COMPARATIVE TOXICOPATHOLOGY OF FIREMASTER BP-6, 2,2',4,4',5,5'-HEXABROMOBIPHENYL AND 3,3',4,4',5,5'-HEXABROMOBIPHENYL AFTER TEN DAYS OF DIETARY ADMINISTRATION TO RATS

By

James A. Render

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Male Sprague-Dawley rats were divided into groups of 6 rats each and fed a diet containing 0, 0.1, 1, 10 or 100 ppm of Firemaster BP-6, 2,2',4,4',5,5'-hexabromobiphenyl (HBB) or 3,3',4,4',5,5'-HBB for 10 days. Lethal effects occurred at 20 days in 2 rats fed 100 ppm of 3,3',4,4',5,5'-HBB. By comparisons of clinical signs, organ weights and histological and ultrastructural changes, 3,3',4,4',5,5'-HBB was more toxic than Firemaster BP-6, which appeared more toxic than 2,2',4,4',5,5'-HBB. Characteristics of 3,3',4,4',5,5'-HBB toxicosis were decreased feed intake eventually leading to a moribund condition, decreased weight gain, hepatomegaly with diffuse hepatocellular hypertrophy, abundant intracytoplasmic lipid droplet accumulation, bile duct proliferation and lymphocytic depletion in the thymus. Alterations in the smooth and rough endoplasmic reticulum progressed to myelin body formation. Administration of Firemaster BP-6 or 2,2',4,4',5,5'-HBB did not cause clinical signs and lesions were mainly confined to the liver.

DEDICATION

to the memory of my father

Ray James Render

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The year 1980 is an election year. A candidate for the degree of Master of Science is similar to a candidate for the office of the President of the United States. Both only achieve their desired goals by the help of supporters.

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INTRODUCTION

Today, 1980, human beings and animals are exposed to more xenobiotics than ever before. More than four million different synthetic compounds, including drug constituents, food additives, pesticides, fungicides, herbicides and insecticides, are present in the environment (Ingelman-Sundberg, 1980). There has been considerable interest in halogenated aromatic hydrocarbons because of their ubiquitous occurrence, environmental persistence, possible magnification in the food chain, and highly toxic isomers (McConnell and Moore, 1979). Within this category are dibenzo-p-dioxins, dibenzofurans, naphthalenes and biphenyls.

The compound 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is formed in significant quantities as an unwanted contaminant under certain conditions during the production of the herbicide, 2,4,5-trichlorophenol (2,4,5-T) (Kociba et al., 1979). Public awareness of TCDD increased when it was incriminated as the toxic agent in Agent Orange, a defoliant used in the Viet Nam War. Reliable reports of TCDD causing mutagenesis or carcinogenesis in humans are lacking (Walsh, 1977), but teratogenesis, fetotoxicity and pathologic changes in the liver, thymus and other organs have been reported in rats (Kimbrough, 1974).

Halogenated naphthalenes and halogenated dibenzofurans may also occur as contaminants. Chlorinated dibenzofurans and chlorinated naphthalenes were present in the commercial Japanese mixture of polychlorinated biphenyls (PCB), Kannechlor 400 (KC-400) (Roach and

Pomerantz, 1974). The KC-400 was implicated as the cause of a peculiar disease in humans called "Yusho" (Kuratsune et al., 1972). Chlorinated naphthalenes, PCB, a few isomers of the chlorinated dibenzodioxins, and chlorinated dibenzofurans have all been reported to cause a human skin condition called chloracne (Kimbrough, 1974). Hyperkeratosis, formerly called X-disease, in cattle was caused by highly chlorinated naphthalenes (Bell, 1953). This skin problem occurred in Germany between 1946 and 1948 on farms where the woodwork of barns had been painted with a certain wood preservative containing the naphthalenes (Wagener, 1951). Outbreaks of the poisoning were also reported in 32 states in the United States (U.S. Department of Agriculture, 1954). Chlorinated naphthalenes, TCDD and PCB have also been reported to cause chick edema in avian species (Kimbrough, 1974).

Contamination of food by PCB is not just limited to Japan but is an international and interspecies problem (Kimbrough, 1974). The Japanese ingested contaminated rice oil but Americans, especially Michigan residents, are ingesting contaminated Great Lakes fish (Cordle et al., 1978). In Michigan this fact has been overshadowed by the attention given polybrominated biphenyls (PBB).

In 1973, Michigan cattle ingested feed contaminated with a commercial brand of PBB, Firemaster (FM), which was manufactured by the Michigan Chemical Corporation, St. Louis, Michigan (they have since merged with the Velsicol Chemical Corporation, Chicago, Illinois) (Dunckel, 1975). Mills in which this feed was handled became contaminated. As a result, cross contamination to other mills occurred. Subsequent consumption of contaminated farm animal produce resulted in widespread human exposure to PBB. Because of the fear of possible adverse health effects in humans, thousands of livestock and chickens

were condemned and slaughtered. Also, large quantities of animal feed, eggs, butter, cheese and dry milk products were buried (Dunckel, 1975).

In the following years, millions of dollars were spent to help compensate farmers for their losses and for research involving PBB. Originally, research was multidirectional. Through epidemiological investigation and dairy product residue examination, researchers explored the area of human exposure. Biochemists were interested in the metabolism of PBB. They examined enzyme inductions and worked on identifying, purifying and structurally characterizing the components of the commercial mixture. Pharmacologists were interested in kinetics and studied clearance, tissue distribution and tissue storage, while other researchers were interested in the extent of feed residues, soil retention, photodegradation and other environmental effects. Neuro-behavioral, clinical, clinical chemical and pathological alterations were characterized. Experiments were done using cattle, swine, poultry, dogs, mink, nonhuman primates, rats, mice and guinea pigs.

As the individual compounds were isolated and purified, the objectives of much of the research involving PBB changed. Researchers from biochemistry and pathology departments, especially at Michigan State University, joined together to determine the toxicopathological effects which can be attributed to the administration of purified congeners. Knowledge gained from this research should provide a better understanding of the toxicosis of the commercial mixture.

In keeping with this idea, the commercial mixture and two purified PBB compounds were each separately administered to different rats so comparisons of their toxic effects could be made. Since the commercial mixture is classified as a phenobarbital-(PB) and a 3-methylchlolanthrene-(MC) type inducer of microsomal drug-metabolizing enzymes (Dent et al.,

1976), a pure PB-type inducer and a pure MC-type inducer were chosen for the comparisons.

LITERATURE REVIEW

Seven years have passed since the accidental contamination of feeds with PBB, and many scientific articles have been written about the subject. Literature reviews (Dunckel, 1975; Carter, 1976; Mercer et al., 1976; Stradfeld, 1976; Getty et al., 1977; Kay, 1977; Di Carlo et al., 1978; Sleight, 1979) allude to the nature of the accident, the extent of the contamination and the effects of the ingestion of PBB in various animal species. This review will concentrate on the biochemistry of the individual congeners and the pathologic effects seen in animals, especially rats, which were given the commercial mixture or purified congeners. Comparisons will be made when appropriate to the biochemistry and pathologic effects associated with administration of PCB or TCDD in various animal species.

Chemical and Physical Properties of Firemaster

Firemaster BP-6 is a registered trademark for an industrial fire retardant manufactured by the Michigan Chemical Corporation from 1970 to 1974. Two forms of FM are mentioned in the literature. One is FM BP-6 and the other is FM FF-1. The latter is a pulverized form of the former with an added anticaking agent (Getty et al., 1977). The anticaking agent is a calcium and silicon mixture and not a reported factor in the toxicity of the commercial mixture. Firemaster BP-6 is a solid which has a melting point at 72 C and will decompose above 300 C (Di Carlo et al., 1978). It is slightly water soluble but very

soluble in nonpolar solvents and is classified as lipophilic. Debromination, especially in the ortho positions, occurs upon exposure to ultraviolet irradiation.

Pomerantz et al. (1978) compared the chemistry of brominated biphenyls to chlorinated biphenyls. Bromine is a better leaving group in chemical reactions and is more efficient in fire retardation (Hutzinger et al., 1976). Bromine is also more labile than chlorine and therefore PBB may be less stable in the environment if exposed to the same physical reactions as PCB. Unlike PCB (Aroclor is the U.S. trade name and it is produced solely by Monsanto), which are liquids, although some commercial preparations are viscous at room temperature, FM BP-6 is a solid. It has been used in thermoplastics, such as typewriter and business machine housings, and it has little tendency to migrate from the thermoplastic into which it is incorporated. These products in which FM BP-6 are incorporated will probably be buried in refuse dumps eventually and not be exposed to light. Chemical reactions are therefore more likely to occur with PCB, since they can be exposed to high temperatures in transformers and capacitors and have the potential of leaking out and being exposed to light.

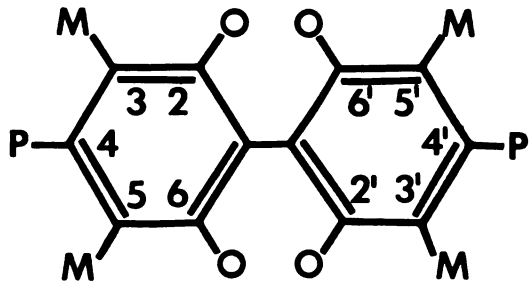
At least 30 brominated biphenyls and other contaminants are present in FM (Moore et al., 1978c). The reported percentage of brominated biphenyl isomers by weight contained in FM BP-6 was tetra- (2.0), penta- (10.6), hexa- (62.8), and hepta- (13.2) (Michigan Chemical Corporation, 1974). Hass et al. (1978) found a total of 13 major congeners in the commercial mixture and reported the percentage of brominated biphenyl isomers by weight to be penta- (4), hexa- (63) and hepta- (33). The latter researchers also reported the presence of approximately 150 ppm of pentabromonaphthalene, 70 ppm of

hexabromonaphthalene, and no evidence at a detection level of 0.5 ppm of either dibenzo-p-dioxins or dibenzofurans. Goldstein et al. (1978) stated that the naphthalenes present are not of a significant quantity to attribute to the toxic effects of FM BP-6.

Of the major congeners, 9 have known structures and are illustrated in a review article by Moore et al. (1980). Possible structures for 3 other congeners are also listed. The congeners (in order of their gas chromatographic elution) are 2,2',4,5,5'-pentabromobiphenyl (PBB_5) (peak 1), 2,3',4,4',5-PBB_5 (peak 2), 2,2',3,4',5',5'-hexabromobiphenyl (HBB) (peak 3), 2,2',4,4',5,5'-HBB (peak 4), 2,2',3,4,4',5'-HBB (peak 5), 2,3',4,4',5,5'-HBB (peak 6), 2,2',3,4,4',5',5- or 2,2',3,4',5,5',6-heptabromobiphenyl (HBB_7) (peak 7), 2,2',3,4,4',5,5'-HBB_7 (peak 8), 2,2',3,3',4,4',5'-HBB_7 (peak 9), 2,2',3,4,4',5,5',6- or 2,2',3,3',4,4',5,6'- or 2,2',3,3',4,5,5',6'-octabromobiphenyl (OBB) (peaks 10 and 11), and 2,2',3,3',4,4',5,5'-OBB (peak 12). As mentioned earlier, 2,2',4,4',5,5'-HBB (peak 4) is the largest peak and constitutes 56% of the mixture by weight (Moore et al., 1978c). The second largest component is 2,2',3,4,4',5,5'-HBB_7 (peak 8), which represents 27% of the mixture by weight (Moore et al., 1978b). The compounds 2,3',4,4',5,5'-HBB (peak 6), 2,2',3,3',4,4',5,5'-OBB (peak 12), 2,2',4,5,5'-PBB_5(peak 1) and 2,2',3,4',5',6'-HBB (peak 3) represent 4%, 1-2% (Besaw et al., 1978), 2% and 1% (Dannan et al., 1978b) of FM BP-6 by weight, respectively.

Conformation and Configuration of PBB Congeners

A biphenyl compound consists of 2 benzene rings covalently linked together, as follows:



There are 2 bridge carbons, 1 within each ring. Adjacent to these atoms are ortho carbons (O) and a substitution of a chemical (halogen) for a hydrogen at this position is called an ortho substitution. The position opposite the bridge carbon is called the para position (P). The position between the ortho and the para positions is called the meta position (M). Each of these positions is important to the metabolism of PBB (Moore et al., 1980).

Also according to Moore et al. (1980), all biphenyl compounds, either substituted or nonsubstituted, may be in a twisted configuration. The biphenyl rings in some can rotate about the long axis of the molecule to a planar configuration. The importance of this fact will be explained later. In general, interference with this rotation occurs as the number of ortho substitutions increase and as a result the molecule spends more time in the twisted configuration.

Biphenyls with one ortho substitution may differ in their ability to rotate, so other factors need to be considered. An ortho substitutent on one benzene ring may bend away from the opposite benzene ring to facilitate rotation. When the meta position adjacent to a substituted ortho position has a substituent, the ortho substituent cannot bend. The meta substituent acts as a buttress to prevent the

bending and thus this is called the buttress effect. Biphenyls in which this occurs tend to stay in the twisted configuration.

In examining the structures of the known congeners of FM, all have ortho substitutions. The ones represented by peaks 2 and 6 (2,3',4,4',5-PBB_5 and 2,3',4,4',5,5'-HBB, respectively) have one ortho substitution. All the others with known structures have 2, except for 2,2',3,4',5',6-HBB (peak 3), which has 3 ortho substitutions. A biphenyl which was mentioned earlier and not reported to be a congener is 3,3',4,4',5,5'-HBB (DeKok et al., 1977). This compound does not have any ortho substitutions and is free to rotate to a planar configuration.

Metabolism of PBB

In general, biphenyl compounds are either metabolized quickly and eliminated or persist in unaltered forms. According to Moore et al. (1980), 3 criteria are involved in PBB metabolism. Metabolism is facilitated when the number of para substitutions decrease, the number of ortho substitutions increase and the total number of substitutions decrease. When all 3 criteria are considered together, the ideal biphenyl structure for the facilitation of metabolism is 2,2'-dibromobiphenyl (DBB). This compound is metabolized at a very rapid rate (Dannan et al., 1978b) and similar findings have been reported for 2,2'-dichlorobiphenyl (DCB) (Greb et al., 1975; Hesse and Wolff, 1977). Only 2 of the major congeners appear to be rapidly metabolized (Aust and Dannan, 1978; Dannan et al., 1978b). These are represented by peaks 1 and 3 (2,2',4,5,5'-PBB_5 and 2,2',3,4',5',6-HBB, respectively). Each compound has one para substitution and their rates of metabolism are slower than that of 2,2'-DBB (Moore et al., 1980).

Except for the 2 congeners just mentioned, all other congeners with known structures have 2 para subsitutions. This seems to be a key factor in the retardation of metablism (Dannan et al., 1978b) and also a factor in the stimulation of microsomal enzyme activity.

Biphenyl compounds can be classified according to the type of microsomal enzyme activity stimulated by their administration. In general, there are 4 types. Non-inducers such as 2,2'-DBB are often compounds which are metabolized quickly (Moore et al., 1979). The ones that do induce enzyme activity are classified as phenobarbital (PB)-type, 3-methylcholanthrene (MC)-type, or as a mixed (PB and MC)-type (Dent, 1978). Of the congeners in the commercial mixture, 2,2',4,4',5,5'-HBB (peak 4) (Moore et al., 1978c), 2,2',3,4,4',5,5'-HBB_7 (peak 8) (Moore et al., 1979), and 2,2',3,3',4,4',5,5'-OBB (peak 12) (Besaw et al., 1978) represent 84-85% of the commercial mixture by weight and are all PB-type inducers. All of these compounds share characteristics which contribute to their induction ability. They all contain at least 2 ortho substitutions, at least 2 meta substitutions (one/benzene ring), and at least one para substitution. A chlorinated analog of the congener represented by peak 4 is 2,2',4,4',5,5'-hexachlorobiphenyl (HCB). It also is a PB-type inducer (Goldstein et al., 1977).

Approximately 3 to 4% of FM by weight is 2,3',4,4',5,5'-HBB (peak 6) and this compound, like the commercial mixture, is classified as a mixed-type inducer (Dannan et al., 1978a,c). To have some MC-type inducing properties it must meet the structural requirements of that type of inducer and also possess the needed qualifications for a PB-type inducer. The amount of MC-type inducing potential may vary among mixed-type inducers and may be dose related.

Robertson et al. (1980) reported that 2,2',3,4',5-PBB_5 (peak 2) is a mixed-type inducer. Dannan et al. (1979) found this compound to be mostly an MC-type inducer and a weak PB-type inducer at different dosage.

The compound 3,3',4,4',5,5'-HBB and its chlorinated analog 3,3',4,4',5,5'-HCB are both strictly MC-type inducers (Poland and Glover, 1977). To be MC-type inducers they must have para substitutions, meta substitutions and a planar configuration. The last 2 requirements are necessary to bind to a cytoplasmic receptor. Binding to the receptor is associated with the induction of aryl hydrocarbon hydroxylase (AHH). An assay for this enzyme is utilized to detect MC-type induction (Poland et al., 1979).

The enzymes (proteins) induced by MC- and PB-type inducers are located on the external surface of the microsomal membrane (Welton and Aust, 1974). Microsomal membranes or microsomes are small vesicles formed by the differential centrifugation of hepatocytes (Blumberg, 1978) and are small portions of endoplasmic reticulum. These enzymes catalyze reactions in which oxidation of xenobiotics is of primary importance and require NADPH and oxygen (Wilkinson, 1980). Since one atom of molecular oxygen is incorporated into the substrate and the other oxygen is reduced to water, the reaction is classified as mixed-function oxidation. This reaction employs 2 enzymes, NADPH-cytochrome P-450 reductase and cytochrome P-450, which is the substrate-binding terminal oxidase. These enzymes are collectively called the mixed-function oxidase (MFO) system.

In a review article by Blumberg (1978), he stated that cytochrome P-450 is an enzyme which contains a heme and a protein. It derived its name from the fact that when carbon monoxide was added to the reduced

form and then compared to the reduced form without carbon monoxide added, the difference of the optical absorption peak occurred at 450 nm. Wilkinson (1980) mentioned that there are multiple forms of cytochrome P-450. When the activity of the cytochromes is induced by the administration of different compounds, they can be divided into 2 major groups. Phenobarbital increases the microsomal content of cytochrome P-450. With 3-methylcholanthrene, the peak of the CO-difference spectrum shifted to 448 nm. This compound induced the activity of cytochrome P-448 (P_1-450).

As mentioned earlier, stimulation of AHH activity is a measure of cytochrome P-448. Poland et al. (1979), in a review article, discussed the structure-function relationships among compounds which are AHH inducers. The classic inducer of this enzyme activity is MC, but TCDD is 30,000 times as potent in causing induction. Both apparently act on the same receptor. Compounds which are approximately stereoisomers of TCDD and have molecular structures that will allow them to fit into a rectangle 3 x 10 Å will bind to this receptor.

A limited number of chlorinated and brominated biphenyl compounds were examined and 3,3',4,4'-tetrachlorobiphenyl (TCB), 3,3',4,4',5,5'-hexachlorobiphenyl (HCB) and 3,3',4,4',5,5'-HBB were found to bind to this receptor (also reported by Poland and Glover, 1977). Apparently, lateral halogens (probably meta substitutions) and a planar configuration are essential requirements for binding to this receptor. This idea was supported by Poland and Glover (1977) when they reported that chlorinated biphenyls that have chlorine atoms substituted in positions 2,2',6 or 6' (which produce nonplanarity) did not induce AHH activity and did not compete for the hepatic cytosolic receptor.

There is also biochemical evidence in inbred strains of mice that the Ah locus is the structural gene for this cytosolic receptor protein (Thorgeirsson and Nebert, 1977). There are 2 regulatory genes referred to as Ah-1 and Ah-2. The latter gene codes for the regulatory protein for the former gene. The Ah-1 gene then codes for the receptor protein. They also mentioned that there is evidence for an Ah locus in humans.

