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Toxicopathological Effects of 2,2',4,4',5,5' and 2,3',4,4'5,5'-Hexabromobiphenyl In White Leghorn Cockerels

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TOXICOPATHOLOGICAL EFFECTS OF 2,2',4,4',5,5'- AND 2,3',4,4',5,5'-HEXABROMOBIPHENYL IN WHITE LEGHORN COCKERELS

By

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ABSTRACT

TOXICOPATHOLOGICAL EFFECTS OF 2,2',4,4',5,5'- AND 2,2',4,4',5,5'-HEXABROMOBIPHENYL IN WHITE LEGHORN COCKERELS

By

Dewa N. Dharma

Toxicopathological effects of 2 congeners of polybrominated biphenyls (PBB), 2,2',4,4',5,5'-hexabromobiphenyl (peak-4) and 2,3',4,4',5,5'-hexabromobiphenyl (peak-6), were compared in White Leghorn cockerels. Chickens were fed diets containing 0, 4 ppm peak-6, 10 ppm peak-6, 10 ppm peak-4, 62 ppm peak-4, 10 or 100 ppm PBB for 28 days. Liver weights increased in cockerels fed diets containing 62 ppm peak-4, 10 or 100 ppm PBB. Hepatocytes were swollen and vacuolated and lymphoid cells of the bursa of Fabricius were depleted in cockerels fed diets containing 10 ppm peak-6, 62 ppm peak-4, 10 or 100 ppm PBB. Ultrastructurally, the hepatocytes showed vacuolation, increase in smooth endoplasmic reticulum, swollen mitochondria and disruption of the mitochondrial cristae. Peak-6 or peak-4 were less toxic than PBB when given in dosages relative to their concentrations in the mixture. Concentrations being equal, PBB was more toxic than peak-6 and peak-6 more toxic than peak-4.

Dedicated with love to my mother, Gusti M. Alus,

and my late father, Dewa P. Kantor

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INTRODUCTION

Polyhalogenated aromatic hydrocarbons such as biphenylenes, naphthalenes, dibenzofurans, dibenzo-p'-dioxins and biphenyls have contaminated the environment since the early 1940's and there is great concern about their potential health hazards. Compared to other families of polyhalogenated aromatic hydrocarbons, polybrominated biphenyls (PBB) received little attention as potential environmental and food contaminants prior to 1973. Meanwhile, polychlorinated biphenyls (PCB), which are closely related to PBB, were studied extensively.

In 1973, a commercial mixture of PBB (Firemaster FF-1), a fire retardant, was mistakenly substituted for magnesium oxide (MgO) and fed to cattle in Michigan (Carter, 1976; Dunckel, 1975; Di Carlo, 1978). Sheep, hogs and chickens were also contaminated because feed handling facilities were exposed to PBB. To avoid further danger to livestock and human health, approximately 30,000 cattle, 6,000 hogs, 1,500 sheep, 1.5 million chickens, 900 tons of animal feed, 18,000 pounds of butter, 34,000 pounds of dry milk products, and 5 million eggs were destroyed (Carter, 1976).

However, it has been estimated that between the onset of contamination in the fall of 1973 and the establishment of the quarantine of affected herds and flocks in the spring of 1974, over 10,000 Michigan residents had consumed PBB-contaminated milk, eggs, meat and other dairy products (Di Carlo, 1978). Meanwhile, the United States Food and

Drug Administration established tolerances for PBB: 0.3 ppm for milk and meat, 0.05 ppm for eggs and feed (Dunckel, 1975). In 1977, the State of Michigan lowered the tolerances to 0.020 ppm for meat (fat basis) and 0.005 ppm for milk.

Even though PBB toxicosis has been studied in cattle (Jackson and Halbert, 1974; Moorhead et al., 1978; Durst et al., 1977, 1978), rats and guinea pigs (Sleight and Sanger, 1976; Akoso, 1977), Japanese quail (Lillie et al., 1975) and chickens (Ringer, 1978; Polin and Ringer, 1978), it is still unclear which of the components of the mixture most contribute to the toxicity. The major objectives of this study were to determine the toxicity of 2 congeners, 2,2',4,4',5,5'- (peak-4) and 2,3',4,4',5,5'- (peak-6) hexabromobiphenyl, in White Leghorn cockerels and to compare their toxicity with the commercial mixture of PBB.

Cockerels were used in this experiment because it was demonstrated that they were sensitive to the mixture of PBB and were good animal models for PBB toxicosis (Ringer and Polin, 1977; Polin and Ringer, 1978; Ringer, 1978).

LITERATURE REVIEW

In an effort to protect people from injury and death due to fires, the Commission on Fire Protection and Control greatly encouraged the discovery, production and application of fire retardant chemicals. Polybrominated biphenyls were introduced as fire retardants in 1970 (Di Carlo, 1978). Michigan Chemical Corporation, St. Louis, Michigan, had developed Firemaster BP-6 and FF-1 (BP-6 plus 2% calcium silicate) to enable resin and synthetic fiber manufacturers to formulate fire retardant products with good physical properties. The production of these fire retardants was stopped by the Federal Government in 1974, after they were mixed in cattle feed and were considered to be a hazard to human health because of their tendency for bioaccumulation.

Chemistry

Since both chlorine and bromine are halogens, the chemistry of PCB and PBB should be similar. Firemaster FF-1 contains approximately 75% bromine and has a low softening point (72 C) and a high decomposition temperature (300 to 400 C). It is insoluble in water but highly soluble in fats and organic solvents such as toluene, benzene and chloroform. Polybrominated biphenyls are chemically inert in most conditions (Michigan Chemical Corporation, 1971; Kay, 1977).

During the production of PBB, direct bromination of biphenyls results in a complex mixture of compounds differing from each other in both number and position of bromine atoms in the molecules (Sundstrom

and Hutzinger, 1976). It has been reported that the mixture of PBB contains at least 30 different congeners and approximately 25 ppm hexabromonaphthalene, 1 ppm pentabromonaphthalene and trace quantities of tetrabromonaphthalene. Neither bromodibenzofurans nor bromodibenzo-p'-dioxins were found in the mixture (Hass et al., 1978).

Moore and Aust (1978) and Moore et al. (1978a) demonstrated 14 major congeners in the mixture of PBB. By using gas chromatography-mass spectrometry (GC-MS) analyses they found that peaks 1 and 2 are penta, 3 to 6 are hexa-, 7 to 9 are hepta, 10 to 12 are octa-,13 is nona- and 14 is decabromobiphenyl. Jacobs et al. (1976) reported that 60 to 70% of the mixture is 2,2',4,4',5,5' hexabromobiphenyl (peak-4) and according to Moore et al. (1978a), 3 to 4% is 2,3',4,4',5,5' hexabromobiphenyl (peak-6).

No evidence of significant degradation of PBB was found after one year incubation in soil, except for one pentabromobiphenyl (peak-1) congener which showed a significant disappearance after 24 weeks (Jacobs et al., 1976). Photodegradation does not appear to be a significant fate of PBB in manure spread in fields. Absence of plant uptake and translocation of PBB have been reported by Chou et al. (1978).

Kinetics

The kinetics of PBB seems to follow the kinetics of PCB (de Freitas and Norstrom, 1974; Hashimoto et al., 1976). There is considerable information on the kinetics of PBB. However, research concerning the kinetics of each congener in the mixture is still very important in order to gain a complete information on the absorption, metabolism, retention and excretion of each congener.

Although the mixture of PBB is poorly absorbed from the intestine, many animal species, including man, cow, pig, dog, guinea pig, rat, mouse, Japanese quail, chicken and fish, have been found to absorb significant quantities of PBB. Willett and Irving (1976) found that approximately 50% of a single intraruminal dose of PBB was cleared by 168 hours. After administration of ^{14}C decabromobiphenyl oxide, Norris et al. (1975) found that all ^{14}C activity was eliminated via the feces within 2 days. A half-life less than 24 hours with an elimination rate of 62% was found after the administration of ^{14}C octabromobiphenyl. When octabromobiphenyl was fed to rats, over 60% was excreted in the feces as the parent compound. Willett and Durst (1978) found that peak-4, the major congener in the mixture of PBB, occurred in the blood plasma within 4 hours after administration. Fries et al. (1976) reported that the average excretion of hexabromobiphenyl in chickens was 9% of intake and that of heptabromobiphenyl was 15% while PBB were fed. Matthews et al. (1978) concluded that there was less absorption of polyhalogenated hydrocarbons with increasing halogenation. Like PCB, PBB which contain 6 or fewer bromine atoms are efficiently absorbed from the gut of higher animals. Consequently, the absorption and the rate of metabolism of hepta-, octa-, nona- and decabromobiphenyl are lower than those of penta- or hexabromobiphenyl.

