AN EIGHTEEN-MONTH STUDY OF POLYBROMINATED BIPHENYL TOXICOSIS IN RATS

By

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An 18-month study designed to assess hepatic vitamin A concentrations and gross morphologic, histologic and ultrastructural changes associated with feeding low dietary levels of polybrominated biphenyls (PBB) was completed. One hundred sixty-eight young male Sprague-Dawley rats were randomly divided into 4 groups each consisting of 42 rats. Each group was assigned to a diet containing 0, 0.1, 1, or 10 ppm of PBB. Six rats from each group were killed at 10, 30, 60, 90, 180, 360, and 540 days.

No clinical signs or mortality referable to PBB toxicosis or hypovitaminosis A occurred. The PBB-fed rats gained weight as well as, and in some instances better than, the control rats. The relative weights of the kidney and brain (when expressed as percent of body weight) were not significantly ($p>0.05$) affected by dietary PBB. Hematologic values, urinalyses and serum proteins were essentially normal. Each of the 3 dietary concentrations of PBB induced significant increases ($p<0.05$) in relative liver weight at 10 and 30 days. Similar significant

increases in relative liver weights occurred for rats consuming diets containing 10 ppm of PBB at 60 days and thereafter. In contrast, relative (μg/g liver) and absolute (mg/liver) hepatic vitamin A concentrations were decreased ($p<0.05$) at one or more dietary levels of PBB throughout the course of the experiment. The most severe depletion of hepatic vitamin A occurred in rats fed diets with 10 ppm of PBB for 540 days.

Histologic lesions were confined to the liver and were related to PBB. Diffuse hypertrophy and hyperplasia of hepatocytes were the major changes in rats killed at 10 days. From 30 to 180 days, swollen hepatocytes and limited midzonal lipid accumulation were the only lesions evident. Severe intracytoplasmic lipid accumulation was the principal change in hepatocytes from rats fed 10 ppm of PBB for 360 and 540 days. In addition, focal necrosis, bile duct proliferation, focal infiltration of inflammatory cells, and portal fibrosis occurred. Ultrastructural changes included a progressive increase of the smooth endoplasmic reticulum (SER) with a concomitant decrease of rough endoplasmic reticulum (RER) and decreased numbers of mitochondria. Myelin body formation was a consistent finding. Some of the myelin bodies enclosed lipid droplets and membrane-bound vacuoles containing whorled figures. Tissue residues of PBB were dose-related, and livers with the highest PBB concentration had the most severe light and electron microscopic changes. The results of the investigation indicate that, even at very low dietary concentrations

of 0.1 ppm, PBB is capable of inducing significant morphologic and biochemical changes in rats.

DEDICATION

Dedicated to my wife, Novelette,

and daughters, Karren and Alison

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INTRODUCTION

The term "economic poison" implies that a toxic agent, either desirable or actually needed in our daily life, is at the same time a hazard to health. In general, insecticides, herbicides, and fungicides comprise this group; however, in the past decade, the toxic potentials of a number of other substances have been recognized. Thoroughly understood and properly used, these chemicals seldom cause adverse effects, and the benefits derived, though often debated, usually justify their use.

In recent years, the halogenated biphenyls, namely polychlorinated biphenyls (PCB) and polybrominated biphenyls (PBB) have attracted much attention, especially concerning their teratogenic and carcinogenic potentials. The PCB are known to be widely distributed in the environment, and many of their toxic effects have been extensively studied in animals (Kimbrough, 1974) and, to a lesser extent, in humans (Kuratsune et al., 1975). Conversely, the brominated analogs have received less attention because of their limited industrial use and the fact that they were not recognized as significant environmental contaminants.

The accidental incorporation of a PBB-containing fire retardant (Firemaster BP-6) into the feed of livestock in Michigan sometime in 1973 precipitated an incident that has been termed the most costly accidental contamination ever to occur in United States agriculture (Isleib and Whitehead, 1975). The consequences of the PBB contamination included condemnation and slaughter of thousands of cattle, poultry, and other livestock, as well as loss of meat, milk, and eggs. In addition, there was considerable concern regarding contamination of food supplies by a toxic substance.

Currently available literature suggests that PBB, like the PCB, are resistant to degradation and therefore result in virtually permanent contamination of soils, water, plants, and animals with which they come in contact. Environmental contamination by this persistent chemical has accordingly become a major concern of conservationists and of legislative and public health officials.

In the past, researchers have been concerned mainly with the effects of excessive amounts of the chemical on animal health. Recently, there is even greater concern regarding "no effect" levels and low-level contamination. Additionally, it would appear from data on Yusho disease (Kuratsune et al., 1975) that long-term public health effects are possible.

The major objectives of this research were:

1. To determine the gross morphologic, histologic and ultrastructural changes associated with feeding low dietary concentrations of PBB.

2. To assess changes in hepatic vitamin A concentrations induced by dietary PBB.

LITERATURE REVIEW

Early literature concerning the chronology of polybrominated biphenyl (PBB) contamination and resultant economic, public health, social and political effects is adequately recorded in reviews by Dunkel (1975), Welborn (1975), Stadtfeld (1976), and Carter (1976). Later reviews by Kay (1977), Getty et al. (1977), and an entire volume of Environmental Health Perspectives (1978) alluded to the contamination incident and summarized the extent of currently available experimental and scientific knowledge on PBB.

This review will be limited primarily to PBB toxicosis in animals. The treatise on chemistry, sources, industrial uses, and metabolism of PBB will be limited to the extent that it permits fundamental understanding. In addition, the effects of chemical toxicosis on vitamin A metabolism will be reviewed.

Chemistry

The PBB-containing fire retardant that was accidentally incorporated into livestock feed in Michigan in 1973 was a complex mixture of various isomers of brominated biphenyls having an average bromine content of 6 atoms

per biphenyl molecule and a total bromine content of 75%. In its natural form, the chemical occurs as a white flake that melts at 72 C, has a low vapor pressure, and is relatively insoluble in water but highly soluble in organic solvents. It is therefore fat soluble. In a monograph report by Michigan Chemical Corporation (1974), the percentage isomeric composition of the various brominated biphenyls was tetrabromo- (2.0), pentabromo- (10.6), hexabromo- (62.8), and heptabromo- (13.2), with the remainder unidentified. Anderson et al. (1974) and Sundstrom et al. (1976) identified the major isomer as 2,2',4,4',5,5' hexabromobiphenyl. Jacobs et al. (1976) estimated the content of the isomer to be 60 to 70%.

The occurrence of hexabromo- (25 ppm), pentabromo- (1 ppm), and traces of tetrabromonaphthalene in the chemical mixture was reported by O'Keefe (1976). A methyl polybrominated furan was detected in the polar fraction of the compound by Moore (1977), but Hass et al. (1978) could not identify brominated furans in PBB using detection methods sensitive to 0.5 ppm.

Although 210 isomeric forms are theoretically possible (Widmark, 1968), the majority of the scientific data available are related to hexabromobiphenyls.

Source

The flame retardant Firemaster BP-6, consisting of a mixture of PBB, was manufactured solely by Michigan Chemical Corporation, St. Louis, Michigan. Approximately

6 million kilograms of PBB were produced in the United States from 1970 through 1976 (Neufeld et al., 1977). About 5.2 million kilograms of the total production was hexabromobiphenyls; the remaining 0.8 million kilograms consisted mainly of octabromobiphenyls and decabromobiphenyls.

Although Firemaster BP-6 production ceased in 1975, 2 companies[a,b] continued production of octabromo- and decabromobiphenyl until 1977. According to Neufeld et al. (1977), no PBB are being imported into the United States in commercial quantities and information is available on the extent of PBB imported in the form of finished plastic products.

Industrial Uses

The chemical mixture was used extensively as a flame retardant for thermoplastics. Kerst (1976) indicated that more than 130 companies in the United States used PBB prior to that year. In 1974, Firemaster BP-6 was used for production of flame-retardant resins of acrylonitrile, butadiene, and styrene and was used in the manufacture of housings for business machines, typewriters, television sets, and other products in which heat resistance was

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[a]White Chemical Company, Bayonne, NJ.

[b]Hexcel Corporation, Sayreville, NJ.

desired. It also found use in coatings and lacquers and in polyurethane foam for automobile upholstery (Neufeld et al., 1977).

Metabolism of PBB

The mammalian body attempts to modify the chemical structure of foreign compounds in order to reduce the physiological activity and enhance excretion. Modification of chemical structure results in the conversion of such compounds into more polar, less lipophilic metabolites that facilitate excretion.

The metabolism of Firemaster BP-6 in birds, laboratory animals, and domestic animals is poorly understood. Kohli and Safe (1976) stated that approximately 1% of the Firemaster BP-6 administered to a pig was excreted as a monohydroxypentabromobiphenyl metabolite. Similarly, Zitko and Hutzinger (1976) suggested the formation of a monohydroxydibromobiphenyl metabolite in fish. Zitko (1977) reported that fish may debrominate the more highly brominated components of PBB. However, in experiments with cows given a single 3 g dose of PBB, Willett and Irving (1976) failed to detect urinary metabolites. Likewise, fecal metabolites were not found in rats given radioactive hexabromobiphenyl intravenously or orally (Matthews et al., 1977). Furthermore, arene oxides, the major intermediates formed by metabolism of lipophilic compounds by hepatic mixed function oxidases, have not been reported to be associated with PBB toxicosis

(Kolbye, 1977). This is surprising, since PBB has been reported by several investigators (Farber and Baker, 1974; Garthoff et al., 1976; Dent et al., 1976a,b, 1977; Moore et al., 1976) to be a potent inducer of hepatic mixed function oxidases.

Jondrof et al. (1955, 1958) noted that chlorinated benzenes having 2 adjacent unsubstituted carbon atoms were metabolized and excreted 3 to 20 times more rapidly than benzenes with similar degrees of chlorination, but without adjacent unsubstituted carbon atoms. Shulte and Acker (1974) suggested that a similar substitution pattern was required for metabolism of the polychlorinated biphenyls (PCB). This was partially confirmed by Jensen and Sundstrom (1974). They indicated that PCB, which did not have 2 adjacent unsubstituted carbon atoms, were found in the highest concentration in the tissues of animals and man previously exposed to PCB. Hexabromobiphenyl, the major congener of PBB, does not have adjacent unsubstituted carbon atoms. The corresponding PCB (hexachlorobiphenyl), which also lacks adjacent unsubstituted carbons, has an extremely long half-life and has been demonstrated to accumulate in tissues of laboratory rats (Matthews and Anderson, 1975). Similar findings have been reported for PBB (Fries, 1978). He suggested that the metabolism of hexabromo- and heptabromobiphenyl, the 2 major isomers of PBB, at rates of practical importance was unlikely because the PCB of equal halogenation were only sparingly metabolized. Furthermore, measurements of

retention of PBB in the edible tissues and excretion in edible products of hens and cattle were found to be similar for hexachloro- and hexabromobiphenyl (Fries and Marrow, 1975; Fries et al., 1976).

In 1975, Lee et al. reported that withdrawal of a diet containing 1,000 ppm of octabromobiphenyl (OBB) caused decline in the levels of OBB in muscle and liver. Concentrations in fat, however, remained unchanged or were somewhat increased. These results suggested that redistribution, and not metabolism, was responsible for OBB removal from the liver and muscle.

After examining tissue residues from cattle originally contaminated in Michigan, Fries (1978) and Fries et al. (1978) reported finding reduction and disappearance of some gas chromatographic peaks characteristic of the parent compound, indicating that either specific *in vivo* metabolic degradation or selective excretion had taken place. Experimental evidence for *in vitro* and *in vivo* metabolism of Firemaster BP-6 was presented by Dannan et al. (1978). They indicated *in vitro* metabolism of 2 minor components, namely 2,4,5,2',5' pentabromobiphenyl and a hexabromobiphenyl, in rats previously treated to maximize their ability to metabolize compounds such as PBB. Drastic reduction of these same components in liver and milk extracts from PBB-treated animals confirmed the *in vitro* results. From their results, they concluded that the availability of a free *para* position was probably more important for metabolism of brominated biphenyls

than either the number of bromines or the presence of 2 adjacent unsubstituted carbon atoms.

Although persistence of PBB in the environment is of major concern, probably of greater significance is the possibility of conversion in plants or in the soil to more toxic products. Jacobs et al. (1976) reported that PBB in soils were not degraded, were not leached, and were not taken up by plants, suggesting that PBB would be a permanent component of contaminated soil. Ruzo and Zabic (1975) obtained tetrabromobiphenyl, pentabromobiphenyl, and methoxylated photoproducts from experimental exposure of Firemaster BP-6 and hexabromobiphenyl to sunlight irradiation. They concluded that PBB exposed to sunlight would photodegrade to lower brominated and hydroxylated biphenyls that should make them more amenable to biological degradation. The possibility of such biological degradation resulting in significant environmental contamination is questionable, since the different end uses of PBB do not predispose the compound to direct exposure to sunlight.

Absorption

Ingested PBB are absorbed from the intestinal tract, are circulated throughout the body, and tend to concentrate in various fatty tissues (Kolbye, 1977). Although the exact site of intestinal absorption has not been determined, several species, namely man, cow, pig, dog, mink, guinea pig, rat, mouse, Japanese quail, gull, chicken and fish, have been demonstrated to absorb PBB.

Direct data on absorption of toxic doses of PBB are limited. Furthermore, the mechanism of absorption has not been elucidated, but results of studies with ^{14}C-OBB (Norris et al., 1974) seem to indicate that there is less absorption with increasing halogenation. The work of Fries and Marrow (1975) tends to support this observation. They indicated that the levels of heptabromobiphenyl in tissues and milk of cattle heavily contaminated with Firemaster BP-6 was barely detectable and that retention and excretion of the hepta-isomer was only 1/10 that of hexabromobiphenyl.