Poland et al. (1979) also gave a model for the mechanism for the induction of AHH. In summary, the inducing compound, for example 3,3',4,4',5,5'-HBB, enters the hepatocyte because it is lipophilic and then binds to the cytosolic receptor. The complex of receptor and compound then translocates to the nucleus and initiates transcription of the gene(s) which code for cytochrome P-448 (AHH activity). The messenger ribonucleic acid (mRNA) leaves the nucleus, moves to the rough endoplasmic reticulum (RER) and translation of new proteins (enzymes) occurs.

Safe et al. (1978) stated that AHH-mediated metabolism involves the formation of an arene-oxide. Epoxides are formed in this manner (Thorgeirsson and Nebert, 1977). Epoxides have been identified as an intermediate in the formation of phenols, trans-dihydrodiols and pre-mercapturic acids (Jerina and Daly, 1974). The rates at which these are formed are related to the steady-state concentrations of epoxides. The epoxides which are not converted to these products are free to covalently bind with macromolecules such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and protein. This provides a molecular basis for the cytotoxicity, mutagenicity and carcinogenicity of AHH inducers (Thorgeirsson and Nebert, 1977).

Bromobenzene metabolism is an example of how an upset in the steady state conditions of an epoxide can lead to pathologic changes (Mitchell et al., 1976). When glutathione is depleted in the hepatocytes, more epoxides will be available for covalent binding to protein. This binding has been associated with hepatic necrosis. In this case, glutathione synthesis is the rate-limiting step in the formation of hepatic lesions due to bromobenzene.

In a review article by Nebert et al. (1976), they mentioned that at least 2 different AHH activities exist, one associated with P-450 and the other associated with P-448. These different forms of P-450 may generate different ratios of metabolites because of different chemical positions hydroxylated on the molecule. The reactivity of intermediates may have marked dissimilarities in their toxicity or carcinogenicity. They also gave an example which is referred to by Blumberg (1978). Cytochrome P-450 induced by PB results in the formation of a p-phenolic derivative of bromobenzene which presumably arose from a 3,4-epoxide intermediate. This intermediate is associated with hepatic necrosis. Cytochrome P-450 induced by MC resulted in the formation of an o-phenolic derivative of bromobenzene. This came from a 2,3-epoxide. No hepatic necrosis was associated with this intermediate. In this case induction of AHH was beneficial.

In 1976, Wyndham et al. reported that the metabolism of 4-chlorobiphenyl proceeds via an epoxide intermediate and the epoxide may bind to RNA and protein. Shimada (1976) indicated that PCB can also bind to DNA. Dannan et al. (1978b) used a radioactively labeled (^{14}C) mixture consisting almost exclusively of 2,2',4,4',5,5'-HBB (peak 4) and 2,2',3,4,4',5,5'-HBB_7 (peak 8) which was incubated with induced microsomes and NADPH. They found no radioactivity associated with

exogenous DNA. They did find that FM-induced microsomes enhanced the binding of radioactively labeled (^{3}H) benzo[a]pyrene metabolites to DNA. Although PBB assists in the binding of compounds to macromolecules and does not bind to them itself, a lot of questions are still unanswered. Dannan et al. (1978b) concluded that if any of the congeners would be possible candidates for being metabolized to DNA-binding derivatives, it would be 2,2',4,5,5'-PBB_5 (peak 1) or 2,2',3,4',5',6'-HBB (peak 3). These 2 congeners are readily metabolized.

Wyndham et al. (1976) reported that 4-chlorobiphenyl was a mutagen to *Salmonella typhimurium* strain TA 1538 in the Ames test (Ames et al., 1973). In 1978, Kohli et al. reported that 4-bromobiphenyl was also a mutagen as classified by the Ames test. This chemical was dependent upon the presence of Aroclor (AR) 1254-induced rat liver microsomal enzymes for metabolic activation. Moore et al. (1980) stated that to date there are no reports of mutagenicity of FM as shown by the Ames test.

Some common MC-type inducing agents possess inherent tumorigenic (initiating, promoting, or both) properties in addition to their enzyme-inducing properties (DiGiovanni et al., 1979). An exception is TCDD; tumorigenic properties are not associated with this chemical. Haroz and Aust (1979) tested FM and 2,2',4,4',5,5'-HBB (peak 4) for these properties. Neither one exhibited either type of activity. They also reported that preliminary results indicated that 2,3',4,4',5,5'-HBB (peak 6) also lacks both of these activities.

Firemaster, as already mentioned, is characterized as a mixed-type inducer in adult and immature rats (Moore et al., 1978a), even though Werner (1979) found no induction of hepatic microsomal enzymes in newborn piglets. Apparently there is age and species variation.

Dent (1978) mentioned that the P-448 associated enzymes are stimulated prior to the P-450 ones. This order of stimulated enzymes is reversed for adult rats. In rainbow trout, FM and PCB only stimulate MC-type induction and the stimulation does not appear to be accompanied by the synthesis of cytochrome P-448 (Elcombe and Lech, 1978). Of the 2 inducing agents, FM is considered more potent on a weight basis than PCB. Both are also known to stimulate MFO activity in the liver and extrahepatic tissues (McCormack et al., 1979).

Toxicity of Firemaster and Related Compounds

Many people have examined the pathologic effects of FM in laboratory animals, especially rats. In 1976, Sleight and Sanger reported that after 30 days of treatment, rats ingesting diets containing 1, 10 or 100 ppm of FM BP-6 did not have clinical signs of toxicosis. Pratt (1979) also reported no clinical signs in rats given FM BP-6. Gupta and Moore (1979) gave 30, 100, 300 or 1000 mg/kg/day of FM FF-1 orally to rats for 5 days/week for 4.5 weeks. They observed the rats for 90 days. Rats given either the 1000 mg or 300 mg dose died before all doses could be given. All female rats and 38% of the male rats died between 41 and 73 days after the last dose was given. Moribund rats had a hunchback posture, rough hair coat, and sunken eyes, and they appeared lethargic, dehydrated and emaciated.

McConnell and Moore (1979), in reviewing the lesions associated with halogenated aromatic hydrocarbons (dibenzofurans, dibenzo-p-dioxins, naphthalenes and PCB), reported that they all produced a similar clinical syndrome in nonhuman primates. Alopecia, edema and acneform eruptions (chloracne) are all a part of this syndrome. Rabbits had acne-like changes but, other than weight loss, very few clinical signs are observed in rats and guinea pigs.

Sleight and Sanger (1976) reported that weight gain and feed efficiency were decreased for rats fed FM BP-6 at a dietary level of 500 ppm. Guinea pigs also had extreme weight loss at the 100 and 500 ppm levels. In 1978, Sleight et al. reported that at the 100 ppm dietary level, rats had slower rates of weight gain. McCormack et al. (1978a,b) also noted decreased weight gains, but Pratt (1979) reported weight gains in rats getting FM BP-6 to be even better than control rats in some cases. Garthoff et al. (1977) compared the effects of FM BP-6 to those of AR 1254 in rats and found both were associated with decreased growth and food efficiency in rats. Only AR 1254 was associated with decreased food intake.

All rats in the experiment by Sleight and Sanger had increased liver to body weight ratios regardless of the treatment level. Kidney to body weight ratios were not affected. Many researchers have reported similar findings (Hinton et al., 1977; Garthoff et al., 1977; Corbett et al., 1978; McCormack et al., 1978a,b; Pratt, 1979; Kluwe et al., 1979; Harris et al., 1978a; McCormack and Hook, 1979). Sleight et al. (1978) reported a smaller increase in liver weight of rats fed an iodine-deficient diet to which FM BP-6 was added. Garthoff et al. (1977) reported that in general FM caused a greater increase in liver weight than AR but that in both changes were time- and dose-related. Sleight and Sanger (1976) and Hall (1980) reported increased liver weights in adult guinea pigs given FM BP-6, but Hall mentioned that FM-treated piglets had decreased liver weights. Increased kidney weights were reported in young rats which got PBB from their dams and then in the diet at a level of 100 ppm (Kluwe et al., 1979).

Other organ weights which are reported to not change as a result of PBB administration in rats are the seminal vesicles, adrenal and

testes (Harris et al., 1978b). Garthoff et al. (1977) and Corbett et al. (1978) also reported no change in the testes weight. McCormack and Hook (1979) reported no changes in the ovary to body weight ratio, uterus to body weight ratio or testes to body weight ratio in rats. Brain weights also were unaffected (Pratt, 1979).

In rats given FM, the erythrocyte count, packed cell volume, hemoglobin, and total and differential leukocyte counts were not affected (Sleight and Sanger, 1976; Garthoff et al., 1977; Sleight et al., 1978; Pratt, 1979; McCormack and Hook, 1979). This is in contrast to the effect of other halogenated aromatic compounds (McConnell and Moore, 1979).

Sleight and Sanger (1976), Garthoff et al. (1977), McCormack et al. (1978a,b) and Sleight et al. (1978) all reported blood urea nitrogen (BUN) values within normal limits. Hall (1980) reported elevated BUN values in newborn guinea pig piglets born from dams ingesting FM BP-6.

Sleight and Sanger (1976) reported hepatocellular swelling with vacuolation, which was confirmed to be lipid, in rats ingesting feed containing 10 and 100 ppm of FM BP-6 for 30 and 60 days. This change appeared to be dose-related. Kimbrough et al. (1978) noticed a similar change but to a lesser degree 24 hours after giving rats FM FF-1 orally at the dosage of 1000 mg/kg. Pratt (1980) also noticed this type of change. In rats ingesting dietary FM BP-6 for 3 or 6 months, the vacuolation appeared to be limited to the midzonal area. With time the vacuolation extended to the periphery of the lobule and occasionally to the centrolobular area. Sleight et al. (1978) stated that the vacuolation of hepatocytes appeared to be most severe in iodine-deficient rats which were fed FM BP-6.

Focal necrosis was seen after months. McCormack et al. (1978a) also reported focal necrosis in the midzonal region of the hepatic lobule. Gupta and Moore (1979) reported that moribund rats which were killed or rats which died had similar hepatic changes, which were primarily around the portal area.

Kimbrough et al. (1978) reported pronounced hepatic changes in livers of rats which were killed 2, 6, 10 or 14 months after being dosed with FM FF-1. The changes consisted of centrolobular hepatocellular enlargement with vacuolation, cytoplasmic inclusions, bi- and multinucleation, pleomorphism, mitotic figures, areas of fibrosis and round cell infiltration. The latter group of researchers described "hyperplastic" or neoplastic nodules which were composed of cells which were enlarged and had cytoplasm which was either clear and eosinophilic or like ground glass in appearance. The normal architecture was absent and individual cells were not well demarcated. Nodules were sometimes multiple, more common in male rats, and seen 10 and 14 months after the treatment. Similar neoplastic nodules were reported by Weltman and Norback (1979) in hepatocytes from rats getting 2,2',4,4',5,5'-HCB at a level of 100 ppm over a 2-year period. Several hyperplastic nodules were also reported in 1 sow fed a diet containing 100 ppm of FM BP-6 (Werner, 1979). Gupta and Moore (1979) noticed that some of the male rats which received either the 30 mg or 100 mg daily dosage of FM FF-1 had atypical liver nodules which were composed of either foamy clear cells or eosinophilic cells. These nodules were clearly demarcated and often compressed the adjacent parenchyma.

Pratt (1979) reported bile duct hyperplasia in 2 of 5 rats which were fed diets containing 1 ppm for 18 months. These livers also had

marked periportal fibrosis. Sleight et al. (1978) reported bile duct hyperplasia and portal fibrosis in iodine-deficient rats which were fed FM BP-6 at a level of 100 ppm for 60 days. Similar changes were associated with PBB toxicosis in a cow (Gutenmann and Lisk, 1975) and in rhesus monkeys (Allen et al., 1978). Kociba et al. (1979) also reported bile duct hyperplasia with periportal inflammation and fibrosis in the livers of rats ingesting diets containing TCDD for 2 years. Proliferative changes in bile ducts have been observed in rats after feeding them mixtures of chlorinated naphthalenes and chlorinated biphenyls (Kimbrough, 1974). McConnell and Moore (1979) mentioned that in chronic exposures to halogenated aromatic hydrocarbons, bile duct hyperplasia was found in varying degrees in all animal species.

Gupta and Moore (1979) observed 2 types of bile duct proliferation. One type was diffuse and similar in appearance to bile duct hyperplasia. The other type was focal. The acini were small and formed by epithelial cells with small and hyperchromatic nuclei located near the basal portion of the cell. The apical portion of these cells contained red granules which were very similar to the zymogen granules of pancreatic exocrine glands. A similar condition has been reported in rats ingesting PCB mixtures and was classified as pancreatic cell metaplasia (Institute of Lsboratory Animal Resources, 1980).

Hepatic changes associated with administration of halogenated aromatic hydrocarbons are conspicuous in mice, rats, rabbits and chickens but minimal in monkeys and guinea pigs (McConnell and Moore, 1979). Hepatomegaly is related to an increase in the endoplasmic reticulum. Focal necrosis of hepatocytes with inflammatory infiltrates,

cytomegaly and fatty change are reported for rats and mice. Hepatocellular necrosis with hemorrhage is the hallmark of acute toxicity in rabbits. Varying degrees of bile duct hyperplasia have been associated with chronic exposures.

Ultrastructurally, Sleight and Sanger (1976) noticed an increase in the size of the mitochondria in hepatocytes of rats fed concentrations of FM BP-6 at the 1 and 10 ppm level. At the 100 ppm level, myelin bodies, proliferation of the SER and abundance of cytoplasmic vacuoles were noticed. These changes were more pronounced at the 500 ppm level. Myelin bodies, proliferation of the SER and abundance of cytoplasmic vacuoles have been reported by many researchers working with PBB (Hinton et al., 1977; Sleight et al., 1978; Pratt, 1979). Myelin bodies have also been reported in guinea pigs and rat pups nursing dams fed a diet containing FM BP-6 (Sleight and Sanger, 1976). Hinton et al. (1976) fed FM to rats at various dietary levels and also reported an increase in the number of liposomes, a marked proliferation of Golgi condensing vesicles containing lipoprotein particles, a decrease in the number of mitochondria and a decrease in lysosomes. These changes appeared to be dose-related. Additional ultrastructural findings by Corbett et al. (1978) consisted of a decreased amount of RER, mitochondrial degeneration, a decreased amount of glycogen, proliferation of microvilli and an increased size and number of nucleoli. Dilatation of the RER, disorganization of the RER, reduction of the RER and mitochondria which were enlarged, reduced in numbers and containing disintegrating cristae were additional findings reported by Pratt (1979).

Gupta and Moore (1979) found that all female rats that survived for 90 days had dark livers. The livers, teeth and bones all had an

intense reddish-pink fluorescence when examined under long wavelength ultraviolet rays. This indicates excess accumulation of porphyrin. None of the ones that died nor the surviving male rats had tissues which were fluorescent.

Kimbrough et al. (1978) also noted that some of the livers from FM-treated female rats had pink fluorescence under ultraviolet light. They also noticed that the Kupffer cells from male and female rats contained a brown pigment histologically.

Many other polyhalogenated hydrocarbons, including TCDD and PCB, are also able to produce porphyria. The most porphyrinogenic compound ever produced by man is considered to be TCDD (McConnell and Moore, 1979), but the commercial PBB mixture is considered more porphyrinogenic than HCB or AR in avian systems (Strik, 1978). Chronic exposure, which is related to liver damage, is needed to produce the porphyria, especially in mammals. This idea is supported by findings of Kimbrough et al. (1978).

In 1973, Strik described experimental porphyria caused by polyhalogenated aromatic compounds in various laboratory animal and avian species. Porphyria caused by PCB, HCB and HBB was detected in the rat and rabbit but not in young rats, guinea pigs, mice, mink and minipigs, even though death was reported for all these species. In the species with porphyria, liver injury, weight loss and tremors were also reported. Kimbrough (1974) reported that porphyria did not occur in mammals until several months after dosing was started. Females and embryos were apparently more susceptible than males.

Heme synthesis is stimulated by HBB in the mouse, rat and Japanese quail (Strik, 1973). This is characterized by an increase in δ-aminolevulinic acid synthesis (ALAS) activity in the liver. A

dose-related increase in ALAS activity is produced by TCDD (Kimbrough, 1974). Sinclair (1980) mentioned that 3,3',4,4',5,5'-HBB produced a strong increase in this activity, whereas 2,2',4,4',5,5'-HBB produced a weaker increase. It is possible that ALAS induction varies with age, sex, species and time.

Strik (1973) noticed that heme synthesis increased as heme content decreased. This was seen for HBB, HCB and PCB. Although heme is needed for the de novo synthesis of cytochromes, the decrease in heme content may be due to a disturbance in protoporphyrin synthesis. Protoporphyrin synthesis is arrested in the stage of decarboxylation and protoporphyrin is no longer produced.

Sinclair (1980) mentioned that protoporphyrin increased when 2,2',4,4',5,5'-HBB was administered but uroporphyrin accumulated when 3,3',4,4',5,5'-HBB was administered. This was due to the inhibition of uroporphyrinogen decarboxylase. A relationship between induction of AHH activity and reduction in uroporphyrinogen decarboxylase activity has been reported by Jones and Sweeney (1977).

As porphyrins accumulate, iron is stored. Sweeney et al. (1979) discovered that porphyria, hepatocellular damage and certain other toxic effects of TCDD did not occur in iron-deficient mice which were given TCDD. The mechanism is not known.

Gupta and Moore (1979) reported thymic involution and small spleens in rats fed FM FF-1 at high doses and which died. Histologically, the normal architecture of the thymus was obliterated, with loss of demarcation between the cortical and medullary regions. In the spleens, there was lack of periarterial lymphoid cells, the capsule of the spleen appeared thickened and irregular, the red pulp was hypocellular and there was occasional necrosis of lymphoblastic cells.

Vos and vanGenderen (1973) reported thymic cortical atrophy and marked depletion of the follicles (humoral immunity) and periarteriolar lymphocyte sheaths (cell-mediated immunity) in guinea pigs given HBB. In 1977, Moorhead et al. reported atrophy of the thymus in cattle given FM BP-6. Kasza (1977) reported some thymic involution, lymph node lymphocyte depletion, especially in the T-cell zone, and a moderate decrease in the lymphocytes in the white pulp of Beagle dogs given FM. Lymphocytic depletion of the bursa of Fabricius, thymus and spleen was reported in chicks by Ringer (1978). Bekesi et al. (1978) reported decreased circulating blood lymphocytes with altered function in Michigan dairy farmers exposed to PBB.

Luster et al. (1978) reported a depressed cell-mediated immunity in both rats and mice which was associated with FM FF-1 administration. In both species there was a decreased spleen and thymus weight, but histologically there were no effects except for a slight decrease in the density of the thymic cortex. The immunosuppression is apparently subtle and occurs at higher levels of PBB administration. They found that at low levels, the immune activity was enhanced. Although FM appears to primarily affect cell-mediated immunity, humoral immune functions were also depressed when mice received a daily dose of 30 mg/kg for 22 treatments in 30 days. In contrast, Fraker and Aust (1979) reported that PBB may have a deleterious effect on B-cells and helper T-cells, but it is still unclear if PBB has an effect on the subsets of T-cells involved in cell-mediated immunity. The mechanism responsible for PBB-induced immunosuppression is still unclear.

In all the laboratory animal species studied which were exposed to different halogenated aromatic hydrocarbons, McConnell and Moore (1979) found loss of body fat and thymic involution to be the most

consistent findings in the literature. The reduction in thymic size was associated with a loss of cortical lymphocytes caused by some degree of lymphocytic necrosis. Numerous macrophages phagocytizing necrotic debris were often present. It was difficult to differentiate the cortex from the medulla. After the involution has occurred, which is in less than 10 days in most species, the necrotic events are less conspicuous. If an animal does not die, the microscopic appearance of the thymus may be relatively normal but the weight may be less than normal. When guinea pigs were given sublethal quantities of TCDD, depletion of lymphocytes was noticed in the thymus, spleen and lymph nodes (Kimbrough, 1974). Kociba et al. (1979) found thymic and splenic atrophy in rats which had received 0.1 µg/kg/day of TCDD for 2 years. Even after a single dose of 25 µg/kg of TCDD, reduced thymic and splenic weights were observed (Kimbrough, 1974). Reduction in the size of the spleen, lymph nodes and gut-associated lymphoid tissue may also be observed with the other halogenated aromatic hydrocarbons.