Besides the number, the position of the halogen atoms is also important in determining the rate of metabolism and excretion. Polychlorinated biphenyls, which have 2 adjacent unsubstituted carbon atoms, are metabolized and excreted more rapidly than PCB having the same degree of chlorination without 2 adjacent unsubstituted carbon atoms (Matthews et al., 1978). Moore et al. (1978a) concluded that peak-1 and peak-3 were metabolized. The presence of an unsubstituted para position was

necessary and sufficient for a congener to be metabolized. The presence of adjacent unsubstituted carbon atoms was not enough for PBB congeners to be susceptible to metabolism. They also proposed that metabolism proceeded via both arene oxide intermediates and by direct hydroxylation.

Urine may be one of the excretion routes of certain polyhalogenated hydrocarbons. Rats given a single dose of a mono-, a di-, and a pentabromobiphenyl excreted approximately 60, 30 and 7% of the compound, respectively, in the urine (Matthews et al., 1978). However, Willett and Durst (1978) failed to detect free PBB in urine in cattle fed PBB.

Considerable quantities of PBB are excreted in milk. Cows fed 10 mg/day of PBB for 60 days had a steady concentration of 3.07 ppm of PBB in their milk fat within 30 days (Fries and Marrow, 1975). When PBB feeding was stopped, the concentration declined 71% in the first 15 days and thereafter the decline was slower with a half-life of 58 days. Willett and Irving (1976) also reported that milk was an important excretion route for PBB in cows. One cow excreted 23% of the dose of PBB in 168 days. These investigators (1975) also reported that in a lactating cow PBB appeared in plasma, milk and feces at 6, 13, and 19 hours, respectively, after the administration. Willett and Durst (1978) reported that in cows previously exposed to PBB, 3 times as much PBB were removed in milk fat as were removed in feces. After parturition, the concentration of PBB in milk fat declined approximately 2-fold in 6 days. Fries and Marrow (1975) found that the concentration of hexabomobiphenyl in milk was approximately 25 times the concentration of heptabromobiphenyl. They also found that the concentrations of hexa- and heptabromobiphenyl reached stable levels in the milk after 20 days of administration and declined rapidly for the first 10 to 15

days thereafter. After 15 days the concentration of hexabromobiphenyl declined much less rapidly and heptabromobiphenyl was undetectable.

In laying hens, PBB are excreted in the eggs. Polin and Ringer (1978) found that the ratio of PBB concentration in whole eggs to the concentration in the diet was 1.5:1, 56 days after withdrawal of PBB from the diet. Fries et al. (1976) concluded that in laying hens the elimination of PBB through the eggs is more important than elimination through the excreta.

Residues of PBB in body tissues have been investigated extensively. Fries et al. (1978) reported that PBB residues were distributed in body tissues proportionally to the concentration of fat in tissues. However, concentrations of PBB residue were very low in brain tissues, even though they have a high content of lipid material. On the other hand, the liver contained residue concentrations that were disproportionally high when compared to the lipid content of the organ (Willett and Durst, 1978). Robl et al. (1978) found that tissue residue concentrations in calves increased with dosage and duration of administration of PBB and the highest concentrations were found in the fat. Fries et al. (1975) and Willett and Irving (1976) found no significant difference in PBB residue concentrations in perianal, omental, and subcutaneous fat. However, Corbett et al. (1978) reported that the tissue concentrations of PBB in rats, in order of highest concentration to lowest concentration, were: perithymic fat, perianal fat, adrenal glands, thymus, liver, and stomach. Analyses of the tissues in rats given octabromobiphenyl revealed a dose-related buildup of bromine, predominantly in the fat and liver (Lee et al., 1975). In Japanese quail, tissue residues of PBB were generally higher in males than in females (Babish et al., 1975). Zitko (1977) found that only

bromobiphenyls with 6 or fewer bromine atoms were accumulated from water by the fish.

Polybrominated biphenyls are transferred to the fetus and may be embryotoxic (Detering et al., 1975). Rickert et al. (1978) and Hall (1980) found that the concentration of PBB in liver of neonates was higher than in the liver of adults nursing them. However, Werner (1979) and Rickert et al. (1978) concluded that transfer of PBB via the milk was much more significant than via the placenta. It has been mentioned earlier that PBB are transferred to eggs and therefore can induce toxicological effects in the offspring (Babish, 1975; Lillie, 1975; Polin and Ringer, 1978).

Toxicity

There was very little information concerning the toxicity of PBB before they were accidentally mixed with cattle feed in Michigan in 1973. Michigan Chemical Corporation had classified PBB (Firemaster BP-6) as nontoxic by ingestion or dermal application. They further classified PBB as not a primary skin irritant, not an eye irritant and not highly toxic by inhalation exposure. They reported that the acute oral LD_{50} for male albino rats was 21.5 g/kg body weight and the acute dermal LD_{50} for albino rabbits was 2.15 to 10.0 g/kg body weight. Since the major concern in public health is the long-term effects of the compound, this information seems to be somewhat irrelevant. Later research has shown the long-term hazards of the compound in a wide variety of animal species.

Clinical Signs

Jackson and Halbert (1974) reported that cows consuming a high dose of PBB had clinical signs such as anorexia, decreased milk

production, increased frequency of urination and lacrimation, shrinking of the udder and early embryonic resorption. Later on, hematomas, abscesses, abnormal growth of the hooves, dystocia, metritis and hydrops amnii were observed. Cows fed 25,000 mg PBB/head/day had general depression, diarrhea, and depressed heart and respiratory rates. The cows died in 33 to 66 days (Durst et al., 1978). Hypospermatogenesis was also observed in male calves fed 0.1, 1.0, 10 or 100 mg PBB/kg body weight (Robl et al., 1978). However, Mercer et al. (1977) and Wastell et al. (1978) reported that cows fed low levels of PBB had no clinical signs except a slower growth rate.

Rats and mice used for experiments with PBB have had few adverse clinical signs. Sleight et al. (1978) reported a slower growth rate in rats fed a diet containing 100 ppm PBB for 30 and 60 days. Sleight and Sanger (1976) reported that guinea pigs fed a diet containing 500 ppm PBB died within 15 days and only 2 of 6 fed a diet containing 100 ppm PBB survived for 30 days. A lethal effect was found in mink fed a diet containing 6.25 ppm PBB for 10 months. When a diet containing 1 to 2.5 ppm PBB was administered to mink, decreased litter size, kit weight at birth, and kit survival were observed (Aulerich and Ringer, 1979).

The toxicosis of PBB in birds has been studied extensively. Decreased feed intake, egg production and hatchability of fertile eggs were reported in chickens and Japanese quail fed a diet containing 45 ppm PBB (Ringer and Polin, 1977; Polin and Ringer, 1978). Subcutaneous edema of the neck and shoulder of hatched chicks from dams fed PBB has been reported (Lillie et al., 1975). Decreased heart rate and voltage amplitude of ECG were reported by Ringer (1978) and Heineman (1976). Cardiac output and cardiac index were reported as decreased in cockerels

fed a diet containing 75 and 150 ppm PBB (Heineman, 1976). The clinical signs induced by PBB seemed to be similar to those induced by PCB (Vos and Koeman, 1970; Vos, 1972).

Clinical Pathology

A considerable amount of research has been conducted concerning the clinicopathological effects of PBB in various kinds of animals. The concentration of blood urea nitrogen (BUN) was reported as considerably increased in cattle (Durst et al., 1978) and slightly increased in pigs (Werner, 1979) and guinea pigs (Hall, 1980). Increased serum glutamic oxaloacetic transaminase (SGOT), serum lactic dehydrogenase (LDH), and serum bilirubin were reported in cattle by Durst et al. (1978). They also reported a decrease in serum calcium and serum albumin. Serum cholesterol was reported increased in rats (Akoso, 1977; Mangkoewidjojo, 1979), and liver vitamin A decreased (Mangkoewidjojo, 1979). Ku et al. (1978) did not find any significant influence on SGOT, serum alkaline phosphatase or serum creatine phosphokinase when pigs were fed diets containing PBB. There was no significant difference in the number of erythrocytes or leukocytes, hematocrit and hemoglobin content when cattle were fed diets containing PBB (Kateley and Bazzell, 1978).