In a quantitative study in which rats were gavaged with ^{14}C-hexabromobiphenyl, at least 90% of the administered dose was shown to be absorbed (Matthews et al., 1977). Following intraruminal administration in cattle, PBB appeared in plasma within 2 to 6 hours, with peak plasma values occurring at 24 to 48 hours (Willett and Irving, 1975). A later report (Willett and Durst, 1978) confirmed the plasma appearance interval (within 4 hours) and indicated that, with continued exposure, plasma concentrations achieved steady state by 15 days.

After absorption, PBB are widely distributed to several tissues, with the largest amounts in the fat of liver, muscle, kidney, and adipose tissue (Fries et al., 1978). Results of various experimental studies (Willett and Irving, 1975; Fries et al., 1978; Matthews et al., 1977) indicate that absorption and subsequent

redistribution of the compound are independent of route of administration.

Retention

Fries et al. (1973) were among the first investigators to report in-depth studies on polybrominated biphenyls. From their studies involving retention and excretion in hens and cows, they concluded that PBB would be resistant to degradation and would persist in tissues. This was confirmed by several investigators (Fries and Marrow, 1975; Corbett et al., 1975; Aftsomis et al., 1972; Norris et al., 1974; Lee et al., 1975), who further pointed out that the lipophilic nature of the chemical made it amenable to storage in fat tissues.

The accumulation of bromine in the fat, liver, and muscle of rats was demonstrated to be related to the concentration of OBB in the diet (Aftsomis et al., 1972; Lee et al., 1975). The kidney, skeletal muscle and testis of rats did not accumulate OBB (Norris et al., 1974); however, studies with PBB in dairy calves (Industrial Biotest Laboratories, Inc., 1975) and lactating cows (Industrial Biotest Laboratories, Inc., 1976) indicated that PBB levels in liver, muscle, fat, kidney and bone marrow increased by increasing the dosage of PBB.

In general, results of studies with cattle (Gutenmann and Lisk, 1975; Willett and Irving, 1975, 1976) suggested that, with the exception of certain organs (most notably the liver and brain), PBB concentration in tissues is

well correlated with fat content. Fries (1978) and Fries et al. (1978) confirmed this observation. They reported finding no significant difference among PBB concentration in the fat of perirenal, omental and subcutaneous adipose tissue, skeletal muscle, cardiac muscle and kidney of cattle originally exposed to the heavily contaminated feed in Michigan. Conversely, concentrations in the fat of the liver were significantly higher, whereas the concentrations in fat of the brain and lungs were significantly lower than concentrations in the fat of other tissues. Gutenmann and Lisk (1975) and Willett and Irving (1976) obtained less uniform tissue distribution in samples obtained from cows soon after PBB ingestion. Fries (1978), however, contended that compared to other halogenated hydrocarbon compounds, overall studies on the distribution of PBB suggest that it reaches steady state concentrations among various tissues more slowly.

Kimbrough et al. (1978) reported that, after an initial decrease, PBB concentration in the blood of rats stabilized and did not further decrease over a 120-day period. Likewise, Willett and Durst (1978) reported steady state plasma levels for PBB after 15 days in cattle subjected to continuous dietary exposure. Hesse (1975) stated that fish apparently bioaccumulated PBB in a comparable manner to PCB. He also pointed out that ducks have considerable capacity for accumulating PBB.

Results of studies involving people from PBB-quarantined farms in Michigan indicated that breast milk

and adipose tissue retained PBB at considerably higher concentrations than the amount present in blood plasma from the same individuals. The concentration in adipose tissue ranged from 61 to 370 times the PBB value found in the blood, with an average ratio of 174:1 (Kolbye, 1977).

In 1975, Babish et al. found higher tissue residues of brominated biphenyls in male than in female Japanese quail. They attributed the difference in tissue retention to deposition and subsequent excretion in the eggs of female quail, a finding also reported for laying hens (Fries et al., 1973, 1976). A similar retention pattern may occur in nonlactating cattle, since lactating cows excrete PBB via the milk (Fries and Marrow, 1975; Gutenmann and Lisk, 1975).

Excretion

Except for egg-laying birds and lactating mammals, PBB has not been shown to be excreted to any appreciable extent. The chemical mixture is excreted by 4 primary pathways: milk, eggs, feces and urine. Fecal elimination is the predominant pathway in nonlactating animals, while excretion in the eggs of laying birds and milk of lactating animals takes precedence over fecal elimination. Free (unconjugated) hexabromobiphenyl is the major excretory product for Firemaster BP-6.

Matthews et al. (1977), using ^{14}C-hexabromobiphenyl in rats, reported that the excretion of the labeled chemical was extremely slow. Over a 6-week period

following intravenous administration of a single dose, only 6.6% of the total amount was excreted in the feces and 0.1% in the urine. On the other hand, rapid fecal elimination of ^{14}C-OBB was reported in rats (Norris et al., 1974). After a single oral dose, the authors detected 62% of the isotope in the feces within 24 hours, and 73% was excreted by the 16th day after administration. It was speculated that poor or incomplete absorption was the reason for the extensive 24-hour elimination.

Evidence for fecal elimination of PBB has been provided for cattle (Willett and Irvin, 1976), pigs (Kohli and Safe, 1976; Ku et al., 1978), rats (Rickert, 1977; Matthews et al., 1977) and chickens (Ringer and Polin, 1977). Urinary excretion has also been recorded for rats (Matthews et al., 1977) and pigs (Kohli and Safe, 1976; Ku et al., 1978), but Willett and Irving (1976) failed to find unconjugated PBB in the urine of cattle.

As mentioned before, egg-laying birds and lactating mammals excreted appreciable quantities of brominated biphenyl in egg yolk and milk, respectively. A biphasic mode of elimination has been observed in cow milk. The half-life (T 1/2) of the first phase was 10.5 days (Gutenmann and Lisk, 1975) and the T 1/2 of the second phase was 58 days (Fries and Marrow, 1975). The T 1/2 of PBB excretion in eggs after cessation of PBB feeding was calculated as 17 days (Ringer and Polin, 1977), but Fries et al. (1976) recorded more specific data on T 1/2 for PBB

elimination in eggs. They found a T 1/2 of 28 days for hexabromo- and 20 days for heptabromobiphenyl.

Although it is well known that PBB are excreted in human breast milk (Eyster, 1976; Kolbye, 1977), no report to indicate urinary or fecal excretion is available in man. Neither are there data to support cutaneous excretion for any species.

Mechanism of PBB Toxicosis

Kay (1977) reviewed the literature and concluded that the current state of knowledge did not allow identity of the toxic components of PBB that may have been responsible for the ill effects observed since 1973 in Michigan farm stock. Results of gross and histopathologic observations of the livers from experimental animals exposed to brominated biphenyls indicate the compound is primarily a hepatotoxin; however, the mechanism of intoxication remains unknown.

Evidence for covalent binding of toxic metabolites to macromolecules and the resultant centrilobular hepatic necrosis demonstrated for ^{14}C-chlorobenzene (Reid and Krishna, 1973) have not been reported for PBB. In fact, Aust (1977) stated that he was unable to demonstrate binding of metabolites *in vitro* under conditions which should maximize binding. Kolbye (1977) suggested that the primary toxicity of PBB in experimental animals revolved around their ability to stimulate or inhibit a variety of enzymes in the mammalian body. In this regard, PBB have been demonstrated to be 3 to 5 times more potent as an inducer

of hepatic mixed function oxidases than PCB (Farber and Baker, 1974; Garthoff et al., 1976). Likewise, on a quantitative basis, PBB appear to cause a greater degree of thyroid hyperplasia (Norris et al., 1974) than PCB. Both compounds, however, are reported to affect a large number of biochemical systems in a similar manner, a finding supported by Garthoff et al. (1976). They noted disruptions of redox and energy state of hepatocytes of rats exposed to dietary PCB (Aroclor) and PBB. From their studies, they speculated that disruptions of energy metabolism were an important phase in the toxicosis associated with these chemicals.

Species and Age Susceptibility to PBB Toxicosis

Although there is an obvious deficiency of published reports concerning lethal doses for PBB among species, there appears to be a definite species and age dependent susceptibility.

In 1977, Kay reviewed the literature and observed that for 8 species investigated, toxicosis resulting in death occurred only in 4 species, namely cattle (Jackson and Halbert, 1974), rodents (Michigan Chemical Corporation, 1974; Jackson and Halbert, 1974; Corbett et al., 1975), Japanese quail (Cecil et al., 1975) and rabbits (Michigan Chemical Corporation, 1974).

Later studies involving mink (Michigan Science in Action, 1976), guinea pigs (Sleight and Sanger, 1976), dogs (Farber et al., 1978) and monkeys (Allen et al., 1978)

suggested that these species may be more sensitive to PBB poisoning. Reasons for species difference in toxicity of PBB have not been elucidated.

In general, PBB administered at relatively high concentrations of 50 ppm or higher to laboratory animals have resulted in detectable toxicosis (Kolbye, 1977). However, an acute lethal oral dose (LD_{50} = 21,500 mg/kg) has been reported only for rats, and acute lethal dermal toxicity (LD_{50} = 2,015 to 10,000 mg/kg) has been reported for rabbits (Michigan Chemical Corporation, 1974). In species in which age was considered, it was found that younger animals were usually more sensitive than older animals (Scientific Panel Report, 1976; Michigan Science in Action, 1976; Preache et al., 1976; Ringer and Polin, 1977).

Systemic Effects of PBB

Hematopoietic and Immunologic System

Results of hematologic studies show a species variation in hematologic response to PBB exposure. Kateley (1977) reported finding no significant difference between the various hematologic values for exposed and unexposed cattle. Similarly, Moorhead et al. (1977) found minimal changes in packed cell volume, hemoglobin content, total erythrocyte and leukocyte counts, and differential leukocyte counts. Hematologic data obtained from cows from one involved herd were inconclusive (Trapp et al., 1975).

Packed cell volumes ranged from 28 to 36%, and total leukocyte counts ranged from 5,700 to 13,300/mm^3.

Sleight and Sanger (1976), Sleight et al. (1978), and Mangkoewidjojo (1979) reported essentially normal hematologic values for rats and guinea pigs. Conversely, Norris et al. (1974) observed a decreased packed cell volume and total erythrocyte count in rats receiving 1% OBB in their diet for 30 days. Likewise, Ringer and Polin (1977) observed decreased hematocrit and hemoglobin values in chickens, a finding they attributed to decreased plasma erythropoietin.

In 1978 Allen et al. found erythropenia in rhesus monkeys. They also recorded leukopenia. Ku et al. (1978) indicated significant decreases in hematocrit and hemoglobin values in pigs 6 weeks after consuming a diet containing 100 ppm of PBB. Werner et al. (1978) and Howard et al. (1978) reported normal hematologic values for sows and their offspring fed diets with 100 or 200 ppm of PBB.

Farber et al. (1978) reported finding marked reduction in hematopoiesis, especially erythropoiesis, in the bone marrow of male dogs given 4 mg/kg Firemaster BP-6 for 61 days. Concurrently, marked extramedullary hematopoiesis, predominantly of an erythropoietic and megakaryocytic nature, and moderate reduction of lymphocytes in the splenic white pulp also occurred.

Studies undertaken to evaluate the immunosuppressive effects of PBB toxicosis have provided variable results. Some investigators have indicated altered immunologic

competence in man (Bekesi et al., 1978), rats and mice (Luster et al., 1978), sows and their offspring (Howard et al., 1978), monkeys (Allen et al., 1978), dogs (Farber et al., 1978) and chickens and guinea pigs (Vos and VanGenderew, 1973), while others have revealed an absence of immunologic effects (Kateley and Bazzell, 1978). Criteria for evaluation of immunologic effects included complete blood counts, identification of peripheral blood T- and B-lymphocyte subpopulations, distribution of plasma cells, serum immunoglobulin levels (IgG, IgM, and IgA), the *in vitro* response of lymphocytes to phytolectins (PHA, Con A, PWM), and determination of autoantibodies and/or immunosuppressive serum factors.

Gastrointestinal System

Getty et al. (1977) reviewed the literature and concluded that a definite palatability problem existed with contaminated feed, with a decrease in feed efficiency at higher levels occurring in most domestic animals. Moorhead et al. (1977) listed diarrhea as a clinical finding in cattle fed 25 g/day; however, they concluded that gastrointestinal changes were minimal.

Fatal gastrointestinal hemorrhage was reported in rats exposed to high levels of PBB (Corbett et al., 1975). Similarly, gastrointestinal hemorrhage was the major gross autopsy finding in a dog that died after ingesting 4 mg/kg PBB for 58 days (Farber et al., 1978).

In 1977, Kay pointed out that gastrointestinal disturbance (stomach cramps and pain) was a common complaint among persons on PBB-quarantined farms. Allen et al. (1978) speculated that the complaints in people may be related to severe and acute hyperplastic and ulcerative gastrointestinal lesions such as he detected in subhuman primates. However, results of physical examination and clinical laboratory tests have so far been unable to establish any effect of PBB exposure in these people.

