppm of 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB the vacuoles were larger and more numerous than those fed 10 ppm of the same congener (Figure 5). Similar histologic changes were seen in rats fed diets containing FM BP-6. However, liver sections from these rats typically had eosinophilic cytoplasmic inclusions (Figure 6). The morphology of

Figure 3. Section of liver from a rat fed a diet containing 10 ppm of 3,3',4,4',5,5'-HBB for 30 days to illustrate numerous and mostly large vacuoles in the centrilobular to midzonal area. (H&E stain, 160X)

Figure 4. Photomicrograph of a liver from a rat fed a diet containing 10 ppm 3,3',4,4',5,5'-HBB for 30 days. Notice an area of necrosis with accumulation of lymphocytes and macrophages. (H&E stain, 300X)

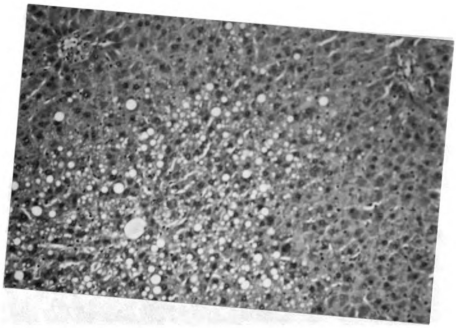

Figure 3

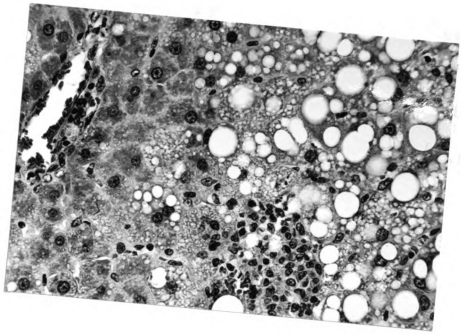

Figure 4

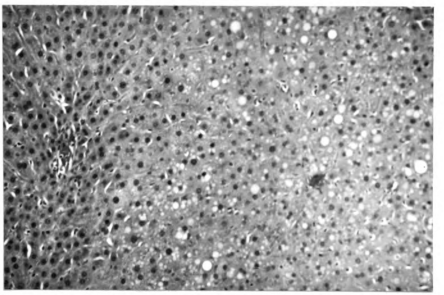

Figure 5. Section of liver from a rat fed a diet containing 100 ppm 2,3',4,4',5,5'-HBB for 30 days. Notice swollen hepatocytes and cytoplasmic vacuoles of various sizes in the central and midzonal regions of the lobules. Similar lesions were observed in rats fed 100 ppm 2,2',4,4',5,5'-HBB. (H&E stain, 160X)

Figure 6. Photomicrograph of a liver from a rat fed 100 ppm FM BP-6 for 30 days. Notice cytoplasmic inclusions and swollen hepatocytes. (H&E stain, 400X)

Figure 7. Photomicrograph of a liver section taken from the same rat as in Figure 6, to illustrate ring-shaped cytoplasmic inclusions within hepatocytes. (Toluidine blue stain, 400X)

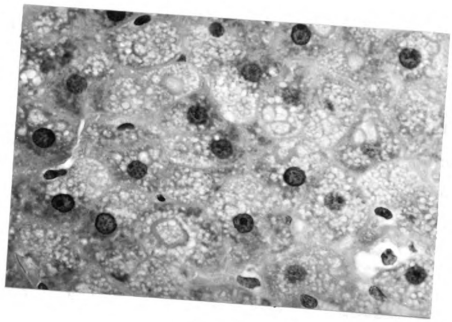

Figure 6

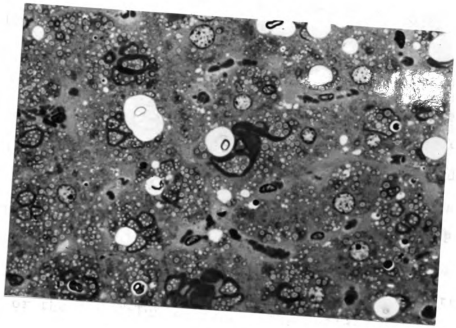

Figure 7

the inclusions was more distinctly seen in epon embedded tissue, sectioned at 1 micron and stained with toluidine blue (Figure 7).

Thyroid gland. Thyroid glands from control rats were essentially normal. Typically, the gland was composed of follicles of varying size lined by a single layer of cuboidal or columnar epithelium with eosinophilic and homogeneous colloid in the lumen (Figure 8). The peripheral follicles were larger than those more centrally situated.

In general, rats fed diets containing FM BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-HBB or 3,3',4,4',5,5'-HBB had similar histologic features at each corresponding dose. The thyroid gland of rats fed 1 ppm of any of the chemicals had a nearly normal morphological structure with slight evidence of an increased number and a decreased size of the follicles, especially at the peripheral location.

Considerable changes were first noticed in rats fed 10 ppm of FM BP-6 or any of the 3 congeners. The follicular epithelium had a tall columnar appearance. Some follicles had papillary projections into the lumen (Figures 9 and 10).

At 100 ppm, there was more extensive hyperplasia and hypertrophy of follicular cells than seen at 10 ppm. Papillary projections were prominent and numerous (Figures 11 and 12). The follicular epithelium was mostly tall columnar instead of cuboidal or the low columnar type. The colloid was scanty or nearly absent, as confirmed by PAS stain (Figure 13) when compared with normal thyroid (Figure 14). There was evidence

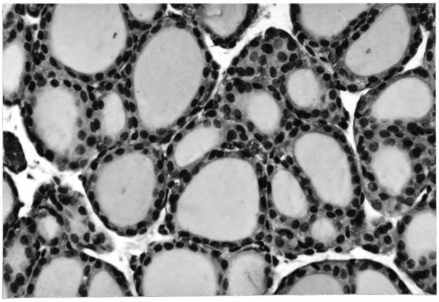

Figure 8. Photomicrograph of normal thyroid gland from a control rat. The follicular cells are composed of a single layer of cuboidal or low columnar epithelium. The lumens contain an abundance of colloid. (H&E stain, 320X)

Figure 9. Photomicrograph of thyroid of a rat fed a diet containing 10 ppm FM BP-6 for 30 days to show evidence of follicular cell hyperplasia. The follicles are mostly small and the colloid was decreased in density. Similar changes were seen in rats fed 10 ppm 2,2',4,4',-5,5'-HBB, 2,3',4,4',5,5'-HBB or 3,3',4,4',5,5'-HBB. (H&E stain, 160X)

Figure 10. Higher magnification of Figure 9 to illustrate early evidence of papillary projection and an increase in follicular connective tissue. Notice the tall columnar appearance of follicular cells. (H&E stain, 320X)

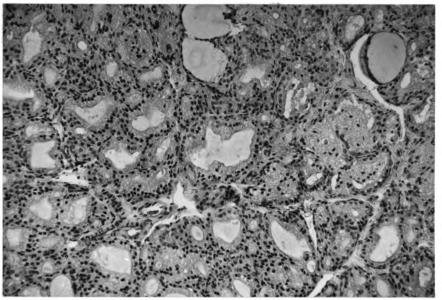

Figure 9

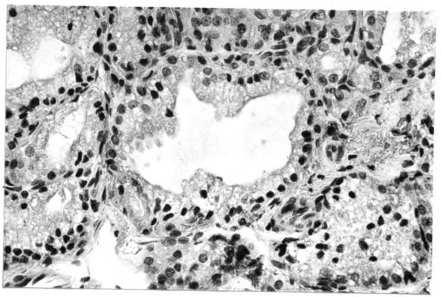

Figure 10

Figure 11. Photomicrograph to illustrate lesions in the thyroid taken from a rat fed a diet containing 100 ppm 2,2',4,4',5,5'-HBB for 30 days. Notice hypertrophy and hyperplasia of the follicular cells as well as depletion of follicular colloid. These changes were typically seen with corresponding doses of 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB. (H&E stain, 160X)

Figure 12. Higher magnification of Figure 11. Notice hypertrophy and hyperplasia of the follicular cells. In addition, there is an increase in the interfollicular connective tissue. (H&E stain, 320X)

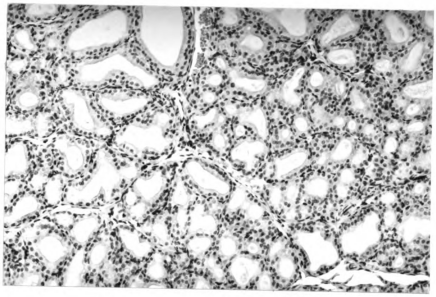

Figure 11

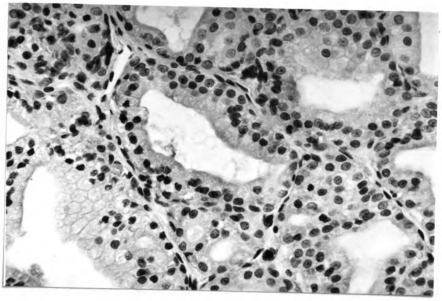

Figure 12

Figure 13. Section of thyroid taken from a rat fed a diet containing 100 ppm FM BP-6 for 30 days. Notice scanty colloid in the follicular lumen. (PAS stain, 300X)

Figure 14. Photomicrograph of a normal thyroid taken from a control rat. Notice abundant colloid in the follicular lumen. (PAS stain, 300X)

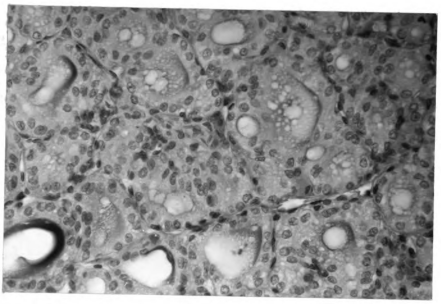

Figure 13

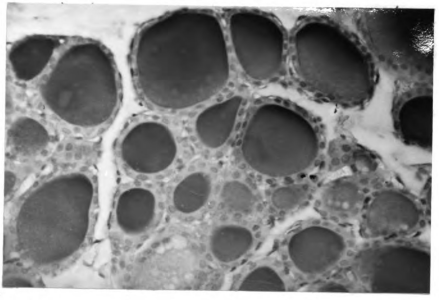

Figure 14

of increased vascularization and interfollicular connective tissue.