Biochemical Pharmacology

Polybrominated biphenyls have been found to be a mixed-type inducer of hepatic microsomal drug metabolizing enzymes with inducing properties similar to phenobarbital (Pb) and 3-methylcholanthrene (3-MC) (Dent et al., 1978; Babish et al., 1975; Moore et al., 1978). Renal microsomal enzymes were also induced by the mixture of PBB (McCormack, 1978). Sidhu and Michelakis (1978) reported that PBB induced adenylate cyclase in rat lung alveoli. Peak-4 and 2,2',3,4,4',5,5'-heptabromobiphenyl

(peak-8) were reported as strictly Pb-type inducers (Moore et al., 1978a,b, 1979). Since those 2 major congeners comprise approximately 80% of the mixture of PBB and are strictly Pb-type inducers, there must be one or more of the other congeners which are 3-MC type inducers. Dannan et al. (1978) reported that peak-6 is a mixed-type inducer and contributes to the properties of PBB as a 3-MC type inducer. Recently, Robertson et al. (1980) reported that 2,3',4,4',5-pentabromobiphenyl (peak-2) was also a mixed type inducer.

Gross Lesions

Gross lesions due to the exposure of PBB have been described in various species of animals. The severity of the lesions depends on the dose, the species and the duration of exposure. Jackson and Halbert (1974) reported that cattle which consumed a diet containing a high dose of PBB had enlargement of the liver. They also reported other findings, such as hematomas, abscesses in peritoneal and thoracic cavities, and sometimes abomasal ulcers. An apparent increased incidence of metritis, retained placenta and adhesions of liver and kidney has been observed in PBB-contaminated cattle (Prewitt et al., 1975). Moorhead et al. (1978) reported some other changes, such as dehydration, subcutaneous emphysema and hemorrhage, atrophy of the thymus, fetal death, mucoid enteritis and enlargement of the kidney and lymph nodes in heifers fed a diet containing 25 g PBB/head/day for 33 to 66 days. Pigs fed a diet containing 20 or 200 ppm PBB for 16 weeks had a marked increase in liver weights. Relative weights of heart, kidney, and adrenals were also increased (Ku et al., 1978).

Hepatic enlargement seems to be a constant finding in rodents fed a diet containing PBB. Sleight and Sanger (1976) found hepatic enlargement in rats fed a diet containing PBB for 30 days at a

concentration of as little as 1 ppm. They also reported enlarged livers in guinea pigs that died when fed a diet containing 500 ppm of PBB. Matthews et al. (1978) failed to observe any gross lesions in rats fed a diet containing 50 ppm PBB for 3 weeks, but hepatic enlargement was observed in rats fed 500 ppm PBB. Increases of hepatic weights were described in mice fed a diet containing 1000 ppm PBB for 11 days (Corbett et al., 1975, 1978). Akoso (1977) observed hepatic enlargement in rats fed a diet containing 10 ppm PBB for 60 days. Hepatic enlargement in rats was also observed after a single oral dose of octabromobiphenyl at 1000 mg/kg body weight (Lee et al., 1975; Aftosmis et al., 1972).

Some workers reported that PBB induced enlargement of thyroids. Akoso (1977) and Magkoewidjojo (1979) observed enlargement of the thyroids in rats fed a diet containing 10 ppm PBB. When sows were fed a diet containing 100 ppm PBB during the last half of gestation, the piglets had enlarged thyroids (Werner, 1979). Ringer (1978) also reported thyroid enlargement in cockerels fed a diet containing 45 ppm PBB.

There have been some reports concerning the gross lesions caused by congeners of the PBB mixture. Dibromobiphenyl (2,2' DBB), a trace congener of the mixture, was not able to induce any lesions in rats given 90 mg/kg body weight, ip. However, peak-4 and peak-8 were found to increase hepatic weights significantly (Moore et al., 1979).

Ringer and Polin (1977) reported general edema, hydropericardium, increased hepatic and thyroidal weights and decreased splenic, bursal and comb weights in chickens fed a diet containing 45 ppm PBB for 60 days. In addition to those changes, Ringer (1978) also reported a decrease in testicular weights in chickens fed a diet containing 50 ppm

PBB for 60 days. In an experiment carried out by Babish et al. (1975), no gross lesions were observed in Japanese quail fed a diet containing up to 100 ppm PBB. Meanwhile, chickens would not eat a diet containing 1000 ppm PBB.

Histopathology

Histologic changes occurred in the liver of most animal species fed PBB or their congeners. Moorhead et al. (1978) reported histopathologic changes in cattle fed a diet containing 25 g PBB/head/day for 33 to 66 days. Fatty degeneration and glycogen depletion were observed in liver sections. They also observed cloudy swelling, hydropic degeneration, and extreme dilatation of collecting ducts and convoluted tubules in the kidney. Hyperkeratosis was reported to occur on the eyelids. Hyperplasia and cystic dilatation of the mucous glands in the lamina propria of the gallbladder were also observed.

Swelling and vacuolation of hepatocytes were observed in rats fed diets containing 100 and 500 ppm PBB for 30 days. At 10 ppm, Sleight and Sanger (1976) found only a slight swelling and vacuolation of hepatocytes. Rats given a single oral dose of 1 g PBB/kg body weight had enlarged and vacuolated hepatocytes at 300 days (Kimbrough et al., 1977). A number of neoplastic nodules were also observed in the liver. Matthews et al. (1978) found only a minimal vacuolation and focal hepatitis in rats fed a diet containing 500 ppm PBB for 3 weeks. Pronounced additive effects of PBB toxicosis were reported when rats were fed 500 mg PBB/kg of body weight by stomach tube and with either 4 or 20% purified fiber in the diet. Steatosis, megalohepatocytes, necrosis and interstitial fibrosis were observed in the liver (Kimbrough et al., 1980). Sleight and co-workers (1978) found that rats fed an iodine deficient diet and 100 ppm PBB for 60 days had hyperplastic bile ducts

and fibrosis of the portal triads of the liver. The size of thyroid follicles in rats fed a diet containing 100 ppm PBB for 30 days was irregular, with increased cellularity, sparse colloid and columnar epithelium. Hyperplasia of thyroids and vacuolation of the colloid were reported in rats fed the mixture of PBB (Akoso, 1977; Mangkoewidjojo, 1979). Rhesus monkeys (*Macaca mulatta*) fed a diet containing 25 ppm PBB for 12 weeks had hyperplastic gastritis (Lambrecht et al., 1978). Recently, McConnell et al. (1979) reported that rhesus monkeys exposed to PCB had squamous metaplasia of several glandular tissues and hypertrophy of the glandular stomach and colon.

There are relatively few descriptions of histopathological changes of PBB toxicosis in avian species. Babish et al. (1975) did not find any gross or microscopic lesions in the Japanese quail fed a diet containing up to 100 ppm PBB. However, Ringer (1978) described lesions in immature chickens fed a diet containing 150 ppm PBB for 6 weeks. He found that the bursa of Fabricius was markedly depleted of lymphocytes in both the cortex and the medulla. Depletion of lymphocytes was also observed in the cortex of the thymus. The spleen showed a loss of germinal centers and a reduction in the diffuse lymphoid tissue (white pulp). Thyroid hyperplasia and vacuolation of the colloid were also reported.

Electron Microscopy

Sleight and Sanger (1976) and Mangkoewidjojo (1979) described swelling of hepatic mitochondria and an increase of smooth endoplasmic reticulum in rats fed a diet containing 100 ppm PBB. They also observed some myelin bodies and numerous vacuoles in the hepatocytes. In addition, a decrease in rough endoplasmic reticulum and glycogen and an increase in lysosomes were reported in the liver of mice fed a diet

containing 1000 ppm PBB for 14 days (Corbett et al., 1978). The livers of rats fed a single oral dose of 1000 mg octabromobiphenyl/kg body weight had a similar electron microscopic change to rats fed the mixture of PBB (Lee et al., 1975a). A comparative study was conducted on the effects of PBB and PCB exposure on the thyroids of rats by Kazsa et al. (1978). They found that PBB and PCB induced similar ultrastructural lesions in the thyroid follicles. Rats fed diets containing 5 ppm PBB or PCB for 5 weeks had an accumulation of colloid droplets and lysosomal bodies within the cytoplasm of follicular cells. The mitochondria were vacuolated and the cristae were disrupted. Blunting and branching or absence of the microvilli were also described. More severe changes were observed when rats were fed diets containing higher doses of PBB or PCB. Akoso et al. (1980) observed less pronounced ultrastructural changes in thyroid follicular cells in rats given a diet containing peak-4 than in those given diets containing PBB or peak-6.