Reproductive System

It appears that PBB has adverse effects on reproduction in a number of species. Parameters employed for assessment of PBB toxicosis on reproduction include: number of fetuses per litter, mortality among fetuses, fertility, hatchability, chick mortality, comb size, kit survival and serum gonadotrophic hormone levels. The chemical mixture has not been demonstrated to be a potent teratogenic agent. In 1 study, exencephaly was noted in the offspring of mice exposed to both 100 (3/121) and 1,000 ppm (2/174) dosages, while cleft palate (4/87) and hydronephrosis were observed in the offspring at the 1,000 ppm level (Corbett et al., 1975). The incidence of anomalies in the treated group was, however, not significantly higher than in control mice. Beaudoin (1977) reported finding cleft palate and diaphragmatic hernia in the offspring of rats gavaged with a single dose (800 mg/kg) of PBB between the 6th and 14th day of pregnancy.

However, Ficsor and Wertz (1976) failed to find teratogenic effects among the offspring of 15 pregnant rats force fed with 100 µg/g between the 6th and 19th day of pregnancy. Likewise, Mercer (1976) found no teratogenic effects in dairy heifers fed diets containing 5,000 ppm of PBB, even though abortions and fetotoxicity occurred.

Reduced comb size in cockerels (Ringer and Polin, 1977) and decreased fertility in mink (Michigan Science in Action, 1976), hens and quail (Ringer and Polin, 1977) have been observed. Fetal resorption in mice (Michigan Science in Action, 1976) and rats (Beaudoin, 1977) and hydrops amnii and abortions in cattle (Jackson and Halbert, 1974; Moorhead et al., 1977) have occurred.

Additionally, PBB has been reported to be responsible for reduced survival rate in newborn calves (Scientific Panel Report, 1976), mink (Michigan Science in Action, 1976), mice (Preache et al., 1976) and chicks, and decreased hatchability of eggs (Ringer and Polin, 1977). Placental transfer has occurred (Willett and Irving, 1976; Fries et al., 1978).

More recently, Allen et al. (1978) recorded prolonged menstrual cycles, decreased concentration of serum progesterone, and excessive postconceptional bleeding in monkeys fed 0.3 ppm PBB for 7 months. Hypoactive seminiferous tubules were also noted by these investigators. Atrophic testicles and spermatozoal abnormalities in a bull were listed by Jackson and Halbert (1974).

Integumentary System

Abnormal hoof growth, matting and hair loss, and thickened skin of the neck and thorax were clinical signs described in the herd of cattle in which the original PBB contamination occurred (Jackson and Halbert, 1974). Furthermore, in a followup calf feeding trial, 2 calves that survived developed marked hyperkeratosis over the entire body. Moorhead et al. (1977) also detected hyperkeratosis of the eyelids of cattle exposed to PBB at the rate of 25 g/day.

Dry scaly skin and alopecia have been reported in man (Chanda, 1977) and in nonhuman primates (Lambrecht et al., 1978; Allen et al., 1978). Generalized subcutaneous edema and loss of eyelashes were also observed by Allen et al. (1978).

Pathology of PBB Toxicosis

Clinical Signs

Jackson and Halbert (1974) first reported on clinical signs of PBB toxicosis. They described signs associated with acute and chronic effects in adult lactating cows exposed to the heavily contaminated feed (4,000 ppm) and in young nonlactating animals deliberately exposed to the same feed. Anorexia, decreased milk production, infertility, elongated hooves, lameness, delayed parturition and thickening of the skin were observed. Feed refusal and grating of teeth were the major clinical findings in the young nonlactating animals, although hyperkeratosis

developed in 2 calves that survived the feeding trial. In 1975, Deming reported on the effects he found in a herd survey involving 72 herds described as low-level contaminated cattle. Decreased milk production, sterility, decreased growth in young stock, excessive calf losses, and reluctance of animals to move, due to soreness and stiffness, were the most consistent abnormalities recorded.

Mercer et al. (1975), in a comparable herd survey involving 31 herds, were unable to find any significant differences in these parameters between low-level contaminated and control cattle. Similarly, Moorhead et al. (1977) failed to find clinical signs in cattle exposed to 0.25 or 250 mg/day for 60 days.

The clinical effects observed in cattle by various investigators have differed according to the levels of PBB ingested. Heifers fed 0.25 or 250 mg/day and cows exposed to single 3 g dose failed to show clinical signs (Moorhead et al., 1977). In contrast, cattle exposed to 1.13 g/day for 15 days (Gutenmann and Lisk, 1975) and 25 g/head/day for 30 to 60 days (Moorhead et al., 1977) developed a variety of nonspecific clinical signs. Feed refusal, especially at higher dosage levels of PBB, was most consistently reported, a finding also reported in rats (Garthoff et al., 1976), guinea pigs (Sleight and Sanger, 1976), and chickens (Ringer and Polin, 1977). Other clinical observations in cattle included diarrhea, emaciation, dehydration, excessive lacrimation and abortions in pregnant heifers fed 25 g/head/day (Moorhead et

comparative lesions of PBB and PCB toxicosis. The gross lesions associated with PBB toxicosis are summarized as: enlarged livers, petechial hemorrhages, gastrointestinal hyperplasia, hemorrhagic gastroenteritis, subcutaneous edema, hydropericardium, thymic atrophy, enlarged kidneys, fetal death, and necrosis of cotyledons.

In 1974, Norris et al. reported on the histopathologic changes associated with OBB toxicosis in rats. Thyroid hyperplasia was detected at all dose levels of OBB, whereas centrilobular cytoplasmic vacuolation of hepatocytes and hyaline degeneration of renal tubular cells occurred only in rats receiving the highest dose levels. Thyroid hyperplasia has since been confirmed in PBB-fed chickens (Ringer and Kowalski, 1977) and in rats exposed to iodine-adequate and iodine-excess diets containing 100 ppm PBB for 30 or 60 days (Mangkoewidjojo, 1979). Enlarged hepatocytes and cytoplasmic vacuolation were noted (Sleight and Sanger, 1976; Aftsomis et al., 1972), and degenerative renal tubular changes have been reported in conjunction with PBB toxicosis in cattle (Jackson and Halbert, 1974; Moorhead et al., 1977).

Gutenmann and Lisk (1975) first described glandular hyperplasia of the major hepatic bile duct of sheep and cattle. Allen et al. (1978) also detected marked hyperplasia of bile ducts in a monkey exposed to 25 ppm PBB for 25 weeks. Likewise, Sleight et al. (1978) found bile duct hyperplasia and portal fibrosis in rats, and Moorhead

et al. (1977) listed cystic hyperplasia of the gallbladder as a major histopathologic finding in PBB-fed cattle.

Farber et al. (1978) reported on the histopathologic effects of PBB toxicosis on bone marrow, spleen, and lymph nodes of dogs. Marked reduction of hematopoiesis, accompanied by focal necrosis and reticuloendothelial hyperplasia, occurred in the bone marrow, whereas depression of lymphocyte numbers and marked extramedullary hematopoiesis were the major histologic features in the lymph node and spleen.

Ultrastructural Changes

From experiments with rats and guinea pigs, Sleight and Sanger (1976) described electron microscopic findings resulting from PBB ingestion. They demonstrated a dose-dependent effect on mitochondrial size and number in PBB-fed rats. In addition, the authors observed vacuolation, marked hypertrophy of smooth endoplasmic reticulum (SER) and myelin body formation. Comparable results were recorded for guinea pigs exposed to lower dose levels of PBB.

Later, published work by Corbett et al. (1978) listed comparable submicroscopic changes in the liver of rats fed 1,000 ppm PBB. Decreased glycogen, increased lysosomes and bile canalicular microvilli proliferation were also recorded by these investigators. These findings have since been confirmed by Mangkoewidjojo (1979). He further reported on submicroscopic findings observed in the thyroid gland in rats fed 10 or 100 ppm PBB.

Effects of Chemical Toxicosis on Vitamin A Metabolism

As early as 1947, Olafson reported finding significant depression of plasma vitamin A levels in cattle affected with hyperkeratosis. Hansel et al. (1951) confirmed this observation. They noted that plasma vitamin A was reduced to extremely low levels within 5 days after the substance (chlorinated naphthalene) that produced the disease was fed. Persistence of low levels of plasma vitamin A for 1 month following cessation of exposure to the toxic agent also occurred. Hoekstra et al. (1954) reported similar findings and noted partial and temporary alleviation of the hyperkeratosis syndrome as a result of vitamin A therapy.

Phillips (1963) first demonstrated that feeding 10 to 100 ppm DDT, [1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane], decreased liver stores of vitamin A in rats. Similar depression of hepatic vitamin A stores was recorded when yearling beef steers consumed forage sprayed with DDT. However, serum vitamin A levels were increased.

In 1969, Tinsley also found that vitamin A stored in the liver was depressed by feeding 100 to 150 ppm DDT to male rats. He indicated that the degree of depression of liver vitamin A content was dependent on the levels of methionine in the diet, suggesting that the nutritional status of animals may be an important factor in the nature of their response to chemical toxins. In this regard, low protein diets have been demonstrated to protect rats from

heptachlor toxicity, whereas the toxicity of DDT, dieldrin and lindane is reduced by increasing the quantity and quality of dietary protein intake (Naber, 1977).

Among the chemicals tested and demonstrated not to affect intestinal carotenoids or plasma and liver vitamin A, even at dosages known to be toxic to domestic animals, are: vanadium, molybdenum, selenium, and copper (Mitchell et al., 1968). The exact effect of dietary nitrate on vitamin A concentration is controversial.

Phillips and Hatina (1972) and Cecil et al. (1973) found no effect of methoxychlor on hepatic storage of vitamin A in rats given a single dietary level (100 ppm), but Davison and Cox (1976) detected reduction of hepatic vitamin A content by the same chemical in rats fed over a wide range of dietary levels. Villeneuve et al. (1971) indicated that vitamin A concentration in the liver was lower in rabbits receiving Aroclor 1254 for 4 weeks than in control rabbits. Similar results were reported in rats exposed to 0.1% dietary PCB (Innami et al., 1976). In a series of experiments, they noted significant reduction of vitamin A concentration in the liver by the 3rd to 6th day of PCB ingestion with no further reduction thereafter. The investigators recorded better growth rates for rats fed a diet containing 0.1% PCB and supplemented with 34,000 IU of vitamin A than rats fed a similar diet without supplementation. Fifty percent reduction in serum retinol binding protein was also listed, suggesting that marked depression of serum vitamin A had occurred. From

these results, Innami et al. (1976) concluded that PCB act to accelerate vitamin A deficiency in rats.

Although clinical signs and histopathologic lesions similar to those observed in hyperkeratosis have been reported for cattle (Jackson and Halbert, 1974; Gutenmann and Lisk, 1975; Moorhead et al., 1977) and in monkeys (Allen et al., 1978), data to indicate decreased hepatic vitamin A storage related to PBB intoxication have only been reported for rats (Mangkoewidjojo, 1979). He reported finding both dose- and time-dependent lowering of liver vitamin A content in rats exposed to 1 or more ppm PBB for 30 or 60 days.

Other xenobiotics known to cause depression of liver vitamin A in rats include phenobarbital, caffein (Magdolna et al., 1969), ethanol (Baumann et al., 1942) and dieldrin (Lee et al., 1964).

The exact mechanism by which the various chemicals precipitate lowered liver vitamin A content is not known. Hansel et al. (1951) noted increased plasma carotene when cattle affected with hyperkeratosis were fed large doses of carotene, but plasma vitamin A was not elevated, suggesting that chlorinated naphthalene exerts its vitamin A depressant effect by interfering with the conversion of carotene to vitamin A. Results of experiments with rats fed DDT in a vitamin A-deficient diet supplemented with either carotene or vitamin A 24 hours before killing do not support such a mechanism. From this, Phillips (1963) concluded that the action of DDT on liver vitamin A content

does not involve conversion of carotene to vitamin A but does involve some stage in metabolism common to both substances.

A common denominator of many chemicals that result in lowered hepatic vitamin A content is induction of hepatic drug metabolizing enzymes (DME). This observation has led some investigators (Innami et al., 1976; Kato et al., 1978) to assume that the liver vitamin A reducing activity of these foreign compounds might be due to the decomposition of vitamin A by DME or by a related metabolic system.

MATERIALS AND METHODS

Experimental Design and Method

One hundred sixty-eight young male Sprague-Dawley rats initially averaging 72 g each were obtained from a commercial source.[a] After 3 days of acclimatization, the rats were randomly divided into 4 groups of 42 rats each. Based on the length of the treatment periods, each major group was further divided into 7 subgroups and fed diets containing different concentrations of PBB (Table 1).

Table 1. Experimental design for 18-month PBB feeding study

Dietary PBB (ppm)	No. of Groups	No. of Rats in Each Group	Treatment Periods (days)
0.0	7	6	10, 30, 60, 90, 180, 360 and 540
0.1	7	6	10, 30, 60, 90, 180, 360 and 540
1.0	7	6	10, 30, 60, 90, 180, 360 and 540
10.0	7	6	10, 30, 60, 90, 180, 360 and 540

[a]Life Science Div. of Mogul Corp., Madison, WI.

The rats were housed 3 per cage in wire top plastic cages. Ground commercial rat food[b] and water were available *ad libitum*. Control rats were maintained in a separate room.

The source of the PBB used in this study was Firemaster BP-6.[c] The test diets were prepared biweekly for the first 6 months; thereafter, they were prepared according to requirements. The rats were observed daily for clinical signs of toxicosis. Body weights were determined twice weekly for the first 2 months, weekly for the next 4 months and biweekly thereafter.

Necropsy and Pathological Examination

Six rats from each group were necropsied at 10, 30, 60, 90, 180, 360 and 540 days. Feed was removed 12 hours before the rats were killed. Blood samples for serum and hematologic examinations were obtained by cardiac puncture following ether anesthesia. The rats were killed by exposure to chloroform. Urine samples for routine analysis were obtained at necropsy.