Thymus. There was a normally dense population of lymphoid cells in the cortex with marked demarcation between the cortex and medulla of the thymuses from control rats (Figure 15). Histologically, FM BP-6, 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB did not affect the thymus even at 100 ppm. However, the thymus of rats fed a diet containing 3,3',4,4',5,5'-HBB had depletion of lymphocytes in the cortex. In some cases there were foci of lymphocytic depletion with some macrophages present in the areas. The demarcation between cortex and medulla was indistinct (Figure 16).

Pituitary. The pituitary glands from control rats were normal (Figure 17). Firemaster BP-6, 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB did not alter the morphological features of the pituitary gland. In contrast, rats fed 3,3',4,4',5,5'-HBB had swollen chromophobe cells in the pars anterior with a marked foamy appearance of the cytoplasm. Some cells had pyknotic nuclei (Figures 18 and 19). The lesions appeared to be dose dependent.

Lungs. Slight to moderate subacute interstitial pneumonia was observed in several rats regardless of treatment. There was thickening of interlobular alveoli with some lymphocytic infiltration, as well as occasional aggregates of macrophages in the alveolar lumen.

Figure 15. Photomicrograph of a normal thymus from control rat. Notice the cellular density and distinct demarcation between the cortex and medulla. (H&E stain, 160X)

Figure 16. Photomicrograph of a section of thymus from a rat fed a diet containing 10 ppm 3,3',4,4',5,5'-HBB for 30 days. There is decreased cellular density and focal lymphocytic depletion in the cortex. Notice the line of demarcation between the cortex and medulla is indistinct. (H&E stain, 160X)

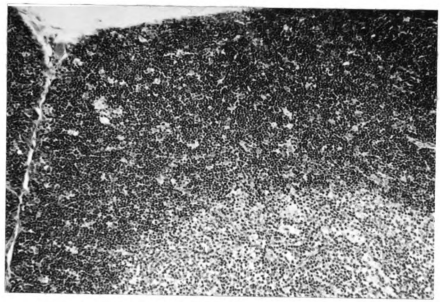

Figure 15

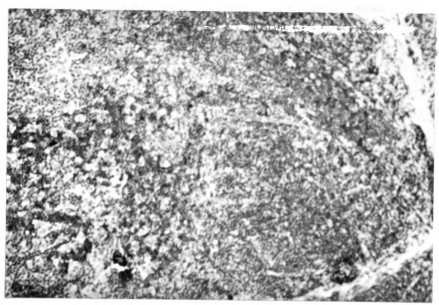

Figure 16

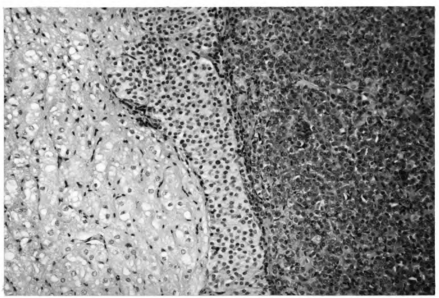

Figure 17. Photomicrograph of a normal pituitary gland from a rat fed a control diet. The chromophobe cells of the pars anterior pituitary (A) are normal with homogeneous and compact cytoplasm. (H&E stain, 160X)

Figure 18. Photomicrograph of a pituitary gland from a rat fed a diet containing 10 ppm 3,3',4,4',5,5'-HBB for 30 days to illustrate swollen and foamy appearance of the chromophobe cells. (H&E stain, 160X)

Figure 19. Higher magnification of Figure 18 to illustrate swollen and foamy appearance of some of the chromophobe cells. Some of the nuclei have undergone pyknosis. (H&E stain, 350X)

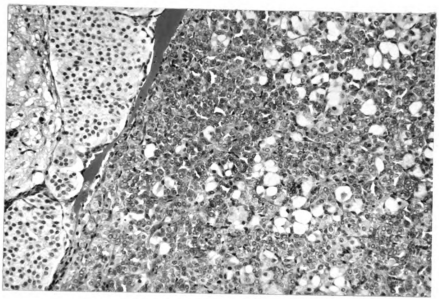

Figure 18

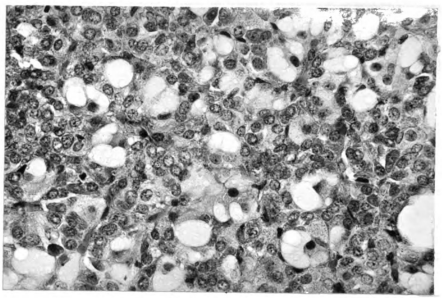

Figure 19

Electron Microscopy

Liver. Ultrastructural features of hepatocytes from control rats were normal (Figure 20). Hepatocytes were polygonal with 2 major parts, the nucleus and the cytoplasm. The nuclei were relatively large, round and centrally located with prominent nucleoli. The mitochondria were round or elongated and had a complex structure with a double membrane. The internal layer invaginated into the matrix. Rough endoplasmic reticulum (RER) had a parallel arrangement with ribosomes studded on the membrane surfaces. Smooth endoplasmic reticulum (SER) was differentiated morphologically from RER by the lack of associated ribosomes. Glycogen granules appeared as dense particles in the cytoplasm. The golgi system was mostly situated close to the bile canaliculi.

The rats fed diets containing 1 ppm FM BP-6, 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB had a mild proliferation of SER and a slight increase in fat droplets. A corresponding dose of 3,3',4,4',5,5'-HBB resulted in slight dilatation of cisternae of endoplasmic reticulum, individualized RER and an increase in fat droplets. In addition, the RER tended to encircle mitochondria (Figure 21).

At 10 ppm of dietary treatment, FM BP-6 produced more severe lesions than those caused by 2,2',4,4',5,5-HBB or 2,3',4,4',5,5'-HBB. Ultrastructural features in the liver from rats fed the latter 2 congeners could not be differentiated from each other. In hepatocytes of rats fed a diet containing FM BP-6, the SER was prominently hyperplastic and

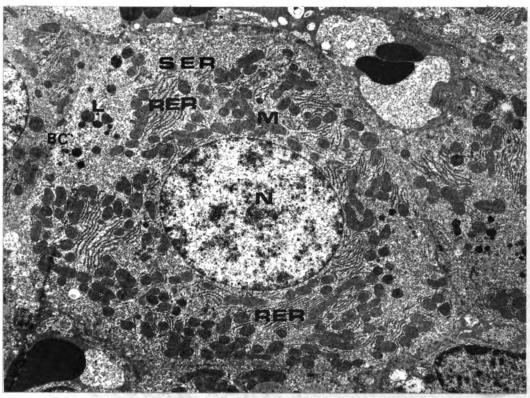

Figure 20. Electron micrograph of a hepatocyte from control rat. The nucleus (N) is centrally located. There are numerous mitochondria (M), well developed rough endoplasmic reticulum (RER) and some smooth endoplasmic reticulum (SER). Lysosomes (L) are present adjacent to bile canaliculi (BC). (Lead citrate and uranyl acetate stain, 4,700X)

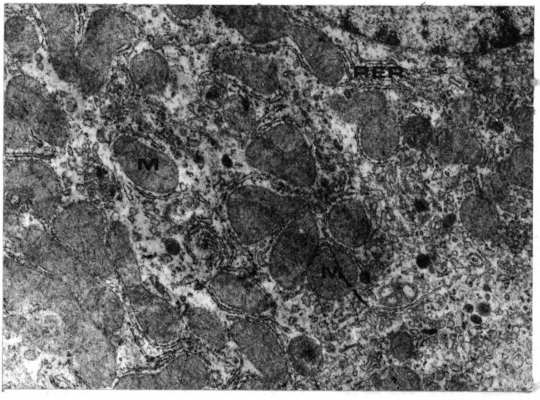

Figure 21. Electron micrograph of a hepatocyte from a rat fed a diet containing 1 ppm 3,3',4,4',5,5'-HBB for 30 days. Notice swollen mitochondria (M) and individualization of rough endoplasmic reticulum (RER). (Lead citrate and uranyl acetate stain, 15,150X)

vesiculated. The RER was disintegrated and some of the ribosomes were detached. The mitochondria were decreased in number and the cisternae were dilated (Figures 22 and 23). The fat droplets were numerous. With 3,3',4,4',5,5'-HBB there was a different pattern of ultrastructural changes (Figure 24). The RER was proliferated, disorganized and most of them encircled mitochondria. The SER was relatively sparse. Mitochondria were swollen and the cristae were disrupted. Fat droplets were numerous and of different sizes.

Figure 25 illustrates ultrastructural features of a hepatocyte from the liver of a rat fed a diet containing 100 ppm of BP-6. The SER was highly proliferated and vesiculated and this caused displacement of other organelles. Mitochondria were swollen, reduced in number and had undergone degeneration. Disruption of the mitochondrial cristae was evident. Furthermore, there was a remarkable reduction in RER, and it was greatly dispersed. There was detachment of ribosomes and many of them appeared free in the cytoplasm. The cisternae were frequently dilated, and fat droplets were numerous. These changes were also observed in rats fed diets containing 2,3',4,4',5,5'-HBB but were less prominent than in rats fed FM BP-6 (Figure 26). In rats fed diets containing 2,2',4,4',5,5'-HBB the proliferation of SER was only mild but dilated cisternae were prominent. Occasionally, swollen mitochondria were observed (Figures 27 and 28). In addition, the RER was nearly unaffected, and its integrity was maintained. The number of fat droplets was increased.