Immunology

The mixture of PBB has been reported to affect lymphoid tissues such as thymus, spleen and bursa of Fabricius. Pathologic changes in these organs would consequently affect the humoral or cellular immune responses. Luster et al. (1978) reported that rats and mice fed diets containing 30 mg PBB/kg body weight for 30 days had depressed cell mediated immunity. They also reported that 30 ppm PBB in the diets of rats and mice depressed humoral immunity. Howard (1979) showed that lymphocytes from sows fed diets containing 100 or 200 ppm PBB for 12 weeks (during the last half of gestation and during lactation) had significantly decreased mitogen responses. Moreover, she found that mitogen responses of lymphocytes from piglets of sows fed PBB were normal at

birth but responses were significantly decreased when the piglets were 4 weeks of age. Cattle with 0.02 to 30 ppm PBB/g fat equivalent had no alteration in lymphocyte surface antigens. Further studies indicated that these levels of PBB in fat tissues did not predispose cattle to autoantibody production or leukotoxic serum factors (Kateley and Bazzel, 1978). Vos and Roij (1972) indicated that PCB had immunosuppressive activity. Guinea pigs fed diets containing 10 or 100 ppm PCB showed a decrease in the number of gamma globulin containing cells in popliteal lymph nodes. Street and Sharma (1975) concluded that lymphatic organs were the most sensitive indicators of immunosuppression. They reported a decrease in plasma cells in popliteal lymph nodes, reduction of germinal centers in the spleen and atrophy of the thymus in rabbits fed diets containing 3, 7, 20, 45.8 or 170 ppm Aroclor 1254.

Oncogenicity

The possibility of neoplasms as a result of exposure to PBB is still a great concern to people. Until the present time, there are no conclusive reports concerning the oncogenicity of PBB in animals or man. However, neoplastic nodules have been observed in the livers of rats fed a single oral dose of 1 g PBB/kg body weight (Kimbrough et al., 1978). An increased incidence of hepatocellular carcinomas and squamous cell carcinomas of the lung, hard palate and tongue was reported in rats fed a diet containing 0.1 µg tetrachlorodibenzo-p'-dioxins/kg/day for 2 years. The incidence of tumors of the pituitary, uterus, mammary glands, pancreas and adrenal glands was reported decreased (Kociba et al., 1978). Other halogenated aromatic hydrocarbons, such as DDT, dieldrin, mirex, kepone, hexachlorobenzene and PCB, were also reported to be able to induce liver tumors in rodents (Kimbrough, 1979). She proposed that not the chemicals themselves but their metabolites are

carcinogenic. Squire and Levitt (1975) reported that these chemicals induced areas of alteration in the liver which later became neoplastic nodules and finally transformed into hepatocellular carcinomas.

MATERIALS AND METHODS

General Experimental Design

In this experiment the toxicity of 2 congeners, 2,2',4,4',5,5'-hexabromobiphenyl (peak-4) and 2,3',4,4',5,5'-hexabromobiphenyl (peak-6), were examined. Peak-4 constitutes 60 to 70% of the mixture of PBB (Jacobs, 1976). If one postulates that peak-4 is the only congener responsible for the toxicity of the mixture, then this congener, when given in dosages relative to its concentration in the mixture, could be expected to produce the same toxicity as the parent mixture. Peak-6 constitutes 3 to 4% of the mixture of PBB, and it was utilized in this experiment because of its ability to cause a mixed type induction of hepatic microsomal drug metabolizing enzymes (Dannan et al., 1978). Likewise, if peak-6 is the only congener responsible for the toxicity of the mixture of PBB, this congener, when given in dosages relative to its concentration in the mixture, should be able to induce the same toxicity as the parent mixture. Based on these assumptions, it was hypothesized that the toxicity of a diet containing 100 ppm of the mixture of PBB was equal to a diet containing 62 ppm peak-4 or 4 ppm peak-6, and likewise a diet containing 62 ppm peak-4 was equally toxic to one containing 4 ppm peak-6. The toxicity of the mixture of PBB, peak-4 and peak-6 was also compared by feeding diets containing 10 ppm of each. At 10 ppm of the diets, the mixture of PBB, peak-4 and peak-6 was hypothesized as being equally toxic. Originally, this experiment was designed to compare the toxic effects of these chemicals at 100 ppm

in the diets, but unfortunately there were not enough chemicals available. It is relatively easy to obtain a sufficient amount of purified peak-4 since it constitutes 60 to 70% of the mixture. On the other hand, it is very difficult to obtain enough purified peak-6 because it constitutes only 3 to 4% of the mixture.

Eighty 2-day-old White Leghorn (WL) cockerels[a] weighing approximately 40.5 g each were randomly allotted into 7 groups. They were reared in conventional electrically heated battery brooders with raised wire floors. The cockerels were fed ground commercial chick starter diets[b] for 28 days. The experimental design is shown in Table 1.

Table 1. Experimental design

Treatments	Concentrations in Feed (ppm)
Control	0
2,3',4,4',5,5'-hexabromobiphenyl (peak-6)[a]	4
	10
2,2',4,4',5,5'-hexabromobiphenyl (peak-4)[a]	10
	62
PBB (Firemaster FF-1)[b]	10
	100

There were 20 cockerels in the control group and 10 cockerels in others.

[a]Department of Biochemistry, Michigan State University.

[b]Michigan Chemical Company, Chicago, IL.

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[a]Reichard's Hatchery, St. Louis, MI.

[b]Clarksville Elevator, Clarksville, MI.

Diet Preparation and Feeding

To obtain the desired concentrations of PBB in the diets, the mixture of PBB was pulverized with a mortar and pestle and mixed with the starter diet to make a 1% premix. The concentrations of 100 or 10 ppm PBB in the diets were then made by mixing the premix with the basal starter diet. In the preparation of the desired concentrations of 2,3',4,4',5,5'-hexabromobiphenyl (peak-6) and 2,2',4,4',5,5'-hexabromobiphenyl (peak-4), as shown in Table 1, the chemicals were dissolved in corn oil by gentle heating and stirring and then mixed with a small amount of the basal diet. More of the basal diet was then added to make certain concentrations of the compounds. Because of the utilization of corn oil in the feed of cockerels fed diets containing peak-6 and peak-4, the same concentration of corn oil was also added to the diets of other groups. To assure uniform distribution, the diets containing the chemicals were tumbled in a feed mixer[c] for 15 minutes in feed cans with a capacity of 3.6 kg. The cockerels were provided with feed and water *ad libitum*. The feeders were half-filled every day to minimize the wastage of the feed. During the feeding, the cockerels were observed for clinical signs of toxicosis. Feed consumption and body weights were recorded weekly.

Collection of Specimens

At 28 days, blood was drawn by cardiac puncture for determination of plasma levels of triiodothyronine (T_3), thyroxin (T_4) and for studies of lymphocyte blastogenesis. Heparin was used as anticoagulant. After blood withdrawal, the cockerels were killed by cervical dislocation.

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[c]Paul G. Abbe, Inc., Little Falls, NJ.

Liver, spleen, thyroids, bursa of Fabricius, testicles and comb were weighed with an electric balance.[d] Specimens of liver and thyroids were minced and placed in Karnovsky's fixative for electron microscopic studies. Specimens of liver were placed in plastic bags and frozen at -24 C for PBB analysis. For histologic examination, specimens of liver, spleen, thymus, thyroids, bursa of Fabricius, cecal tonsils, kidneys, lungs, testicles, brain and comb were placed in 10% neutral buffered formalin.

Examination of Specimens

Stimulation of Lymphocyte Blastogenesis[e]

For studies of lymphocyte blastogenesis, RPMI 1640 media with 25 mM HEPES and 2 mM l-glutamic[f] were utilized for all suspensions, mitogen dilutions and isotope dilutions. Concanavalin A[g] was used for lymphocyte blastogenesis stimulation. The isotope used in this study was tritiated thymidine (^{3}H).[h] Cells were counted in an Isocap/300 liquid scintillation counter.[i] Results were reported as counts per minute (CPM) or disintegrations per minute (DPM).