McConnell and Moore (1979) reported that reproductive difficulties were a part of the clinical syndrome associated with halogenated aromatic hydrocarbon toxicosis observed in nonhuman primates. In rhesus monkeys prolonged menstrual cycles, decreased concentrations of serum progesterone and excessive postconceptional bleeding were all associated with FM FF-1 administration (Allen et al., 1978; Lambrecht et al., 1978). Infants born to these females were small and failed to gain weight as rapidly as controls. Decreased birth numbers and decreased kit survival are aspects of PBB toxicosis in mink (Aulerich and Ringer, 1979). Ringer and Polin (1977) reported decreased egg production and hatchability. Hatched chicks had subcutaneous edema. Fetal deaths, abnormal fetal size and fetal

malformation were not associated with exposure of rats to FM BP-6. Length of estrous cycle was unaffected (McCormack and Hook, 1979). However, Corbett et al. (1975) reported the mean fetal weight was decreased in rats and mice and inversely related to the dosage of FM BP-6.

Although PBB may be transferred via the egg in avian species (Fries et al., 1976) and via the placenta in mammalian species, nursing appears to be a much more important mechanism of PBB transfer to mammalian neonates (Werner, 1979). Dent (1978) reported that the mammary gland of pregnant rats had higher concentrations of PBB than the liver. McCormack et al. (1979) reported that in comparing PCB to PBB, rat milk contained higher concentrations of PCB than PBB, indicating that this route of excretion may be more important for the former. Excretion of PBB in the milk is the topic of research and discussion in other articles in the literature (Fries and Marrow, 1975; Willett and Irving, 1976; Murata et al., 1976; Fries, 1978; Fries et al., 1978; Cook et al., 1978; Robl et al., 1978; Willett and Durst, 1978).

Werner (1979) discovered that swine piglets consumed a somewhat different group of PBB than the PBB in the mixture given their dams. The PBB present in the milk apparently came directly from the sow's adipose tissue without being metabolized in the liver. Rickert et al. (1978) reported that rat neonatal livers contained higher concentrations of PBB than the livers from their dams. This was also reported by Hall (1980) in guinea pig piglets. Rickert et al. (1978) speculated that since the levels are higher, neonates may not be more susceptible to the toxic effects of PBB, as suggested by Kluwe et al. (1979) and Moore et al. (1978a).

Kasza et al. (1978) compared the histological and ultrastructural changes seen in the thyroid in association with the administration of PBB or PCB. Both compounds produced similar and dose-dependent changes as a result of daily dietary administration and appeared to interfere with the synthesis and secretion of thyroxine. In general, follicular cells were tall columnar with a basally placed nucleus. Occasionally papillae and cytoplasmic processes extended into the luminal colloid.

Sleight et al. (1978) reported that thyroid weights were increased in rats fed FM. At a dietary level of 100 ppm, rats which were getting iodine-deficient diets or iodine-excess diets had thyroids with irregularly-sized follicles. They were hypercellular and colloid was sparse. Thyroid changes similar to those reported by Kasza et al. (1978) were also described by Sleight and Akoso (1979) and Akoso et al. (1980).

Sleight et al. (1978) reported an interference with vitamin A metabolism in rats fed diets containing an excess of iodine and FM. Pratt (1979) reported decreases in liver vitamin A content in rats given diets containing FM which were apparently iodine-adequate. Mangkoewidjojo (1979) reported a dose-dependent depression of hepatic vitamin A content in rats fed 1 or more ppm of FM. Pratt (1979) stated that PCB, DDT, methoxychlor and chlorinated naphthalenes have all been associated with depression of hepatic vitimin A content. He suggested that all these halogenated hydrocarbons, including PBB, decrease the vitamin A content by inducing microsomal enzymes which increase the decomposition of the vitamin.

The type of diet may affect the toxicosis of PBB. Kimbrough et al. (1980) reported that liver lesions were more severe when rats were ingesting synthetic diets in comparison to commercial rat feeds.

Kluwe et al. (1979) reported dilatation of the renal pelvis in young rats getting PBB from their dams and then in the diet. No treatment-related lesions were found in the kidney, brain, heart, lung, adrenals, stomach, small intestine, colon, testes and urinary bladder by Sleight et al. (1978) and Pratt (1979) in rats given FM BP-6. Sleight et al. (1978) also found no changes in the pancreas and skeletal muscle and Pratt (1979) also found no changes in the salivary glands. Hyperplasia and squamous metaplasia of the epithelial cells lining the ductus deferens were reported in some male rats that died after getting treated with FM FF-1 (Gupta and Moore, 1979). Ku et al. (1978) reported gastric hyperplasia in pigs given FM BP-6 and proliferative lesions of the gastrointestinal tract are reported in monkeys exposed to halogenated aromatic hydrocarbons (McConnell and Moore, 1979). Gastrointestinal lesions in other laboratory animal species are occasionally found terminally in association with these compounds.

Toxicity of Individual Compounds

Non-Inducers

The toxicity of a biphenyl compound apparently depends upon how it is metabolized. Moore et al. (1979) reported that 2,2'-DBB was nontoxic when administered to rats in an amount which was 5000 times the amount of the dibromobiphenyl present in the commercial PBB mixture. Hansell and Ecobichon (1974) reported that 2,2'-DCB administration had no effect on the ultrastructural morphology of rat livers. Both of these compounds are metabolized quickly. Other

compounds which are metabolized in this category, like 2,2',4,5,5'-PBB_5 (peak 1) and 2,2',3,4',5',6-HBB (peak 3), should be nontoxic, too. Reports are still lacking to prove or disprove this hypothesis.

Phenobarbital-Type Inducers

In 1978, Besaw et al. examined the pathologic effects of one of the PB-type inducers, 2,2',3,3',4,4',5,5'-OBB (peak 12). They found that rats, after being given 90 mg/kg by intraperitoneal (IP) injection 1 week earlier, had histologically normal appearing livers, even though there was an increase in the liver to body weight ratio.

Earlier Lee et al. (1975a,b) described the pathologic changes and tissue distributions associated with administration of OBB. This product was a mixture of hepta-, octa-, nona- and decabromobiphenyls, which was referred to as OBB because it averaged approximately 8 bromine atoms per molecule. In one experiment they fed young adult rats diets containing 1, 10, 100 and 1000 ppm of OBB for 2 or 4 weeks. In another experiment they gave rats either a single oral administration of 1000 mg/kg or 2 consecutive doses of 3000 mg/kg and then killed them at certain time intervals. No significant histological changes were reported in the liver in rats fed diets containing 1 and 10 ppm, although there was a dose-related accumulation of bromine in fat, liver and muscle at the 10 ppm level and higher levels. After 2 weeks the amount of bromine in the fat and the liver was approximately the same and greater than the amount in the muscle at the 100 and 1000 ppm levels. After 4 weeks the amount of bromine in the fat was greater than that in the liver at these same levels. After withdrawal of OBB from the diet, the amount of bromine in the liver decreased but the amount in the fat persisted.

The latter researchers mentioned that hepatic changes were dose- and time-related at the 100 and 1000 ppm level. Hepatocellular swelling and obliteration or narrowing of sinusoidal spaces was first noticed in the centrolobular area of the hepatic lobule and extended to the periphery of the lobule at the 1000 ppm level and only to the midzonal area for the 100 ppm level with time. The cytoplasm of the cells had basophilic clumping which was limited to a zone adjacent to the cell membrane. The perinuclear cytoplasm was pale, foamy, granular or vesicular. Cytoplasmic inclusions were visible and fine cytoplasmic lipid droplets were confirmed by oil red O stain. After withdrawal of OBB from the diet, the changes in the peripheral areas began to subside. After 18 weeks the livers from rats formerly given 100 ppm appeared almost normal. The livers from rats formerly getting a diet containing 1000 ppm still had mild hepatocellular changes at that time. Lee and associate described ultrastructural changes. Proliferation of the smooth endoplasmic reticulum (SER) was seen in the perinuclear cytoplasm. The normally parallel rough endoplasmic reticulum cisternae were dilated, denuded of ribosomes and transformed into vesicular profiles of various sizes that contained moderately electron-dense granular material. As the amount of SER became more abundant, the amount of RER became diminished. Lipid bodies of various sizes were enclosed by several laminated smooth membranes, some of which contained ribosomes. Others did not, but the outer membrane pairs were continuous with the SER. The inclusions seen histologically were concentrically laminated paired arrays of smooth membranes called myelin figures. The outer membrane pairs of these figures were continuous with either the RER or the SER. The inner portion of the inclusions was usually occupied by several lipid droplets. These increased in

size and internal complexity with prolonged treatment or increased dosage. The mitochondria appeared normal but lysosomes and membrane-limited vacuoles containing whorled figures increased in hepatocytes after the rats were withdrawn from the treated diets.

Hansell and Ecobichon (1974) gave rats 2,2',3,3',4,4',5,5'-octachlorobiphenyl (OCB) for 3 consecutive days at a daily IP dose of 50 mg/kg. Four days after the injection they were killed. The hepatic changes included cytoplasmic vacuolization and small foci of hepatic necrosis located in the periphery of the lobule. Ultrastructurally there was an increase in the SER, lipid droplets and microbodies.

Moore et al. (1979) examined the pathologic effects of another PB-type inducer, 2,2',3,4,4',5,5'-HBB_7 (peak 8). After a single IP injection (90 mg/kg), rats developed hepatocellular swelling with vacuolization that disappeared in approximately 3 weeks. No changes were in the brain, heart, stomach, small intestine, pancreas, testes, thymus, spleen and kidneys.

As already mentioned, 2,2',4,4',5,5'-HBB (peak 4) is also a PB-inducer. Because it was the major congener in FM, it was used in many experiments in an attempt to describe its pathologic effects. Moore et al. (1978c) gave this congener in a single IP injection (90 mg/kg) to rats. After 2 days the rats had enlarged livers with swollen and vacuolated hepatocytes. The swelling was diffusely distributed with the midzonal region most severely affected. The vacuolization was confirmed to be fat by an oil red O stain. No changes were apparent in the brain, heart, stomach, small intestine, pancreas, thymus, spleen and kidneys when examined up to 14 days after the injection.

In 1979, Akoso and Sleight (also Akoso et al., 1980) described the changes in the liver and thyroid of fats fed levels of 1, 10 and

100 ppm for 30 and 60 days. Again, hepatocellular swelling with cytoplasmic vacuolization which appeared to be lipid droplets ultrastructurally were noted. Also ultrastructurally there was proliferation of the SER and mitochondrial swelling. The thyroid had follicular cell hyperplasia and loss of or scanty colloid. Some follicular cell hypertrophy, increased amount of dense bodies, prominent cytoplasmic projections, and dilated RER were seen.

Also in 1979, Ecobichon et al. reported increased liver weights, hepatocellular hypertrophy as confirmed by light microscopy and a decreased amount of hepatic DNA and RNA. Some necrotic foci occurred throughout the lobules and hepatocellular cytoplasm was vacuolated, especially in the periphery of the lobule. Ultrastructurally, proliferation of the SER and lipid droplets were observed. These changes were all associated with the administration of 2,2',4,4',5,5'-HBB (peak 4) in rats.

In earlier work with rabbits and using the chlorinated analog 2,2',4,4',5,5'-HCB, Vos and Notenboom-Ram (1972) reported hepatocellular necrosis, focal cytoplasmic hyaline degeneration and a peripheral and perinuclear shift of all organelles. The hyalinized cytoplasm was recognized as tightly packed tubules of proliferating SER, which was considered to be hypertrophic and hypoactive. With this change there was also displacement of the RER and mitochondria.

In 1979, Weltman and Norback reported the pathologic effects of 2,2',4,4',5,5'-HCB in rats which were fed 100 ppm over a 2-year period. They reported pigmentation, centrolobular to midzonal hepatocellular hypertrophy, hepatocellular hyperplasia and neoplastic nodules, in sequence. Ultrastructurally, there were interdigitations of the plasmalemma, proliferation of the SER, disorganization of the RER,

many polyribosomes, numerous large and lucent mitochondria, pigmented lysosomes, convoluted nuclei and multiple nucleoli. Cysts lined by ductal epithelium were also reported. In comparison to 2,2',5,5'-TCB, 2,2',4,4',5,5'-HCB produced more hepatic changes. This was also shown by Ecobichon et al. in 1979.

Hansell and Ecobichon (1974) found similar hepatic alterations for 2,2',4,4',5,5'-HCB as it did for 2,2',3,3',4,4,'5,5'-OCB. These ultrastructural findings were also similar to those seen with 2,2',4,4',6,6'-HCB, o,p'-DDT, 2,2',3,3',5,5'-HCB, AR 1254, AR 1260 and p,p'-DDT. The last 4 chemicals were the only ones to significantly increase liver weights.

Bairstow et al. (1978) examined the effects of 2,2',4,4',5,5'-HBB (peak 4), 2,2',4,4',5,5'-HCB and FM FF-1 on C3H/10T 1/2 mouse embryo fibroblasts. The HCB was more toxic than FM FF-1, which was more toxic than the HBB. Abnormal findings consisted of loss of post-confluent inhibition of cell division and increased numbers of lysosomes and autophagic vacuoles. The cells treated with FM FF-1 also had a decrease in the surface villi, which probably was why they attached poorly to plastic.

The toxic effects of both 2,2',4,4',5,5'-HBB (peak 4) and 2,2',4,4',5,5'-HCB have been examined in chickens. Polin et al. (1980) compared the effects of 2,2',4,4',5,5'-HBB to the effects of FM. Although the chicks hatched from eggs laid by hens exposed to FM had edema, this was not true for those chicks with 2,2',4,4'5,5'-HBB exposure. Hatchability was decreased by FM but not by 2,2',4,4',5,5'-HBB.

Dharma (1980) examined the effects of feeding 2,2',4,4',5,5'-HBB (peak 4) mixed in the diet of chicks. He observed no clinical

signs of toxicosis and no effect on feed consumption. Slight hepatocellular swelling and vacuolation were noticed. Lymphoid cells in the bursa of Fabricius were depleted. In comparison to FM BP-6, 2,2',4,4',5,5'-HBB produced less severe changes and thus was considered less toxic.

In 1976, McKinney et al. assessed the toxicosis associated with 2,2',4,4',5,5'-HCB in chicks. He reported liver enlargement, decreased body weight, decreased spleen weight, slight thymic involution, and a moderate amount of hepatic single cell necrosis. He also noticed that the liver changes were more marked for 2,2',4,4',6,6'-HCB.

Mixed-Type Inducers

Dharma (1980) also examined the pathologic effects of a mixed-type inducer, 2,3',4,4',5,5'-HBB (peak 6), in young cockerels. Similar changes were seen with the administration of this chemical as were seen with the administration of 2,2',4,4',5,5'-HBB (peak 4), only the changes were seen at a lower level. He concluded that FM BP-6 was more toxic than 2,3',4,4',5,5'-HBB (peak 6), which was more toxic than 2,2',4,4',5,5'-HBB (peak 4).

Akoso and Sleight (1979) and Akoso et al. (1980) evaluated the effects of 2,2',4,4',5,5'-HBB (peak 6) in rats. They reported changes were similar but more severe or pronounced than those associated with 2,2',4,4',5,5'-HBB (peak 4) administration. An additional change was the presence of intracytoplasmic myelin figures in the hepatocytes. This change was also seen with FM BP-6 administration. They concluded that 2,3',4,4',5,5'-HBB and FM BP-6 are more toxic than 2,2',4,4',5,5'-HBB.

The other known mixed-type inducer is 2,3',4,4',5-PBB_5 (peak 2). Dannan et al. (1979) administered this compound to rats in a single IP injection of 90 mg/kg. The rats were killed 1 or 2 weeks later.

The liver to body weight ratios were increased in rats killed at 1 or 2 weeks, but the spleen to body weight ratios and thymus to body weight ratios were decreased only in rats killed after 2 weeks. There was diffuse hepatocellular swelling and vacuolization, which was confirmed to be fat. The periportal areas were the most affected area of the hepatic lobule and this was more pronounced after 2 weeks. The adrenal, heart, lung, trachea, thyroid, small intestine, pancreas, thymus, spleen and kidney all failed to reveal changes. Ultrastructurally, there was a proliferation and dilatation of the SER after both time periods. After 1 week the mitochondria were swollen and lighter in appearance but after 2 weeks the mitochondria appeared normal.

3-Methylcholanthrene-Type Inducers

A clearly MC-type inducer is 3,3',4,4',5,5'-HBB. Ecobichon et al. (1979) compared the effects of this compound in rats to 2,2',4,4',5,5'-HBB (peak 4) and to FM BP-6. Although they reported similar results for all 3 compounds, all their rats were killed within 1 week.

Others have compared the pathologic effects of chlorinated analogs in chickens. McKinney et al. (1976) reported that 3,3',4,4',5,5'-HCB caused decreased food intake, death, thymic involution, depletion of lymphoid elements in the thymus and spleen, mild liver necrosis, fatty infiltration of the liver, loss of adipose tissue, subcutaneous edema, ascites and hydropericardium. These changes were similar to those seen with 2,3,7,8-tetrachlorodibenzofuran (TCDF), except for the liver changes. They characterized this compound as being more toxic than 2,2',4,4',5,5'-HCB and 3 other PCB congeners.

MATERIALS AND METHODS

Experimental Design

This project was designed to study the gross, histological and ultrastructural changes associated with a 10-day administration of FM BP-6, 2,2',4,4',5,5'-HBB (peak 4) or 3,3',4,4',5,5'-HBB at various dietary treatment levels in rats. Groups of 6 rats each were fed a diet containing 0, 0.1, 1, 10 or 100 ppm of each compound. Rats were killed on day 10. This plan was executed with one exception. Only 4 rats were killed in the group given 100 ppm of 3,3',4,4',5,5'-HBB. It was decided to continue feeding this diet to the remaining 2 rats until they died or became moribund. Consequently, 2 rats were maintained for an additional 10 days.

Another alteration in the original design was the addition of a modified paired-feeding study. All rats which ingested feed containing 100 ppm of 3,3',4,4',5,5'-HBB had an apparent decreased feed intake. To help clarify whether lesions were associated with 3,3',4,4',5,5'-HBB ingestion or with decreased feed intake at this dietary level, 6 rats were restricted to 13 g/day of control feed. This amount was approximately the mean daily feed intake/rat for those consuming feed containing 100 ppm 3,3',4,4',5,5'-HBB. The amount of daily feed intake for the other rats was approximately 23 g/day, so 6 other rats were restricted to this daily amount for 10 days. A third group was fed *ad libitum* to serve as a control group for the other 2 groups.

Three groups of 2 rats each were fed either the control feed *ad libitum* or 23 g/day for 20 days. The data obtained from these studies aided in evaluating the data from the 2 rats given 100 ppm 3,3',4,4',5,5'-HBB for 20 days.

A total of 114 rats was used. Because of limited space, the experiment was performed in segments. First the 10- and 20-day studies with 3,3',4,4',5,5'-HBB were done. This was followed in order by studies of FM BP-6, 2,2',4,4',5,5'-HBB and finally the 10- and 20-day paired-feeding study. Rats were observed daily for changes in their usual behavior or appearance. Feed intake was recorded daily and body weights were measured at the beginning, every other day, prior to fasting, and prior to euthanasia. Water was always available and, except for the restricted feed intake studies and during the night before euthanasia, feed was continually available to the rats.

All rats were euthanatized by inhalation of carbon dioxide and were necropsied. Selected organs were weighed. Brain weights were used in assessing organ weight changes in rats whose body weights were significantly altered. Other desired tissues were collected for chemical analysis, histopathologic examination and electron microscopic examination. All data were analyzed statistically by using the Student's t-test for 2 sample means and the 5% level, $p<0.05$, was used as the minimal level of statistical significance.