Following systematic examination of organs of each carcass for gross pathologic changes, the liver, kidney and brain were weighed with a top-loading balance.[d] Portions of the liver, kidney, spleen, testicles, heart, small intestine, stomach, urinary bladder, brain, lung, salivary

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

[c]Michigan Chemical Corp., St. Louis, MI.

[d]Mettler Series P, Model 163, Mettler Instrument Corp., Hightstown, NY.

gland, thyroid gland, pituitary gland, and adrenal gland were fixed in 10% buffered formalin for light microscopic examination. Samples of liver, kidney, brain, thyroid gland, and pituitary gland were fixed in 3% glutaraldehyde for transmission electron microscopy. Liver, kidney, adipose tissue and brain were collected for PBB and vitamin A analysis. These tissues were stored at -70 C until analyzed.

Clinical Pathologic Tests

Hematologic Examination

Blood samples were collected by cardiac puncture following ether anesthesia. Heparin[e] was the anticoagulant. Duplicate blood smears were made, fixed in methanol, stained by Wright's stain in an automatic stainer[f] and examined microscopically. Packed cell volume was determined by use of microhematocrit tubes[g] and hemoglobin was determined by standard cyanmethemoglobin method described by Benjamin (1969). Total red and white blood cells per deciliter were obtained by an electronic counter.[h]

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[e]Sherwood Medical Industries, Evanston, IL.

[f]Ames Hematek, Division of Miles Laboratories, Inc., Elkhart, IN.

[g]Capillary Tubes, Scientific Products, Evanston, IL.

[h]Coulter Electronic, Inc., Hialeah, FL.

Urinalysis

Urine samples were collected in plastic syringes at necropsy. Color was recorded and specific gravity determined by refractive index as measured by a refractometer.[i] Urinary glucose, blood, pH and protein content were determined by reagent strips.[j]

Serum Protein Electrophoresis

Total protein of frozen sera was determined by refractive index as measured by a refractometer, prior to electrophoresis. Protein electrophoresis was performed in a chamber[k] containing a buffer[l] (pH 8.6-9.0) for 15 minutes at 180 volts. Proteins were stained with a special stain.[m] Following dehydration in methanol for 2 minutes, the plates were cleared in 25% acetic acid in methanol for 5 to 10 minutes. The plates were then oven dried at 50 to 60 C for 5 to 10 minutes. Within 24 hours of electrophoresis, strips were quantitated by densitometry.[n] Serum protein peaks were recorded in percentage and in grams per deciliter.

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[i]Goldberg Refractometer, American Optical Co., Buffalo, NY.

[j]Labstix, Ames Co., Elkhart, IN.

[k]Deluxe Chamber, Gelman Instrument Co., Ann Arbor, MI.

[l]Helena Laboratory Corporation, Beaumont, TX.

[m]Ponseau S., Helena Laboratory Corp., Beaumont, TX.

[n]Quick Quant II, Helena Laboratory Corp., Beaumont, TX.

Vitamin A Analysis

Liver vitamin A concentration of the experimental rats was determined by the fluorometric and silicic acid column chromatographic technique as described by Garry et al. (1970) and Harris and Navia (1977).

In preparation for vitamin A analysis, 1 gram of liver was homogenized in 4 ml of distilled water. One milliliter of the homogenate was saponified with 2 ml of 1% ethanol pyrogallic acid and 1 ml of 50% KOH. The mixture was heated at 60 C for 30 minutes and cooled at room temperature for 10 minutes, after which 4 drops of ethanol were added to each sample.

The samples were then extracted with 4 ml of glass-distilled petroleum ether (PE). The emulsion was centrifuged at 1,000 rpm for 5 minutes and the petroleum ether (upper) layer transferred to 10 ml volumetric flasks for assay for vitamin A derivatives (retinol). The extraction was repeated once and the final extracts made up to 10 ml by addition of PE.

Chromatographic microcolumns were prepared by inserting a small amount of glass wool into regular disposable pasteur pipettes (14.6 cm in length). Alternate layers of sand, glass beads, and 130 to 135 mg of silicic acid were then added to the column. To the prepared columns 0.2 ml sample of extract was transferred. Following washings of the column with 1 ml of PE, the residue on the column was eluted by 0.8 ml of isopropanol.

The eluate was collected in disposable tubes, made up to 1 ml by addition of isopropanol, and read in a fluorometer[o] against a blank. The results were calculated using a standard curve that was prepared from known amounts of purified vitamin A.[p]

PBB Analysis

Liver and adipose tissue from PBB-fed rats was analyzed for 2,2',4,4',5,5' hexabromobiphenyl, the major congener of Firemaster BP-6 (Anderson et al., 1976; Sundstrom et al., 1976). Five-tenths gram of wet pooled tissue was ground with sand and anhydrous sodium sulfate and then extracted by boiling with petroleum ether. The extracts were filtered into 100 ml volumetric flasks and made up to 100 ml by addition of hexane. Twenty milliliter aliquots were then transferred to screw-cap tubes and PBB residue separated from the remaining lipids by florisil[q] column chromatography by elution with hexane.

Polybrominated biphenyl residues in the eluate were analyzed by gas-liquid chromatography on a gas chromatograph[r] equipped with electron capture detector with nitrogen as the carrier gas. Operating conditions were column

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[o]Turner Fluorometer, Arthur H. Thomas Co., Philadelphia, PA.

[p]Retinol, Crystalline Synthetic Type X, Sigma Chemical Co., St. Louis, MO.

[q]Florisil, Fisher Scientific Co., Cleveland, OH.

[r]Varian Instrument Division, Palo Alto, CA.

250 C, detector 310 C, injector 280 C, and carrier gas 30 ml per minute. Gas-chromatograph readings were recorded, and concentrations of PBB were calculated by multiplication of the gas chromatograph reading by a calibration factor derived from standards containing 50, 100 and 200 ppm PBB prepared from Firemaster BP-6.

Histopathologic Procedure

Following fixation in 10% buffered formalin, tissues for histologic examination were embedded in paraffin, sectioned at 6 μ in thickness, and stained with hematoxylin and eosin. Special stains for glycogen or fat were used as indicated.

Transmission Electron Microscopy

Sections of liver obtained at necropsy were fixed in Karnovsky's fixative and washed in Zetterqvist's solution (Pease, 1964) at pH 7.4. They were then postfixed in 1% osmium tetroxide in Zetterqvist's solution. After dehydration in graded alcohol (50, 70, 95% and absolute), they were transferred to propylene oxide. Selected specimens were then embedded in a mixture of Epon and Araldite.

To facilitate correlation between light and electron microscopic examination, semithin sections of the embedded material were stained with toluidine blue and viewed with a light microscope. Thin sections were stained with uranyl acetate and lead citrate and examined with an electron microscope.[S]

[S]EM 952, Carl Zeiss, Germany.

RESULTS

General

Clinical signs of toxicosis or hypovitaminosis A were not observed throughout the course of the experiment. In general, PBB-fed rats ate well, maintained a healthy appearance and gained weight as well as, and in some cases better than, control rats (Figure 1). During the course of the experiment, 4 rats died, 2 developed posterior paralysis and were killed 12 days prior to date of scheduled necropsy, and a female rat was eliminated from the study.

Development of posterior paralysis or death of the experimental rats was not related to PBB toxicosis. Necropsy and histopathologic findings on 1 control and 2 PBB-fed rats in the 540-day group indicated that all 3 rats succumbed to age-related renal failure. The fourth rat from the same group had broken upper central incisors and died from starvation. Of the 2 rats that had posterior paralysis, 1 had a tumor (meningioma) involving the thoracic spinal cord and 1 had been traumatized.

Laboratory Findings

Urinalysis

No consistent abnormality in values for specific gravity, pH, glucose, ketone, bilirubin or blood was

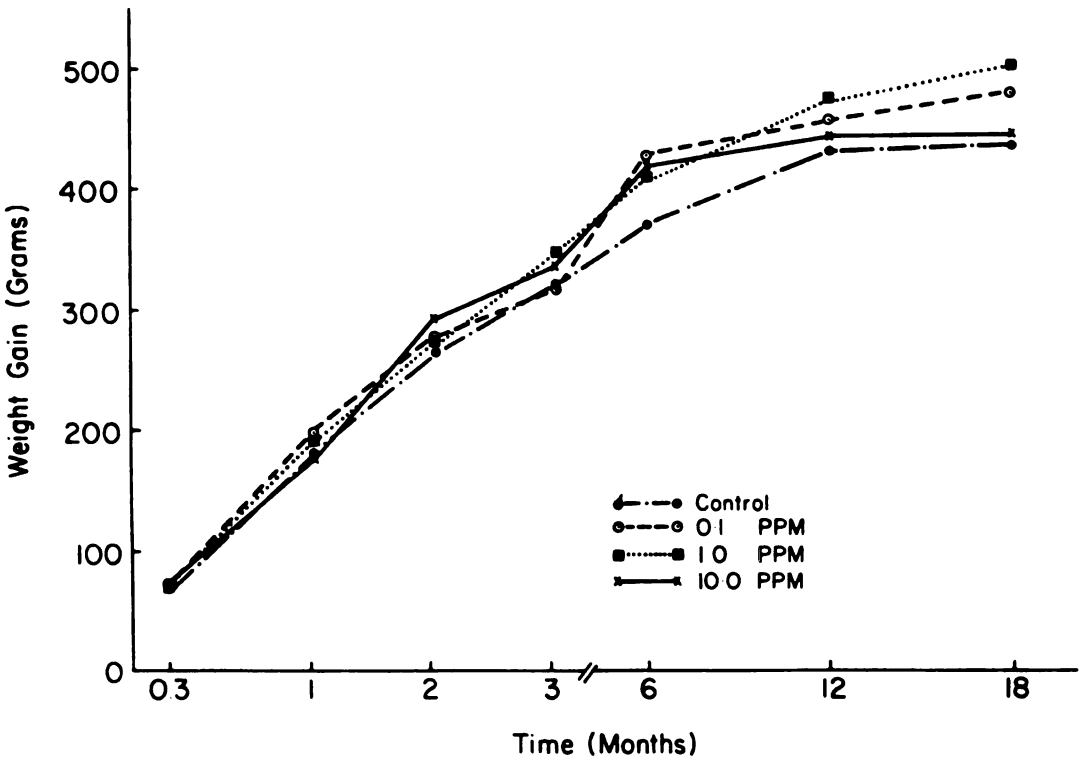

Figure 1 Mean Weight Gain in Rats Fed PBB

observed in the experimental rats. Proteinuria (30 to 100 mg/dl) was a constant feature in control and PBB-fed rats up to 360 days. At 540 days, urinary proteins were increased (300 to greater than 1,000 mg/dl) in control and PBB-fed rats.

Hematology

Results of hematologic studies are given in Table 2. Although sporadic significant differences ($p \leq 0.05$) in hematocrit, hemoglobin, and total white and red blood cell counts occurred at 10, 60, 360 and 540 days, all values excepting total red blood cells were well within the normal range. With few exceptions, mean total red blood cell counts were consistently lower than normal values in control and PBB-fed rats. In addition, morphologic appearance of erythrocytes, leukocytes and platelets was essentially normal.

Serum Electrophoresis

The mean total serum protein values were all within normal range. Component protein fractions were, however, altered resulting in changes in the albumin:globulin (A/G) ratio. Mean albumin values for control and PBB-fed rats were within normal range at 10, 30 and 60 days. The values thereafter decreased to below normal from 90 to 540 days. Concomitant relative and occasional absolute increases in serum globulin fractions, especially alpha 2 ($\alpha 2$) globulins, accompanied the changes in albumin. The resultant effect was reduction in the A/G ratio, which

Table 2. Mean hemoglobin, hematocrit, total leukocyte and erythrocyte counts, and differential leukocyte counts in rats fed PBB

Days on Diet	Dietary PBB (ppm)	Hct (%)	Hb (g/dl)	WBC ($X10^3/\mu l$)	RBC ($X10^6/\mu l$)	WBC Differential (%)			
						Neut	Lymph	Mono	Eosin
10	0.0	37.6[a]	13.2	9.7	3.9	13	84	3	0
10	0.1	39.8[b]	13.3	8.8	4.2	11	84	5	0
10	1.0	37.8	12.4	10.0	3.8	9	89	2	0
10	10.0	38.8	12.3	9.3	4.0	12	83	4	0
30	0.0	47.2	14.7	9.9	5.0	14	80	4	2
30	0.1	46.2	14.8	10.6	4.5	15	80	3	2
30	1.0	45.7	14.4	9.2	4.6	7	89	3	1
30	10.0	46.2	14.4	9.3	5.0	12	85	3	0
60	0.0	48.8	14.8	8.4	4.7	16	76	4	4
60	0.1	47.0	14.9	7.4[b]	5.3[b]	10	85	4	1
60	1.0	48.8	14.9	6.1[b]	5.8[b]	15	79	3	3
60	10.0	46.7	14.3	6.0[b]	5.2	15	79	4	2
90	0.0	46.9	15.7	8.1	5.0	14	80	4	2
90	0.1	47.8	15.7	8.7	5.0	13	78	7	2
90	1.0	47.5	15.2	8.4	5.0	12	78	8	2
90	10.0	45.8	15.0	7.5	4.3	18	74	5	3
180	0.0	46.2	15.4	8.9	4.4	27	68	3	2
180	0.1	46.7	15.6	7.3	4.6	25	71	2	2
180	1.0	47.2	15.7	8.9	4.8	18	78	2	2
180	10.0	45.8	15.2	8.8	4.5	30	66	2	2

Table 2 (cont'd.)