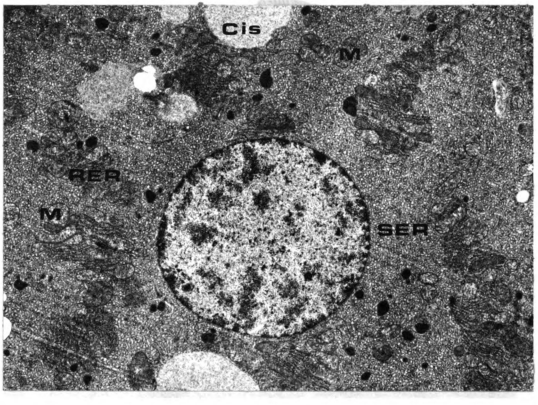

Figure 22. Electron micrograph of a hepatocyte from a rat fed 10 ppm FM BP-6 for 30 days. The smooth endoplasmic reticulum (SER) is markedly hyperplastic and has displaced other organelles. The rough endoplasmic reticulum (RER) is relatively sparse and mitochondria (M) are decreased in number. Some of the cisternae (Cis) are dilated. (Lead citrate and uranyl acetate stain, 5,450X)

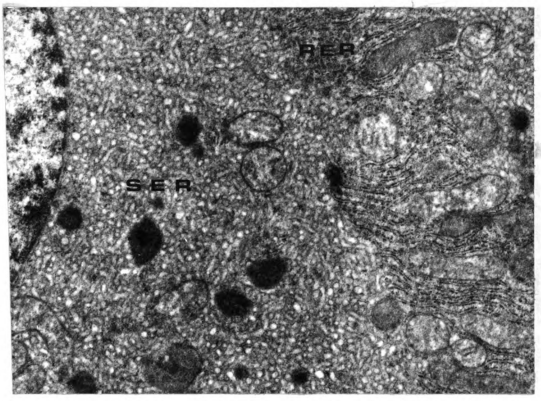

Figure 23. Higher magnification of Figure 22. The smooth endoplasmic reticulum (SER) is prominently proliferated and rough endoplasmic reticulum (RER) is disintegrated. (Lead citrate and uranyl acetate stain, 16,160X)

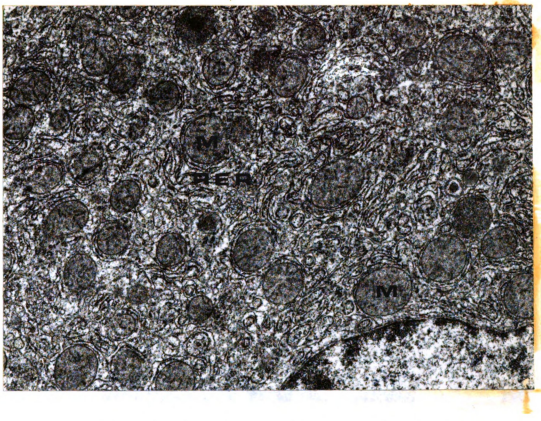

Figure 24. Electron micrograph of a hepatocyte from a rat fed a diet containing 10 ppm 3,3',4,4',5,5'-HBB for 30 days. Notice widely dispersed rough endoplasmic reticulum (RER) which tends to encircle mitochondria (M). (Lead citrate and uranyl acetate stain, 15,150X)

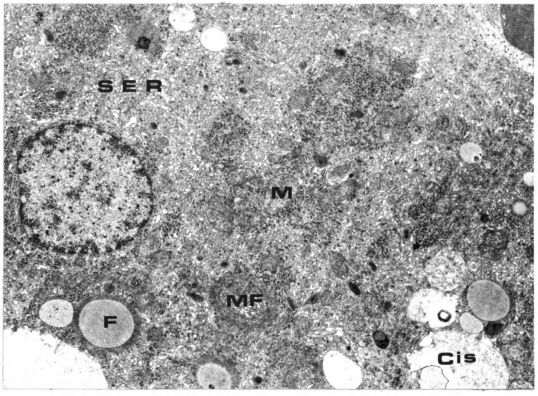

Figure 25. Electron micrograph of a hepatocyte from a rat fed a diet containing 10 ppm FM BP-6 for 30 days. Notice proliferation of smooth endoplasmic reticulum (SER), dilated cisternae (Cis), increase in fat droplets (F), decrease in number of mitochondria (M) and prominent myelin figures (MF). (Lead citrate and uranyl acetate stain, 6,000X)

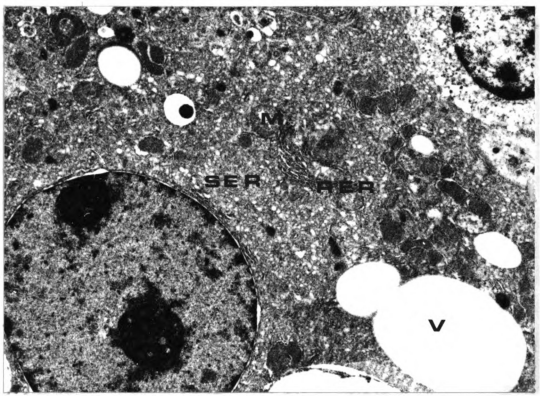

Figure 26. Electron micrograph of a hepatocyte from a rat fed a diet containing 100 ppm 2,3',4,4',5,5'-HBB for 30 days. Notice an increase of smooth endoplasmic reticulum (SER), sparse rough endoplasmic reticulum (RER) and vacuoles (V). Few mitochondria were present (M). Lead citrate and uranyl acetate stain, 7,170X.

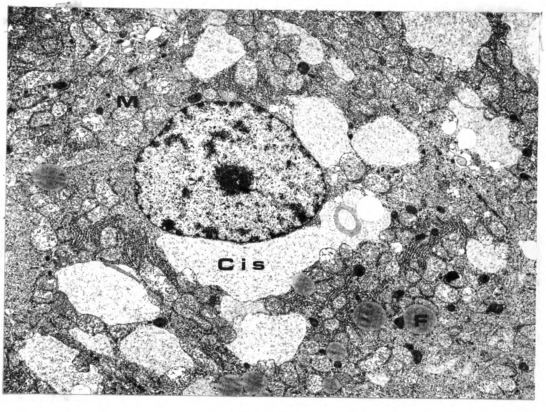

Figure 27. Electron micrograph of a hepatocyte from a rat fed a diet containing 100 ppm 2,2',4,4',5,5'-HBB for 30 days, to illustrate dilated cisternae (Cis), increase in fat droplets (F) and normally abundant mitochondria (M). (Lead citrate and uranyl acetate stain, 4,600X)

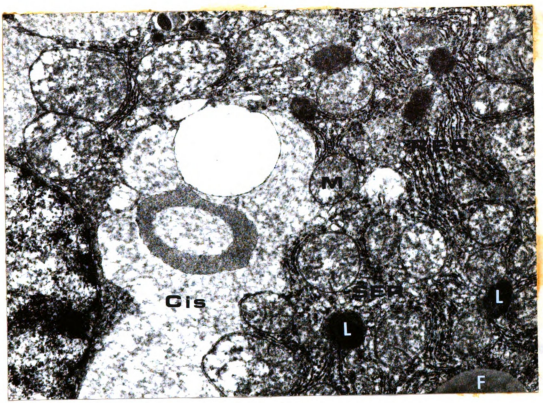

Figure 28. Higher magnification of Figure 27. Notice prominent dilatation of cisternae (Cis) and swollen mitochondria (M). The smooth endoplasmic reticulum (SER) is normally developed and the rough endoplasmic reticulum (RER) is maintained intact. Lysosomal bodies (L) and fat droplets (F) are present. (Lead citrate and uranyl acetate stain, 15,650X)

Concentrically laminated structures called myelin bodies were consistently seen in hepatocytes from rats fed diets containing 100 ppm FM BP-6 (Figure 25). The myelin bodies were of various sizes; many of them were large. In some instances they formed a connection with RER (Figure 29). Some of the myelin bodies encircled SER, fat, mitochondria or other organelles. In contrast, there were no myelin bodies observed in hepatocytes of rats fed diets containing 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB.

Thyroid gland. Ultrastructurally, the thyroid glands from control rats were normal (Figure 30). The epithelium was of low coumnar or cuboidal type with the apex toward the colloid. The apical portion was irregular with numerous fingerlike projections of microvilli penetrating into the colloid. The apical vesicles were enclosed by a fine membrane and contained a substance analogous in density to the follicular colloid. The nucleus was centrally or basally located, rounded or oval. Mitochondria were numerous, elongated or ovoid and distributed throughout the cytoplasm. Ocasionally, there were colloid droplets in the cytoplasm as well as dense bodies. The endoplasmic reticulum was pleomorphic. The base of the cell was toward the margin of the follicle and came in contact with sinusoids.