Chemicals

Firemaster BP-6, as mentioned in the Introduction, was manufactured by the Michigan Chemical Corporation. The 2,2',4,4',5,5'-HBB was isolated from FM BP-6 by repeated fractional recrystallization. The 3,3',4,4',5,5'-HBB was purchased from the RFR Corporation, Hope, Rhode Island, and purified by repeated alumina chromatography until >99% pure.

Both procedures were performed under the direction of Steven D. Aust, Department of Biochemistry, Michigan State University.

Animals, Diet and Environment

Outbred male Sprague-Dawley rats with initial body weights between 250 and 300 g were purchased from Spartan Research Animals, Haslett, Michigan. After a 2-day acclimatization period in which they were fed a commercial rat feed (Wayne Lab-Blox, Allied Mills, Inc., Chicago, IL), which had been ground to a powdery consistency, the rats were randomly grouped into cages. There were 3 rats/cage for the PBB studies and 1 rat/cage for the paired-feeding study. All cages were the plastic shoebox type with a wire top. Heat-treated wood chips manufactured by the Northeastern Products Corporation, Warrensburg, NY, were used as bedding. All cages were shelved in a filtered laminar flow system (Contamination Control, Inc., Lansdale, PA). Filters were changed and the stainless steel shelves wiped with acetone prior to each of the 4 segments of the experiment.

Mazola^{R} corn oil was thoroughly mixed with ground feed at a level of 1 ml/100 g for 15 min and used as the control feed. The corn oil was used as the vehicle for dissolving or suspending the individual PBB compounds before mixing in feed. Each chemical was slowly mixed with warm (40 C) corn oil until it went into solution. This took less than 1 hour for FM BP-6, approximately 12 hours for 2,2',4,4',5,5'-HBB and it never occurred for 3,3',4,4',5,5'-HBB. The last chemical stayed as a fine suspension. Feed was given to the rats in porcelain feed cups covered with stainless steel perforated discs.

The laminar flow system was kept in a room with a constant temperature of 23.3 C. The lights were automatically adjusted for 12.5 light hours/day.

Necropsy and Tissue Samples

Necropsies consisted of a systematic examination of the organs for gross pathologic changes. The brain, kidneys, liver, spleen, thymus, and thyroids were removed and weighed with a top-loading balance (Mettler Series P, Model 163, Mettler Instrument Corporation, Hightstown, NY). The adrenals, colon, esophagus, heart, lungs, pancreas, pituitary, prostate, small intestine, stomach, testes, trachea, urinary bladder and the sublingual, submaxillary and parotid salivary glands were removed. Portions of the removed tissues were fixed in 10% buffered formalin for histological examination. Samples of body fat, brain, kidney and liver were collected, wrapped in aluminum foil and stored at -20 C for future chemical analysis. Another portion of liver was sliced into pieces approximately 1 mm in thickness, fixed in Karnovsky's fixative (Karnovsky, 1965) and stored at 4 C until further preparation for ultrastructural examination.

Histologic Preparation

The formalin-fixed tissues were trimmed to appropriate size, automatically processed (Histomatic, Model 166, Fisher Scientific Company, Pittsburgh, PA), embedded in paraffin and sectioned at 6 μ. Tissue sections were then stained with hematoxylin-eosin.

Other formalin-fixed liver sections were stained with oil red O for lipid identification.

Ultrastructural Preparation

Fixed liver tissues were washed in Zetterqvist's osmium fixative (Pease, 1964) at pH 7.4 and then postfixed in 1% osmium tetroxide in Zetterqvist's fixative. They were dehydrated in graded alcohol (50,

70, 90 and 100%) and then transferred to propylene oxide. A mixture of Epon and Araldite was used for embedding.

First, thick sections (1 μ) were stained with toluidine blue and viewed with the light microscope. Areas for further examination were selected and thin sections (approximately 900 Å) were made. They were stained with uranyl acetate and lead citrate and viewed by using an electron microscope (EM 952, Carl Zeiss, Germany).

Chemical Analysis

The standardized procedure utilized by the personnel of the research laboratory within the Department of Pathology at Michigan State University was used in the chemical analysis of the tissues collected from rats in the FM BP-6 and 2,2',4,4',5,5'-HBB_6 studies. Tissue samples from each rat in a cage were pooled for the procedure. The total amount of tissue weighed approximately 0.5 g as measured on an analytical balance (Type H15, Mettler Instrument Corporation, Hightstown, NJ). The weighed samples were rinsed into a stainless steel beaker with petroleum ether. Washed and ignited sand (Mallinckrodt, Inc., Paris, KY) was added and the sand-tissue mixture was then ground. This mixture was next dehydrated by the addition of approximately 10 to 20 g of granular anhydrous sodium sulfate (Mallinckrodt, Inc., Paris, KY). Approximately 15 ml of glass distilled hexane (J. T. Baker Chemical Company, Phillipsburg, NJ), which was used in the extraction of all solvents, was added. The entire mixture was brought to a boil over an 80 C water bath and then filtered into a 100 ml volumetric flask. The hexane washes and subsequent filtrations were repeated until a total of 4 extractions had been completed. After the fourth extraction, the volume was brought to

100 ml by the addition of hexane. Two 20 ml quantities of the extract were saved in screw-cap tubes at -20 C for lipid determination and chemical quantitation.

The 20 ml aliquot to be used for lipid determination was condensed to approximately 2 ml by evaporation (N-Evap, Model III, Meyer Oranomation Assoc., Inc., Shrewsbury, MA) and then rinsed with petroleum ether into preweighed foil pans.

A heated water bath was used to evaporate the solvent in the pans. Upon completion, the pans were placed in a desiccator and a vacuum was applied to promote drying. The pans were again weighed and the lipid weight recorded.

The columns (Chromaflex, 200 mm x 7 mm ID), which were prewashed with acetone, were plugged at the tapered end with a small amount of glass wool. The columns were filled with 1.6 g of activated magnesium silicate (Florisil, 60-100 mesh, Fisher Scientific Company, Cleveland, OH) and then approximately 2 cm of granular anhydrous sodium sulfate was added. The column was washed with 5 ml of hexane, which was discarded as the first 5 ml of eluent. Next the sample which had been condensed from 20 ml to approximately 2 ml was added and then repeatedly rinsed with hexane. The sample eluent was condensed to 0.5 ml and then brought up to 2 ml with the addition of iso-octane (2,2,4-trimethylpentane, Burdick and Jackson Laboratories, Inc., Muskegon, MI). The sample was then ready for the gas-liquid chromatographic analysis.

The gas chromatograph (G. C. Model 3700, Varian Instrument Division, Palo Alto, CA) was equipped with an electron capture detector which operated with an injector temperature of 280 C, a column temperature of 250 C and a detector temperature of 310 C. Gaseous nitrogen which was 99.99% pure was used as the carrier at a flow rate of 30 ml/min.

Gas-chromatographic readings were recorded and the tissue concentrations of FM BP-6 and 2,2',4,4',5,5'-HBB were compared to standards containing 0.05 and 0.1 µg FM BP-6/ml. Control samples of calf liver were also used to monitor extraction recovery.

Modifications of the procedure were made under the direction of Ghazi A. Dannan from the Department of Biochemistry, Michigan State University, for the 3,3',4,4',5,5'-HBB tissue analysis. Glass distilled toluene (J. T. Baker Chemical Company, Phillipsburg, NJ) was used instead of hexane as the extraction solution. The injection volume of 1 µl was injected into the gas chromatograph as in the standardized procedure.

Isolation of Microsomes

Under the direction of Dr. Steven D. Aust from the Department of Biochemistry, Michigan State University, rat hepatic microsomes were isolated and enzyme activity evaluated by graduate students working in his laboratory. Briefly, the procedure is first one of differential centrifugation. A weighed portion of the liver was collected and kept in cold potassium chloride. The liver was sliced into small pieces and then homogenized. The homogenate was centrifuged 2 times using the first supernatant for the second centrifugation. The first centrifugation was at 10,000 x g for 20 min and the second was at 105,000 x g for 90 min. The microsomes were washed to remove ribosomes and absorbed proteins and then stored at -20 C in 0.05 M Tris-HCl, pH 7.5, containing 50% glycerol and 0.01% butylated hydroxytoluene (BHT) until used (Welton and Aust, 1974).

Microsomal Enzyme Assays

These assays were also performed by graduate students in the Department of Biochemistry, Michigan State University, under the direction of Dr. Steven D. Aust. The amount of microsomal protein and cytochrome P-450 and the activity of demethylase and benzopyrene hydroxylase were determined. The cytochrome P-450 CO difference spectrum maximum also was determined. The methods are described by Moore et al. (1978c).

RESULTS

Clinical Signs

Sneezing occurred in control and treated rats from various treatment groups in the 3,3',4,4',5,5'-HBB study. Swelling of one or both eyelids with red pigment encrustations was observed.

The 2 rats which ingested feed containing 100 ppm of 3,3',4,4',5,5'-HBB for 20 days gradually became less active. They were emaciated, with a roughened hair coat and sunken eyes, and after 16 days they stopped eating. One of them died on day 20 and, since the other rat was extremely weak, it was killed on the same day.

Feed Intake

The mean daily feed intake for rats in each cage is given in Table 1 for the 10-day studies. The values for the rats given 0.1 ppm of FM BP-6 in the feed are smaller than the values for the other rats in the FM BP-6 study ($p<0.025$). Values for the rats given 10 and 100 ppm dietary concentrations of 3,3',4,4',5,5'-HBB are smaller than the values for the control rats, respectively ($p<0.05$ and $p<0.0005$). There is also a dose-related decrease in the values for rats consuming 1, 10, or 100 ppm in the 3,3',4,4',5,5'-HBB study ($p<0.005$).

Values for the rats fed feed containing 1 ppm ($p<0.005$) and 10 ppm ($p<0.05$) of 2,2',4,4',5,5'-HBB are larger than the values from control rats and thus indicate an increase in feed consumption.

Table 1. Mean daily feed intake of 3 rats/cage given dietary treatment for 10 days[a]

Concentration of Chemical in Rats' Diet (ppm)	Chemical Added to Diet		
	Firemaster BP-6	2,2',4,4',5,5'-HBB	3,3',4,4',5,5'-HBB
0	68.8±7.1	70.0± 7.9	69.8±16.0
0.1	62.7±8.5[d]	71.4± 7.1	70.7±15.5
1	69.9±6.9	78.6± 7.4[c]	74.2± 9.6
10	68.6±6.4	76.4±11.0[e]	60.7±10.8[e]
100	66.1±9.2	69.5± 8.3	41.2±18.0[b]

[a]Data in grams represent the mean ± SD for pooled values for the 2 cages of rats in each study; N = 18 for all groups except 0.1 and 1 ppm 3,3',4,4',5,5'-HBB and 100 ppm Firemaster BP-6, in which N = 17.

[b,c,d,e]Values significantly different from control values ($p<0.0005$, $p<0.005$, $p<0.025$, $p<0.05$, respectively).

Body Weights

Listed in Table 2 are the mean body weights for the rats in various treatment groups prior to feed removal. The mean body weights for rats fed diets containing 10 ppm of 3,3',4,4',5,5'-HBB ($p<0.05$) and 100 ppm of 3,3',4,4',5,5'-HBB ($p<0.005$) were decreased in comparison to the values from controls. There was a decrease in body weights for rats given 10 ppm in comparison to those fed 1 ppm 3,3',4,4',5,5'-HBB ($p<0.005$). Rats given feed containing 100 ppm of 3,3',4,4',5,5'-HBB did not have significantly lower body weights than those given 10 ppm of the same chemical ($p<0.05$). There was no statistical difference between the body weights of rats given FM BP-6 or 2,2',4,4',5,5'-HBB, even though rats consuming feed with 1 ppm of 2,2',4,4',5,5'-HBB had increased body weights in comparison to those rats getting 0, 0.1 and 10 ppm of the same chemical.

The mean body weights for the rats in the 10-day paired-feeding study are listed in Table 3. There were no significant differences between weights for rats fed *ad libitum* and those rats restricted to 23 g/day of feed, but there was a difference ($p<0.0005$) between mean body weights of rats in these 2 groups when compared to weights of rats restricted to feed intake of 13 g/day.

Table 4 lists the body and organ weights for the 2 rats in each of the 20-day study groups. It appears obvious that the body weights of rats fed diets containing 3,3',4,4',5,5'-HBB or restricted to feed intake of 13 g/day were less than the body weights of rats fed control diet *ad libitum*. Rats consuming the 3,3',4,4',5,5'-HBB also had body weights less than the rats consuming 13 g/day of the control diet.

Table 2. Mean body weights of rats after 9 days of dietary treatment[a]

Concentration of Chemical in Rats' Diet (ppm)	Chemical Added to Diet		
	Firemaster BP-6	2,2'4,4',5,5'-HBB	3,3',4,4',5,5'-HBB
0	332.0±13.3	342.1±18.4	350.2±14.7
0.1	323.2±27.0	347.9±15.6	343.2±26.9
1	339.9±15.0	356.2± 9.5	359.2±28.2
10	326.5±21.2	345.7± 7.9	312.2±18.9[c]
100	323.3± 6.9	344.7±13.7	292.6± 8.7[b]

[a]Data in grams represent the mean ± SD; N = 6 for all groups except for 10 ppm Firemaster BP-6, in which N = 5, and for all groups of 3,3',4,4',5,5'-HBB, in which N = 3.

[b,c]Values significantly different from control values ($p<0.005$, $p<0.05$, respectively).

Table 3. Mean body and organ weights from rats in the 10-day modified paired-feeding study[a]

Dietary Allowance per Rat	Weights (g)						
	Body	Brain	Liver	Kidney	Thymus	Thyroid	Spleen
ad libitum	345.9±17.6	1.80±0.04	11.79±2.15	2.54±0.12	0.667±0.155	0.014±0.003	0.841±0.078
23 g/day	339.9±13.4	1.77±0.04	10.13±0.83	2.43±0.21	0.687±0.124	0.013±0.002	0.806±0.104
13 g/day	296.4±16.6[b,f]	1.76±0.05	8.11±0.36[d,f]	2.06±0.16[b,f]	0.541±0.108[h]	0.011±0.003[e]	0.671±0.078[c,g]

[a]Data represent the mean ± SD; N = 8.

[b,c,d,e]Values significantly different from values from rats fed *ad libitum* ($p<0.0005$, $p<0.005$, $p<0.025$, $p<0.05$, respectively).

[f,g,h]Values significantly different from values for rats fed 23 g/day ($p<0.0005$, $p<0.025$, $p<0.05$, respectively).

Table 4. Body and organ weights from rats in the 20-day studies[a]

Concentration of 3,3',4,4',5,5'-HBB in Rats' Diets (ppm)	Dietary Allowance per Rat	Rat No.	Weights (g)						
			Body	Brain	Liver	Kidney	Thymus	Thyroid	Spleen
100	*ad libitum*	1	210.0	1.70	12.05	2.10	0.064	0.010	0.236
		2	198.3	1.53	11.90	1.72	0.057	0.008	0.348
0	*ad libitum*	1	399.6	1.88	14.01	3.02	0.632	0.013	0.825
		2	386.7	1.79	14.20	3.35	0.889	0.015	0.898
0	23 g/day	1	347.9	1.74	10.73	2.47	0.692	0.014	0.806
		2	370.8	1.76	10.87	2.83	0.709	0.012	0.909
0	13 g/day	1	274.0	1.68	7.26	2.01	0.325	0.011	0.725
		2	282.0	1.60	7.64	2.13	0.500	0.008	0.525

[a]Rats fed a diet containing 100 ppm of 3,3',4,4',5,5'-HBB were in 1 study and all other rats were in a separate study.

Brain Weights

Brain weights generally are uniform for all rats of a certain age. However, there were increases in the brain weights of the rats in 3 of the 4 groups treated with dietary levels of 2,2',4,4'5,5,'-HBB ($p<0.05$).

When either body weights or brain weights from treated rats were statistically different ($p<0.05$) from the weights of control rats, these values were not used as standards for making comparisons of other organ weights.

Other Organs Weights

The liver weights from rats fed 10 or 100 ppm of each of the 3 chemicals were increased in comparison to weights from control rats (Table 5). Liver weights progressively increased as the dietary treatment level increased. For rats fed FM BP-6, the weights of the livers from rats getting 10 ppm were greater than the weights of those getting 1 ppm ($p<0.025$). The weights of those getting 100 ppm were greater than the weights of those getting 10 ppm ($p<0.0005$). This dose-related increase was seen in the weights of livers from rats fed a diet containing 2,2',4,4',5,5'-HBB. The liver weights of rats getting 10 ppm were greater than the weights of those getting 1 ppm ($p<0.01$) and the weights of those getting 100 ppm were greater than the weights of those getting 10 ppm ($p<0.01$). This same pattern was seen for those rats getting dietary 3,3',4,4',5,5'-HBB. The liver weights of rats fed a diet containing 10 ppm were greater than the weight of those fed a diet containing 1 ppm ($p<0.05$). The liver weights of those getting 100 ppm were greater than the liver weights of those consuming a diet containing 10 ppm ($p<0.005$).

Table 5. Mean liver weights of rats after 10 days of dietary treatment[a]

Concentration of Chemical in Rats' Diet (ppm)	Chemical Added to Diet		
	Firemaster BP-6	2,2',4,4',5,5'-HBB	3,3',4,4',5,5'-HBB
0	10.69±0.49	11.25±0.31	10.32±0.96
0.1	9.87±1.00	10.89±0.70	10.18±0.77
1	10.85±0.87	11.59±1.54	11.29±1.04
10	12.50±1.42[d]	12.65±1.54[e]	12.08±0.73[d]
100	16.69±0.90[b]	14.30±1.21[e]	14.19±1.23[c]

[a]Data in grams represent the mean ± SD; N = 6 for all groups except for 100 ppm 3,3',4,4',5,5'-HBB, in which N = 4.

[b,c,d,e]Values significantly different from control values ($p<0.0005$, $p<0.005$, $p<0.01$, $p<0.05$, respectively).

The mean liver weights for the rats restricted to 13 g/day of control diet were decreased ($p<0.025$) in comparison to the rats fed *ad libitum* (Table 3). The values listed for the rats fed control feed *ad libitum* or in restricted amounts for 20 days were lower than the values for the rats with identical dietary allowances at 10 days (Table 4). The liver weights for the rats fed 23 g/day and the rats fed diets containing 100 ppm of 3,3',4,4',5,5'-HBB were lower than values from rats fed the control diet *ad libitum*, but the liver weights of those rats fed 13 g/day were approximately half the values for those fed *ad libitum*.

None of the kidney weights of treated rats were significantly different than the weights of control rats except at the 0.1 ($p<0.01$), 1 ($p<0.05$) and 100 ppm ($p<0.0005$) dietary levels of 3,3',4,4',5,5'-HBB. The respective values were 2.24 ± the standard deviation of 0.14, 2.71 ± 0.27, and 1.89 ± 0.15 g. The controls had 2.52 ± 0.09 g for their mean kidney weight. At 1 ppm of this chemical, the kidney weights were larger ($p<0.0005$) than the weights of the controls. Statistically, there was no dose-related difference between the mean kidney weight of rats getting 1 ppm and the mean kidney weight of rats getting 10 ppm. Also, there was no difference between the mean kidney weight of those getting 10 ppm and the mean kidney weight of those getting 100 ppm.

The mean kidney weight of rats fed 13 g of control diet/day for 10 days was less than the *ad libitum* fed rats' mean kidney weight ($p<0.0005$) and the value for the rats ingesting 23 g/day ($p<0.005$) (Table 3).

The mean kidney weights for rats in the 20-day studies are shown (Table 4). Those rats getting 13 g/day had apparently lighter kidneys

than the kidneys from the rats fed *ad libitum*. Those fed 23 g/day of control diet and those fed 3,3',4,4',5,5'-HBB at a dietary level of 100 ppm also had kidneys of less weight.

Mean thymus weights of treated rats were not affected by any of the chemicals except 3,3',4,4',5,5'-HBB at dietary levels of 10 and 100 ppm. The mean weights were, respectively, 0.547 ± 0.094 and 0.178 ± 0.063 g. The weight of the controls was 0.659 ± 0.063 g.