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[d] Mettler A 30 with 0.0001 g readability.

[e] Dr. R. K. Ringer, Department of Poultry Science, Michigan State University.

[f] GIBCO 380-2400.

[g] Miles Laboratories 79-003-5.

[h] New England Nuclear NET-027.

[i] Searle Analytic 6872.

Thyroid Hormone Analyses[j]

Concentrations of T_3 and T_4 in the plasma of the cockerels were determined by radioimmunoassay (Chopra et al., 1971, 1972). The results were expressed in ng/100 ml plasma.

Electron Microscopy[k]

Specimens for electron microscopic examination were washed with Sorensen's phosphate buffer (pH 7.2) and post-fixed in 1% osmium tetroxide. After dehydration with graded ethanol, specimens were transferred into propylene oxide and then embedded in epoxy. Ultrathin sections were cut and stained with uranyl acetate and lead citrate and examined with an electron microscope.

Analysis of Polybrominated Biphenyls

In this experiment, 2 or 3 specimens of liver from each group were pooled. Procedures for the extraction of PBB from the liver and for the gas chromatographic analysis of PBB in the extract have been previously described (Werner, 1979). Results were compared to standard samples containing 50 ppb PBB/g liver and expressed as ppm PBB on a fat basis and on a whole weight basis.

Histopathology

Specimens for histopathologic examination were processed in an automatic processor, embedded in paraffin, cut at 5 µm and stained with hematoxylin-eosin (H&E). Hepatic sections were stained with oil red O to demonstrate fat.

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[j]Dr. R. F. Nachreiner, Department of Large Animal Surgery and Medicine, Michigan State University.

[k]Dr. Esther Roege, Department of Pathology, Michigan State University.

Statistical Analysis

Data were statistically analyzed by analysis of variance followed by comparison of the means by using the Bonferroni t-test.

RESULTS

Clinical Signs

There were no adverse clinical signs observed in any of the groups of cockerels. Feed consumption was apparently not affected and body weights were not significantly different (Tables A1 and A2, Appendix). One cockerel from the group fed a diet containing 10 ppm PBB died at 5 days of the experiment. The cause of death was not determined; it was probably not related to PBB toxicosis.

Organ Weights

Six organs were weighed at necropsy. The weights of thyroids, spleen, testicles and comb were not significantly different in all groups (Table A3, Appendix). The relative hepatic weights are presented in Table 2. Hepatic relative weights of cockerels fed diets containing 62 ppm peak-4 were significantly increased when compared to the controls. When the relative hepatic weights of cockerels fed a diet containing 62 ppm peak-4 were compared to those given 10 ppm peak-4 or 4 ppm peak-6, they were significantly higher ($p<0.05$). At 100 ppm PBB, the relative hepatic weights were significantly higher when compared to those given 10 ppm PBB, 62 ppm peak-4 or 4 ppm peak-6. The relative hepatic weights of cockerels fed a diet containing 10 ppm PBB were significantly higher ($p<0.05$) when compared to those given 10 ppm peak-6 or 10 ppm peak-4.

Table 2. Relative hepatic weights of cockerels fed diets containing 2,3',4,4',5,5'-hexabromobiphenyl (peak-6), 2,2',4,4',5,5'-hexabromobiphenyl (peak-4) or the commercial mixture of polybrominated biphenyls (PBB) for 28 days

Treatments	Concentrations	Relative Hepatic Weights (g/kg body weight)
Control	0	24.69 ± 2.28
Peak-6	4	24.30 ± 2.64
	10	25.84 ± 3.36
Peak-4	10	23.06 ± 2.08
	62	27.14 ± 3.20[a]
PBB	10	27.49 ± 2.65[b]
	100	31.42 ± 2.85[c]

[a]Cockerels fed a diet containing 62 ppm peak-4 had significantly higher ($p<0.05$) relative hepatic weights when compared to those given diets containing 0, 4 ppm peak-6 or 10 ppm peak-4.

[b]Cockerels fed a diet containing 10 ppm PBB had significantly higher ($p<0.05$) relative hepatic weights when compared to those given diets containing 0, 10 ppm peak-6 or 10 ppm peak-4.

[c]Cockerels fed a diet containing 100 ppm PBB had significantly higher ($p<0.05$) relative hepatic weights when compared to those given diets containing 0, 4 ppm peak-6, 62 ppm peak-4 or 10 ppm PBB.

Data are presented as mean ± SD.

Relative bursal weights of the cockerels are presented in Table 3. The relative bursal weights of cockerels fed a diet containing 100 ppm PBB were significantly lower ($p<0.05$) compared to those given 0, 10 ppm PBB, 62 ppm peak-4 or 4 ppm peak-6. Also, the relative bursal weights of cockerels fed a diet containing 10 ppm PBB were significantly lower ($p<0.05$) when compared to those given 0, 10 ppm peak-4 or 10 ppm peak-6.

Lymphocyte Blastogenesis

Data on stimulation of lymphocyte blastogenesis using Concanavalin-A were inconsistent (Table A4, Appendix).

Thyroid Hormone Analysis

There were no significant differences in the concentrations of T_3 in the plasma of the cockerels (Table A5, Appendix). Data on the concentrations of T_4 in the plasma were inconsistent (Table A6, Appendix).

Analysis of Polybrominated Biphenyls

Data for concentrations of PBB in the hepatic tissues are presented in Table 4. A concentration of 2.25 ± 1.5 ppm of PBB in the liver tissues of the control cockerels may be due to the contamination of the room where the experiment was conducted or could be due to the contamination of the equipment during the gas-chromatographic analysis. The concentrations of the mixture of PBB in the liver tissues were approximately 8 times higher than the concentrations of the mixture in the diets. Meanwhile, the concentrations of peak-4 and peak-6 in the liver tissues were 10 to 11 times higher than the concentrations in the diets.

Table 3. Relative bursal weights of cockerels fed diets containing 2,3',4,4',5,5'-hexabromobiphenyl (peak-6), 2,2',4,4',5,5'-hexabromobiphenyl (peak-4) or the commercial mixture of polybrominated biphenyls (PBB) for 28 days

Treatments	Concentrations	Relative Bursal Weights (g/kg body weight)
Control	0	6.37 ± 1.14
Peak-6	4	6.18 ± 0.77
	10	6.32 ± 1.33
Peak-4	10	6.22 ± 0.96
	62	6.05 ± 1.79
PBB	10	5.74 ± 1.06[a]
	100	4.08 ± 1.51[b]

[a]Cockerels fed a diet containing 10 ppm PBB had significantly lower ($p<0.05$) relative bursal weights when compared to those given diets containing 0, 10 ppm peak-6 or 10 ppm peak-4.

[b]Cockerels fed a diet containing 100 ppm PBB had significantly lower ($p<0.05$) relative bursal weights when compared to those given diets containing 0, 4 ppm peak-6, 62 ppm peak-4 or 10 ppm PBB.

Data are presented as mean ± SD.

Table 4. Concentrations of 2,3',4,4',5,5'-hexabromobiphenyl (peak-6), 2,2',4,4',5,5'-hexabromobiphenyl (peak-4) or the commercial mixture of polybrominated biphenyls (PBB) in liver tissues after 28 days of dietary treatment

Treatments	Concentrations in Diets (ppm)	Concentrations in Liver Tissue (ppm)
Control	0	2.5 ± 1.5
Peak-6	4	32.5 ± 4.3
	10	132.1 ± 16.4
Peak-4	10	104.8 ± 16.3
	62	751.1 ± 105.9
PBB	10	83.8 ± 4.2
	100	751.5 ± 77.3

Data are expressed as mean ± SD.

Histopathology

Liver

There were no apparent lesions in the liver of the cockerels fed diets containing 0, 4 ppm peak-6 or 10 ppm peak-4 (Figure 1). Moderate swelling and vacuolation of the hepatocytes were observed in cockerels fed a diet containing 100 ppm PBB (Figure 2). Similar but less severe changes were also found in cockerels fed diets containing 62 ppm peak-4, 10 ppm peak-6 or 10 ppm PBB. At 10 ppm PBB the lesions appeared to be slightly more severe when compared to those at 62 ppm peak-4 or 10 ppm peak-6. Liver sections from cockerels fed diets containing 10 ppm peak-6, 62 ppm peak-4, 10 or 100 ppm PBB were oil red O positive.