In general, FM BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-HBB or 3,3',4,4',5,5'-HBB produced similar and dose-dependent lesions with some differences in the severity of the alterations. In rats fed diets containing 1 ppm of FM BP-6,

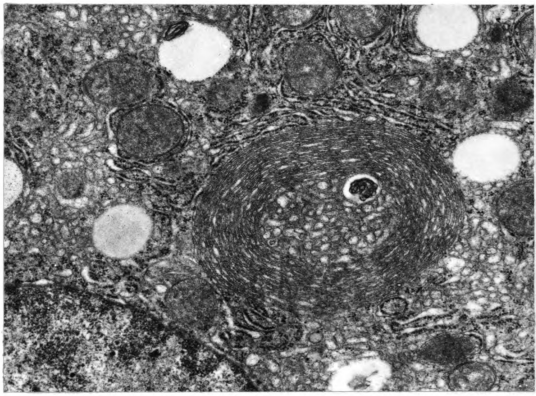

Figure 29. Higher magnification of a myelin body from Figure 25. Notice concentrically laminated structure and transition between myelin figure and rough endoplasmic reticulum. Some of the rough endoplasmic reticulum tends to encircle mitochondria. (Lead citrate and uranyl acetate stain, 24,250X)

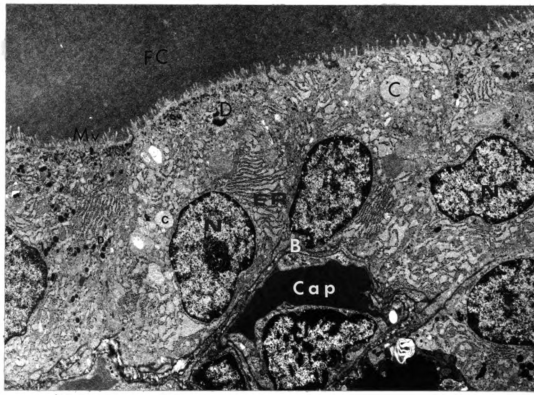

Figure 30. Electron micrograph of thyroid follicular cells from a control rat. Notice follicular colloid (FC) in the lumen, microvilli (MV) on the apical surface, relatively few dense bodies (D), small number of colloid droplets (C), endoplasmic reticulum (ER), nuclei (N), basal membrane (B) and capillaries (Cap). (Lead citrate and uranyl acetate stain, 4,600X)

2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB there was evidence of a slight increase in the number of dense bodies and colloid droplets in the follicular thyroid cells. Figure 31 is an electron micrograph of follicular cells from a rat fed a diet containing 2,3',4,4',5,5'-HBB and is representative for lesions due to 1 ppm of FM BP-6, 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB. More severe alterations were seen in rats fed 1 ppm 3,3',4,4',5,5'-HBB. This chemical caused a moderately increased number of dense bodies and colloid droplets. In addition, the microvilli were reduced in number and the follicular lumens were irregular due to protrusions from the apical portion of the cells. The cisternae were dilated (Figures 32 and 33).

In thyroids of rats fed diets containing 10 ppm FM BP-6 there was a moderate increase in colloid droplets and dense bodies, as well as an increased cellular height and dilated cisternae (Figure 34). The apical portion of the cells had protrusions toward the colloid and the apical vesicles were increased in density (Figure 35). The golgi apparatus was hypertrophied and the microvilli were decreased in number. Similar but less severe lesions were seen in rats fed 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB. In rats fed 3,3',4,4',5,5'-HBB, the lesions were also similar to those due to FM BP-6 but the dense bodies were relatively more numerous (Figure 36).

The most severe changes were seen with the 100 ppm treatment. In rats fed diets containing 100 ppm FM BP-6, the basic alterations were similar but lesions were more severe

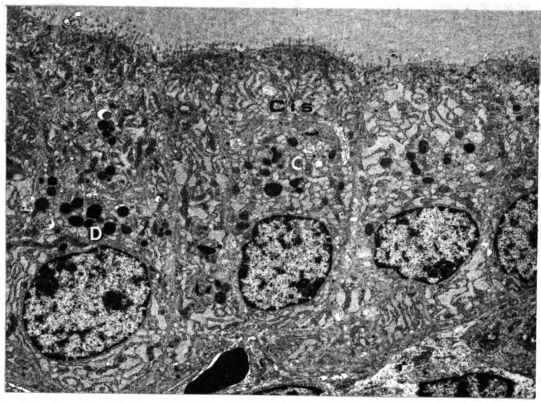

Figure 31. Electron micrograph of thyroid follicular cells from a rat fed a diet containing 2,3',4,4',5,5'-HBB for 30 days to illustrate early changes observed at 1 ppm. There is a mild increase in dense bodies (D) and colloid droplets (C) in the cytoplasm. The cisternae (Cis) are dilated. Similar lesions were observed in rats fed FM BP-6 or 2,2',4,4',5,5'-HBB. (Lead citrate and uranyl acetate stain, 4,300X)

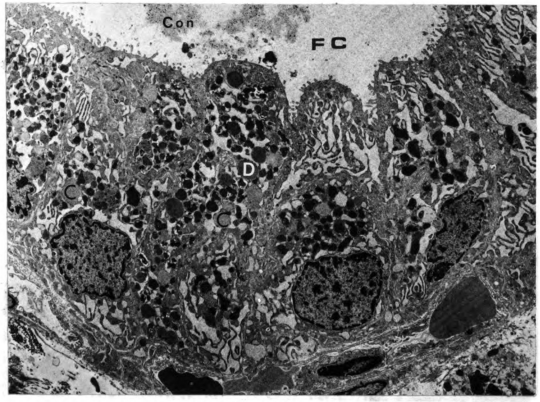

Figure 32. Electron micrograph of thyroid follicular cells from a rat fed a diet containing 1 ppm 3,3',4,4',5,5'-HBB for 30 days. There are increases in cellular height, number of dense bodies (D) and colloid droplets (C). Notice the apical surfaces are bulging up toward the follicular colloid (FC). A few concretions (Con) are present. (Lead citrate and uranyl acetate stain, 4,300X)

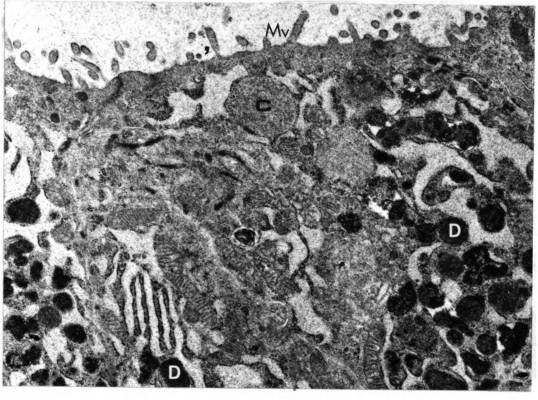

Figure 33. Higher magnification of Figure 32. Notice there is an increase in number of dense bodies (D) and colloid droplets (C). The number of microvilli (Mv) is decreased. (Lead citrate and uranyl acetate stain, 15,150X)

Figure 34. Electron micrograph of thyroid follicular cells from a rat fed a diet containing 10 ppm FM BP-6. There were remarkable increases in cellular height, increased dense bodies (D) and increased colloid droplets (C). Notice dilated cisternae (Cis) throughout the cytoplasm. (Lead citrate and uranyl acetate stain, 3,800X)

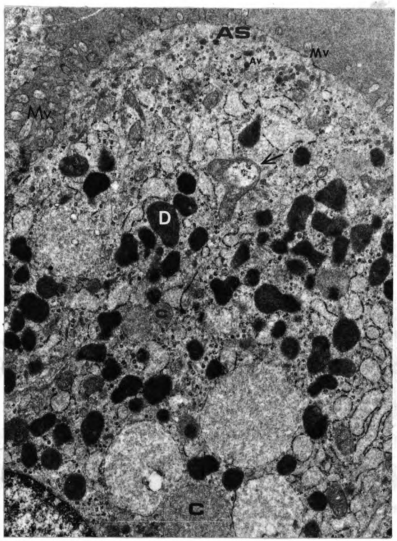

Figure 35. Electron micrograph of apical portion of a thyroid follicular cell from a rat fed a diet containing 10 ppm FM BP-6 for 30 days. Notice bulging up of the apical surface (AS) toward the lumen due to hypertrophy of the cell. Also notice numerous dense bodies (D), numerous colloid droplets (C), vacuolization of mitochondria (arrow), an increase in density of apical vesicles (AV), as well as very few and short microvilli (Mv). (Lead citrate and uranyl acetate stain, 13,600X)

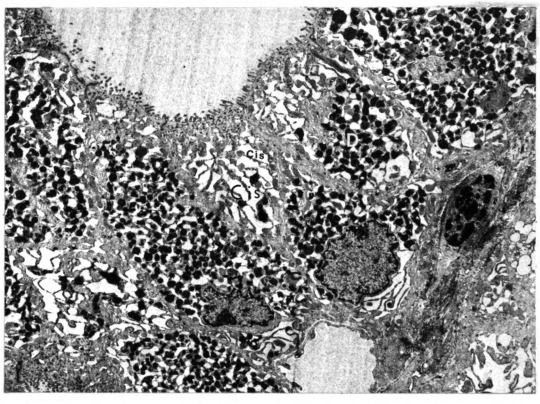

Figure 36. Electron micrograph of thyroid follicular cells from a rat fed a diet containing 10 ppm 3,3',4,4',5,5'-HBB for 30 days. Notice dramatic increases in dense bodies (D) and colloid droplets (C). The cisternae are dilated (Cis) and the cellular height is increased. (Lead citrate and uranyl acetate stain, 4,300X)

than at 10 ppm (Figure 37). The follicular lumens were irregular due to a protrusion of the apical surfaces of cells as a result of hypertrophy (Figure 38). Additionally, there were cytoplasmic projections toward the follicular lumen, and the golgi apparatus was hypertrophied (Figure 39). In rats fed diets containing 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB the thyroid changes included increased cellular height, increase in density of apical vesicle, increased colloid droplets and increased dense bodies. These changes in rats fed diets containing 2,3',4,4',5,5'-HBB were similar to those due to FM BP-6 but were slightly less severe (Figures 40 and 41). Furthermore, the changes due to 2,2',4,4',5,5'-HBB were similar but relatively less severe than those due to 2,3',4,4',5,5'-HBB (Figures 42 and 43).