Severe decreases in thymic weights of rats fed 100 ppm 3,3',4,4',5,5'-HBB in comparison to the weights from those fed control diets *ad libitum* were noticed after 20 days (Table 4). The thymic weights of these treated rats were also less than the thymic weights of those fed 13 g of control feed/day. The weights from this latter group of rats were less than the weights of those fed *ad libitum*. After 10 days, rats restricted to 13 g of control diet/day had a mean thymus weight less than those fed 23 g/day ($p<0.05$) but not statistically different from the value for those fed *ad libitum*.

Rats getting 100 ppm of dietary FM BP-6 were the only treated ones with an increase in mean thyroid weight ($p<0.01$). The value for the control rats was 0.014 ± 0.002 g and the value for the treated rats was 0.018 ± 0.003 g. Rats fed 13 g/day had a smaller mean thyroid weight in comparison to those fed the control diet *ad libitum* for 10 days ($p<0.05$) but not a statistically different weight from those fed 23 g/day (Table 3). After 20 days, rats fed 3,3',4,4',5,5'-HBB and those restricted to 13 g of control feed/day had thyroid weights which were less than rats fed control diet *ad libitum* (Table 4).

At the dietary levels of 10 and 100 ppm of 3,3',4,4',5,5'-HBB, mean spleen weights were 0.761 ± 0.068 g and 0.683 ± 0.118 g, respectively, and less than 0.866 ± 0.045 g, the value for the controls

($p<0.025$). This was a dose-related decrease in mean spleen weights ($p<0.05$).

Rats fed 1 ppm of dietary 2,2',4,4',5,5'-HBB had a mean spleen weight of 0.915 ± 0.105 g, which was heavier than the weight for controls of 0.818 ± 0.052 g ($p<0.05$). The value was not statistically different for the mean spleen weights of rats getting 0.1 and 10 ppm of the same chemical.

In the 20-day studies, rats fed 13 g of control diet/day and rats given 100 ppm of dietary 3,3',4,4',5,5'-HBB *ad libitum* both had smaller spleen weights than those given control diet *ad libitum* (Table 4). The treated rats had values obviously less than those which were restricted to 13 g/day.

Chemical Analysis

Results of analyses are given in Table 6. Values are on a fat basis and were determined by dividing the chemical concentration in parts per million (ppm) on a whole weight basis by the fractional amount of lipid in the tissues.

In viewing Table 6, several general tendencies are apparent. First, there appears to be a dose-related increase in the tissue chemical concentration as the dietary level increases. At the 100 ppm dietary level of chemical, the concentration of 2,2',4,4',5,5'-HBB is greater than the concentration of FM BP-6 in all 4 tissues. Also at the 100 ppm dietary level, the greatest concentration for both chemicals appears to be in the liver, followed by the adipose tissue, kidney and brain, in descending order. This order changes to liver, kidney, adipose tissue and brain for the 1 and 10 ppm levels.

Table 6. Mean chemical concentrations in pooled samples of tissues of 3 rats/cage after 10 days of dietary treatment[a,b]

Chemical Added to Diet	Concentration of Chemical in Rats' Diet (ppm)	Cage Number	Tissue Concentrations (ppm) Liver	Adipose Tissue	Brain	Kidney
Firemaster BP-6	0	1	0.5	0	0.2	0.4
		2	0.5	0	0.3	0.1
	0.1	1	1.7	0.2	0.5	0
		2	1.3	0.3	0.4	0.3
	1	1	11.3	1.7	1.7	10.0
		2	5.2	1.6	1.8	0
	10	1	156.5	27.4	11.9	65.0
		2	113.1	26.6	12.6	52.9
	100	1	1342.0	271.1	98.5	351.4
		2	1084.4	230.3	108.5	378.8
2,2',4,4',5,5'-HBB	0	1	0.3	0	0.2	0.5
		2	0.2	0	0.1	0.3
	0.1	1	1.2	0.2	0.2	1.9
		2	2.2	0.2	0.3	1.8
	1	1	11.4	2.4	1.4	9.4
		2	11.5	3.8	0.7	8.8
	10	1	152.1	31.0	10.2	54.9
		2	210.2	31.4	12.8	59.8
	100	1	2186.1	450.7	150.0	485.2
		2	2929.6	421.2	136.8	617.1
3,3',4,4',5,5'-HBB	0	1	0	0	0	NS[c]
		2	0	0	0	0
	0.1	1	0.9	0	0	0
		2	5.6	0	0	0
	1	1	128.7	0.4	0	0
		2	73.2	0.3	0	3.2
	10	1	1666.8	1.7	0	19.9
		2	6759.9	2.0	0	9.1
	100	1	298.8	30.4	0	183.0
		2	1897.0	14.7	0	249.6

[a] Values are expressed on a fat basis.

[b] N=3 for all cages except cage 2 (100 ppm 3,3',4,4',5,5'-HBB), in which N=1.

[c] NS = no sample.

This disruption of the general trend seen at low dietary levels of FM BP-6 in the brain tissue is also observed in the comparison of different chemical concentrations at the same dietary level. In general, all 4 tissues at the different levels tend to concentrate more 2,2',4,4',5,5'-HBB than FM BP-6 when there is a clear distinction between concentrations. The exception to this trend is the brain. At dietary levels of 0.1 and 1 ppm, the concentration of FM BP-6 is greater than the concentration of 2,2',4,4',5,5'-HBB.

The tissue concentrations from the dietary study of 3,3',4,4',5,5'-HBB are listed in Table 6. In general, as the dietary level increased, the tissue concentration increased, but the liver concentrations varied at the 10 and 100 ppm level and make interpretation difficult. The liver was the only tissue to have detectable levels at the 0.1 ppm level. Kidney and adipose tissue concentrations were first seen at the 10 ppm level. The brain never had any detectable concentrations of chemical.

The greatest concentration of chemical on a fat basis appears to be in the liver, then in descending order kidney, adipose tissue, and brain. Liver concentrations for the 3,3',4,4',5,5'-HBB-treated rats at the 10 and 100 ppm levels were greater than those for 2,2',4,4',5,5'-HBB or for FM BP-6.

The 2 rats fed 100 ppm of dietary 3,3',4,4',5,5'-HBB for 20 days had equivalent samples of tissue pooled togehter. The respective concentrations for the liver, adipose tissue, brain and kidney were 2179.729, 76.828, 0, and 529.669 ppm. These values, except for the brain, were increased in comparison to the values for the tissue concentrations at 10 days, indicating a time-dependent response.

Analysis for FM BP-6 was done on liver and adipose tissues from every rat in the 10- and 20-day paired-feeding studies to determine if there was a significant amount of contamination. All values in ppm of FM BP-6 on a fat basis were <0.8 and thus contamination was minimal.

Hepatic Drug Metabolism Parameters

There was a dose-related increase in the amount of cytochrome P-450 at dietary levels of 1, 10 and 100 ppm 3,3',4,4',5,5'-HBB ($p<0.0005$, $p<0.0005$, $p<0.025$, respectively, Table 7). The delta (λ) max. gradually shifted from 449 to 448 nm in a dose-related fashion. The λ max. was never at 450 nm.

At the dietary levels of 0.1 and 1 ppm, aminopyrine demethylase activity was increased ($p<0.01$) but at higher dosage levels there was no statistical difference between the values and those from the control rats. In contrast, benzopyrene hydroxylase activity was increased at the dietary levels of 1, 10 and 100 ppm ($p<0.0005$) and the increase was 4-, 9- and 9-fold, respectively, in comparison to values from controls. The increase was also dose-related for the 1 and 10 ppm levels but there was no statistical difference between the values for dietary levels of 10 and 100 ppm.

Gross Findings

The lungs from some rats in the groups getting dietary FM BP-6 and 2,2',4,4',5,5'-HBB and some of those in the paired-feeding studies were abnormal. They had up to 50 multiple, diffusely distributed, raised, white foci which were approximately 1 mm in diameter. Occasionally, adjacent areas were irregular, dark and sunken. Focal areas of emphysema were also present.

Table 7. Effects of 10-day dietary administration of 3,3',4,4',5,5'-HBB on several liver hepatic drug metabolism parameters in rats[a]

Concentration of 3,3',4,4',5,5'-HBB in Rats' Diet (ppm)	Parameters			
	Cytochrome P-450 (nmoles/mg prot.)	λ max. (nm)	Aminopyrine Demethylase[b] (nmoles/mg prot./min)	Benzopyrene Hydroxylase[c] (nmoles/mg prot./min.)
0	0.95±0.07	450	11.2±1.5	1.2±0.4
0.1	0.96±0.13	448-449.0	13.5±1.2[f]	1.5±0.4
1	1.19±0.05[d]	448-449.0	13.2±0.8[f]	4.3±0.5[d]
10	2.40±0.37[e]	448-448.5	11.0±0.9	10.5±1.1[d]
100	2.88±0.18[g]	448	10.5±1.9	10.1±1.8[d]

[a]Data represent the mean ± SD; N = 6 for all groups except 100 ppm, in which N = 4.

[b]Indicator of PB-type induction.

[c]Indicator of MC-type induction.

[d,e,f,g]Values significantly different from control values ($p<0.0005$, $p<0.005$, $p<0.01$, $p<0.025$, respectively).

Two of the rats given dietary levels of 100 ppm of FM BP-6 had apparently enlarged thyroids. All rats from this treatment group had enlarged, friable, yellow livers with a prominent centrolobular pattern. The livers from the rats fed diets containing 10 and 100 ppm 3,3',4,4',5,5'-HBB were similar in appearance. The livers from the 2 rats in the 20-day 3,3',4,4',5,5'-HBB study were also similar. These rats had less apparent body fat and no fat was found around the heart or in the mesentery. There was obvious thymic atrophy, smaller spleens and larger adrenals.

Histological Findings

No differences were observed in sections of cerebellum, cerebrum, epididymis, esophagus, heart, kidney, pancreas, prostate, salivary glands (parotid, sublingual, submaxillary), small intestine, spleen, stomach, testis, urinary bladder, colon, trachea, lung and thyroid from treated rats in comparison to the tissues from the control rats. Also, no differences in these tissues were observed among the rats in the modified paired-feeding study. Some tissue sections from the latter 4 tissues had changes, but the findings had no consistent pattern and occurred in both control and treated rats.

Cross sections of parasites, some containing ova, were observed in the lumens of some sections of colon. They were adjacent to the mucosa which had no lesions. This organism appeared to be a nematode and is probably the rat pinworm, *Syphacia muris*.

The respiratory tract also had changes not related to PBB administration. Tracheal changes were mainly variations in the thickness of the mucosal epithelium. In some sections it appeared thickened and hypercellular. The hypercellularity was due to mucosal epithelial hyperplasia and/or infiltration of lymphocytes and macrophages. In

other areas the normal appearance of pseudostratified epithelium was gone and the cells appeared to be low cuboidal.

Approximately 50% of the rats had pulmonary changes which varied from congestion and thickened alveolar walls to consolidation. Large peribronchiolar, perivascular and individualized foci of lymphocytes, plasma cells and mast cells were seen (mast cells were common in many tissues) along with alveolar emphysema. Some bronchioles contained a bluish material which probably represented mucus. In total, these findings represented a subacute interstitial pneumonitis, most likely caused by *Mycoplasma pulmonis*.

None of the thyroids examined had a normal appearance. None was entirely composed of follicles with cuboidal to low columnar epithelium filled with homogeneous, or peripherally vacuolated, eosinophilic colloid. Instead, the follicles had one of 3 general appearances.

The first type had low cuboidal to almost squamous epithelium. They were quite large and in a range of sizes which are twice the size range of normal thyroid follicles of a rat. The colloid had a normal appearance. These follicles were located along the periphery and/or within the center of the gland and comprised 5 to 50% of the total number of follicles present.

The second type of follicle was generally the major type. These were approximately within the range of normal follicles and had cuboidal to low columnar epithelium. The colloid was normal, lighter in color and foamy, or absent. Basophilic material was clumped together in the colloid of some follicles.

The third general type of follicle did not have a follicular appearance. The nuclei were smaller, darker and some were pyknotic. Some nuclei remained in the periphery of the follicles, while others

were located randomly within the center mixed with a pale, granular material. Cell outlines were indistinct. These follicles comprised 5 to 50% of the total follicles present in a thyroid section and were located along the periphery and usually within a group.

The large type of follicles are suggestive of inactivity, while the second type are suggestive of hyperactivity. The third type is a degenerative type leading to necrosis. In general, these changes indicate a problem with iodine metabolism which may be dietary related, even though the feed was labeled "complete diet."

The thymus, spleen, lymph nodes and liver were the only organs that had alterations which were related to PBB ingestion. All thymuses were indistinguishable except for those from rats getting 10 and 100 ppm of dietary 3,3',4,4',5,5'-HBB for 10 days. The thymus from control rats had a smooth capsule, a definite line of demarcation between the cortex and medulla, and a cortex to medulla thickness ratio of approximately 60:40 (Figure 1). At the dietary level of 10 ppm this ratio changed to 50:50 and the cortex had a slight starry-sky effect, indicating the presence of macrophages.

At the dietary level of 100 ppm, dramatic changes were observed (Figure 2). The cortex to medulla ratio was approximately 30:70. The cortex had prominent macrophages, giving it the appearance of a starry sky, and was composed of fewer mature lymphocytes. The border between the cortex and medulla was indistinct and irregular. The capsule was irregular in some areas.

The thymuses from rats fed 100 ppm of this chemical for 20 days were lost and thus not available for histological examination.

Figure 1. Photomicrograph of a section of thymus from a control rat. Notice the definite line of demarcation between the cortex and medulla and the relatively thick cortex containing densely packed mature lymphocytes. Hematoxylin and eosin stain; X160.

Figure 2. Section of thymus from a rat fed a diet containing 100 ppm of 3,3',4,4',5,5'-HBB for 10 days. Notice the irregular and indistinct line of demarcation between the cortex and medulla and the relatively thin cortex with prominent macrophages (arrow) and relatively few mature lymphocytes. Hematoxylin and eosin stain; X160.

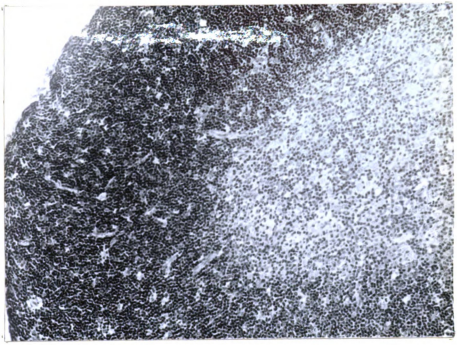


Figure 1

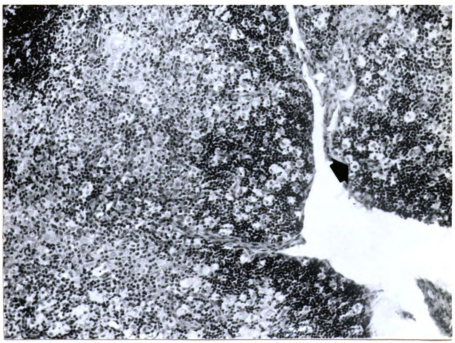


Figure 2

The spleen and lymph nodes from the rats getting 100 ppm of dietary 3,3',4,4',5,5'-HBB for 10 and 20 days generally had an increased number of macrophages intermixed with the mature lymphocytes in comparison to those from control rats. They had a similar appearance to the starry-sky appearance of the thymus but not as pronounced.

The liver was altered by all 3 chemicals. The livers of rats fed 0.1 and 1 ppm of FM BP-6 were not distinguishable from those of control rats (Figures 3, 4 and 5). At the dietary level of 10 ppm all hepatocytes appeared slightly swollen with sinusoidal spaces still visible (Figures 6 and 7). The midzonal region to periportal regions were most affected.

At 100 ppm of dietary FM BP-6, changes were more advanced, especially in the centrolobular and midzonal regions (Figures 8, 9 and 10). In the midzonal area sinusoidal spaces were obliterated and hepatocytes were very swollen and vacuolated. Small vacuoles were abundant in this region but much larger in the centrolobular area, even though hepatocytes were not as swollen.

Oil red O stain confirmed the presence of fat droplets in the liver section, but only occasional lipid droplets could be seen.

In the study of rats given 2,2',4,4',5,5'-HBB, dietary levels of 0.1 and 1 ppm failed to produce hepatic changes. At 10 ppm, some livers had slightly swollen and vacuolated hepatocytes in the centrolobular and midzonal region, although many were indistinguishable from those from control rats.

As with FM BP-6, livers from rats fed 2,2',4,4',5,5'-HBB at the 100 ppm level had more pronounced lesions (Figure 11). There was a

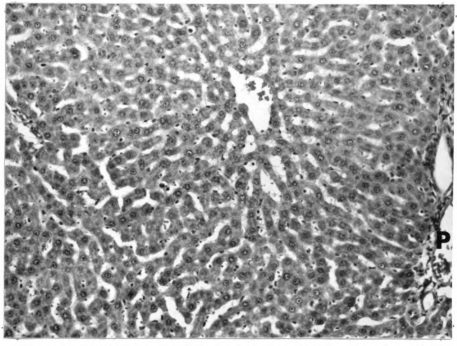


Figure 3. Section of liver from a control rat. Notice obvious sinusoidal spaces throughout lobule, even though less distinct in periportal areas (P). Hematoxylin and eosin stain; X160.

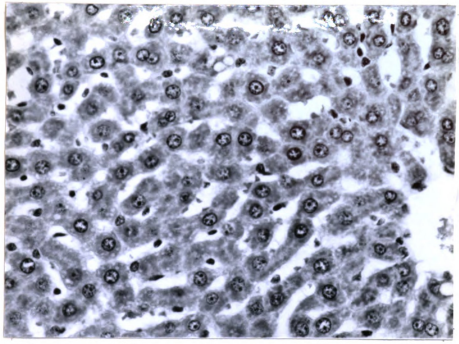


Figure 4. Section of liver from control rat at a higher magnification than Figure 3, to show the centrolobular to midzonal area. Hematoxylin and eosin stain; X400.

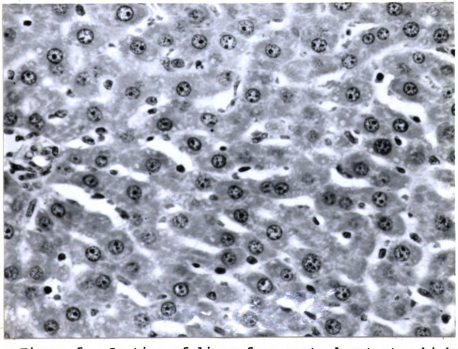


Figure 5. Section of liver from control rat at a higher magnification than Figure 3, to show the portal to midzonal area. Notice the sinusoidal space is less distinct. Hematoxylin and eosin stain; X400.

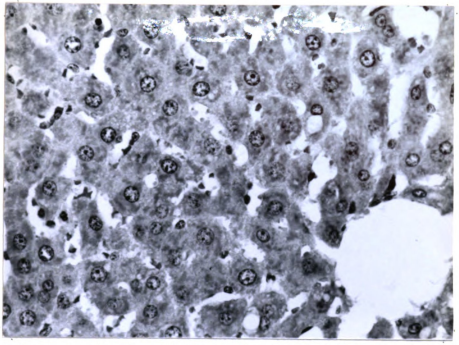


Figure 6. Section of liver from a rat fed a diet containing 10 ppm of FM BP-6 for 10 days, to show the centrolobular to midzonal area. Notice the swollen hepatocytes and decreased amount of sinusoidal space. Hematoxylin and eosin stain; X400.

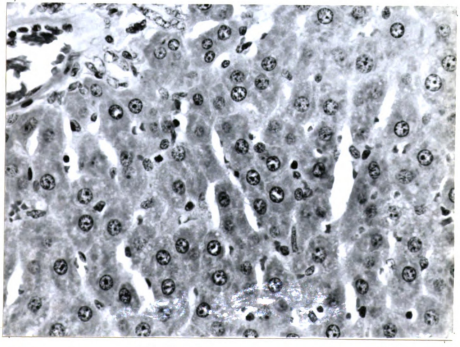


Figure 7. Section of liver from a rat fed a diet containing 10 ppm of FM BP-6 for 10 days, to show the portal to midzonal area. Notice that swollen hepatocytes and decreased amount of sinusoidal space appears more pronounced than in Figure 6. Hematoxylin and eosin stain; X400.