Days on Diet	Dietary PBB (ppm)	Hct (%)	Hb (g/dl)	WBC ($X10^3/\mu l$)	RBC ($X10^6/\mu l$)	WBC Differential (%) Neut	Lymph	Mono	Eosin
360	0.0	48.3	16.9[b]	5.8	ND	17	73	7	3
360	0.1	46.0[b]	16.0[b]	8.0	ND	18	74	5	3
360	1.0	45.8[b]	15.6[b]	8.0	ND	16	77	4	3
360	10.0	44.2[b]	15.6[b]	7.4	ND	11	81	5	3
540	0.0	43.7	15.9	7.8	ND	31	64	4	1
540	0.1	43.4	16.0	9.3	ND	29	66	3	2
540	1.0	36.7[b]	13.4[b]	7.5	ND	35	61	2	2
540	10.0	41.7[b]	15.0[b]	8.8	ND	32	64	3	1

[a]Values are means for N=3 to 6 rats.

[b]Significantly different from control values ($p \leq 0.05$).

ND = not done, Neut = neutrophils, Lymph = lymphocytes, Mono = monocytes, Eosin = eosinophils.

was especially noticeable in control and PBB-fed rats at 90 through 540 days (Table 3).

Organ Weights

The mean body and liver weights, relative liver weights and statistical significance of differences are given in Table 4. The mean (g) and relative (% of body weight) liver weights of rats receiving 10 ppm dietary PBB were significantly increased ($p<0.05$) over control values at all treatment periods (10 through 540 days). In contrast, the mean and relative liver weights of rats receiving either 0.1 or 1.0 ppm PBB were only significantly different from control values at 10 and 30 days. These results suggest that after 30 days a dosage greater than 1 ppm PBB was necessary to maintain the stimulus responsible for increased liver size. Neither mean nor relative kidney or brain weights were significantly affected ($p>0.05$) by dietary PBB (Appendix A).

Polybrominated Biphenyl Content of Tissues

The mean PBB concentrations of pooled samples from the liver and adipose tissue expressed as ppm PBB (fat basis) are given in Tables 5 and 6. A definite dose-related increase in residual PBB occurred. Similarly, with few exceptions, a time-dependent increase in PBB content occurred in tissues from rats fed 1 or 10 ppm PBB. Samples from the liver had higher concentrations of PBB than samples from adipose tissue.

Table 3. Serum proteins (total protein, albumin, α2 globulin values and A/G ratios) of representative control rats and rats fed PBB

Time (days)	Number of Samples	Dietary PBB (ppm) 0	0.1	1.0	10
		Total Protein (g/dl)			
10	2	4.60	5.00	5.30	5.40
30	2	6.70	6.40	7.10[a]	6.50
60	2	8.70	7.00[a]	7.80	7.20
90	2	6.80[a]	6.40	7.00	6.00
180	2	5.50	7.00	6.40	7.20
360	3	7.20	6.90	6.50	6.50
540	4	7.50	7.10	6.00	6.00
		Albumin (g/dl)			
10	2	2.47	2.87	2.77	2.82
30	2	3.03	2.79	2.77[a]	2.93
60	2	3.88	3.14	3.33	2.91
90	2	2.60	2.59[a]	2.59	2.69
180	2	ND	2.46	2.57	2.36
360	5	2.25	2.19	2.26	2.08
540	4	2.00	1.94	1.81	1.78
		α2 Globulin (g/dl)			
10	2	1.01	1.04	1.20	1.28
30	2	1.99	1.88	1.86[a]	1.73
60	2	2.45	1.85	1.98	2.10
90	2	2.05	2.12[a]	2.22	1.71
180	2	ND	2.56	1.73	2.08
360	4	2.67	2.55	2.35	1.75
540	4	2.88	2.95	2.21	1.73
		A/G Ratio			
10	2	1.30	1.35	1.13	1.15
30	2	0.83	0.79	0.66[a]	0.82
60	2	0.84	0.82	0.75	0.69
90	2	0.65	0.67[a]	0.59	0.84
180	2	ND	0.55	0.67	0.49
360	4	0.46	0.47	0.54	0.49
540	4	0.37	0.37	0.44	0.43

[a]Values for N=1.

ND = not done.

Table 4. Mean body, liver and relative liver weights in rats fed PBB

Days on Diet	Mean Body Weights (g)				Mean Liver Weights (g)				Relative Liver Weights (%)			
	0 ppm	0.1 ppm	1 ppm	10 ppm	0 ppm	0.1 ppm	1 ppm	10 ppm	0 ppm	0.1 ppm	1 ppm	10 ppm
10	139	140	141	144[a]	6.3	7.9[c]	7.3[c]	7.6[c]	4.6	5.6[c]	5.2[c]	5.3[c]
30	254	271[b]	260	252	9.5	12.0[c]	11.3[c]	11.8[c]	3.7	4.5[c]	4.4[c]	4.7[c]
60	336	346	347	362	11.2	11.4	11.9	15.3[c]	3.3	3.3	3.4	4.2
90	391	388	419	410	12.3	12.3	13.4	13.9[c]	3.1	3.2	3.2	3.4[c]
180	440[b]	498	482[b]	487	14.8	16.7	15.4	19.6[c]	3.4	3.4	3.2	4.1[c]
360	503	527	543	512	17.8	17.7	18.0	25.6[c]	3.5	3.3	3.3	5.4[c]
540	505[b]	550[b]	575[b]	521[b]	16.1	17.8	19.5	26.6[c]	3.2	3.2	3.4	5.1[c]

[a]Values are mean for N=6.

[b]N=5.

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

Table 5. The concentrations (ppm) of PBB in the fat of liver from rats fed diets containing PBB

Dietary PBB (ppm)	Days on Diet					
	30	60	90	180	360	540
0.0	0.93[a] (1.7)[b]	0.50 (1.6)	0.82 (1.7)	1.10 (2.3)	0.43 (2.1)	0.68 (2.4)
0.1	0.58 (2.2)	1.00 (2.3)	4.80 (1.7)	2.30 (2.9)	6.70 (2.3)	4.70 (2.6)
1.0	6.70 (1.7)	7.70 (2.3)	18.50 (2.1)	24.40 (2.5)	40.00 (2.5)	70.40 (1.6)
10.0	55.90 (1.8)	59.70 (2.6)	186.10 (3.0)	404.40 (3.0)	726.60 (6.7)	627.20 (6.6)

[a]The values are means of 2 pooled samples from 2 or 3 rats each.

[b]Numbers in parentheses represent percent fat.

Table 6. The concentrations (ppm) of PBB in the fat of adipose tissue from rats fed diets containing PBB

Dietary PBB (ppm)	Days on Diet					
	30	60	90	180	360	540
0.0	0.04[a] (69)[b]	0.04 (71)	0.06 (65)	0.05 (72)	0.86 (53)	0.06 (57)
0.1	0.41 (66)	0.72 (75)	1.80 (63)	1.60 (70)	7.10 (60)	4.90 (58)
1.0	6.20 (64)	15.20 (69)	12.10 (72)	20.30 (68)	36.70 (67)	63.40 (63)
10.0	59.90 (68)	84.80 (70)	142.40 (70)	169.90 (68)	542.60 (66)	630.00 (62)

[a]The values are means of 2 pooled samples from 2 or 3 rats each.

[b]Numbers in parentheses represent percent fat.

Residual concentrations of PBB detected in the samples from control rats are probably the result of technical problems associated with the extraction and analysis of PBB.

Liver Vitamin A Content

Liver vitamin A content was assayed for all experimental rats except rats from the 10-day group. The mean vitamin A values expressed on a microgram per gram (µg/g) or milligram per whole liver (mg/liver) wet weight basis are given in Tables 7 through 12. The mean hepatic vitamin A of control rats was 190.1 µg/g and 1.8 mg/liver at 30 days and 827.0 µg/g and 13.3 mg/liver at 540 days. In rats receiving 10 ppm dietary PBB for 30 days, hepatic vitamin A content was 96.9 µg/g and 1.1 mg/liver, both values being significantly lower than the corresponding control values (Table 7). Although the liver vitamin A concentrations of rats fed either 0.1 or 1 ppm of PBB were similarly depressed, the differences were only significant when the data were calculated on a µg/g basis. In fact, calculation of the data on a mg/liver basis indicated that rats receiving 1 ppm of PBB had slightly higher hepatic vitamin A levels (1.9 mg/liver) than control rats (1.8 mg/liver).

At 60 days, rats fed diets containing 1 or 10 ppm of PBB had significantly lower liver vitamin A values than control rats (Table 8). In contrast, the relative (µg/g) hepatic vitamin A value for rats fed diets

Table 7. Liver vitamin A content in rats fed PBB for 30 days

Dietary Level (ppm)	Liver Weight (g)	Vitamin A	
		µg/g Liver	mg/Liver
0.0	9.5 ± 0.8[a]	190.1 ± 12.2[b]	1.8 ± 0.2
0.1	12.0 ± 1.0[bc]	122.0 ± 26.4[c]	1.5 ± 0.4
1.0	11.3 ± 1.0[ae]	164.5 ± 11.3[d]	1.9 ± 0.3
10.0	11.8 ± 1.9[af]	96.9 ± 22.1[c]	1.1 ± 0.3[d]

[a]Values are mean ± SD; N=6.

[b]N=5.

[c,d,e,f]Significantly different from control value ($p<0.001$, $p<0.005$, $p<0.01$ and $p<0.025$, respectively).

Table 8. Liver vitamin A content in rats fed PBB for 60 days

Dietary Level (ppm)	Liver Weight (g)	Vitamin A	
		µg/g Liver	mg/Liver
0.0	11.2 ± 1.0[a]	294.9 ± 25.7	3.3 ± 0.4
0.1	11.4 ± 1.1	328.1 ± 23.6[e]	3.7 ± 0.4
1.0	11.9 ± 1.8	222.7 ± 37.5[c]	2.6 ± 0.3[d]
10.0	15.3 ± 1.8[b]	144.6 ± 16.5[b]	2.2 ± 0.4[c]

[a]Values are mean ± SD; N=6.

[b,c,d,e]Significantly different from control value ($p<0.001$, $p<0.005$, $p<0.025$ and $p<0.05$, respectively).

containing 0.1 ppm of PBB for the same time period was increased over the value for control rats. Only in rats receiving 10 ppm of PBB in their diets was there significant depression of hepatic vitamin A concentration at 90 days (Table 9). Hepatic vitamin A content was, however, decreased in rats ingesting 0.1, 1 or 10 ppm of dietary PBB in the 180-day experimental group (Table 10). Likewise, rats consuming feed containing 0.1, 1 or 10 ppm PBB for 360 days had significantly lower vitamin A levels in their liver compared to control rats (Table 11). Reduction in hepatic vitamin A concentration was especially marked for rats receiving 10 ppm PBB in the 360-day group, the decrease in vitamin A concentration being 10-fold on a μg/g and 6-fold on a mg/liver basis, compared to control values. Similarly, marked reduction in vitamin A levels (17- and 10-fold) was recorded for rats fed 10 ppm dietary PBB for 540 days (Table 12).

Miscellaneous Laboratory Findings

Thyroid hormones. Serum thyroxine (T_4) and triiodothyronine from randomly selected rats from the 10-, 90- and 540-day groups were evaluated by radioimmunoassay. Although a single significant decrease in T_4 (Appendix B) was found for rats fed 0.1 ppm for 10 days, the toxicological significance was not evident. The hormone values were quite variable, lacked a dose response relationship, and showed consistent overlapping of individual values from control and PBB-fed rats.

Table 9. Liver vitamin A content in rats fed PBB for 90 days

Dietary Level (ppm)	Liver Weight (g)	Vitamin A	
		µg/g Liver	mg/Liver
0.0	12.3 ± 1.1[a]	351.8 ± 69.7	4.3 ± 1.0
0.1	12.3 ± 0.9	346.1 ± 57.8	4.2 ± 0.6
1.0	13.4 ± 1.3	351.5 ± 45.2	4.7 ± 0.6
10.0	13.9 ± 0.6[c]	222.5 ± 45.4[b]	3.1 ± 0.6[d]

[a]Values are mean ± SD; N=6.

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

Table 10. Liver vitamin A content in rats fed PBB for 180 days

Dietary Level (ppm)	Liver Weight (g)	Vitamin A	
		µg/g Liver	mg/Liver
0.0	14.8 ± 1.7[b]	478.6 ± 54.5	7.1 ± 0.8
0.1	16.7 ± 2.0[a]	401.9 ± 32.4[d]	6.8 ± 1.2
1.0	15.4 ± 0.8[b]	334.1 ± 23.9[c]	5.1 ± 0.3[c]
10.0	19.6 ± 1.2[ac]	159.8 ± 37.1[c]	3.1 ± 0.6[c]

[a]Values are mean ± SD; N=5.

[b]N=6.

[c,d]Significantly different from control value ($p<0.001$ and $p<0.025$, respectively).

Table 11. Liver vitamin A content in rats fed PBB for 360 days

Dietary Level (ppm)	Liver Weight (g)	Vitamin A	
		µg/g Liver	mg/Liver
0.0	17.8 ± 3.2[a]	626.6 ± 54.0	11.0 ± 1.5
0.1	17.7 ± 2.6	421.9 ± 35.8[b]	7.4 ± 1.0[b]
1.0	18.0 ± 1.9	352.1 ± 66.8[b]	6.2 ± 0.7[b]
10.0	25.6 ± 3.4[c]	59.6 ± 12.2[b]	1.5 ± 0.4[b]

[a]Values are mean ± SD; N=6.