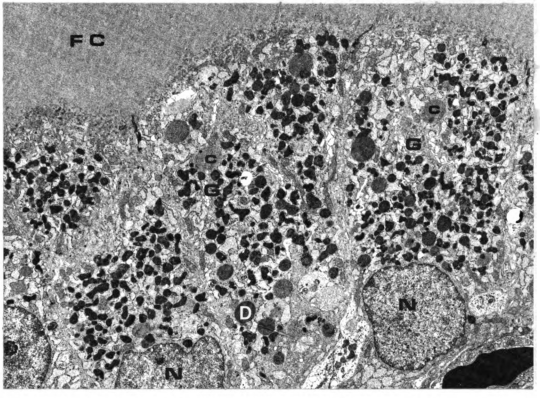

Figure 37. Electron micrograph of thyroid follicular cells from a rat fed a diet containing 100 ppm FM BP-6 for 30 days. Notice an increase in cellular height, dilated cisternae (Cis), numerous dense bodies (D) and colloid droplets (C). Golgi apparatuses (G), nuclei (N) and follicular colloid (FC) are also present. (Lead citrate and uranyl acetate stain, 4,600X)

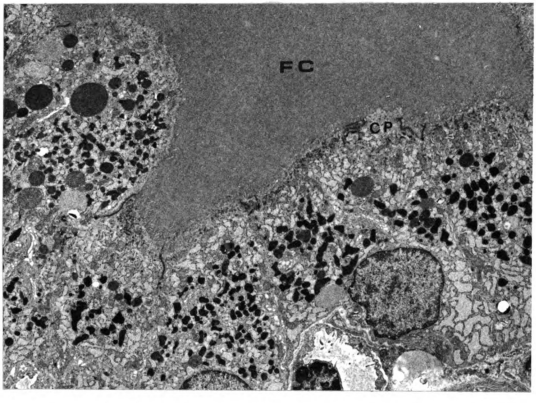

Figure 38. Electron micrograph of a thyroid follicular cell in a rat fed a diet containing 100 ppm FM BP-6 for 30 days. Notice irregularity of the lumen, bulging up of apical portion toward the follicular colloid (FC) and cytoplasmic projection (CP). (Lead citrate and uranyl acetate stain, 4,300X)

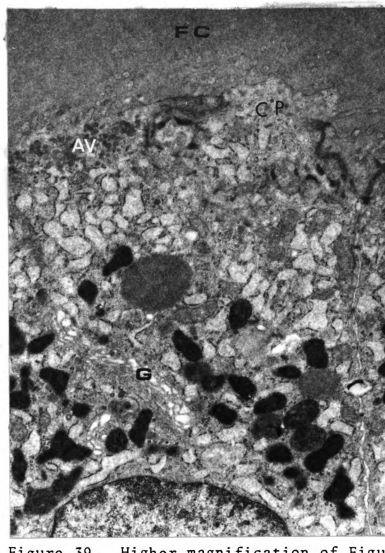

Figure 39. Higher magnification of Figure 38. Notice cytoplasmic projection (CP) extended toward the follicular colloid (FC). The apical vesicles (AV) are darkly stained and the golgi apparatus (G) is hypertrophied. (Lead citrate and uranyl acetate stain, 12,600X)

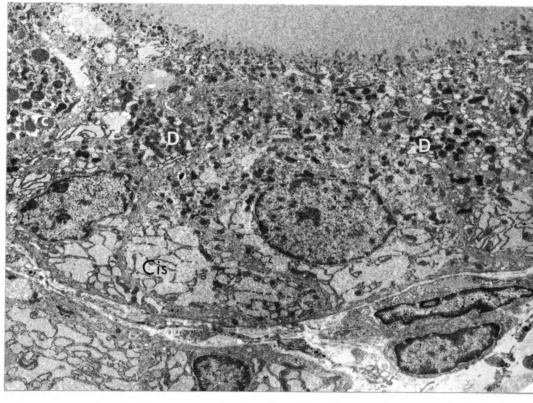

Figure 40. Electron micrograph of thyroid follicular cells from a rat fed a diet containing 100 ppm 2,3',4,4',5,5'-HBB for 30 days. The changes include increased cellular height, colloid droplets (C), dense bodies (D) and dilated cisternae (Cis) similar to those due to FM BP-6 but of slightly less severity. (Lead citrate and uranyl acetate stain, 4,450X)

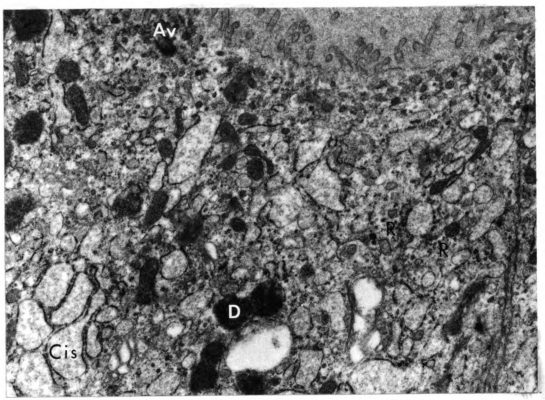

Figure 41. Higher magnification of Figure 40. Notice an increase in dense bodies (D), density of apical vesicles (Av) and dilated cisternae (Cis) endoplasmic reticulum as well as detachment of ribosomes (R). (Lead citrate and uranyl acetate stain, 15,150X)

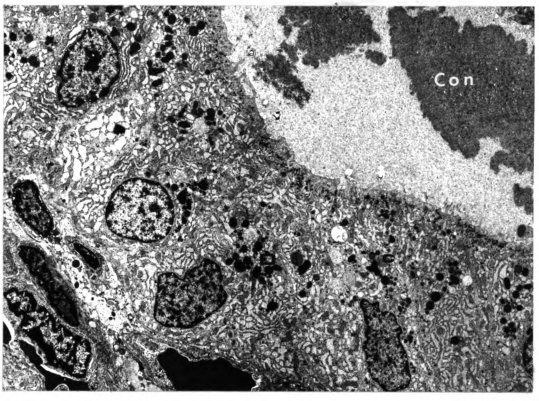

Figure 42. Electron micrograph of thyroid follicular cells from a rat fed a diet containing 100 ppm 2,2',4,4',5,5'-HBB for 30 days. Lesions are similar to those seen with FM BP-6 or 2,3',4,4',5,5'-HBB with less numerous dense bodies (D) and colloid droplets (C). Notice the presence of concretions (Con) in the follicular colloid (FC). (Lead citrate and uranyl acetate stain, 4,000X)

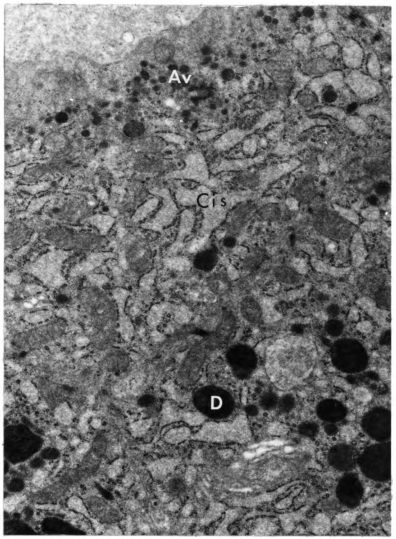

Figure 43. Higher magnification of Figure 42. Notice numerous dense bodies (D) and an increase in the density of apical vesicles (AV). The cisternae are markedly dilated. (Lead citrate and uranyl acetate stain, 15,150X)

DISCUSSION

Toxicological assessment of FM BP-6 is complicated by the fact that the product is a mixture of several different brominated biphenyls. Investigators are becoming more aware that the toxicity caused by the PBB mixture may be due to synergistic or additive effects from several different brominated biphenyls. The congeners have been well purified, the chemical structures have been characterized, and the type of microsomal enzyme induction related to specific congeners has been determined (Dent, 1976; Aust et al., 1981).

The present study was designed to assess whether specific biochemical and structural characteristics of selected purified PBB congeners could be correlated with pathologic changes in the target organs.

Clinical signs of toxicosis were not observed in rats fed diets containing FM BP-6, 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB for 30 days. Firemaster BP-6, 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB did not alter daily feed intake or body weight. Rats given 1 ppm 3,3',4,4',5,5'-HBB had an increased feed intake but the body weight was no different than the controls. This may indicate that the food consumed was not utilized efficiently by the body. Furthermore, after 20 days of dietary treatment, the body weight gains and food consumption

of rats fed 10 ppm 3,3',4,4',5,5'-HBB were less than the controls. These data provided more evidence that this congener affected food consumption and body weight.

Hematologic values were not affected by FM BP-6 or any of the 3 congeners. Other investigators did not find changes in hematologic values in rats given diets containing PBB (Sleight and Sanger, 1976).

Hepatomegaly was produced by each of the chemicals, and the responses were directly proportional to the dose. In laboratory animals, increased liver weight due to administration of the PBB mixture or its congeners has been widely reported. Hepatomegaly was reported with PBB (Babish et al., 1975; Corbett et al., 1975; Sleight et al., 1978); 2,2',4,4',5,5'-HBB; 2,3',4,4',5,5'-HBB (Dharma, 1980) or 3,3',4,4',5,5'-HBB (Render, 1980).

Although 2,2',4,4',5,5'-HBB increased liver weight at 1, 10 or 100 ppm, similar changes with 2,3',4,4',5,5'-HBB were only seen at 100 ppm. However, at 100 ppm the liver weights were nearly identical for each of the 2 chemicals. Apparently, at the low doses 2,2',4,4',5,5'-HBB contributes significantly to the production of hepatomegaly. Similar findings have been reported in cockerels (Dharma, 1980). The cause of the stimulatory effect on the liver weight at the low dose of 2,2',4,4',5,5'-HBB is not known. However, increases in liver weight by xenobiotics do not always directly reflect the toxicity of compounds. Hepatomegaly was not observed in rats fed diets containing TCDD, but histologically hepatic lesions were severe (Gasiewicz et al.,

1980). The FM BP-6 increased liver weight to body weight ratios at 10 or 100 ppm. At 100 ppm, the liver weight of rats fed FM BP-6 was heavier than with either 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB. The increasing weight of the liver in rats fed PBB was thought to be due to the increase in amount of fat, protein, water or other constituents and not caused by hyperplasia.