Bursa of Fabricius

As in the liver, there were no apparent lesions in the cockerels fed diets containing 0, 4 ppm peak-6 or 10 ppm peak-4 (Figure 3). Moderate depletion of lymphoid cells was observed in the cortex and the medulla of cockerels fed a diet containing 100 ppm PBB (Figure 4). Lymphoid cells of cockerels fed diets containing 10 ppm PBB, 10 ppm peak-6 or 62 ppm peak-4 were also slightly depleted.

Transmission Electron Microscopy

Electron microscopic examination was conducted of liver tissues, but thyroids were not examined because there were no changes in the histologic sections. Cockerels fed diets containing 0, 4 ppm peak-6 or 10 ppm peak-4 (Figure 5) had no apparent ultrastructural changes. Vacuolation, swelling of mitochondria and disruption of mitochondrial cristae and hyperplasia of smooth endoplasmic reticulum were observed in hepatocytes of cockerels fed a diet containing 100 ppm PBB (Figure 6).

Figure 1. A liver section of a control cockerel fed a basic diet for 28 days. Hematoxylin and eosin staining; X300.

Figure 2. A liver section of a cockerel fed a diet containing 100 ppm polybrominated biphenyls (PBB) for 28 days. Notice swelling and vacuolation of the hepatocytes. Hematoxylin and eosin staining; X300.

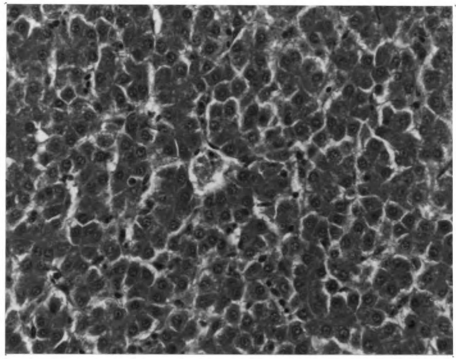

Figure 1

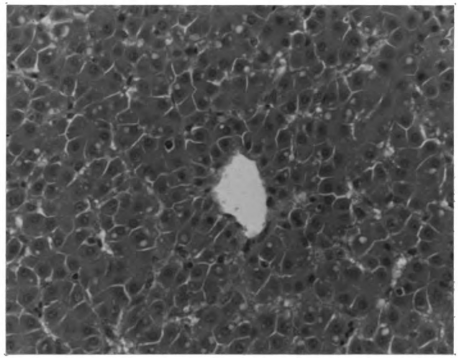

Figure 2

Figure 3. A section of the bursa of Fabricius of a control cockerel fed a basic diet for 28 days. Hematoxylin and eosin staining; X120.

Figure 4. A section of the bursa of Fabricius of a cockerel fed a diet containing 100 ppm polybrominated biphenyls (PBB) for 28 days. Notice depletion of lymphoid cells, particularly in the medulla. Hematoxylin and eosin staining; X120.

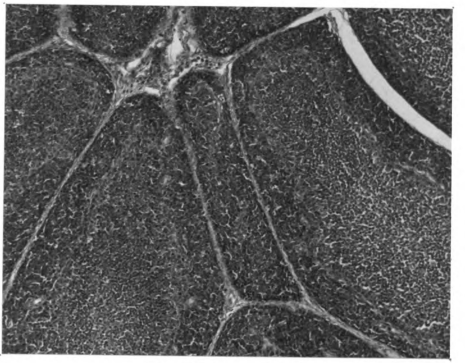

Figure 3

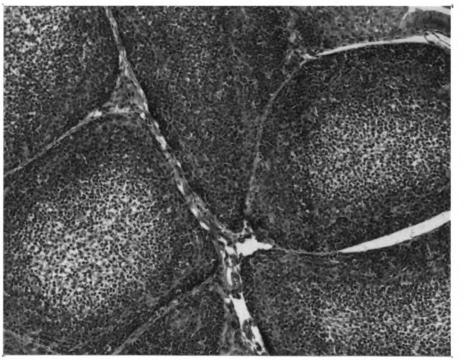

Figure 4

Figure 5. Hepatocyte of a control cockerel fed a basic diet for 28 days. Uranyl acetate and lead citrate staining; X11,600.

Figure 6. Hepatocyte of a cockerel fed a diet containing 100 ppm polybrominated biphenyls (PBB) for 28 days. Notice vacuolation, increase in smooth endoplasmic reticulum (arrow), swollen mitochondria and disruption of the mitochondrial cristae. Uranyl acetate and lead citrate staining; X11,600.

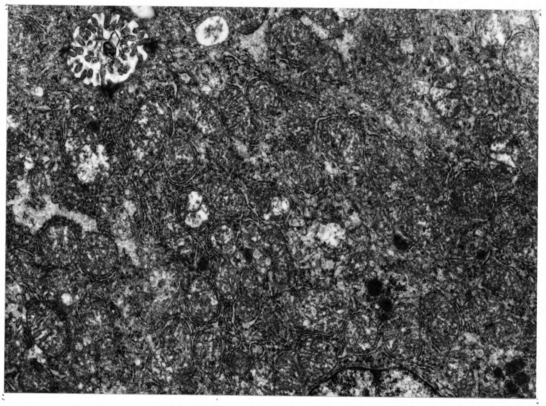

Figure 5

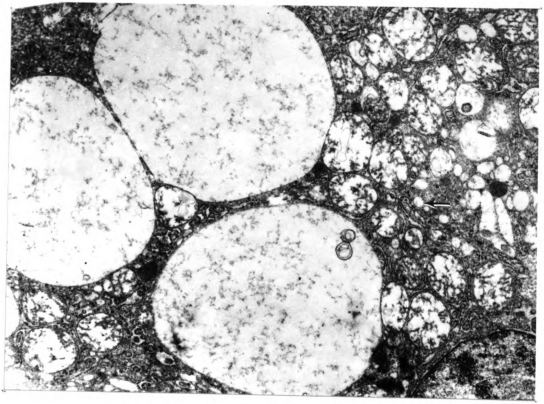

Figure 6

Similar but less severe lesions were observed in cockerels fed diets containing 10 ppm peak-6 or 62 ppm peak-4 (Figures 7 and 8). Hepatic lesions at 10 ppm PBB (Figure 9) appeared to be slightly more severe than at 10 ppm peak-6.

Figure 7. Hepatocyte of a cockerel fed a diet containing 10 ppm 2,3',4,4',5,5'-hexabromobiphenyl (peak-6) for 28 days. Notice increase in smooth endoplasmic reticulum (arrow), swollen mitochondria and disruption of the mitochondrial cristae. Uranyl acetate and lead citrate staining; X11,600.

Figure 8. Hepatocytes of a cockerel fed a diet containing 62 ppm 2,2',4,4',5,5'-hexabromobiphenyl (peak-4) for 28 days. Notice increase in smooth endoplasmic reticulum (arrow), swollen mitochondria and disruption of the mitochondrial cristae. Uranyl acetate and lead citrate staining; X11,600.

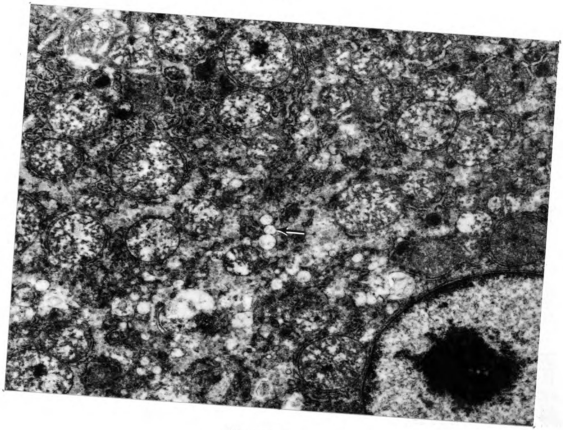

Figure 7

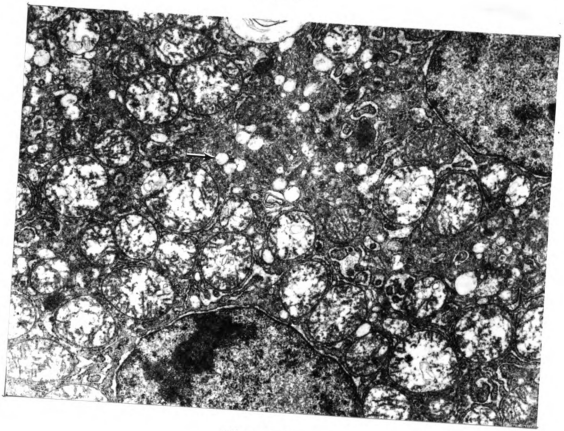

Figure 8

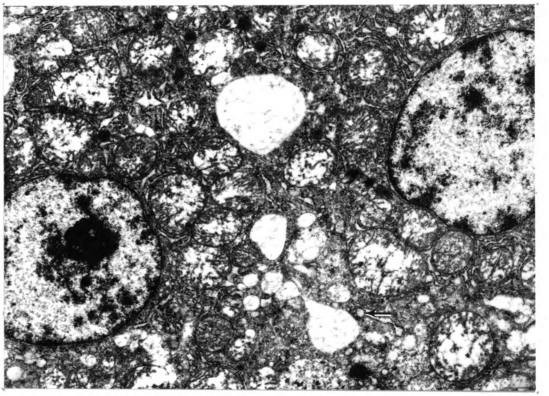

Figure 9. Hepatocytes of a cockerel fed a diet containing 10 ppm polybrominated biphenyls (PBB) for 28 days. Notice vacuolation, increase in smooth endoplasmic reticulum, swollen mitochondria and disruption of the mitochondrial cristae. Uranyl acetate and lead citrate staining; X11,600.