[b,c]Significantly different from control value ($p<0.001$ and $p<0.005$, respectively).

Table 12. Liver vitamin A content in rats fed PBB for 540 days

Dietary Level (ppm)	Liver Weight (g)	Vitamin A	
		µg/g Liver	mg/Liver
0.0	16.1 ± 1.6[a]	827.0 ± 178.7	13.3 ± 2.9
0.1	17.8 ± 1.7	763.2 ± 208.3	13.6 ± 3.6
1.0	19.5 ± 4.4	441.0 ± 79.2[c]	8.4 ± 1.4[d]
10.0	26.2 ± 3.5[b]	46.2 ± 18.8[b]	1.2 ± 0.4[b]

[a]Values are mean ± SD; N=5.

[b,c,d]Significantly different from control value ($p<0.001$, $p<0.005$ and $p<0.01$, respectively).

Testosterone. Mean serum testosterone values for representative control rats and rats fed PBB for 10, 30, 60, 90 and 180 days are given in Appendix C. There was no significant effect of dietary PBB on testosterone concentrations.

Pathologic Findings

Gross Lesions

Gross lesions were confined to the liver and kidney.

Liver. Except for enlarged livers in the PBB-fed rats, no significant lesions were seen at 10 and 30 days. The livers from 3 of 6 rats fed diets containing 1 ppm of PBB for 60 days had a somewhat prominent lobular pattern. These livers were also quite pale. A slightly mottled appearance was evident in the liver from 1 of 6 rats fed a diet with 10 ppm of PBB for 90 days.

Likewise, the livers from 3 of 6 rats consuming a diet with 10 ppm of PBB for 180 days were yellowish in color and had several small (2 mm) whitish foci irregularly distributed over the dorsal surface. Rounding of the edges and increased friability were evident in 1 of these livers. A control rat from this group also had some of these small white foci. The lesions observed in rats killed at 360 days were present in 6 of 6 rats fed diets with 10 ppm of PBB. The changes included moderate to marked mottling, yellowish to copper-colored appearance, and rounded edges. Similar changes were noted in the

livers from 5 of 5 rats fed diets containing 10 ppm of PBB for 540 days. In addition, a moderately large (1.5 cm x 0.5 cm), white, papillary growth involving the middle hepatic lobe was present in 1 of these rats. The livers from 2 of 5 rats fed diets containing 1 ppm of PBB for 540 days also showed some mottling of the dorsal surface.

Kidney. Pitting of the renal capsule, paleness, and a few darkly discolored kidneys were the major gross findings. Occasional small cysts were also seen. One or more of the above changes were seen in the kidneys from PBB-fed and control rats.

Incidental gross findings. In rats killed at 30 days, right testicular hypertrophy with an associated vestigial left testicle was found in 1 of 6 rats fed 10 ppm dietary PBB. Additionally, prominent Peyer's patches were evident in the small intestines from the control and PBB-fed rats.

Histopathologic Findings

Significant histologic alterations were mainly limited to the liver. At 10 days, all PBB-fed rats had a moderate to marked increase in mitotic activity of hepatocytes. The mean numbers of mitotic figures observed in 10 consecutive (25X objective) microscopic fields of hepatocytes from 3 of 6 rats fed diets with 0.1, 1 and 10 ppm PBB were 8.7, 8, and 3.7, respectively. In contrast, the mean mitotic index derived from the same number of microscopic fields from 3 of 6 control rats was 0.7. Diffuse cytoplasmic

vacuolation and swollen hepatocytes were also prominent features in the livers from these rats (Figures 2 and 3). The cytoplasmic vacuoles varied in size but were generally large and extended from the centrally located nuclei to the periphery of individual cells. These vacuoles did not stain with oil red O. Except for moderately swollen hepatocytes, the livers from rats fed 1 or 10 ppm PBB for 30 and 60 days were essentially normal. Similarly, the livers from all control rats and all rats fed diets with 0.1 ppm of PBB from 30 through 540 days were normal. In the livers from rats receiving 1 or 10 ppm PBB for 90 and 180 days, there was prominent swelling of hepatocytes and limited midzonal vacuolation that was particularly noticeable in the livers from rats receiving 10 ppm of PBB for 180 days.

At 360 and 540 days, the changes were most severe in rats consuming 10 ppm dietary PBB. The hepatic alterations included intracytoplasmic lipid accumulation, advanced degenerative hepatocellular changes and focal areas of necrosis. Individual hepatocytes were enlarged, resulting in obliteration of sinusoidal spaces. The nuclei of some affected hepatocytes were karyorrhectic or absent. Occasinal nuclei were pyknotic. The cytoplasm of the nonvacuolated cells varied from granular to an increased homogeneous and eosinophilic. Occasional foci of necrosis were partially replaced by infiltration of inflammatory cells that were mainly mononuclear (Figure 4). In addition, occasional clusters of yellowish-brown pigmented macrophages were observed in livers from rats receiving 10 ppm PBB for 540

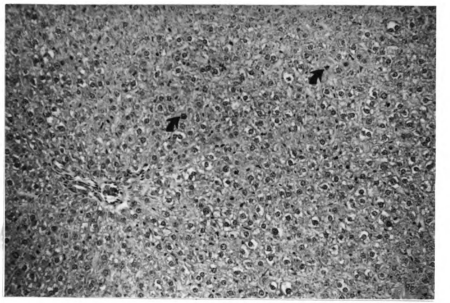

Figure 2. Diffuse perinuclear vacuolation and increased number of mitotic figures (arrows) in the liver from a rat fed a diet containing 0.1 ppm of PBB for 10 days. H&E stain; X200.

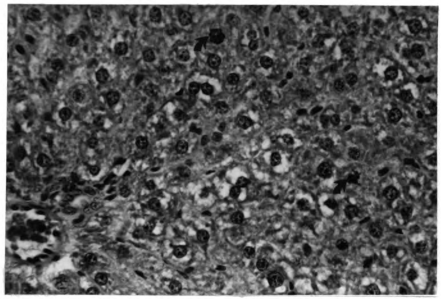

Figure 3. Higher magnification of the liver from the rat in Figure 2, to show the absence of sinusoidal spaces and prominent mitotic figures (arrows). H&E stain; X500.

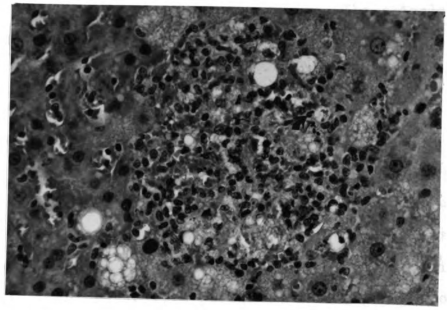

Figure 4. Focal areas of necrotic hepatocytes were replaced by inflammatory cells that were mainly mononuclear. Occasional neutrophils (arrow) were present in some of these foci. Tissue from a rat fed a diet with 10 ppm of PBB for 540 days.

days. These pigmented macrophages were located adjacent to vacuolated areas, central veins and portal triads.

Intracytoplasmic lipid accumulation, confirmed by oil red O staining, was the most consistent lesion. The vacuoles varied in size from large vacuoles that practically occupied the entire cytoplasmic space to the more abundant fine vacuoles that resulted in a foamy appearance of the affected hepatocytes (Figure 5). The cytoplasmic vacuolation was irregular in distribution but was more commonly midzonal with extensions to the peripherolobular and occasionally to the centrolobular areas (Figure 6). Approximately 50 to 80% of the hepatocytes in individual hepatic lobules were affected in rats receiving 10 ppm PBB for 360 or 540 days. In the livers from rats fed diets containing 1 ppm PBB for 360 and 540 days, a similar pattern of lipid accumulation occurred. A more typical midzonal involvement with extension to the peripherolobular areas, as illustrated in Figure 7, was evident. In addition, marked periportal fibrosis (Figure 8) with accompanying focal lymphocytic infiltration was observed in 2 of 5 rats fed 1 ppm PBB for 540 days. Bile duct hyperplasia was also evident in these livers.

Other Histopathologic Findings

Lung. Varying degrees of focal interstitial pneumonitis were observed in the lungs from several control and PBB-fed rats. These lesions were evident in rats necropsied from 90 days onwards. In no case was there involvement of

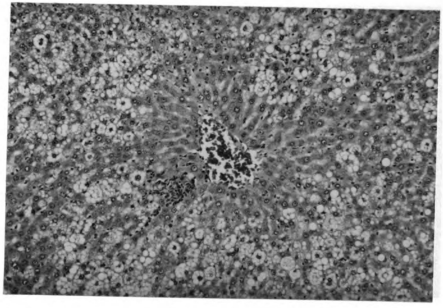

Figure 5. Typical small, multiple intracytoplasmic lipid droplets gave a foamy appearance to the hepatocytes located in the midzonal area of the hepatic lobule. Tissue from a rat fed a diet with 10 ppm of PBB for 360 days. H&E stain; X200.

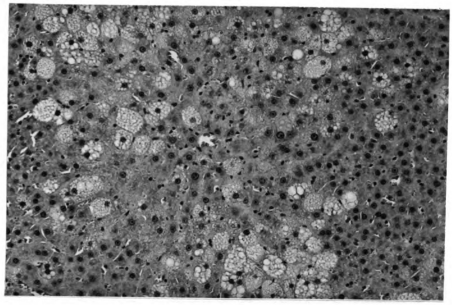

Figure 6. Tissue from a rat fed a diet with 10 ppm of PBB for 540 days. There is intracytoplasmic lipid accumulation involving some hepatocytes adjacent to the central vein. H&E stain; X200.

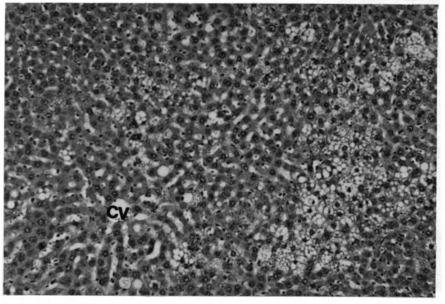

Figure 7. Intracytoplasmic lipid accumulation involving the mid- and peripheral zones in the liver from a rat fed a diet containing 1 ppm of PBB for 540 days. Relatively normal hepatocytes are adjacent to the central vein (CV). H&E stain; X200.

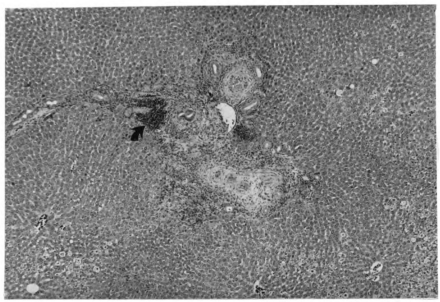

Figure 8. Portal fibrosis and bile duct hyperplasia with associated focal infiltration of lymphocytes (arrow) in the liver from a rat fed a diet with 1 ppm of PBB for 540 days. H&E stain; X80.

more than 25 to 30% of a particular lung lobe. A limited amount of hyperplasia of bronchiolar-associated lymphoid tissue also occurred.

Pituitary gland. Multiple pituitary cysts were observed in the pituitary gland from a rat exposed to 10 ppm PBB for 180 days. The interior of the cysts contained proteinaceous material and was lined by elongated, flattened cells. There was also an adenoma involving the anterior hypophysis from a rat fed 1 ppm PBB for 540 days.

Thyroid gland. The microscopic changes observed in thyroid glands included marked follicular dilatation, flattening and atrophy of follicular lining cells, resulting in hyalinization and thinning of the follicular basement membrane. Mild hyperplasia and vacuolation of follicular epithelium were also evident. These changes were present in the thyroid glands from control rats and PBB-fed rats. Additionally, a thyroid adenoma occurred in the thyroid gland from a rat fed 10 ppm PBB for 360 days.

Kidney. Age-related progressive nephrosis characterized by the accumulation of eosinophilic renal tubular casts, atrophy of tubular epithelium and tubular dilatation was observed in control and PBB-fed rats necropsied as early as 180 days. In some kidneys, occasional foci of calcification and lymphocytic infiltration accompanied the nephrosis. The cause of dark discoloration observed grossly was not evident histologically.

Liver. Microscopically, the papillary growth seen in the middle hepatic lobe from a rat fed a diet containing 10 ppm of PBB for 540 days was a cystic mucoadenoma originating from bile duct epithelium. Accumulation of glycogen was not seen in any of the Carnoy's-fixed and periodic acid-Schiff (PAS)-stained liver sections obtained from selected control and PBB-fed rats killed at 30, 60, 90, 180, 360 and 540 days. Histologic findings in the livers from rats killed at 60 days were poorly correlated with the gross observations.

Spleen. Increased hematopoietic activity was commonly observed in the spleens from several control rats and rats fed diets with PBB. Moderate vascular congestion and increased amounts of hematogenous pigment were also evident in some spleens.

Adrenal glands. Occasional foci of vacuolation were evident in the adrenal cortex of many rats, including control rats and PBB-fed rats.

Small intestines. Increased numbers of globular leukocytes and occasional intestinal nematode parasites were seen in the intestines from several rats. There was also focal hyperplasia of Peyer's patches leading to atrophy of intestinal smooth muscle and elevation of the overlying serosal surface. One or more of the above observations were present in representative numbers of control rats and PBB-fed rats.

Other tissues. No spontaneous or PBB-related lesions were found in the testicle, heart, stomach, urinary bladder, brain or salivary glands from any rat.