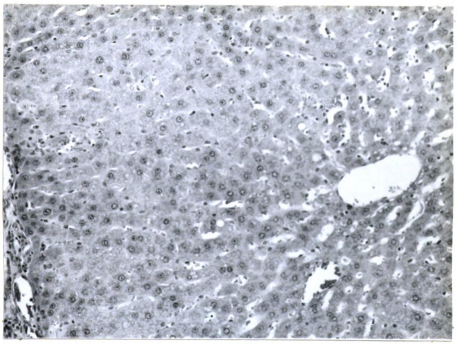


Figure 8. Section of liver from a rat fed a diet containing 100 ppm of FM BP-6 for 10 days. Notice diffuse hepatocellular swelling and decreased sinusoidal space, especially in midzonal area. Also notice vacuolization in centrolobular and midzonal areas. Hematoxylin and eosin stain; X160.

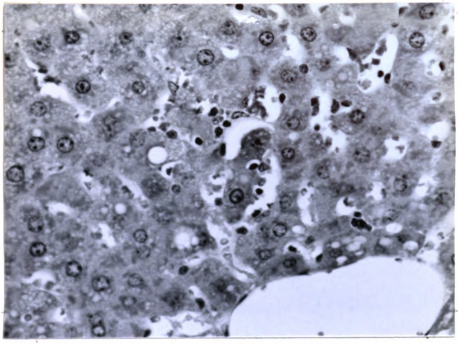


Figure 9. Section of liver from a rat fed a diet containing 100 ppm of FM BP-6 for 10 days, to show centrolobular to midzonal area. Notice swollen hepatocytes, decreased sinusoidal space and vacuoles. Hematoxylin and eosin stain; X400.

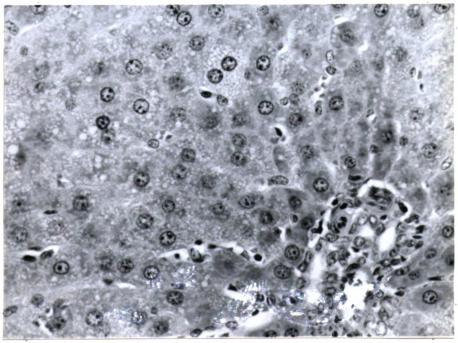


Figure 10. Section of liver from a rat fed a diet containing 100 ppm of FM BP-6 for 10 days, to show portal to midzonal area. Notice swollen hepatocytes, obliteration of sinusoidal space and fine vacuolization. Hematoxylin and eosin stain; X400.

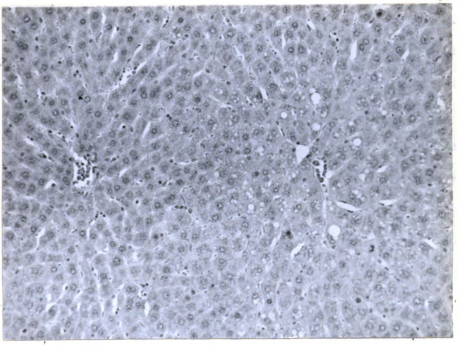


Figure 11. Section of liver from a rat fed a diet containing 100 ppm of 2,2',4,4',5,5'-HBB for 10 days. Notice hepatocellular swelling, decreased sinusoidal space and vacuolization, especially in the centrolobular to midzonal area. Hematoxylin and eosin stain; X160.

diffuse pattern of hepatocyte swelling with prominent vacuolization in the centrolobular to midzonal region.

Oil red O stain confirmed the presence of fat droplets in those at the 10 ppm dietary level but failed to confirm the presence of fat at the 100 ppm level.

Liver sections from rats fed dietary 3,3',4,4',5,5'-HBB at the level of 0.1 ppm looked like those from control rats. At the 1 ppm dietary level, some hepatocellular swelling was observed in the periportal region and at the 10 ppm level this observation was more evident. The centrolobular areas appeared relatively normal except for the presence of variably-sized vacuoles in one-third of the liver sections (Figure 12). Vacuolization extended into the midzonal region.

More advanced and consistent findings occurred at the 100 ppm dietary level. All hepatocytes were swollen, sinusoidal spaces were decreased in size to nonexistent, and midzonal to centrolobular vacuolization was obvious (Figures 13 and 14). Nucleoli were prominent and slightly larger than those present in hepatocytes of control rats.

Hepatic changes appeared to be time dependent. At the 20-day, 100 ppm dietary level of 3,3',4,4',5,5'-HBB all hepatocytes were swollen with a relatively distinct rounded appearance and many binucleated cells were observed (Figures 15, 16 and 17). Nucleoli were enlarged and the cytoplasm had a foamy appearance with variably-sized vacuoles. The largest vacuoles seemed to be located in the centrolobular areas, whereas the portal areas appeared hypercellular. The hypercellularity was primarily due to cells with a round and vesicular nucleus and indistinct cytoplasmic borders. Their general appearance was similar to the epithelial cells lining the bile ducts.

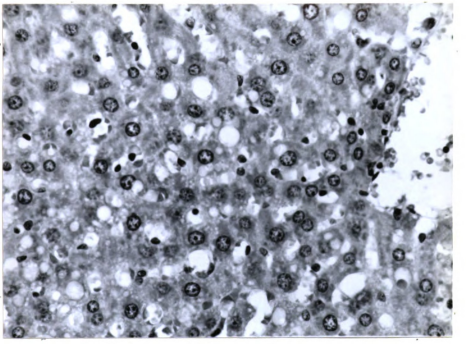


Figure 12. Section of liver from a rat fed a diet containing 10 ppm of 3,3',4,4',5,5'-HBB for 10 days, to show vacuolization in the centrolobular to midzonal area. Notice that some hepatocytes are relatively normal. Hematoxylin and eosin stain; X400.

Figure 13. Section of liver from a rat fed a diet containing 100 ppm of 3,3',4,4',5,5'-HBB for 10 days. Notice diffuse swelling of hepatocytes, decreased sinusoidal space and prominent vacuolization in the centrolobular to midzonal area. Hematoxylin and eosin stain; X160.

Figure 14. Section of liver from a rat fed a diet containing 100 ppm of 3,3',4,4',5,5'-HBB for 10 days, to show the centrolobular to midzonal area. Notice swollen hepatocytes, decreased sinusoidal space and variably-sized vacuoles. Hematoxylin and eosin stain; X400.

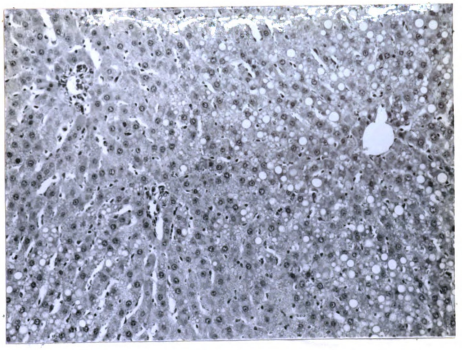


Figure 13

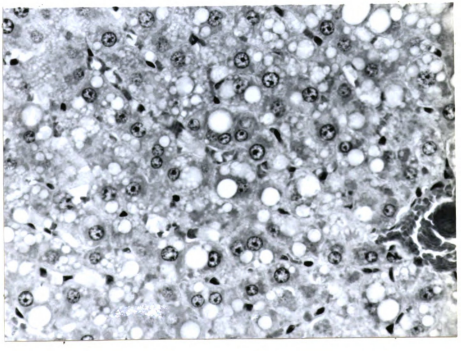


Figure 14

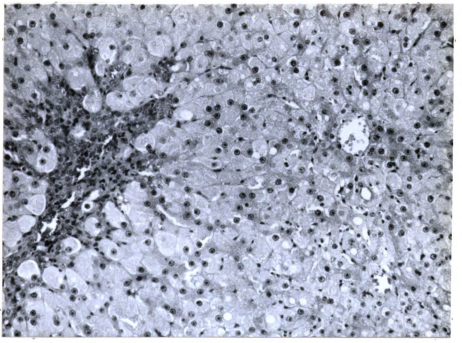


Figure 15. Section of liver from a rat fed a diet containing 100 ppm of 3,3',4,4',5,5'-HBB for 20 days. Notice diffusely swollen and rounded hepatocytes, vacuolization in centrolobular to midzonal area and hypercellularity of portal area. Hematoxylin and eosin stain; X160.

Figure 16. Section of liver from a rat fed a diet containing 100 ppm of 3,3',4,4',5,5'-HBB for 20 days, to show the centrolobular to midzonal area. Notice rounded and swollen hepatocytes with prominent and enlarged nucleoli and foamy cytoplasm with variably-sized vacuoles. Binucleated hepatocytes are present and sinusoidal space is also obliterated. Hematoxylin and eosin stain; X400.

Figure 17. Section of liver from a rat fed a diet containing 100 ppm of 3,3',4,4',5,5'-HBB for 20 days, to show the portal area. Notice portal vein (V), bile duct (BD) and proliferating epithelial cells (arrow). Hematoxylin and eosin stain; X400.

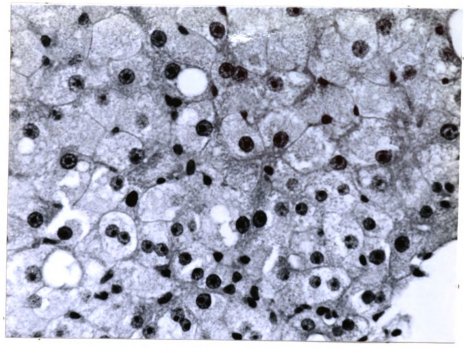


Figure 16

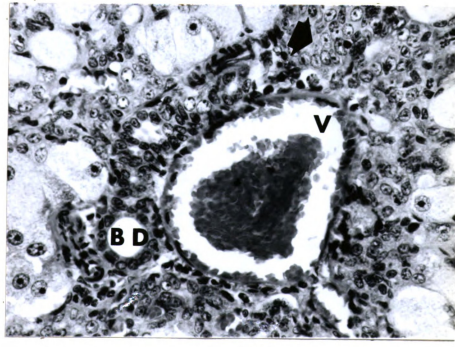


Figure 17

Structures similar to ducts were seen occasionally, but in general no ductule formation occurred.

The presence of lipid droplets within hepatocytes was confirmed with oil red O stain. The liver from rats at the 1 ppm dietary level had only occasional droplets. The amount of lipid droplets progressively increased as the dietary level of chemical increased. The liver from those rats getting the chemical for 20 days had more lipid droplets than the liver from those getting fed the chemical for 10 days, indicating a time-dependent relationship, too.

All livers from rats in the modified paired-feeding study appeared the same.

Ultrastructural Findings

Variations occurred in the hepatocytes from control rats. Hepatocytes from control rats in the 2,2',4,4',5,5'-HBB study had a slight proliferation of smooth endoplasmic reticulum (SER) and indistinct cytoplasmic borders, indicating slight changes from normal (Figure 18). Mitochondria from these cells were generally the same as ones from other control rats and had a rounded, oval or elongated and dark appearance. An exception was the hepatocellular mitochondria from control rats in the 3,3',4,4',5,5'-HBB study (Figure 19). They were lighter but of the same size as those from other control rats. Light and dark staining hepatocytes were seen and appeared normal. Cell outlines were apparent and 2 types of nuclei were present. One had a smooth circular outline and the other type was irregular and suggestive of a degenerative change. Whorled figures which represent phospholipid were seen within some of the occasional lipid droplets (Figure 20). Hepatocellular mitochondria from a control rat in the

Figure 18. Electron micrograph of a hepatocyte from control rat. Notice parallel aggregations of rough endoplasmic reticulum (arrow) near the nucleus (N). Mitochondria (M) appear dark, and there is slight proliferation of smooth endoplasmic reticulum (SER) and indistinct cellular borders. Uranyl acetate-lead citrate stain; X3600.

Figure 19. Hepatocyte from control rat. Notice lighter appearance of mitochondria (M) than those in Figure 19. Lipid droplets (arrow) are present in lighter-appearing cell and in adjacent darker cell. Uranyl acetate-lead citrate stain; X3600.

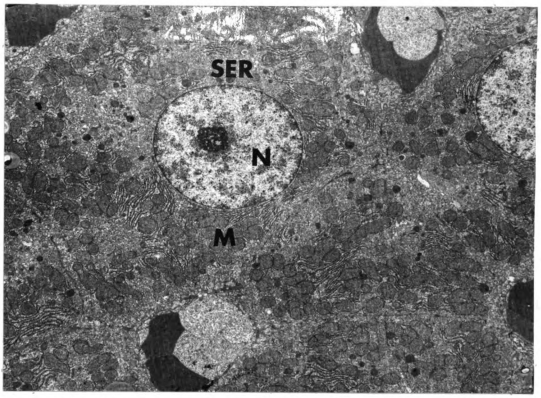


Figure 18

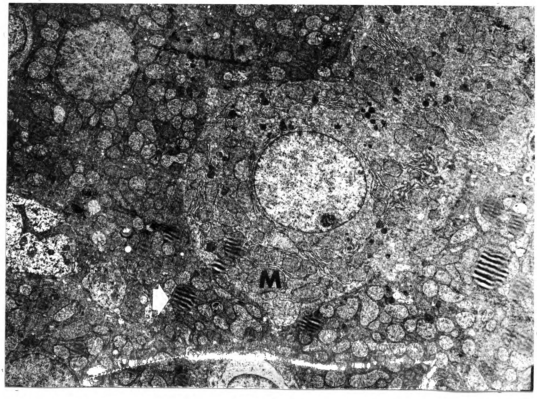


Figure 19

2,2',4,4',5,5'-HBB study were smaller than ones from control rats in other studies (Figure 21). Lysosomes also varied in size and number among control rats.

No changes were observed in the hepatocytes from rats fed diets containing 0.1 and 1 ppm of dietary FM BP-6. At the 10 ppm level, mitochondria were lighter and there was a proliferation of the SER (Figure 22). These changes were also observed at the 100 ppm level, but also many intracytoplasmic lipid droplets with variable size and internal density were present (Figure 23). Other hepatocytes from the same rat had many nuclei with irregular borders and degenerative organelle changes (Figure 24). In some cells mitochondria were swollen with clear spaces in their centers (Figure 25). The rough endoplasmic reticulum (RER) and SER appeared dilated and non-membrane-bound ribosomes were free within the cytoplasm. Free ribosomes may also occur within the cytoplasm of hepatocytes from control rats (Figure 26).

Rats given 0.1, 1 and 10 ppm dietary levels of 2,2',4,4',5,5'-HBB did not have ultrastructural changes in rat hepatocytes. At the 100 ppm level, proliferation of the SER was observed (Figure 27). Cell margins were indistinct and the mitochondria had a lighter appearance.

Dose- and time-dependent changes were observed in the hepatocytes from rats given diets containing 3,3',4,4',5,5'-HBB. As with the other 2 chemicals, no changes were observed at the 0.1 ppm level. At the 1 ppm level, there appeared to be a slight proliferation and dilatation of the SER. Free ribosomes were present within the cytoplasm and there was an increased incidence of double membrane RER

Figure 20. Portion of hepatocyte from a control rat. Whorled figure (arrow) present within lipid droplets. Uranyl acetate-lead citrate stain; X12,600.

Figure 21. Portion of hepatocyte from a control rat. Note mitochondria appear smaller than those in Figure 21. Rough endoplasmic reticulum (RER) appears in normal parallel aggregations. Uranyl acetate-lead citrate stain; X12,600.

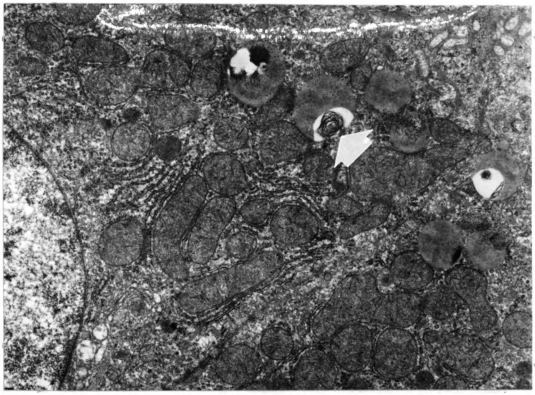


Figure 20

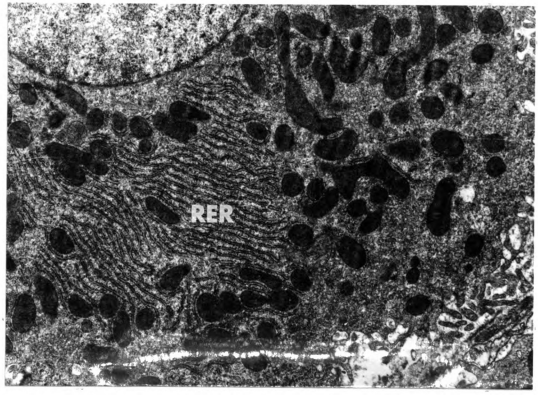


Figure 21

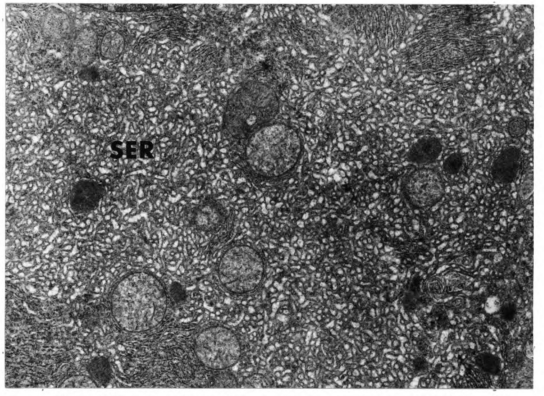


Figure 22. Portion of a hepatocyte from a rat fed a diet containing 10 ppm of FM BP-6 for 10 days. Notice proliferation of the smooth endoplasmic reticulum (SER). Mitochondria appear light staining. Uranyl acetate-lead citrate stain; X12,600.

Figure 23. Hepatocyte from a rat fed a diet containing 100 ppm of FM BP-6 for 10 days. Notice irregular nuclear outline (white arrow), light-staining mitochondria (clear arrow) and lipid droplets (LP). Uranyl acetate-lead citrate stain; X3600.

Figure 24. Hepatocytes from a rat fed a diet containing 100 ppm of FM BP-6 for 10 days, to show degenerative changes. Notice irregular outline of nuclei, swollen and light-staining mitochondria (clear arrow) and dilatation of endoplasmic reticulum (white arrow). Uranyl acetate-lead citrate stain; X3600.

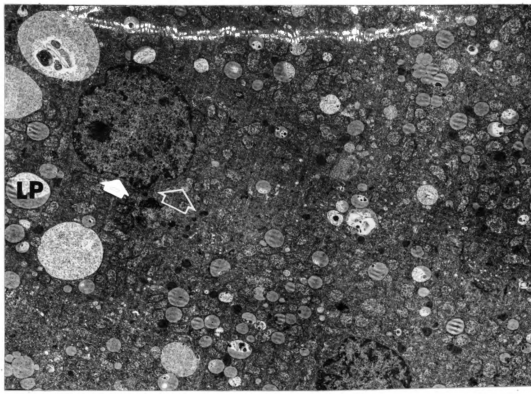


Figure 23

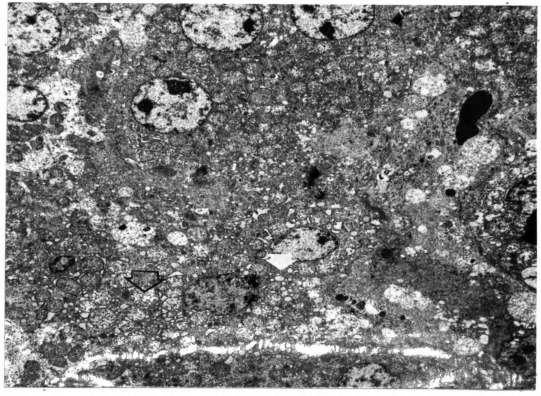


Figure 24

Figure 25. Portion of hepatocyte from a rat fed a diet containing 100 ppm of FM BP-6 for 10 days. Notice higher magnification of the swollen mitochondria observed in Figure 24. Routh endoplasmic reticulum (RER) and smooth endoplasmic reticulum (SER) appear dilated. Uranyl acetate-lead citrate stain; X22,800.