Liver lesions mainly included swelling of hepatocytes and cytoplasmic vacuoles which were seen mostly in the centrilobular or midzonal regions. The basic lesions produced by all 4 chemicals were similar. However, 3,3',4,4',5,5'-HBB produced more numerous and larger vacuoles within hepatocytes than the other 3 chemicals. At 10 ppm, 3,3',4,4',5,5'-HBB caused highly vacuolated hepatocytes. The architectural structure of the lobules was abnormal. Some of the central veins were not identifiable due to extensive cytoplasmic vacuolation and severe cellular destruction in these areas.

Rats fed diets containing 100 ppm of FM BP-6, 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB had markedly swollen hepatocytes and moderate cytoplasmic vacuolation. The architectural structure of the lobules was not greatly affected such as was seen at 10 ppm 3,3',4,4',5,5'-HBB. Unlike rats fed 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB, rats given 100 ppm FM BP-6 had ring-shaped cytoplasmic inclusions within hepatocytes. These inclusions later were identified by electron microscopy as myelin bodies.

Ultrastructural features of the hepatocytes included proliferation and vesiculation of SER, increased fat droplets

and dilated cisternae of the endoplasmic reticulum. It appears that the proliferation of SER and the increase in fat droplets are mainly responsible for hypertrophied hepatocytes and thereby contributed to the increased liver weight. The amount of increase in SER appeared to be directly proportional to the dose of treatment. Proliferation of SER was followed by diminution and displacement of RER and other organelles mostly to the periphery of the cells. Hansell and Ecobichon (1974) postulated that the proliferation of SER is a structural response from stimulation of enzyme activity. Meanwhile, Remmer and Merker (1963) defined these changes as a nonspecific adaptation to a drug administration. Blumberg (1978) and Fowler (1980) suggested that the induction of microsomal enzymes first occurs in the RER. When RER is saturated with enzyme, this organelle will lose its ribosomes and become SER. According to Trump and Jones (1978), proliferation of SER is an initial response, which is reversible when the induction is ceased. Lesions involving proliferation of SER have been reported in rats fed diets containing the commercial PBB mixture or its congeners (Lee et al., 1975a; Sleight and Sanger, 1976; Akoso and Sleight, 1979; Render, 1980). When rats were fed diets containing 3,3',4,4',5,5'-HBB, the RER was severely altered. The RER was individualized and proliferated and lacked normal organization. Proliferation of SER was not as prominent as with the other 3 chemicals. The changes due to 3,3',4,4',5,5'-HBB could be clearly differentiated from those caused by the other 3 chemicals. Changes in hepatocytes in rats fed 3,3',4,4',5,5'-HBB were

similar to those described for rats fed diets containing TCDD (Gasiewicz, 1980; Kociba et al., 1978).

Rats fed 100 ppm FM BP-6 had concentrically laminated myelin bodies within hepatocytes. Usually these bodies encircled mitochondria, SER, fat globules or other organelles. The presence of myelin bodies was readily identified by light microscopy as ring-shaped eosinophilic inclusions. Myelin bodies have been reported in rats fed 100 ppm 3,3',4,4',5,5'-HBB for 20 days or 2,3',4,4',5,5'-HBB for 60 days but were not seen in rats fed 2,2',4,4',5,5'-HBB for 60 days (Akoso and Sleight, 1979; Render, 1980). There has been considerable controversy regarding the nature and formation of myelin bodies. Several terms have been used to designate these bodies, such as myelin figures, whorls, fingerprints, myeloid bodies, glycogen bodies and inclusion bodies (Hruban, 1965; Ortega, 1966; Lee et al., 1975a). Myelin bodies have also been reported in rats after administration of dimethylnitrosoamine, aflatoxin, carbon tetrachloride, DDT and phenobarbitone (Ghadially et al., 1975; Herdson et al., 1964). It has been postulated that the first step in myelin body formation is an alteration of endoplasmic reticulum (Steiner and Baglio, 1963; Norback and Allen, 1972). The cisternae may become disoriented, lose ribosomes and then encircle mitochondria. Myelin bodies are formed after the altered cisternae are layered around mitochondria or other organelles (Steiner and Baglio, 1963). Hruban (1965) speculated that myelin bodies arise through sequestration of myeloid membranes which are formed from RER or SER.

The significance of myelin bodies is not clearly understood. They have been observed in normal or pathological conditions in several tissues (Ghadially et al., 1975). However, myelin bodies were not found in normal hepatocytes (Steiner et al., 1976). Myelin bodies have been associated with toxic chemicals as well as with carcinogens in man and animals (Steiner and Baglio, 1963; Ghadially et al., 1975). Herdson et al. (1964b) reported that myelin bodies may persist during exposure with xenobiotics, but these changes are reversible.

Lee et al. (1975) speculated that myelin bodies may represent modified secondary lysosomes resulting from a focal cytoplasmic degradation. In contrast, Hruban (1965) stated earlier that myelin bodies were not positive for acid phosphatase, hence could not be considered as "typical" lysosomes.

Concentrations of vitamin A in the liver of rats fed diets containing FM BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-HBB or 3,3',4,4',5,5'-HBB were decreased. Decreases in liver vitamin A concentration have been reported in rats given PBB (Mangkoewidjojo, 1979), PCB (Innami et al., 1976; Kato et al., 1978), DDT (Phillips, 1963), dieldrin (Lee et al., 1964), methoxychlor (Davison and Cox, 1976), and chlorinated naphthalene (Hansel et al., 1951). Innami et al. (1976) studied the reduction of vitamin A content in the liver of rats fed PCB or DDT. These 2 chemicals are known to induce microsomal drugs metabolizing enzymes. Innami et al. (1976) speculated that during the hydroxylation reaction catalyzed by cytochrome P-450 an active oxygen is generated. The active oxygen is

coupled to the superoxide generating system and may be responsible for the reduction of vitamin A. Mangkoewidjojo (1979) suggested that a similar mechanism may apply for PBB, since PBB are also potent inducers of microsomal drug metabolizing enzymes.

Results of microsomal enzyme assays clearly indicated the type of microsomal enzyme induction caused by each of the 4 chemicals. Results are similar to those described by Aust et al., 1981. Benzo(α)pyrene hydroxylation, which is used to assess MC-type induction, was caused by 3,3',4,4',5,5'-HBB, while aminopyrene demethylation, a standard test for Pb-type induction, was associated with 2,2',4,4',5,5'-HBB. In addition, FM BP-6 and 2,3',4,4',5,5'-HBB caused both benzo(α)-pyrene hydroxylation and aminopyrene demethylation. These results further confirmed that FM BP-6 and 2,3',4,4',5,5'-HBB are Pb- and MC-type inducers, 2,2',4,4',5,5'-HBB is a Pb-type and 3,3',4,4',5,5'-HBB is an MC-type inducer.

Histological changes in the thyroid glands were dose dependent but were similar for all the treatments. The characteristic lesions were first noticed at 10 ppm. Hypertrophy and hyperplasia of follicular cells as well as diminution of follicular colloid were prominent features seen in the thyroid gland. Thyroid hyperplasia has been reported in rats given PCB (Yamane et al., 1975), aminotriazole (Strum and Karnovsky, 1971), DDD (Fregly et al., 1968), 4,4-oxidianiline (Hayden et al., 1978) and PBB (Sleight et al., 1978).

Ultrastructurally, the follicular cells were hypertrophied with increased colloid droplets and dense bodies and dilated

cisternae of endoplasmic reticulum. These changes were similar in rats fed any of the diets, but there were some differences in severity of lesions. More severe lesions were seen with 3,3',4,4',5,5'-HBB than with FM BP-6, while the latter chemical caused more extensive changes than 2,3',4,4',5,5'-HBB. The least severe lesions were seen in rats fed 2,2',4,4',5,5'-HBB. Lesions similar to those produced by these chemicals have been reported in rats fed an iodine deficient diet (Feldman, 1961; Lupulescu, 1970), PCB (Collin et al., 1977) and PBB (Kasza et al., 1978; Akoso and Sleight, 1979). Kasza et al. (1978) concluded that ultrastructural features of thyroid glands from rats fed PBB or PCB are similar.

The existence of colloid droplets in the thyroid follicular cells of iodine deficient rats or rats fed goitrogenous substance have created considerable discussion. It is generally believed that the colloid droplets are resorbed colloid which originated from the follicular colloid. It has been demonstrated by using radiolabeled isotopes that the number of colloid droplets increases after administration of TSH while follicular colloid is diminished (Lupulescu and Petrovici, 1968). The colloid droplets migrate from the apical portion of the cells toward the base (Seljelid, 1966). In the meantime, the dense bodies containing esterase and acid phosphatase as lysosomes will migrate from the base of the cells toward the apical portion (Wollman, 1964; Wetzel et al., 1965). The colloid droplets encounter and fuse with the dense granules (Seljelid, 1965). The enzymes acid phosphatase and esterase are incorporated into the colloid droplets.

Intracellular hydrolysis occurs and T_3 and T_4 are liberated into the serum as free hormones. Subsequently, the granules become denser and smaller and migrate toward the base. However, the final steps in secretion of these hormones are still not clearly understood.

Results of thyroid hormone analysis revealed a decreased concentration of serum T_3 and T_4 in rats fed diets containing FM BP-6 or 3,3',4,4',5,5'-HBB. In addition, the ratio of T_3 and T_4 was decreased in the serum of rats fed either of the 4 chemicals. Decreases in serum T_4 have been reported in rats fed 50 or 500 ppm PCB for 4 weeks (Collins et al., 1977). The T_4 concentration was back to normal at 35 weeks after PCB administration had been discontinued. Similarly, the thyroid gland was of normal size. A decrease in T_3 and T_4 as well as an increase in T_3/T_4 ratios have been reported in rats fed an iodine deficient diet and goitrogenic agents (Studer and Greer, 1965; Mayberry, 1968; Lupulescu, 1969).