Electron Microscopic Findings

Electron microscopic studies were carried out on selected livers from rats exposed to dietary PBB for 10, 90, 180, 360 and 540 days. The ultrastructural features of hepatocytes from a control rat are illustrated in Figure 9. Except for occasional cytoplasmic vacuoles and mild proliferation of the smooth endoplasmic reticulum (SER), hepatocytes from control rats were basically normal. In general, the livers from PBB-fed rats showed time-dependent progressive hepatocellular changes that were especially prominent in rats fed diets with the highest dosage (10 ppm) of PBB. The ultrastructural lesions were characterized by proliferation and dilatation of SER (Figure 10) with accompanying disorganization and reduction of rough endoplasmic reticulum (RER). Mitochondria were enlarged and reduced in numbers and had undergone degenerative changes. In some instances, the degenerative mitochondrial changes appeared to involve imbibition of fluid resulting in swelling and disintegration of cristae (Figure 11). Many intracytoplasmic vacuoles that varied in size, shape and internal density were observed, especially in the hepatocytes from rats killed at 360 and 540 days (Figures 12 and 13). Myelin body formation was a consistent finding, although these bodies were more

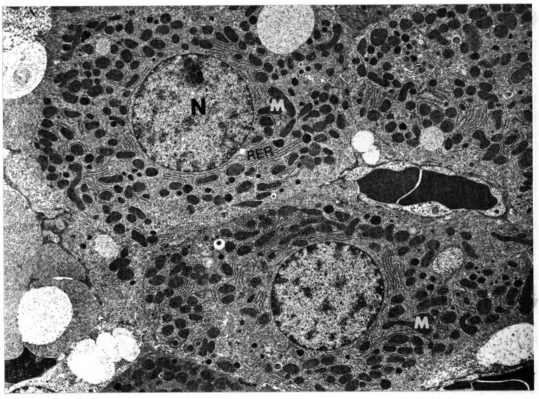

Figure 9. Electron micrograph of hepatocytes from a control rat. Notice the normal complement of mitochondria (M) randomly distributed within the cytoplasm. Parallel aggregations of rough endoplasmic reticulum (RER) show normal orientation towards the nucleus (N). Occasional cytoplasmic vacuoles are present. Lead citrate and uranyl acetate stain; X4,700.

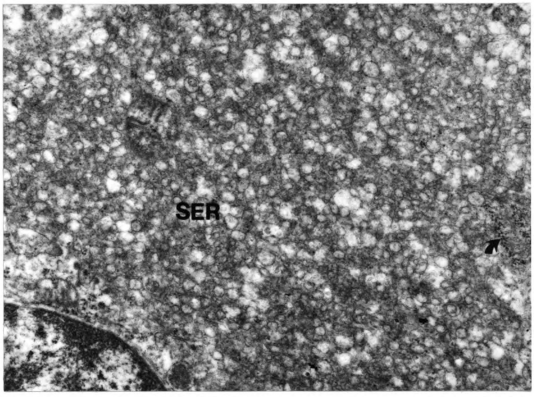

Figure 10. Electron micrograph of a portion of a hepatocyte from a rat fed a diet containing 10 ppm of PBB for 540 days. There is an abundance of vesiculated smooth endoplasmic reticulum (SER) and some free ribosome (arrow). There is also a relative absence of other cytoplasmic organelles. Lead citrate and uranyl acetate stain; X21,000.

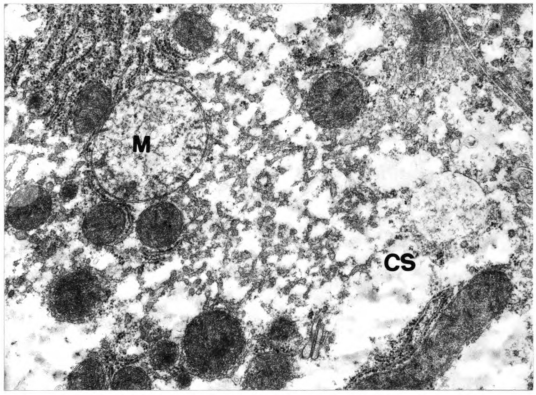

Figure 11. Electron micrograph of part of a hepatocyte from a rat fed a diet containing 1 ppm of PBB for 10 days. Notice the swollen mitochondrion (M) with disruption of the cristae and numerous clear spaces (CS) within the cytoplasm. Lead citrate and uranyl acetate stain; X21,000.

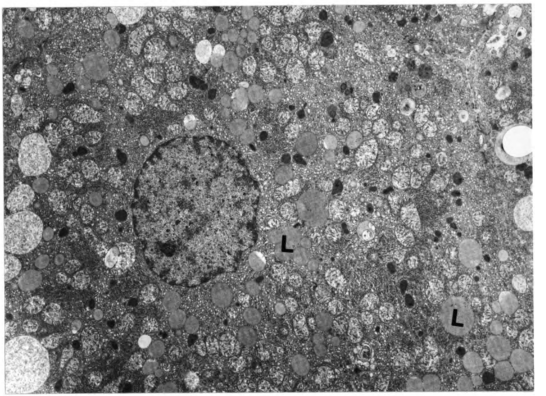

Figure 12. Electron micrograph of a hepatocyte from a rat fed a diet containing 10 ppm of PBB for 360 days. Medium density lipid droplets (L) predominate. Lead citrate and uranyl acetate stain; X4,700.

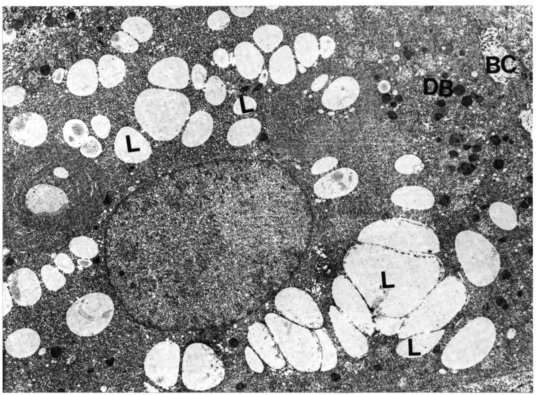

Figure 13. Electron micrograph of a hepatocyte from a rat fed a diet containing 10 ppm of PBB for 540 days. Notice the variation in size and shape of the cytoplasmic lipid droplets (L). Increased numbers of dense bodies (DB) are present within the vicinity of the bile canaliculus (BC). Lead citrate and uranyl acetate stain; X5,060.

numerous in hepatocytes from rats receiving 10 ppm PBB for 540 days (Figure 14). In some cases the myelin bodies had vacuoles or cytoplasmic organelles trapped within the interior (Figure 15). Some of the trapped vacuoles contained small whorled figures (Figure 15) and had an outer double-layered membrane that appeared to be continuous with the SER forming the myelin body. Some of the larger myelin bodies were formed by aggregation of smaller bodies. Definite continuity between myelin bodies and the SER or RER was evident in some hepatocytes. Other ultrastructural lesions included swollen hepatocytes with alternating clear and finely granular spaces between segregated and adjacent organelles and increased amounts of free ribosomes that were usually adjacent to disrupted or denuded RER. In addition, moderate increases in lysosomes were also observed.

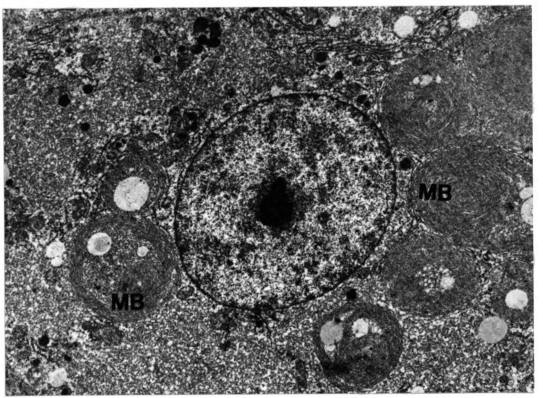

Figure 14. Electron micrograph of a hepatocyte from a rat fed a diet containing 10 ppm of PBB for 540 days. There are multiple myelin bodies of different sizes. Lead citrate and uranyl acetate stain; X5,200.

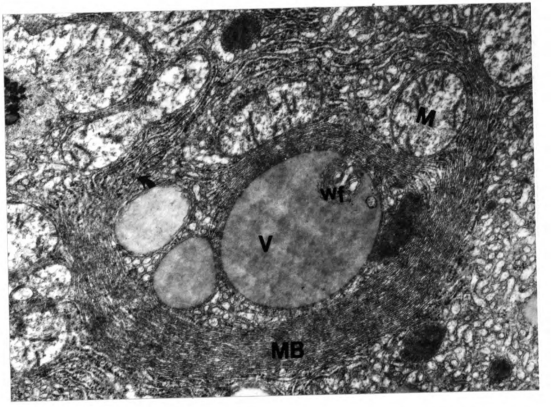

Figure 15. Electron micrograph of a hepatocyte from a rat fed a diet containing 10 ppm of PBB for 360 days. Notice the large myelin body (MB) with a membrane-bound vacuole (V) containing a whorled figure (Wf) and a partially trapped mitochondrion (M). The core of the myelin body is made up of paired smooth membranes that show continuity with the RER (arrow). Lead citrate and uranyl acetate stain; X20,600.

DISCUSSION

There is enough scientific evidence to implicate PBB as a primary hepatotoxin. With few exceptions, the majority of the data available have been derived from experimental animals subjected to medium or large doses of PBB. Part of the rationale for using such doses was based on data that suggested that Firemaster BP-6 was of relatively low toxicity. For example, the acute LD_{50} in rats was reported to be 21.5 g/kg (Michigan Chemical Corporation, 1974). The results of the present research clearly point out that even at very low dietary levels of 0.1 ppm, PBB is capable of precipitating significant biochemical and morphologic tissue alterations in rats. Further, the results of the present study also indicate that thorough morphologic and biochemical evaluation of experimental animals is necessary before a given chemical can be considered toxicologically and environmentally safe for production. Based on the customary screening tests, such as weight gain, urinalysis, mortality rate and hematologic parameters, the rats in the study reported here could be classified as normal. In spite of such clinical normalcy throughout the course of the experiment, detailed biochemical and morphologic evaluations revealed

distinct hepatic alterations, indicating that these rats were not normal.

The most important finding obtained from the present investigation was the statistically significant increase ($p<0.05$) in relative liver weight recorded at all 3 dietary levels of PBB at 10 and 30 days. Although liver to body weight ratios were significantly increased in rats subchronically fed 1 or more ppm PBB (Sleight and Sanger, 1976; Dent et al., 1976a,b), data to indicate such effects at lower dosages have not been reported for any experimental animals.

Morphologically, the hepatomegaly appeared to be the result of hepatocellular hypertrophy and hyperplasia, since prominent mitotic activity was evident in the hepatocytes from the rats killed at 10 days. In addition, fluid imbibition, as recognized by perinuclear vacuolation that failed to stain with oil red O and by clear cytoplasmic spaces ultrastructurally, may have contributed to the increased relative liver weight. Although fluid imbibition was not mentioned by Lee et al. (1975), the observations in the rats killed at 10 days in the present study are in good agreement with their results. They reported finding markedly increased mitotic activity in the hepatocytes from rats gavaged with a single oral dose of either 1000 or 3000 mg/kg of OBB. Gradual decline in mitotic activity occurred in both treated groups at the 5th and 13th day after treatment. The investigators indicated that the continued decline in mitotic activity

could account for the absence of such lesions in a previous 28-day study. A similar explanation may be applicable to the present study, since neither the cytoplasmic vacuolar changes nor increased mitotic activity were seen in hepatocytes obtained from the rats killed at 30 days. The significant increases ($p<0.05$) in relative liver weights of rats consuming diets containing 10 ppm for 60 days and thereafter are probably the result of increased hepatic lipid content (Table 5). Garthoff et al. (1976) indicated that all liver lipid fractions, including total fat, cholesterol, phospholipid and neutral lipids, were increased in rats fed 50 and 500 ppm of PBB. In a more recent report, Harris et al. (1978) also concluded that increased liver size observed in rats fed 50 or more ppm of PBB was due to increased amounts of fat, water or other constituents and not to increased number or size of cells. It is possible that several mechanisms may be functional in bringing about increased liver size in rats consuming dietary PBB. Obviously, hyperplasia seems to be a prominent feature, at least in the early stages, and the age of the experimental animals, the time the animals are killed and the dosage of PBB may be important determinants of which mechanism or mechanisms predominate. In the meantime, further controlled studies are needed to clarify the exact morphogenesis of the hepatomegaly.

The most interesting clinical data derived from this study were the significant decreases ($p<0.05$) in liver vitamin A content. Data on vitamin A content at 10 days

are not available because the livers from these rats were inadvertently misplaced or discarded. Relative (μg/g) and absolute (mg/liver) decreases in liver vitamin A concentration were evident at one or more dietary levels of PBB throughout the course of the experiment. Mangkoewidjojo (1979) reported a dose-dependent depression of hepatic vitamin A content in rats fed 1 or more ppm of PBB. Depression of hepatic vitamin A storage has also been reported for experimental animals treated with PCB (Cecil et al., 1973; Innami et al., 1976; Kato et al., 1978), DDT (Phillips, 1963), methoxychlor (Davison and Cox, 1976) and chlorinated naphthalenes (Olafson, 1947; Hansel et al., 1951). Although there is a paucity of information concerning the association between liver vitamin A content and PBB, the association between the closely-related chlorine analog PCB and the chlorinated hydrocarbon DDT has been extensively studied, and possible mechanisms by which these compounds bring about reduction of liver vitamin A content have been proposed. A common biochemical property of PCB and DDT is their ability to induce liver drug metabolizing enzymes (DME). Concurrent lowering of liver vitamin A concentration occurred when DME were induced (Innami et al., 1976). They speculated that active oxygens generated by hydroxylation reactions catalyzed by cytochrome-P_{450} coupled to the superoxide generating system probably led to the decomposition of vitamin A and reduction of vitamin A concentration in the liver. A similar mechanism is probably responsible for reduction

of hepatic vitamin A by PBB, since the compound is well recognized as a potent inducer of DME, including cytochrome-P_{450}.