Figure 26. Portion of hepatocyte from a control rat at same magnification as Figure 25. Notice normal appearance of mitochondria and rough endoplasmic reticulum (white arrow). Free ribosomes are also present (clear arrow). Uranyl acetate-lead citrate stain; X22,800.

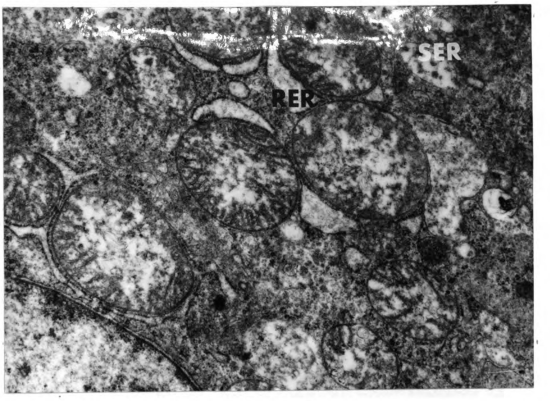


Figure 25

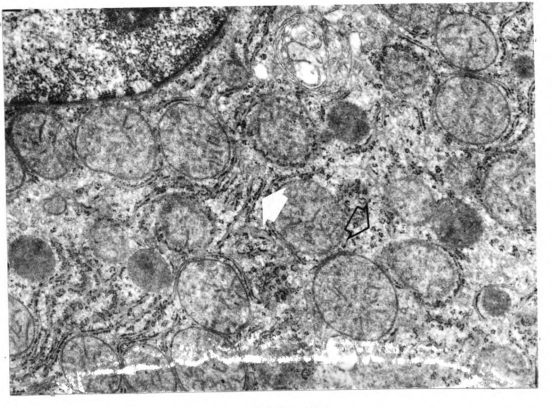


Figure 26

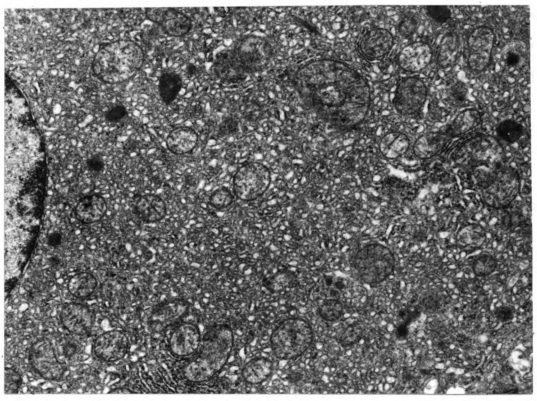


Figure 27. Portion of hepatocyte from a rat fed a diet containing 100 ppm of 2,2',4,4',5,5'-HBB for 10 days. Notice proliferation of smooth endoplasmic reticulum. Uranyl acetate-lead citrate stain; X12,600.

adjacent to mitochondria. These changes were subtle. More obvious changes were found associated with the 10 ppm level.

At 10 ppm, hepatocytes had an abundance of SER and an increased amount of individualized double-membrane RER which was often located around mitochondria (Figure 28). There was also an increased number of variably-sized lipid droplets. Four types of lipid droplets were seen. One was dark but usually of lighter density than mitochondria (Figure 29). Some were light and granular. Others were light and contained whorled figures and some were completely clear.

More severe changes were observed at the 100 ppm level. An increased amount of lipid droplets, SER proliferation, and disorganization of the RER were obvious (Figure 30). The individualized double-membraned RER encapsulated mitochondria and the lipid droplets (Figure 31). Free ribosomes were also present in the cytoplasm (Figure 32).

Time-dependent changes were seen in the hepatocytes of rats fed diets containing 100 ppm of 3,3',4,4',5,5'-HBB for 20 days: enlarged nucleoli, even more fat droplets, swollen mitochondria with irregular outlines, disorganized RER and multilayered myelin bodies which are continuous with the endoplasmic reticulum (Figures 33 and 34). In the hepatocytes adjacent to the portal area abundant lipid droplets filled the cytoplasm and very few mitochondria were observed in comparison to hepatocytes from a similar area of the hepatic lobule in a control rat (Figures 35 and 36).

The majority of the cells contributing to the hypercellularity of the portal area were bile duct cells. Some of the cells comprising bile ducts with lumens contained intracytoplasmic lipid droplets (Figures 36 and 37). Other cells which were more individualized and

Figure 28. Portion of a hepatocyte from a rat fed a diet containing 10 ppm of 3,3',4,4',5,5'-HBB for 10 days. Notice characteristics of rough endoplasmic reticulum (arrow). It appears to have a double membrane, is individualized, and is surrounding mitochondria. Uranyl acetate-lead citrate stain; X12,600.

Figure 29. Portion of a hepatocyte from a rat fed a diet containing 10 ppm of 3,3',4,4',5,5'-HBB for 10 days, to show variations in lipid droplet density. Notice lipid droplets (LP). Uranyl acetate-lead citrate stain; X12,600.

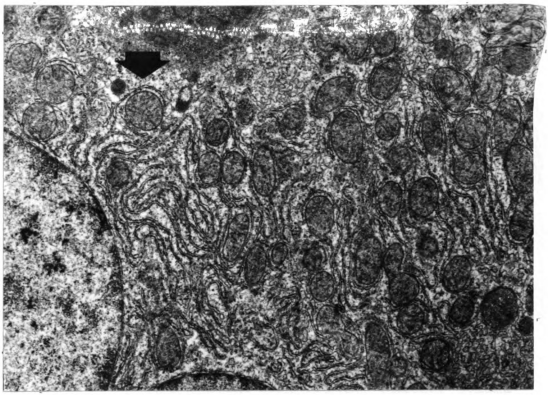


Figure 28

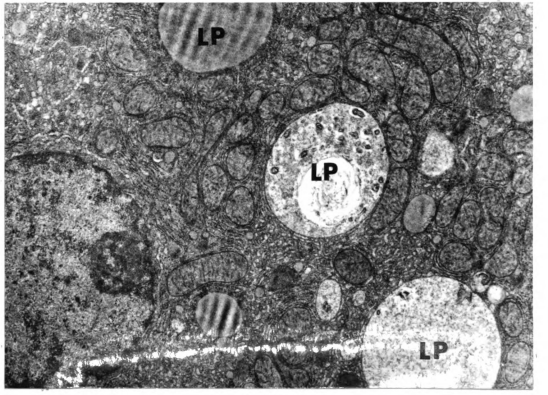


Figure 29

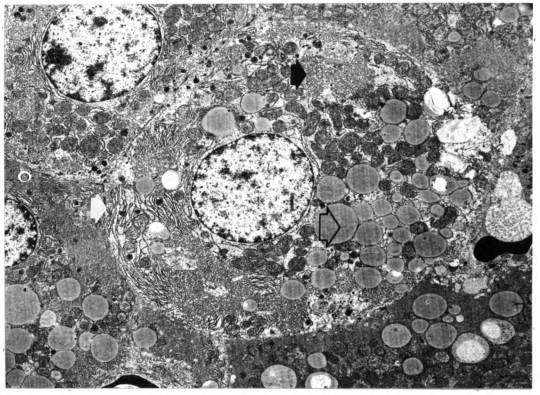


Figure 30. Hepatocyte from a rat fed a diet containing 100 ppm of 3,3',4,4',5,5'-HBB for 10 days. Notice proliferation of smooth endoplasmic reticulum (black arrow), disorganization of the rough endoplasmic reticulum (white arrow), and abundant lipid droplets (clear arrow). Changes are in a light cell. Uranyl acetate-lead citrate stain; X3600.

Figure 31. Portion of hepatocyte from a rat fed a diet containing 100 ppm of 3,3',4,4',5,5'-HBB for 10 days. Notice increased amount of lipid droplets. Individualized, rough endoplasmic reticulum (arrow) has encircled mitochondria and lipid droplets. Uranyl acetate-lead citrate stain; X12,600.

Figure 32. Portion of hepatocyte from a rat fed a diet containing 100 ppm of 3,3',4,4',5,5'-HBB for 10 days, to show proliferation of smooth endoplasmic reticulum and increased amount of lipid droplets. Uranyl acetate-lead citrate stain; X12,600.

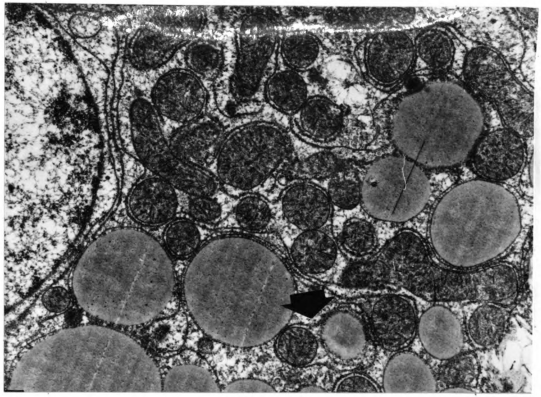


Figure 31

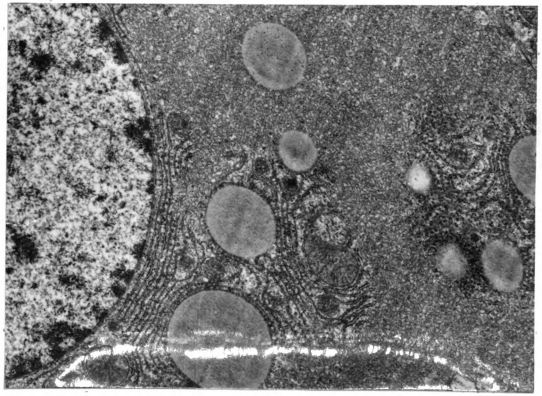


Figure 32

Figure 33. Hepatocyte from a rat fed a diet containing 100 ppm of 3,3',4,4',5,5'-HBB for 20 days. Notice enlarged nucleolus (black arrow), increased amount of lipid droplets (L), swollen mitochondria (clear arrow) and myelin body (white arrow). Uranyl acetate-lead citrate stain; X3600.

Figure 34. Portion of a hepatocyte from a rat fed a diet containing 100 ppm of 3,3',4,4',5,5'-HBB for 20 days. Notice degenerating mitochondria (M) encircled by membranes of endoplasmic reticulum and 2 lipid droplets surrounded by a multilayered myelin body (MB). Uranyl acetate-lead citrate stain; X12,600.

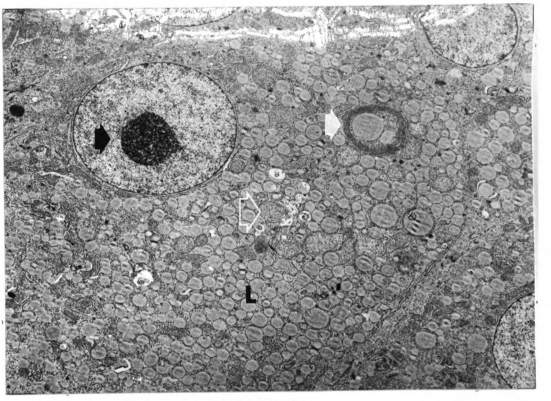


Figure 33

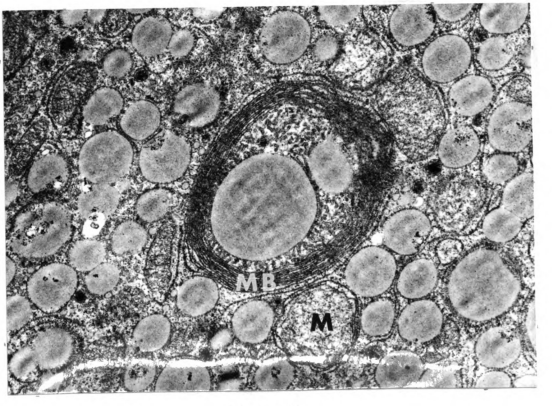


Figure 34

Figure 35. Portion of portal area of liver from a control rat. Notice the bile duct epithelium (arrow), macrophage (black M) and abundant mitochondria of a hepatocyte (white M). Uranyl acetate-lead citrate stain; X4300.

Figure 36. Portion of portal area of liver from a rat fed a diet containing 3,3',4,4',5,5'-HBB for 20 days. Notice bile duct epithelial cell with fat droplets (B), lumen of bile duct (L) and abundant lipid droplets filling the cytoplasm of a hepatocyte (H). Uranyl acetate-lead citrate stain; X4300.

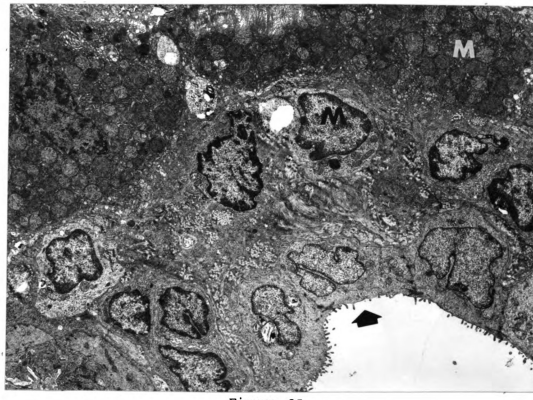


Figure 35

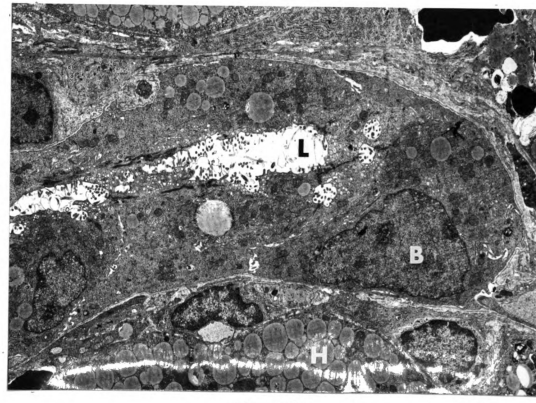


Figure 36

Figure 37. Portion of portal area of liver from a rat fed a diet containing 3,3',4,4',5,5'-HBB for 20 days. Notice bile duct epithelial cell (B) containing lipid droplets (L), lumen of bile duct (arrow) and fibroblast (F). Uranyl acetate-lead citrate stain; X4300.

Figure 38. Portion of portal area of liver from a rat fed a diet containing 3,3',4,4',5,5'-HBB for 20 days. Notice bile duct epithelial cell with fat droplets (B), portion of plasma cell (P), neutrophil (N), endothelial cell (E), fibroblast (F), erythrocytes (arrow) and cytoplasm of a hepatocyte filled with lipid droplets (H). Uranyl acetate-lead citrate stain; X4300.

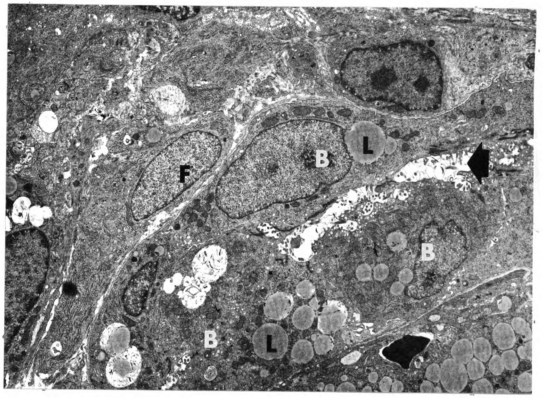


Figure 37

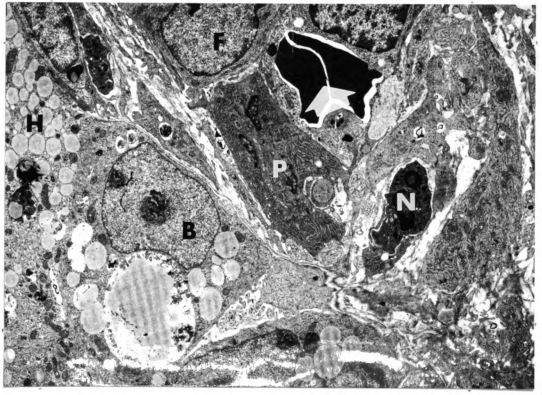


Figure 38

had the appearance of bile duct cells also contained lipid droplets (Figure 38). Inflammatory cells and fibroblasts also added to the hypercellularity (Figures 38 and 39). The majority of the inflammatory cells were macrophages. Some of them contained lipid droplets and dense bodies suggesting phagocytosis (Figure 40).

Figure 39. Portion of portal area of liver from a rat fed a diet containing 3,3',4,4',5,5'-HBB for 20 days. Notice bile duct epithelial cell (B), macrophage (M), and fibroblast (F). Uranyl acetate-lead citrate stain; X4300.

Figure 40. Portion of portal area of liver from a rat fed a diet containing 3,3',4,4',5,5'-HBB for 20 days. Notice macrophages (M). Two contain lipid droplets (L) and circular black dense bodies. Uranyl acetate-lead citrate stain; X4300.

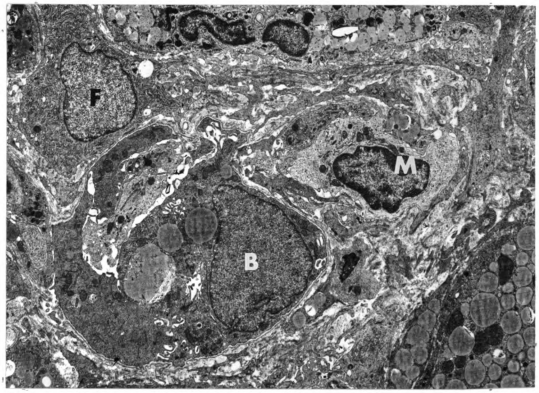


Figure 39

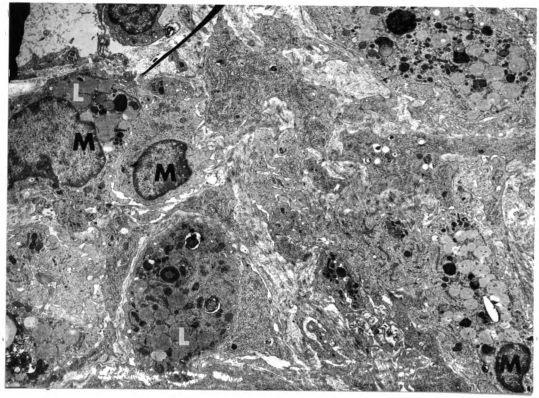


Figure 40

DISCUSSION

The author agrees with Witschi (1980) when he emphasized the importance of not just identifying pathogenic agents but also understanding the mechanisms by which they cause cell and tissue lesions. The author also agrees with Moore et al. (1980) when they state that the understanding of the nature and mechanisms of the toxicity of PBB will not be gained unless there is an understanding of the effects of individual compounds. Understanding the toxicity of an individual chemical begins with the knowledge of the chemical structure. Eventually, a theory for its toxicity based on structure-function relationships is established.

The earliest papers about PBB described research conducted with FM. With time many papers appeared describing the effects of the major congener, 2,2',4,4',5,5'-HBB (peak 4). In general this congener, 2,2',3,4,4',5,5'-HBB (peak 8) and 2,2',3,3',4,4',5,5'-OBB (peak 12), all of which are PB-type inducers, produced less severe effects than the commercial mixture. The author agrees with Akoso et al. (1980) that it appears other congeners contribute significantly to the toxicity of the FM.

Reports in the literature indicate that MC-type inducers such as TCDD are quite toxic. Moore et al. (1980) mentioned that the toxicity of PBB congeners appears to be closely related to their ability to produce this type of induction, but reports are lacking about the

effects of a pure MC-type inducer which is also a highly brominated biphenyl.