Based on the pathologic features as well as thyroid hormone determinations, there is reason to believe that FM BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-HBB and 3,3',4,4',5,5'-HBB have a potential to be goitrogenic. It appears that duration of exposure also plays a role in the severity of the lesions. Studies in our laboratory indicated that morphological and functional disturbances became more intense after 60 days (Akoso and Sleight, 1979; Akoso and Sleight, unpublished data, 1979).

The exact mechanism as to how polyhalogenated aromatic hydrocarbons such as PBB affect thyroid function is not clearly

defined. Thyroid hyperplasia due to octabromobiphenyl was associated with competitive binding between iodine and bromine (Norris et al., 1975). Feeding of bromine to rats reduced ^{131}I uptake by the thyroid gland and resulted in goiter (Underwood, 1977). Rats fed diets containing PCB had enhanced biliary excretion of thyroxine (Yamane et al., 1975; Bastomsky and Murthy, 1976). Similarly, DDD, 3,4-benzopyrene or 3-methylcholanthrene have been shown to enhance thyroid metabolism or clearance of thyroxine by the liver (Fregly et al., 1968; Bastomsky, 1973).

There were dose-dependent decreases of thymus and spleen weight in rats fed diets containing 3,3',4,4',5,5'-HBB. Decreased thymus and spleen weights have also been reported in rats given TCDD (Kimbrough, 1974; Kociba et al., 1979). Thymus weights were not affected by FM BP-6 or 2,3',4,4',5,5'-HBB. Histological examination of thymuses from rats which died after feeding FM FF-1 at high doses (up to 100 mg/kg body weight/day) revealed thymic atrophy with obliteration of normal architectural structure, loss of demarcation between the cortical and medullary region and disappearance of cortical thymocytes (Gupta and Moore, 1979). Similar changes were seen in this study in rats given 3,3',4,4',5,5'-HBB and were also described by Render (1980). In Beagle dogs given PBB, there were thymic involution, lymphocytic depletion in lymph nodes particularly in the T-cell region, and a decrease in the lymphocytes in the white pulp of the spleen (Kasza, 1977). Firemaster FF-1 was reported to depress cell-mediated immunity in rats and mice (Luster et al., 1978). The spleen and thymus

weights were decreased, but histologically only a slight decrease in the density of the thymic cortex was described.

The increase in brain weight to body weight ratios in rats fed diets containing 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB was unexplained. The increasing weights appeared to be dose dependent. However, there were no histological changes observed in the brains of the rats fed these congeners at any given dose. There were no signs of neurological disorder, and the animals' behavior was not affected. An increase in brain weight associated with 2,2',4,4',5,5'-HBB was also reported by Render (1980).

Concentrations of FM BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-HBB or 3,3',4,4',5,5'-HBB in tissues were generally directly proportional to the concentration in the feed. The highest value was in the liver. In contrast, Harris et al. (1978a) stated that in rats fed 100 ppm of PBB for 10 weeks, the PBB concentration in the fat was 30 times greater than in the liver. Interestingly, in our findings the concentration of 3,3',4,4',5,5'-HBB in the liver was much greater, about 220 times (at 1 ppm) or 65 times (at 10 ppm) than in the fat. In addition, the chemical residues in the kidney and thymus were higher than in the fat. Among 3 congeners studied, it appears that 3,3',4,4',5,5'-HBB is accumulated the least in tissues, whereas 2,2',4,4',5,5'-HBB accumulates to the greatest extent.

In general, the objectives of this research were fulfilled. The toxicologic effects of feeding diets containing FM BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-HBB or 3,3',4,4',5,5'-HBB

for 30 days were determined. The magnitude of toxicity starting with the most toxic is 3,3',4,4',5,5'-HBB; FM BP-6; 2,3',4,4',5,5'-HBB; and 2,2',4,4',5,5'-HBB. It is also clear that 3,3',4,4',5,5'-HBB, an MC-type inducer with structure and activity analogous to TCDD, produces a similar toxic syndrome. The least effects were seen with 2,2',4,4',5,5'-HBB, which was strictly a Pb-type inducer. Firemaster BP-6 and 2,3',4,4',5,5'-HBB, which have properties of both MC- and Pb-type inducers, were more toxic than 2,2',4,4',5,5'-HBB. In addition, although 2,3',4,4',5,5'-HBB is also a mixed-type inducer, the congener is less toxic than the parent compound FM BP-6.

The results indicate that very little of the toxicity associated with FM BP-6 is caused by its major constituent 2,2',4,4',5,5'-HBB. Therefore, signs of toxicity such as loss of weight, thymic and splenic atrophy, severe liver damage and death are most likely caused by the congeners which are MC-type inducers. These congeners are also most likely responsible for any severe alterations in thyroid function or in vitamin A metabolism.

SUMMARY

Seventy-eight male Sprague-Dawley rats initially weighing 148 ± 16 g were assigned into groups of 6 and were fed diets containing either 0, 1, 10 or 100 ppm of FM BP-6, 2,2',4,4',5,5'-hexabromobiphenyl (HBB) or 2,3',4,4',5,5'-HBB. The other groups of 6 rats each were fed either 0, 1 or 10 ppm of 3,3',4,4',5,5'-HBB. The rats were killed on the 30th day of each experiment.

There were no clinical signs of toxicosis in rats fed FM BP-6, 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB for 30 days. In rats fed 10 ppm 3,3',4,4',5,5'-HBB the feed intake was decreased and the growth rate was depressed starting from the 20th day.

Results of urinalysis and hematologic examinations were essentially normal in all rats. Dose-dependent increase in liver weight to body weight ratios was seen in rats given any of the 4 chemicals.

Histologic changes in hepatocytes, including swollen hepatocytes and cytoplasmic vacuolation, were observed in rats fed diets containing any of the chemicals but were seen most prominently with 3,3',4,4',5,5'-HBB. Ultrastructurally, rats given FM BP-6 had hepatic lesions including proliferation of smooth endoplasmic reticulum (SER), decreased numbers of mitochondria and increased fat droplets. Similar but less

severe changes were seen with 2,3',4,4',5,5'-HBB or 2,2',4,4',5,5'-HBB. The latter chemical produced the least severe changes. Hepatocytes of rats fed 10 ppm 3,3',4,4',5,5'-HBB had extensive proliferation and disorganization of rough endoplasmic reticulum (RER), increased fat droplets and some proliferation of SER. These changes were comparable to those reported with TCDD. Firemaster BP-6 at 100 ppm produced myelin bodies in the hepatocytes, but 2,2',4,4',5,5'-HBB and 2,3',4,4',5,5'-HBB did not.

Concentration of liver vitamin A was decreased by each of the 4 chemicals. Although there were no clinical signs that could be attributed to vitamin A deficiency, these chemicals may possibly aggravate clinical or subclinical nutritional problems associated with hypovitaminosis A.

Histologically, the thyroids of rats fed any of the 4 chemicals had follicular cell hyperplasia and hypertrophy with scanty or absent colloid. Ultrastructurally, there were increases in dense bodies and colloid droplets, and the cisternae were dilated. Serum thyroid hormone analysis indicated decreases in T_3 and T_4 concentrations and increased T_3/T_4 ratios. The morphological and functional alterations suggested that FM BP-6 and the 3 congeners are goitrogenic.

The results indicated that 3,3',4,4',5,5'-HBB, which is an MC-type inducer, is the most toxic congener among the 3 congeners studied. Firemaster BP-6, a mixed (MC and Pb)-type inducer, is more toxic than either 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB. However, 2,3',4,4',5,5'-HBB (mixed-type inducer) is more toxic than 2,2',4,4',5,5'-HBB (Pb-type

inducer). Apparently, 2,2',4,4',5,5'-HBB, which is the major congener in FM BP-6, contributes very little to its toxicity. Toxicity of FM BP-6 can be mostly attributed to its congeners with MC-type induction capability.

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APPENDIX

Table A-1. Values of blood urea nitrogen, serum aspartate aminotransferase and serum alkaline phosphatase in rats fed diets containing FM BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-HBB or 3,3',4,4',5,5'-HBB for 30 days

Group	Modification of Dietary Treatment	Chemical Concentration in Feed (ppm)	BUN (mg/dl)	AST (IU/l)	SAP (IU/l)
I[a]	Control	0	18.7±2.08	132.0±15.72	130.3±11.68
	Firemaster BP-6	1	16.3±1.16	193.3±56.86	155.7±31.53
		10	15.3±0.58[c]	150.0±43.58	136.0±12.29
		100	19.0±2.00	170.0±26.46[c]	171.0±46.52
	2,2',4,4',5,5'-hexabromobiphenyl	1	19.0±2.00	203.7±17.79[e]	152.7±22.05
		10	21.0±1.00	150.7±40.15	171.3±56.31
		100	20.3±3.15	168.0±25.06	119.7±22.68
	2,3',4,4',5,5'-hexabromobiphenyl	1	15.7±1.16[d]	140.3± 7.77	126.3± 8.51
		10	20.3±2.52	98.7±18.56[c]	112.0±12.12
		100	20.3±1.53	136.7±31.57	150.3±63.11
II[b]	Control	0	f	66.0± 2.55	224.8±66.24
	3,3',4,4',5,5'-hexabromobiphenyl	1	f	71.0±10.12	284.0±52.63
		10	f	142.2±102.6	453.8±351.8

[a]Data are expressed as mean ± SD (n=2 pooled samples of 3 rats each).

[b]Data are expressed as mean ± SD (n=6 rats).