In spite of the marked reductions in hepatic vitamin A storage (as much as 10-fold in rats fed 10 ppm for 540 days) caused by feeding diets containing PBB, no clinical signs of vitamin A deficiency or histologic lesions suggestive of hypovitaminosis A were observed.

Mangkoewidjojo (1979) reported prominent squamous metaplasia in bronchiolar epithelium, alveoli and interlobular ducts of salivary glands in rats fed diets containing excessive dietary iodine and 100 ppm of PBB. Metaplastic changes were also reported in tarsal glands of heifers (Moorhead et al., 1977) and hyperkeratosis was a clinical observation in 2 calves in a Michigan herd (Jackson and Halbert, 1974). The lack of clinical signs or histologic lesions in rats in the present study may be explained by the fact that the rats were fed a vitamin A-adequate diet. A second possible explanation may be that critical levels at which signs are likely to appear were probably not achieved, the lowest level of liver vitamin A concentration recorded being 46.2 µg/g compared to 7.95 µg/g observed by Mangkoewidjojo (1979). Although no ill effects were observed, the data seem to suggest that, in animals receiving a marginal level of vitamin A in their diet, exposure to PBB could possibly reduce liver vitamin A content to the extent that clinical signs might occur.

The occasional significant increases and decreases obtained for some hematologic parameters were not considered as evidence of PBB toxicosis because the values were all within the normal range for rats. The reason for the consistently lower total red blood cell values is not clear. However, the values for hematocrit and hemoglobin, 2 more reliable indicators of erythron abnormalities, were all within the normal range, suggesting that the lowered values were probably not an effect of dietary PBB. The generally negative effect of dietary PBB on hematologic values is in agreement with previous reports for rats (Sleight et al., 1978; Sleight and Sanger, 1976), as well as for other species (Kateley and Bazzell, 1978; Howard et al., 1978; Werner et al., 1978).

Residual PBB concentrations in tissues were dose-related and were higher (fat basis) in the liver than in adipose tissue. Steady state concentration apparently was not achieved in either tissue and livers from rats with the highest concentrations of PBB (726 and 627 ppm in livers from rats fed 10 ppm for 360 and 540 days, respectively) had the most severe histopathologic and ultrastructural lesions. The higher concentration of residual PBB in the liver fat is in agreement with reports in cattle (Fries, 1978; Fries et al., 1978). Inability of tissue concentrations of PBB to achieve steady-state may have been due to the continued increase in body weight, deposition of fat in various tissues, or both.

Many of the histopathologic changes seen in the livers from the rats consuming diets containing 10 ppm of PBB for 360 or 540 days were similar to those previously reported in association with either PBB or PCB in rats and other species. The predominantly midzonal lipid accumulation, with extensions to the central and peripheral zones, agrees with the results of Kasza et al. (1976) and Kimbrough et al. (1972) in rats fed PCB. However, Sleight and Sanger (1976) found fatty changes that were diffusely distributed throughout the hepatic lobule and Mangkoewidjojo (1979) reported changes that were either midzonal or centrolobular. Lee et al. (1975) reported on the location of pathologic changes including fatty metamorphosis in rats dosed with OBB. At 7 days after treatment, changes in rats dosed with 1000 mg/kg were centrolobular and midzonal, whereas in rats dosed with 3000 mg/kg, extensions to the peripheral zone occurred. These findings seem to suggest that the location of fatty changes in the livers of rats exposed to polyhalogenated compounds are likely to vary with the dosage of the chemical administered and probably account for the differences in observations among investigators.

The cause of the degenerative changes and focal hepatic necrosis is undetermined. According to Allen et al. (1974) and Allen and Abrahamson (1972), both lesions occur commonly in rats and monkeys exposed to PCB. These investigators postulated that the mechanism responsible for the focal necrosis may be directly related to

PCB-induced changes within the hepatic cells. They further indicated that the cells that undergo necrosis were invariably those that had shown the most pronounced hyperplastic changes in their organelles. A similar explanation is suggested for the cause of the focal necrosis associated with PBB in this study, since ultrastructural observations from hepatocytes from the involved livers also had the most severe organelle changes. No histologic lesion to suggest any likely carcinogenic properties for PBB were evident in this study. In fact, the incidence of spontaneous tumorogenesis was surprisingly low. The following factors could be responsible for this: the sex of the rats, the age (approximately 19 months), and possibly the low numbers of rats used in the study.

Ultrastructural lesions involving progressive increase and dilatation of SER and myelin body (figure) formation are in good agreement with the reports of Corbett et al. (1978), Sleight and Sanger (1976) and Lee et al. (1975). Of 3 types of myelin figures classified by the latter investigators, the smooth membrane-associated body was the common type observed. A similar type of myelin body has been found in the livers from rats treated with DDT (Ortega, 1966), 3-allyl-5-isobutyl-2-thiohydantoin (Herdson et al., 1964a) and phenobarbital (Herdson et al., 1964b), suggesting that myelin body formation might be nonspecific evidence of toxic cell injury. However, the myelin figures found in this study differ from other smooth membrane-associated bodies by having internal

membrane-bound vacuoles containing small whorled figures (Figure 15). These membrane-bound vacuoles containing whorled figures also differ from autophagic vacuoles found in the liver of rats treated with glucagon (Arstila and Trump, 1968) and carbon tetrachloride (Stenger, 1963) in the following ways. The membrane-bound vacuoles lack sequestered organelles. Secondly, there is direct continuity between the outer paired membrane and the cisternae of the SER forming the myelin body. Lee et al. (1975) described somewhat similar vacuoles in the hepatocytes from rats dosed with OBB, suggesting that these features may be a characteristic of myelin figures induced by brominated biphenyls.

Although further studies in rats and other species are necessary to elucidate the morphogenesis of PBB-induced hepatomegaly, the results of this investigation provided information that may be useful to public health and regulatory personnel as a basis for establishing guidelines designed to protect the public from excessive exposure to chemicals in food.

SUMMARY

One hundred sixty-eight male Sprague-Dawley rats initially averaging 72 g were used to study the toxicity of low dietary concentrations of polybrominated biphenyls (PBB). Four groups of 42 rats each were fed 0, 0.1, 1 or 10 ppm PBB. Clinically, there were no ill effects. The PBB-fed rats ate well, maintained a healthy appearance and gained weight as well as, and in some instances better than, the control rats.

Six rats from each group were killed at 10, 30, 60, 90, 180, 360 and 540 days. The results of routine hematologic values and urinalyses were essentially normal. Significant depletion of liver vitamin A storage occurred. Residual PBB concentrations in the liver and adipose tissue were dose-related, and the concentrations in the liver were higher (fat basis) than concentrations in adipose tissue.

Significant increases in relative liver weight were fairly well correlated with hepatocellular hyperplasia and hypertrophy in rats killed at 10 days, or with increased lipid content in rats killed at 360 and 540 days. Bile duct hyperplasia and portal fibrosis occurred in rats fed diets with 1 ppm PBB for 540 days. At 360

and 540 days, histopathologic and ultrastructural changes were most severe in rats fed the highest (10 ppm) dietary level of PBB.

The results indicate that low dietary concentrations of PBB cause bile duct hyperplasia in rats, deplete liver vitamin A to the extent that it could cause avitaminosis, and induce hepatomegaly related to hepatocellular hyperplasia and hypertrophy. Furthermore, the results of this investigation may be useful to public health and regulatory personnel, by providing information that could serve as a basis for establishing guidelines designed to protect the public from excessive exposure to chemicals in food.

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VITA

VITA

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APPENDICES

APPENDIX A

MEAN AND RELATIVE KIDNEY AND BRAIN WEIGHTS FOR PBB-FED RATS

Table A-1. Mean and relative kidney and brain weights for PBB-fed rats

Days on Diet	Dietary PBB (ppm)	Kidney Weight (g)	Brain Weight (g)	Kidney/Body Weight (%)	Brain/Body Weight (%)
10	0.0	1.36±0.15[a]	1.53±0.06	0.97±0.05	1.07±0.07
10	0.1	1.32±0.11	1.56±0.08	0.94±0.05	1.11±0.06
10	1.0	1.29±0.07	1.50±0.03	0.91±0.07	1.06±0.06
10	10.0	1.31±0.14	1.53±0.04	0.90±0.06	1.06±0.06
30	0.0	2.36±0.23[b]	1.69±0.11[b]	0.93±0.05[b]	0.66±0.06[b]
30	0.1	2.64±0.16[b]	1.75±0.10[b]	0.97±0.03[b]	0.65±0.08[b]
30	1.0	2.52±0.14	1.76±0.05	0.97±0.06	0.67±0.02
30	10.0	2.39±0.26	1.71±0.09	0.94±0.06	0.67±0.02
60	0.0	2.84±0.16	1.77±0.17	0.85±0.07	0.53±0.06
60	0.1	2.96±0.51	1.75±0.11	0.85±0.15	0.50±0.03
60	1.0	2.61±0.31	1.74±0.06	0.76±0.07	0.50±0.03
60	10.0	2.84±0.23	1.76±0.09	0.78±0.05	0.49±0.02
90	0.0	3.00±0.18	1.78±0.08	0.77±0.06	0.46±0.04
90	0.1	3.23±0.28	2.14±0.27	0.83±0.06	0.55±0.08
90	1.0	3.44±0.29	2.34±0.06	0.82±0.06	0.56±0.02
90	10.0	3.26±0.19[b]	2.28±0.06[b]	0.80±0.04[b]	0.56±0.04[b]
180	0.0	3.49±0.27[b]	1.93±0.09[b]	0.79±0.03[b]	0.44±0.04[b]
180	0.1	3.78±0.26[b]	2.00±0.10[b]	0.76±0.07[b]	0.40±0.04[b]
180	1.0	3.26±0.18[b]	1.96±0.04[b]	0.68±0.05[b]	0.41±0.02[b]
180	10.0	3.26±0.23	1.91±0.09	0.67±0.05	0.39±0.02
360	0.0	3.77±0.48	1.86±0.08[b]	0.75±0.07	0.38±0.02[b]
360	0.1	3.83±0.26	1.76±0.32[b]	0.73±0.05	0.33±0.06[b]
360	1.0	3.62±0.23	1.90±0.06	0.67±0.06	0.35±0.02
360	10.0	3.48±0.31[b]	1.86±0.06[b]	0.69±0.12[b]	0.37±0.05[b]
540	0.0	3.69±0.32[b]	1.89±0.07[b]	0.73±0.06[b]	0.38±0.03[b]
540	0.1	4.01±0.47[b]	1.91±0.08[b]	0.75±0.10[b]	0.38±0.03[b]
540	1.0	4.46±0.84[b]	1.91±0.07[b]	0.78±0.04[b]	0.33±0.03[b]
540	10.0	3.89±0.35[b]	1.87±0.09[b]	0.75±0.12[b]	0.36±0.03[b]

[a]Values are mean ± SD; N=6. [b]N=5.

APPENDIX B

MEAN THYROID HORMONE CONCENTRATIONS IN RATS FED PBB FOR 10, 90, AND 540 DAYS

Table B-1. Mean thyroid hormone concentrations in rats fed PBB for 10, 90, and 540 days

Days on Diet	Number of Rats	Dietary PBB (ppm)	Hormone Concentrations T_3	T_4
			(ng/ml)	
10	2	0.0	1.07±0.04	46.2± 1.8
10	2	0.1	1.06±0.13	33.9± 3.8[a]
10	2	1.0	1.24±0.22	42.9± 5.7
10	2	10.0	1.19±0.16	47.4± 8.6
90	3	0.0	0.85±0.25	35.7± 9.2
90	3	0.1	1.08±0.43	36.4± 7.2
90	3	1.0	0.86±0.22	35.0± 6.1
90	2	10.0	0.90±0.06	29.7± 0.9
540	5	0.0	0.59±0.13	16.0± 4.0
540	5	0.1	0.29±0.40	15.3± 4.7
540	5	1.0	0.66±0.30	20.0±10.2
540	5	10.0	0.77±0.20	14.6± 6.6

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

APPENDIX C

MEAN SERUM TESTOSTERONE CONCENTRATIONS IN RATS FED PBB FOR 10, 30, 60, 90 AND 180 DAYS

Table C-1. Mean serum testosterone concentrations in rats fed PBB for 10, 30, 60, 90 and 180 days

Days on Diet	Dietary PBB (ppm)	Testosterone Concentration (ng/ml)
10	0.0	1.0 ±0.6[a]
10	0.1	1.6 ±1.3
10	1.0	2.9 ±0.9
10	10.0	2.0 ±1.4
30	0.0	2.3 ±0.4
30	0.1	3.3 ±2.4
30	1.0	2.3 ±0.1
30	10.0	1.7 ±0.2
60	0.0	3.6 ±2.6
60	0.1	3.8 ±1.8
60	1.0	2.5 ±0.4
60	10.0	0.95±0.2
90	0.0	1.6 ±0.5
90	0.1	1.3 ±0.7
90	1.0	6.4 ±3.0
90	10.0	3.6 ±1.9
180	0.0	5.8 ±2.5
180	0.1	3.3 ±0.9
180	1.0	1.7 ±0.1
180	10.0	3.3 ±0.2

[a]Values are mean ± SD; N=2.