DISCUSSION AND CONCLUSIONS

Research concerning the individual congeners of the mixture of polybrominated biphenyls (PBB) is very important for a better understanding of the nature and mechanisms of the toxicity of PBB. If there is no single congener able to induce the same toxicity as the parent mixture, then further studies should be conducted regarding the synergistic or additive effects of a combination of more than one congener. At present, the structures of most of the major congeners are known. It is important to understand structure-function relationships of the congeners, since it is becoming apparent that the chemical structure has a profound influence on metabolism and biological and toxicopathological effects.

In this experiment cockerels were used to study the toxicopathological effects of 2 congeners in the mixture of PBB. The effects were compared with those caused by the parent mixture.

Organ Weights

Statistically, hepatic and bursal weights were significantly affected. Hepatic weights were increased in cockerels fed diets containing 62 ppm peak-4, 10 or 100 ppm PBB, and bursal weights were decreased in cockerels fed diets containing 10 or 100 ppm PBB. Increases in hepatic weights caused by PBB were reported in cattle (Moorhead et al., 1978), pigs (Ku et al., 1978), rodents (Sleight and Sanger, 1977; Akoso, 1977) and poultry (Ringer and Polin, 1977;

Kowaleski, 1976). It seemed that peak-4 contributed significantly to the increased hepatic weight induced by the mixture of PBB in the diets of the cockerels, but peak-6 did not. At dietary concentrations of 10 ppm, only the mixture of PBB increased hepatic weights, whereas peak-4 and peak-6 did not. Akoso et al. (1980) reported that rats fed diets containing 10 ppm peak-4 for 30 days had significantly increased hepatic weight to body weight ratios. However, rats fed peak-6 did not.

In the bursa of Fabricius, diets containing 62 ppm peak-4 or 4 ppm peak-6 failed to decrease the weights, whereas a diet containing 100 ppm PBB decreased bursal weights significantly. At 10 ppm in the diets, only the mixture of PBB decreased the bursal weights significantly. This concentration in the diet was less than that reported by Ringer (1978), who fed cockerels diets containing 50 ppm PBB for 60 days and decreased bursal weights significantly. Decrease of the bursal weights was likely related to the depletion of lymphoid cells. Since the bursa of Fabricius is the place where lymphoblasts are transformed into B lymphocytes, defects in B lymphocyte formation may cause suppression in humoral immunity.

Hepatic Residues

The higher the concentrations of PBB in the diets of the cockerels, the higher the residues found in the liver (Table 4). The liver of the cockerels fed a diet containing the mixture of PBB had approximately 8 times the concentrations in the diets after 28 days. The hepatic concentrations of peak-4 and peak-6 seemed to be slightly higher than with the mixture of PBB. Absence of or low absorption of PBB which have more than 6 bromine atoms and the metabolism of peak-1 and peak-3 may contribute to this phenomenon (Moore et al., 1978a). There were apparently no differences in amounts of peak-4 and peak-6 in the liver.

This could be explained by the molecular structure of the 2 congeners. Both congeners have 6 bromine atoms. As far as the positions of the bromine atoms on the biphenyl rings is concerned, neither peak-4 nor peak-6 has adjacent unsubstituted carbon atoms or unsubstituted para positions of the carbon atoms. According to Moore et al. (1978a), the above conditions are required for the congeners to be metabolized. Peak-4 and peak-6 are absorbed from the intestine, and apparently accumulate in the liver without being metabolized.

Light Microscopy and Ultrastructure

Hepatic changes such as vacuolation and swelling of the hepatocytes have been reported to occur in various species of animals given PBB. Cockerels fed a diet containing 100 ppm PBB for 28 days had moderate changes in the livers. Meanwhile, less severe lesions were observed in cockerels fed diets containing 10 ppm PBB, 62 ppm peak-4 or 10 ppm peak-6. There were no lesions found in the cockerels fed diets containing 0, 10 ppm peak-4 or 4 ppm peak-6. Hepatic lesions were slightly more severe in cockerels fed a diet containing 10 ppm PBB when compared to those given 10 ppm peak-6. Poland et al. (1979) suggested that the toxicity of these compounds is mediated through receptors in the target organs. They also proposed that there is a correlation between the toxicity of these compounds and the ability of these compounds to induce aryl hydrocarbon hydroxylase (AHH).

Vacuolation, swelling of the mitochondria, disruption of the mitochondrial cristae, and hyperplasia of the smooth endoplasmic reticulum were observed electron microscopically in the hepatocytes. Those changes have been reported by some investigators in other species. Changes such as depletion of glycogen, detachment of the ribosomes from rough endoplasmic reticulum, the appearance of myelin figures, and an

increase of lysosomes have been reported in rats (Sleight and Sanger, 1976; Mangkoewidjojo, 1979; Akoso, 1980) but were not observed in this experiment. Electron microscopic changes in the livers of cockerels fed diets containing 10, 100 ppm PBB, 62 ppm peak-4 or 10 ppm peak-6 were similar except for differences in severity. At 100 ppm PBB the hepatocytes had larger vacuoles and more swollen mitochondria.

In conclusion, neither 4 ppm peak-6 nor 62 ppm peak-4 induced the same toxicity as 100 ppm of the mixture of PBB. It seemed that diets containing 62 ppm peak-4 are more toxic than those containing 4 ppm peak-6. However, when dietary concentrations were 10 ppm, PBB appeared to be more toxic than peak-6 and peak-6 was more toxic than peak-4. There are apparently more than one congener involved in the toxicity of the mixture of PBB and synergistic or additive effects among two or more congeners may account for the toxicity. Other congeners such as 2,3',4,4',5-pentabromobiphenyl (peak-2) are known to be mixed-type inducers (Robertson et al., 1980) and their toxicity needs to be studied. Moreover, contaminants such as bromonaphthalenes, even though in minute concentrations, should also be considered to contribute to the toxicity of the mixture of PBB.

SUMMARY

This experiment was conducted to determine the toxicity of two congeners of a mixture of polybrominated biphenyls (PBB) and to compare their toxicity to that of the commercial mixture of PBB. The two congeners were 2,2',4,4',5,5'-hexabromobiphenyl (peak-4), comprising 60 to 70% of PBB, and 2,3',4,4',5,5'-hexabromobiphenyl (peak-6), comprising 3 to 4% of PBB. Various doses of peak-4, peak-6 or the mixture of PBB were added to the diets and fed to cockerels for 28 days.

Feed consumption and body weight were not affected in any of the groups. There were no apparent toxic effects in cockerels fed diets containing 0, 4 ppm peak-6 or 10 ppm peak-4. Increased hepatic weights were evident in cockerels fed diets containing 62 ppm peak-4, 10 or 100 ppm PBB. Hepatocytes of cockerels fed diets containing 10 ppm peak-6, 62 ppm peak-4 or 10 ppm PBB were slightly swollen and vacuolated. Similar but more severe changes were found in livers of cockerels fed a diet containing 100 ppm PBB. Ultrastructurally, vacuolation, hyperplasia of smooth endoplasmic reticulum, swollen mitochondria and disruption of mitochondrial cristae were observed in the hepatocytes. Decreased relative bursal weights were evident in cockerels fed diets containing 10 or 100 ppm PBB. There was marked depletion of lymphoid cells in the medulla of the bursa of Fabricius in cockerels fed a diet containing 100 ppm PBB. Similar but less severe lesions were observed in cockerels fed diets containing 62 ppm peak-4, 10 ppm peak-6 or 10 ppm PBB.