[c,d,e]Significantly different from control value ($p<0.05$, $p<0.025$, $p<.0005$, respectively).

[f]Not done.

Table A-2. Serum protein fractions from rats fed diets containing FM BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-HBB, or 3,3',4,4',5,5'-HBB for 30 days

Group	Modification of Dietary Treatment	Chemical Concentration in Feed (ppm)	Albumin (g/dl)	α-Globulin (g/dl)	γ-Globulin (g/dl)	Total Protein (g/dl)	Albumin/ Globulin Ratio
I	Control	0	2.6±0.40	2.0±0.25	1.4±0.32	5.4±0.58	0.76
	Firemaster BP-6	1	2.7±0.12	2.3±0.15	1.5±0.10	6.5±0.36[a]	0.71
		10	2.3±0.30	2.1±0.36	1.7±0.30	6.1±0.36	0.60
		100	2.6±0.12	2.0±0.06	2.0±0.12	6.6±0.25[b]	0.65
	2,2',4,4',5,5'-hexabromobiphenyl	1	2.8±0.32	1.9±0.12	1.6±0.53	6.4±0.59	0.80
		10	2.7±0.17	2.2±0.06	1.6±0.10	6.5±0.25[a]	0.71
		100	2.9±0.06	2.5±0.06	1.7±0.12[b]	7.2±0.06	0.69
	2,3',4,4',5,5'-hexabromobiphenyl	1	2.7±0.15	2.1±0.10	1.3±0.15	6.1±0.25[c]	0.79
		10	2.6±0.06	2.1±0.27	1.2±0.06	5.9±0.27	0.79
		100	2.4±0.15	2.0±0.12	1.5±0.17	5.8±0.42	0.69
II	Control	0	2.9±0.35	2.0±0.27	1.7±0.00	6.6±0.20	0.78
	3,3',4,4',5,5'-hexabromobiphenyl	1	3.1±0.21	2.3±0.21	1.7±0.10	7.1±0.46	0.78
		10	2.8±0.12	2.2±0.10	2.0±0.06	7.0±0.06	0.67

Data are expressed as mean ± SD (n=3).

[a,b,c]Significantly different from control value ($p<0.05$, $p<0.025$, $p<0.005$, respectively).

Table A-3. Serum lactic dehydrogenase isoenzymes in rats fed FM BP-6, 2,2',4,4',5,5'-HBB or 2,3',4,4',5,5'-HBB for 30 days

Group	Modification of Dietary Treatment	Chemical Concentration in Feed (ppm)	Lactic Dehydrogenase Isoenzymes (IU/1)					
			LDH-1	LDH-2	LDH-3	LDH-4	LDH-5	Total LDH
I	Control	0	115 ±7	185 ±50	90 ±57	55 ±50	1005 ±346	1455 ±519
	Firemaster BP-6	1	245 ±64	325 ±78	130 ±57	32 ±40	1015 ±177	1746 ±415
		10	160 ±71	205 ±92	105 ±50	55 ±35	930 ±354	1460 ±594
		100	130 ±14	150 ±57	100 ±14	105 ± 7	1190 ± 28	1680 ± 28
	2,2',4,4',5,5'-hexabromobiphenyl	1	270 ±28[b]	275 ±64	110 ±57	70 ±14	895 ±247	1620 ±113
		10	195 ± 7[c]	180 ± 0	85 ± 7	60 ± 0	730 ±113	1255 ±120
		100	190 ±7.07[c]	245 ±21	115 ±92	130 ±28	965 ±191	1645 ±106

Table A-3 (continued)

Group	Modification of Dietary Treatment	Chemical Concentration in Feed (ppm)	Lactic Dehydrogenase Isoenzymes (IU/l) LDH-1	LDH-2	LDH-3	LDH-4	LDH-5	Total LDH
	2,3',4,4',5,5'-hexabromobiphenyl	1	110 ±14	60 ±14[a]	80 ±14	65 ± 7	1230 ± 57	1535 ± 35
		10	115 ± 7	95 ± 7	35 ±21	20 ±14	745 ± 92	1005 ± 54
		100	170 ±57	185 ±78	75 ±64	30 ±14	610 ±184	1060 ± 28

Data are expressed as mean ± SD (n=2).

[a,b,c]Significantly different from control value ($p<0.05$, $p<0.01$, $p<0.005$, respectively).

Table A-4. Organ weights and final body weights in rats fed HBB or 3,3',4,4',5,5'-HBB for 30 days

Group	Modification of Dietary Treatment	Chemical Concentration of Feed (ppm)	Final Body Weight (g)
I	Control	0	362.8±26.1
	Firemaster BP-6	1	336.7±16.5
		10	342.8±12.8
		100	325.0±17.9
	2,2',4,4',5,5'-hexabromobiphenyl	1	347.0±26.6
		10	325.7±29.6
		100	336.3±20.8
	2,3',4,4',5,5'-hexabromobiphenyl	1	335.8±20.9
		10	338.7±25.3
		100	340.2± 7.4
II	Control	0	353.0±27.4
	3,3',4,4',5,5'-hexabromobiphenyl	1	356.8±18.0
		10	308.5±10.3

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

[a]Not done.

diets containing FM BP-6, 2,2',4,4',5,5'-HBB, 2,3',4,4',5,5'-

Brain weight (g)	Spleen weight (g)	Thymus weight (g)	Liver weight (g)	Thyroid weight (mg)
1.78±0.06	0.89±0.10	0.80±0.06	14.30±0.88	21.63±3.94
1.77±0.07	0.82±0.08	0.87±0.13	13.55±1.15	18.27±2.65
1.70±0.05	0.72±0.10	0.64±0.13	24.56±2.34	23.33±2.98
1.70±0.05	0.72±0.10	0.64±0.13	24.56±2.34	23.33±2.98
1.85±0.08	a	0.86±0.17	16.31±1.10	23.93±3.44
1.76±0.08	a	0.90±0.14	17.30±2.40	22.85±5.08
1.82±0.05	a	0.97±0.16	22.06±3.94	22.50±3.12
1.80±0.05	0.08±0.11	0.87±0.16	14.88±2.72	18.43±2.03
1.79±0.05	0.86±0.10	0.90±0.14	15.65±1.30	18.32±2.86
1.88±0.10	0.82±0.07	0.90±0.11	21.68±2.05	20.45±3.30
1.83±0.06	0.92±0.08	0.85±0.09	12.19±1.47	14.97±0.58
1.84±0.04	0.90±0.14	0.80±0.07	14.19±1.18	18.18±1.89
1.74±0.04	0.71±0.05	0.43±0.07	17.01±1.52	18.98±1.87

VITA

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The author was born in Klaten, Central Java, Indonesia, on November 7, 1945. He graduated from the Faculty of Veterinary Medicine, Gadjah Mada University, in April 1973.

Following graduation he was employed by the Directorate of Animal Health of Indonesia in Jakarta. He was then appointed to the Laboratory of Disease Investigation Center in Ujung Pandang in a project of joint cooperation between the Indonesian government and the Food and Agriculture Organization of the United Nations in July 1973.

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Papers presented at scientific meetings:

1. Akoso, B. T., Mangkoewidjojo, S., and Sleight, S. D.: Pathologic effects of polybrominated biphenyls (PBB) in rats fed an iodine deficient diet for 30 or 60 days. Presented at the 58th Conference of Research Workers in Animal Diseases, Chicago, Illinois, November 28 and 29, 1977.

2. Akoso, B. T., and Sleight, S. D.: Histologic and ultrastructural features of the liver and thyroid gland of rats given purified congeners of polybrominated biphenyls for 60 days. Presented at the 60th Conference of Research Workers in Animal Diseases, Chicago, Illinois, November 26 and 27, 1979.

3. Akoso, B. T., Sleight, S. D., Aust, S. D., and Nachreiner, R.: Comparative study of the toxicopathology of purified congeners of polybrominated biphenyls in rats. Presented at the 19th Annual Meeting, Society of Toxicology, Washington, D.C., March 9-13, 1980.

4. Sleight, S. D., Mangkoewidjojo, S., Akoso, B. T., and Sanger, V. L.: Toxicosis of polybrominated biphenyls (PBB) in rats fed an iodine deficient, iodine adequate or iodine surplus diet. Presented at Workshop on Scientific Aspects of Polybrominated Biphenyls, Michigan State University, East Lansing, Michigan, October 24-25, 1977.

5. Mangkoewidjojo, S., Akoso, B. T., and Sleight, S. D.: Pathologic effects of polybrominated biphenyls (PBB) in rats fed an iodine surplus diet for 30 or 60 days. Presented at the 58th Conference of Research Workers in Animal Diseases, Chicago, Illinois, November 28 and 29, 1977.

6. Pearson, A. M., Sleight, S. D., Cornforth, D. P., and Akoso, B. T.: Effects of nitrosamines, nitrite and secondary amines on tumor development in mice. Proc. Europ. Meeting Meat Res. Workers 2 (1980): 216.

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8. Sleight, S. D., Render, J. A., Akoso, B. T., and Nachreiner, R.: Comparative toxicopathology of Firemaster BP-6, 2,2',4,4',5,5'-hexabromobiphenyl (HBB) and 3,3',4,4',5,5'-HBB after 10 and 30 days of dietary administration to rats. Presented at "Toxicology in Michigan Today", the Center for Environmental Toxicology, Michigan State University, East Lansing, Michigan, May 8, 1981.

Master of Science thesis: Pathologic Effects of Polybrominated Biphenyls in Iodine Deficient Rats. Michigan State University, 1977.

Article published: Sleight, S. D., Mangkoewidjojo, S., Akoso, B. T., and Sanger, V. L.: Polybrominated biphenyl toxicosis in rats fed an iodine-deficient, iodine-adequate or iodine-excess diet. Environ. Health Perspect. 23 (1978): 341-346.

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