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Polybrominated Biphenyls (PBB) Toxicosis In Sows And Piglets Caused By Feeding Diets Containing PBB To Sows During Pregnancy And Lactation

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POLYBROMINATED BIPHENYLS (PBB) TOXICOSIS IN SOWS AND PIGLETS CAUSED BY FEEDING DIETS CONTAINING PBB TO SOWS DURING PREGNANCY AND LACTATION

By

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ABSTRACT

POLYBROMINATED BIPHENYLS (PBB) TOXICOSIS IN SOWS AND PIGLETS CAUSED BY FEEDING DIETS CONTAINING PBB TO SOWS DURING PREGNANCY AND LACTATION

By

Pedro Ribas Werner

Twelve pregnant sows and their offspring were used to investigate the toxicity and the distribution of polybrominated biphenyls (PBB). The sows were fed diets containing 0, 10, 100 or 200 ppm of PBB during the last half of gestation and during lactation. Approximately 1/3 of each litter was killed and necropsied immediately after birth. The remainder of the litters and the sows were killed and necropsied 4 weeks later.

Transplacental passage of PBB to the fetuses occurred, but far more PBB were transferred to the piglets through the milk. Consequently, on a body weight basis, the piglets consumed PBB in concentrations similar to the concentrations given to the sows. On a fat basis, highest concentrations of PBB were in the liver of sows and piglets, followed by the adipose tissue, kidney, and brain, in decreasing order. Chromatographic analysis indicated that piglets consumed a somewhat different PBB mixture than the PBB mixture given to the sows. The PBB present in the

milk apparently came directly from the sows' adipose tissue without being metabolized in the liver, since the proportions of the PBB isomers were nearly identical in the sows' adipose tissue and in the milk.

Newborn piglets were clinically unaffected by PBB. There was a higher mortality among piglets nursing sows fed diets containing PBB, but a cause-effect relationship could not be established. The weight gain of surviving piglets was not affected. Hematologic values for sows, newborn, and 4-week-old piglets were not affected by PBB.

Concentrations of 10 ppm of PBB induced an increase in the concentration of triiodothyronine and thyroxine, and in serum alkaline phosphatase (SAP) and serum glutamic pyruvic transaminase (SGPT) of piglets, whereas concentrations of 100 or 200 ppm of PBB induced a decrease in those values.

Thyroid weight to body weight ratios were increased in newborn piglets from sows fed diets containing 100 or 200 ppm of PBB. The thyroid gland of those piglets was slightly hyperplastic and the colloid was scant and vacuolated.

Concentrations of blood urea nitrogen were increased in the serum of newborn piglets from sows fed diets containing 200 ppm of PBB. However, there were no histopathologic changes in the kidney of those piglets or in the kidney of sows and 4-week-old piglets.

Measuring the serum concentrations of ornithine carbamyl transferase was the most effective clinical test

in assessing the severity of PBB-induced liver damage. Hepatic damage was not detected by analysis of serum cholesterol, SAP and SGPT, and serum electrophoresis of proteins, lipoproteins, and lactic dehydrogenase isoenzymes.

The PBB caused a dose-related increase in the liver weight to body weight ratios of 4-week-old piglets only. Microscopically, the liver changes were more severe in the liver of sows than in 4-week-old piglets. Swelling of hepatocytes and centrolobular necrosis were the most prominent lesions observed. There were no changes in the liver of newborn piglets. One sow fed a diet containing 100 ppm of PBB had several hyperplastic nodules in the liver. Hepatic concentrations of vitamin A were not affected in the piglets.

There was an increase in microsomal protein, cytochrome P_{450}, and in the activity of hexobarbital hydroxylase, ethylmorphine demethylase, and ethoxycoumarin deethylase in the liver of sows fed diets containing PBB and in the liver of piglets nursing those sows. The activity of arylhydrocarbon hydroxylase was measured only in the kidney of 4-week-old piglets and the activity was increased by PBB. There was no induction of drug-metabolizing enzymes in newborn piglets.

DEDICATION

to the memory of my father

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INTRODUCTION

Polybrominated biphenyls (PBB) are stable, relatively inert chemicals employed in the manufacture of certain hard, heat resistant plastics. The PBB belong to the same class of compounds as the polychlorinated biphenyls (PCB), responsible for the "Yusho" incident in Japan, when hundreds of people consumed rice oil contaminated with PCB (Takagi et al., 1976).