It is concluded that neither of the two congeners, when given in dosages relative to its concentration in the mixture, was able to induce the same toxicity as the parent mixture. When the concentrations of the chemicals were equal in the diets, the mixture of PBB was more toxic than peak-6 and peak-6 was more toxic than peak-4. The results suggest synergistic or additive effects between two or more congeners. Further studies concerning the congeners of the mixture, either individually or in combination, are needed.

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APPENDIX

Table A1. Feed consumption of cockerels fed diets containing 2,3',4,4',5,5'-hexabromobiphenyl (peak-6), 2,2',4,4',5,5'-hexabromobiphenyl (peak-4) or the commercial mixture of polybrominated biphenyls (PBB) for 28 days (g/bird/day)

Week	Control	4 ppm peak-6	10 ppm peak-6	10 ppm peak-4	62 ppm peak-4	10 ppm PBB	100 ppm PBB
1	6.89	6.06	7.59	7.37	6.67	6.03	7.06
2	21.36	20.53	22.41	20.66	22.40	21.14	23.00
3	27.36	28.67	30.39	24.26	28.11	28.68	25.26
4	34.88	33.70	33.83	30.99	32.60	33.82	30.29

Feed consumption was apparently not affected in any of the groups of cockerels.

Table A2. Weights of cockerels fed diets containing 2,3',4,4',5,5'-hexabromobiphenyl (peak-6), 2,2',4,4',5,5'-hexabromobiphenyl (peak-4) or the commercial mixture of polybrominated biphenyls (PBB) for 28 days (g)

Week	Control	4 ppm peak-6	10 ppm peak-6	10 ppm peak-4	62 ppm peak-4	10 ppm PBB	100 ppm PBB
0	40.65	40.50	40.30	40.80	40.90	40.50	40.50
	±2.39	±2.07	±2.71	±2.30	±2.38	±2.64	±2.64
1	78.05	76.70	79.80	80.50	75.50	76.50	75.10
	±6.78	±7.72	±4.83	±5.91	±7.41	±5.60	±5.34
2	140.50	136.10	144.40	146.00	134.10	140.30	132.20
	±12.35	±13.86	±9.09	±10.90	±12.61	±10.17	±11.98
3	229.65	224.20	242.00	240.50	221.70	191.10	212.50
	±20.30	±19.11	±14.40	±17.18	±18.55	±17.15	±17.28
4	324.91	320.00	338.15	328.99	329.03	327.16	299.32
	±28.55	±28.70	±32.24	±30.98	±31.57	±25.47	±25.07

Data are expressed as mean ± SD.

There were no significant differences in any of the groups of cockerels.

Table A3. Relative organ weights of cockerels fed diets containing 2,3',4,4',5,5'-hexabromobiphenyl (peak-6), 2,2',4,4',5,5'-hexabromobiphenyl (peak-4) or the mixture of polybrominated biphenyls (PBB) for 28 days

	Control	4 ppm peak-6	10 ppm peak-6	10 ppm peak-4	62 ppm peak-4	10 ppm PBB	100 ppm PBB
Spleen weights g/kg body weight	2.02	1.60	1.98	1.57	1.42	1.46	1.10
	±1.51	±0.48	±0.62	±0.34	±0.19	±0.37	±0.26
Thyroid weights mg/100 g body weight	6.12	5.88	4.52	6.37	6.43	5.57	6.55
	±1.88	±2.27	±1.44	±4.06	±2.51	±2.43	±1.88
Comb weights g/kg body weight	3.96	4.50	3.39	4.30	4.39	4.17	2.45
	±2.09	±1.82	±1.54	±4.01	±1.75	±2.11	±1.41
Testicular weights mg/100 g body weight	30.64	31.87	24.32	30.87	29.92	34.45	30.68
	±8.25	±5.98	±7.88	±16.94	±5.80	±13.13	±6.83

Data are expressed as mean ± SD.

There were no significant differences in any of the groups of cockerels.

Table A4. Stimulation of lymphocyte blastogenesis using Concanavalin A in cockerels fed diets containing 2,3',4,4',5,5'-hexabromobiphenyl (peak-6), 2,2',4,4',5,5'-hexabromobiphenyl (peak-4) or the mixture of polybrominated biphenyls (PBB) for 28 days

Treatment	Concanavalin A Level (µg/culture well)				
	10.0	5.0	2.5	0.5	0.0
Control	2242	24624	14952	361	211
	2005	10235	8071	825	1045
	6929	13811	44224	601	405
	17717	82480	50216	5934	473
	1986	5652	15799	532	629
	853	4691	857	342	305
4 ppm	20171	98969	62477	332	344
peak-6	302	727	1306	207	192
	15236	31012	44815	7124	647
10 ppm	588	2812	108	118	131
peak-6	2598	11999	22547	342	260
	276	648	737	268	571
10 ppm	12999	62018	38806	501	567
peak-4	2293	20576	25905	220	217
	251	515	306	400	625
62 ppm	4927	8031	18341	943	1176
peak-4	1434	2966	2037	283	304
	292	916	886	800	843
10 ppm PBB	310	313	209	133	349
	346	2323	742	196	219
	301	398	348	265	278
100 ppm PBB	3868	28714	13776	1126	817
	3569	28762	3271	1034	397
	12630	52404	43863	2697	1189

Data are presented as disintegrations per minute for each specimen. Each number represents the average of 2 counts.

The inconsistency of the data might be due to the age factor. Lymphocytes of cockerels 4 weeks old or less do not appear to respond well to blastogenic stimulation (Ringer, personal communication).

Table A5. T_3 levels in blood plasma of cockerels fed diets containing 2,3',4,4',5,5'-hexabromobiphenyl (peak-6), 2,2',4,4',5,5'-hexabromobiphenyl (peak-4) or the mixture of polybrominated biphenyls (PBB) for 28 days (ng/100 ml)

Control	4 ppm peak-6	10 ppm peak-6	10 ppm peak-4	62 ppm peak-4	10 ppm PBB	100 ppm PBB
2.82	3.70	10.47	2.74	2.84	2.30	1.97
2.02	2.80	3.02	3.77	3.33	2.70	2.41
2.57	1.78	1.66	1.91	2.50	1.83	0.88
3.99	1.66	1.79	1.77	2.44	3.41	1.48
4.62	1.17	2.88	1.80	1.97		3.00
2.21	2.43		3.34	2.82		
1.75						
1.82						
1.73						
3.24						
2.67[a]	2.26	3.96	2.56	2.65	2.56	1.95
±1.00[b]	±0.91	±3.69	±0.86	±0.46	±0.67	±0.82

Bonferroni t-test revealed no significant differences between any of the groups.

[a] Mean

[b] SD

Table A6. T_4 levels in blood plasma of cockerels fed diets containing 2,3',4,4',5,5'-hexabromobiphenyl (peak-6), 2,2',4,4',5,5'-hexabromobiphenyl (peak-4) or the mixture of polybrominated biphenyls (PBB) for 28 days (ng/100 ml)

Control	4 ppm peak-6	10 ppm peak-6	10 ppm peak-4	62 ppm peak-4	10 ppm PBB	100 ppm PBB
1.15	3.00	0.72	8.79	2.51	5.78	13.90
14.54	6.10	0.74	4.62	11.92	3.36	16.13
5.05	11.24	7.44	11.61	7.03	14.77	16.54
9.63	8.25	8.24	8.53	6.24	7.89	13.80
2.19	11.35	0.74	7.00	8.87		16.49
4.44	7.84		13.71	8.66		
12.10						
8.12						
14.07						
8.06[a]	7.96	3.58	9.04	7.54	7.95	15.37
±4.75[b]	±3.18	±3.90	±3.24	±3.15	±4.91	±1.40

[a] Mean

[b] SD

VITA

VITA

The author was born in Gianyar, Bali, Indonesia, on June 22, 1951. He graduated from the Faculty of Veterinary Medicine, Gadjah Mada University, in August 1976. Following graduation he was employed by the Directorate of Animal Health, Agricultural Department of Republic of Indonesia. He was appointed to the Laboratory of Veterinary Disease Investigation Center in Denpasar, Bali.

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