The basic hypothesis is that a compound such as 3,3',4,4',5,5'-HBB, which is a pure MC-type inducer, is more toxic than a mixed-type inducer, which would be more toxic than a PB-type inducer. Through an integrated approach involving many techniques, this premise was borne out in the results of this study.

Evidence that 3,3',4,4',5,5'-HBB is a strict MC-type inducer included a shift in the λ max. from 450 to 448, a 4- to 9-fold increase in benzopyrene hydroxylase activity. Poland and Glover (1977) reported similar results. Since there was a less than 2-fold increase in aminopyrine demethylase activity, there was no indication of PB-type induction (Moore et al., 1978c).

Dannan et al. (1978b) mentioned that benzo[a]pyrene is a well characterized compound which is metabolized into a diol epoxide. The epoxide is capable of binding to DNA. They showed that there was increased binding catalyzed by MC-induced microsomes. The binding was greater than that catalyzed by microsomes induced by PBB, which was greater than that catalyzed by PB-induced microsomes. This provides further evidence that a MC-type inducer is potentially more toxic than a mixed-type inducer, which is more toxic than a PB-type inducer. It also suggests that MC-type inducers are toxic because they enhance the toxic qualities of other compounds like benzo[a]pyrene.

Gupta and Moore (1979) described clinical signs in rats which were associated with high treatment levels of FM FF-1. These signs were similar to those seen in rats fed 100 ppm of 3,3',4,4',5,5'-HBB in the diet for 20 days in the present investigation. Gupta and Moore reported deaths in young rats given FM FF-1 in a cumulative oral dose

of 385 mg or greater. Considering that approximately 84% of FM FF-1 by weight is composed of pure PB-type inducers (Moore et al., 1980), at most only 16% of the mixture can be mixed-type inducers. The least amount of mixed-type inducers that was given to Gupta's and Moore's rats which died was approximately 61.5 mg. The rats that died in the present investigation consumed approximately 19.5 mg of pure MC-type inducer each. It is likely that the rats died which were given FM FF-1 because they were given lethal quantities of compounds which have MC-type inducing qualities.

In general, feed intake for rats fed FM BP-6 was not significantly different than the feed intake by control rats. Of more importance is the decreased feed intake by rats fed 3,3',4,4',5,5'-HBB at dietary levels of 10 and especially at the 100 ppm level ($p<0.0005$) for 10 days. After 16 days, the rats fed 100 ppm of 3,3',4,4',5,5'-HBB stopped eating and 1 died 4 days later. Gupta and Moore reported significant ($p<0.05$) decreases in feed consumption at dosages of 100, 300, and 1000 mg FM FF-1/kg/day within 10 days from the start of treatment. The feed consumption remained depressed in these rats until they died. Decreased feed intake alone does not account for the clinical signs seen with 3,3',4,4',5,5'-HBB administration because the rats consuming 13 g/day of the control diet did not have clinical signs other than a depressed rate of gain or body weight.

In contrast, rats fed dietary 2,2',4,4',5,5'-HBB had an increased mean feed intake at the dietary levels of 1 and 10 ppm. By this criterion, this compound is obviously less toxic than the other two.

If feed intake is decreased, then decreased body weight is logically expected. This was true for 3,3',4,4',5,5'-HBB administration at the dietary levels of 10 and 100 ppm. The rats getting 13 g/day of

control diet/day also had decreased body weights after 10 days. After 20 days the rats getting 13 g/day had decreased body weights as well as the rats getting dietary 3,3',4,4',5,5'-HBB. The values for the treated rats were less than the rats getting 13 g/day and indicate a treatment effect. Goldstein et al. (1977) found marked decreases in weight gain in rats getting 3,3',4,4',5,5'-HCB in comparison to control rats, rats fed 2,2',4,4',5,5'-HCB and rats getting AR 1260 via IP administration.

Garthoff et al. (1977) stated that for AR and FM, impaired food utilization rather than decreased intake appeared to be the primary cause of slow rate of body weight gain. They noticed a marked decrease in rate of body weight gain after 10 days of treatment, even though feed intake was normal at the 500 ppm dietary level but not at the 50 or 5 ppm levels. At the dietary level of 100 ppm of FM BP-6, rats had normal growth with normal feed intake in the present investigation.

With increased feed intake, increased body weight is expected. The increase in body weight of rats getting 1 ppm of 2,2',4,4',5,5'-HBB, even though it is not significant, is probably due to the increased feed intake.

After 10 days, rats getting 10 and 100 ppm of dietary FM BP-6 had increased liver weights which were dose-related. Pratt (1979) reported a similar finding at the 10 ppm level but found increased relative liver weights in rats at the 0.1 and 1 ppm dietary levels. Sleight and Sanger (1976) reported increased liver to body weight ratios for male rats fed diets containing 1, 10 or 100 ppm of FM BP-6 for 30 days. The dietary administration of 2,2',4,4',5,5'-HBB at the levels of 10 and 100 ppm also resulted in increased liver to body

weight ratios. Moore et al. (1978c) reported an increase in the ratio with a 25% increase within 2 days after IP injection. This increase was still pronounced 12 days later. Goldstein et al. (1977) injected 2,2',4,4'5,5'-HCB or 3,3',4,4',5,5'-HCB into rats at 2 different doses. An increased liver to body weight ratio was noticed in rats given the high dose at 2,2',4,4',5,5'-HCB, but both the high and the low dose of 3,3',4,4',5,'-HCB caused an increased ratio.

Many chemicals are reported to increase the liver weight of animals. Results from this present investigation indicate 3,3',4,4'5,5'-HBB should be added to this list of compounds. The results also confirm previous reports in the literature that FM and 2,2',4,4'5,5'-HBB increase liver weights in rats. Between 10 and 20 days the liver size of rats getting 3,3',4,4',5,5'-HBB at a dietary level of 100 ppm did not increase but decreased. However, at 20 days the livers were still relatively enlarged but the decreased weight was probably associated with decreased feed intake. For example, rats limited to either 13 g/day or 23 g/day for 20 days had smaller livers than those fed *ad libitum*.

During starvation the liver decreased in size because of the protein depletion (Krustev, 1976). Ultrastructurally, there were fewer mitochondria, decreased amount of RER, loss of parallel arrangement of the RER and an increased amount of intracytoplasmic lipid droplets in hepatocytes from rats which were protein deficient. Although these changes were seen in the hepatocytes of the rats given 100 ppm of dietary 3,3',4,4',5,5'-HBB for 20 days, they were also seen after 10 days of treatment in the cells of livers which were not decreased in size from rats which were still eating. Starvation did not fully explain the hepatic lesions in the rats given the chemical

for 20 days, but it may help explain why they had the most prominent and advanced changes.

Although both hypertrophy and hyperplasia have been associated with the increase in liver size, an increased number of mitotic figures and binucleated cells was generally not evident histologically in the present investigation. This is in contrast to the findings of Pratt (1979) in which there was a marked increase in mitotic activity of hepatocytes of rats at the dietary levels of 0.1, 1 and 10 ppm FM BP-6 for 10 days.

In general, hepatocellular changes were seen at a lower dietary level and were more advanced at the highest level in the rats given 3,3',4,4',5,5'-HBB in comparison to the changes associated with the administration of either of the other 2 chemicals. The hepatocytes from the rats given 2,2',4,4',5,5'-HBB had the least severe changes.

In this investigation hepatocellular swelling and proliferation of SER were prominent changes. Swollen hepatocytes are reported to result from stimulation of the endoplasmic reticulum (ER) (Trump and Jones, 1978; Lee et al., 1975a). Proliferation of the SER is an early and reversible (Trump and Jones, 1978) response to a variety of chemicals and is considered to be a structural reflection of the enhanced enzyme activity (Hansell and Ecobichon, 1974). However, proliferation of RER and SER may not be associated with increased enzyme content (Smuckler and Ancasoy, 1969; Fowler, 1980). Synthesis of the microsomal enzymes occurs in the RER, and when the RER becomes saturated with enzyme it appears to lose its ribosomes and to become SER (Blumberg, 1978; Fowler, 1980). If the stimulus for microsomal enzyme induction is eliminated, then the amount of SER decreases. Subsequently, there is an increase in autophagic vacuoles (Fowler, 1980).

After prolonged administration of inducers, whorls of SER or RER are found in the cytoplasm (Smuckler and Arcasoy, 1969). Both the proliferation of SER and the whorl formation represent proliferative responses by the hepatocyte. Histologically, the whorls appear as ring-shaped inclusions (Lee et al., 1975a; Akoso et al., 1980). They have been called whorls, fingerprints, myeloid bodies, glycogen bodies, or myelin bodies (Lee et al., 1975a). These whorls are apparently species related, as such structures were found in rats and mice, rarely in rabbits and not found in monkeys given PCB (Hansell and Ecobichon, 1974).

In the present study the whorls were only seen in the hepatocytes of rats after 20 days of dietary administration of 3,3',4,4',5,5'-HBB at a level of 100 ppm. Akoso and Sleight (1979) and Akoso et al. (1980) reported finding them after 30 days of dietary administration of FM BP-6 or 2,2',4,4',5,5'-HBB (peak 6). Both of these are mixed-type inducers. They have also been seen in hepatocytes of rats given chlorinated aromatic hydrocarbons (Norback and Allen, 1972). These compounds are either mixed- or MC-type inducers. Dilatation of the cisternae of the SER and/or RER is associated with altered (usually decreased) functional states (Smuckler and Arcasoy, 1969) and correlated with the inward movement of water and ions into the cisternae (Trump and Jones, 1978). This type of change was seen in hepatocytes from rats given FM BP-6 at a dietary level of 100 ppm. Histologically there is a decreased staining intensity of the hepatocytes (Smuckler and Arcasoy, 1978). This rearrangement of RER into sinusoidal arrays is believed to be the first stage in whorl production (Norback and Allen, 1972). Shortly after the development of annular formations, concentric annuli composed of variable numbers of paired membranes

form. The other membrane pairs of the lamellae are continuous with elements of the SER and RER since ribosomes are occasionally attached to the surfaces of the outer concentric membrane pairs (Norback and Allen, 1972). These concentric membrane arrays increase in size and complexity in direct relationship to the time the rats are exposed to the chemical.

The parallel cisternae of the RER reorganize and encompass islands of cytoplasm, mitochondria and lipid droplets. By surrounding lipid droplets that contain lipophilic chemicals, the exposure of these chemicals to degradative enzymes in these membranes is enhanced (Norback and Allen, 1972; Lee et al, 1975a). These membranes also have an increased amount of phospholipid which also enhances the ability of the membrane to sequester chemicals. Trump and Jones (1978) mentioned that these membranes may be enzyme-poor. Lee et al. (1975a) stated that the significance of whorled figures or myelin bodies is still speculative.

Intracytoplasmic lipid vacuolation was apparent in the present study and in previous studies associated with administration of PBB. Hepatocytes take up free fatty acids which are esterified to triglycerides, appear in the cisternae of the ER as small liposomes and emerge via the Golgi complex as very low density lipoproteins (Cheville, 1976). Hinton et al. (1977) stated that there is a blockade in the transport of lipid upon exposure to PCB and/or PBB. This blockade appears to be at the Golgi apparatus or between the Golgi and the endoplasmic reticulum. Liposomes were not seen in the cisternae of the ER in the hepatocytes from rats given any of the 3 chemicals, but many lipid droplets were found in the cytoplasm. Lipids which are occasionally found in the cytoplasm of normal hepatocytes are globules

of triglycerides or neutral lipids. They are not membrane-bound but exist free within the cytoplasmic matrix due to high surface tension of the external phospholipid monolayer surrounding the lipid (Cheville, 1976).

Lipid droplets varied in ultrastructural appearance. Some were clear, some were partially clear, some appeared granular and some had a medium gray appearance. Occasionally small whorled figures (not to be confused with myelin bodies) were found inside these lipid droplets. More were found when more lipid droplets occurred in the hepatocyte. Lee et al. (1975a) reported similar findings and described them as unmasked phospholipid. Lee and fellow workers observed these figures in autophagic vacuoles. The ones observed in this study were clearly within lipid droplets. These whorled figures may be associated with degradative or autophagic changes within the lipid droplets, but their significance and the mechanism by which the lipid droplets accumulate are still speculative.

At the 100 ppm level, hepatocytes from rats fed FM BP-6 for 10 days and rats getting dietary 3,3',4,4',5,5'-HBB for 20 days had large and lighter-staining mitochondria. Similar changes were seen in hepatocytes of rats which were injected with peak 2 one week after the injection (Dannan et al., 1979). After 2 weeks, the mitochondria did not have the swollen appearance but appeared relatively normal. Mitochondrial swelling is associated with loss of the capacity to synthesize ATP at the inner membrane and is a reversible change (Cheville, 1976). Cheville also mentioned that the presence of flocculation of protein is an absolute sign of irreversible injury. In this experiment none of the mitochondria examined contained such material.

Rats given 3,3',4,4',5,5'-HBB for 20 days at 100 ppm had enlarged hepatocellular nucleoli. Although this change is frequently seen in neoplastic cells, Cheville (1976) mentioned that it can be seen in association with cytotoxic reactions. Mitochondria were diminished in periportal hepatocytes from these treated rats. Since mitochondria are supposedly numerous in the periportal area, the lack of them in this area is even more significant.

Variations were noticed between the ultrastructural appearance of hepatocytes from control rats. Some of these variations may be due to the normal variation within a hepatic lobule. Trump and Jones (1978) reported the differences between hepatocellular organelles within the hepatic lobule of a human being. Mitochondria appeared round to oval in the centrolobular area but were more numerous, larger and oval to oblong in the periphery. The small mitochondria reported in this investigation were from the centrolobular area in comparison to the others, which were from the midzonal area. The reason why some appeared lighter than others may just have been due to staining.

Generally, the midzonal area was most severely affected. Prominent changes in the midzonal area were also reported by Pratt (1979) in rats given dietary FM BP-6 and by Moore et al. (1978c) in rats given dietary 2,2',4,4',5,5'-HBB. For the rats given FM BP-6 and 3,3',4,4',5,5'-HBB, there were some changes in the midzonal to peripheral area at the lowest dietary levels in which changes were seen. At the higher levels of these chemicals and in the hepatocytes of rats given 2,2',4,4',5,5'-HBB, the centrolobular to midzonal areas were most affected. Histologically, hepatocellular swelling and lipid vacuolation were the prominent changes common to all 3 chemical toxicoses.

A prominent change observed after 20 days of dietary administration of 100 ppm 3,3',4,4',5,5'-HBB was the proliferation of bile ducts. This change was similar to the diffuse type described by Gupta and Moore (1979) in rats given multiple high doses of FM FF-1. Synonyms for bile duct proliferation include adenofibrosis, atrophic hepatic cell cords and oval cell hyperplasia (Institute of Laboratory Animal Resources, 1980). In this study, the bile duct changes most closely resemble bile duct hyperplasia as depicted in this publication. An additional finding was the presence of intracytoplasmic lipid droplets in bile duct cells. The significance of this finding was undetermined.

The thymus weights and the spleen weights were both decreased in comparison to controls for only the rats getting 10 or 100 ppm of 3,3',4,4',5,5'-HBB. These decreases appeared to be dose-related and were espeically severe in the thymus of rats given 100 ppm for 20 days. Rats given 13 g/day also had decreased thymus and spleen weights in comparison to those fed *ad libitum*. However, only rats given 3,3',4,4',5,5'-HBB had histological changes in the thymus and spleen. The findings from the present investigation are similar to the reports mentioned earlier in the literature review for halogenated aromatic hydrocarbons and give support to the idea that 3,3',4,4',5,5'-HBB is more toxic than FM BP-6 or 2,2',4,4',5,5'-HBB.

On a fat basis the liver concentrations for all 3 chemicals, including FM, are greater than the adipose tissue concentrations. In general, the amount of 2,2',4,4',5,5'-HBB was higher in the tissues than the amount of PBB from FM. This is probably because the rats were given pure 2,2',4,4',5,5'-HBB in comparison to those given FM, which contains approximately 56% 2,2',4,4',5,5'-HBB. Liver concentrations of 3,3',4,4',5,5'-HBB appear higher than the respective values

of PBB from FM or 2,2',4,4',5,5'-HBB, with the exception of the 100 ppm level. These values were very inconsistent and indicate that the procedures need refinement. It is of interest that the chemical was never recovered from brain tissue.

Moore et al. (1980) mentioned that 3,3',4,4',5,5'-HBB is a potent MC-type inducer, that its toxicity has not been established. The results from this present investigation are a start at establishing the toxicity of 3,3',4,4',5,5'-HBB. This chemical is an MC-type inducer and, in general, it appeared to be more toxic than the mixed-type inducer, which appeared to be more toxic than the PB-type inducer. The results of this investigation also bring researchers closer to the goal of being able to predict the toxicity of a polybrominated biphenyl or any halogenated aromatic hydrocarbon by knowing its molecular structure.

SUMMARY

Male Sprague-Dawley rats were fed diets containing 0, 0.1, 1, 10 or 100 ppm of FM BP-6, 2,2',4,4',5,5'-HBB or 3,3',4,4',5,5'-HBB for 10 days to compare toxicopathologic effects. At the 2 highest dietary concentrations, the rats fed 3,3',4,4',5,5'-HBB had decreased feed intake and body weights in comparison to control rats. At the 10 or 100 ppm dietary levels, liver weights were increased for rats getting any of the 3 chemicals. Histologically, these livers had evenly distributed and enlarged hepatocytes. Lipid vacuolation was most prominent in the centrolobular to midzonal area. This change was dose-related and most marked in the livers from rats getting 3,3',4,4',5,5'-HBB. Ultrastructural lesions consisted of proliferation of SER, disorganization of RER and increased amounts of free ribosomes and lipid droplets.

Alterations in extrahepatic organ weights were observed in rats fed 3,3',4,4',5,5'-HBB. At the 100 ppm level, kidney weights were decreased and thyroid weights were increased in comparison to controls. At 10 or 100 ppm, the thymus and spleen weights were decreased. At the 10 ppm level the thymus had a thinner than normal cortex. At the 100 ppm level the thymus was severely depleted of mature lymphocytes and the cortex was very thin. To a lesser degree, the spleen and lymph nodes were depleted of mature lymphocytes.

Two rats were fed 100 ppm of 3,3',4,4',5,5'-HBB for 20 days, but they quit eating after 16 days. At 20 days, 1 rat died and the other

was moribund and was killed. Both rats had hepatocytes which were rounded, containing large nucleoli and intracytoplasmic lipid droplets. Bile duct epithelium had also proliferated and contained intracytoplasmic lipid droplets. Myelin bodies were also present which were not seen in hepatocytes from rats after 10 days for any of the chemicals at any of the dietary levels. Further reductions in thymic and splenic weights had occurred between 10 and 20 days.

To help differentiate changes which were associated with decreased feed intake by rats given dietary 100 ppm 3,3',4,4',5,5'-HBB, rats were restricted to 13 g/day of control feed. These rats had lower body, liver, kidney and thyroid weights in comparison to the control rats. No histological changes were observed.

It appears that 3,3',4,4'5,5'-HBB, an MC-type inducer, is more toxic than FM BP-6, a mixed-type inducer, which is more toxic than 2,2',4,4',5,5'-HBB, a PB-type inducer.

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VITA

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The author was born in Pontiac, Michigan, on August 13, 1951. He lived with his parents on their modest farm, surrounded by an expanding wealthy suburban community until 1970. Upon graduating from Wylie E. Groves High School in Birmingham, Michigan, in 1969, he attended Wayne State University in Detroit, Michigan, for one year as an art major.

In 1970 he enlisted in the United States Air Force and spent the majority of four years stationed at England Air Force Base, Alexandria, Louisiana. During this time he worked as a medic and attended classes at Northwestern State University, Natchitoches, Louisiana, and Louisiana College, located in Pineville, Louisiana.

In 1974 he received an honorable discharge from the service and began attending classes at Michigan State University. He graduated with high honors as a Bachelor of Science in Dairy Science in 1976 and immediately entered the professional program in the College of Veterinary Medicine.

Upon graduating as a Doctor of Veterinary Medicine with honors, he began graduate work in the College of Veterinary Medicine and the pursuit of a Master of Science degree in Pathology.