Polybrominated biphenyls would not have been of much concern had not Michigan livestock become contaminated accidentally. In 1973 PBB were used mistakenly in place of magnesium oxide in the preparation of dairy feed. As a consequence, thousands of farm animals and animal products had to be destroyed (Carter, 1976).

The PBB are hepatotoxic. Studies have indicated that PBB are potent inducers of hepatic drug-metabolizing enzymes and may alter the biological response to other xenobiotics (Getty et al., 1977). Some authors claim that PBB are teratogenic to rodents when given in high doses (Corbett et al., 1978). Others found neoplastic nodules in the liver of rats given one oral dose of 1 g of PBB (Kimbrough et al., 1977).

Since PBB can cross the placenta and are eliminated through the milk, concern was expressed about the possible

harmful effects of PBB on the developing fetus and on the nursing infant. It is known that for PCB, young animals are more sensitive than adults, and females more sensitive than males (Kimbrough et al., 1978a). This is relevant when one considers the similarities between PCB and PBB.

There was a need for more research on the transplacental and lactational effects of PBB. Most of the work done in these areas had involved the use of small laboratory animals and little work had been done on farm animals. Although the cow may be the animal of choice to study excretion through the milk, the sow offers many advantages. Sows are less costly than cows, have a shorter gestation period, and have multiple offspring.

The objectives of the present research were:

1. To determine the pathologic effects of PBB in the sow and in the fetus.

2. To determine the pathologic effects on the nursing piglets of PBB eliminated through the milk.

3. To correlate the concentrations of PBB in the sow's diet with the concentrations of PBB in the milk and with the concentrations of PBB in the tissues of the sow, fetus and nursing piglet.

4. To determine any change in the proportions and concentrations of the different components of PBB, as they were metabolized by the sow, excreted through the milk, and metabolized by the piglet.

LITERATURE REVIEW

Introduction

Polybrominated biphenyls are stable, relatively inert chemicals that were extensively used to increase fire retardance in industrial and consumer products (MSU Experiment Station, 1976). The PBB were physically blended into products in concentrations up to 15% by weight (Norris et al., 1974) to attain flame retardant properties.

The PBB and PCB are polyhalogenated aromatic compounds that share similar physical and chemical properties (Pomerantz et al., 1978). The PBB were introduced into industrial use because they are more stable and were considered to be environmentally safer than PCB (Norris et al., 1974).

The PBB were manufactured and sold by the Michigan Chemical Company, St. Louis, MI, under the trade name of FireMaster BP-6.[a] Their use was restricted to those applications where the end-product was not exposed to animal feeds. They were not used as flame retardants in

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[a]Unless otherwise specified, PBB as used in this dissertation is FireMaster BP-6.

fabrics where human exposure would occur (Cordle et al., 1978). The production of PBB was stopped in 1974, after the 1973 PBB contamination in Michigan (Di Carlo et al., 1978).

Physical and Chemical Properties

The PBB may contain as many as 30 brominated biphenyl isomers and other contaminants (Moore et al., 1978). The gas chromatographic profile of PBB shows at least 12 different components (peaks). The peaks are numbered in order of appearance during gas chromatographic analysis. The chemical structure of 8 of the 12 major components of PBB has been determined (Moore and Aust, 1978). According to Dannan and co-workers (1978a), the two major components of PBB (peaks 4 and 8 in the gas chromatogram) are 2,4,5,2',4',5'-hexa- and 2,3,4,5,2',4',5'-heptabromobiphenyl. Together they comprise 83% of the total mixture. Further chromatographic analysis of partially purified fractions of PBB would reveal many more components (Moore et al., 1978). Among the probable contaminants in PBB, the brominated dibenzofurans and brominated naphthalenes are most important, according to the DHEW Subcommittee on Health Effects of PCBs and PBBs (1978). Brominated naphthalenes have been found at concentrations of 220 ppm in PBB (Haas et al., 1978). There has been an unconfirmed report of a methyl brominated furan in a fraction of PBB (Kay, 1976).

Kinetics and Metabolism of Polybrominated Biphenyls

The PBB are poorly absorbed from the intestine of ruminants and 50% of the ingested dose is eliminated intact in the feces (Willett and Irving, 1976; Willett and Durst, 1978). The available data imply that PBB containing 6 or less bromine atoms per molecule are readily absorbed from the gastrointestinal tract of higher animals (DHEW, 1978). Rats absorbed 90% of the oral dose of 2,4,5,2',4',5'-hexabromobiphenyl, whereas 65% of the oral dose of octabromobiphenyl was present in the feces during the first day after dosing (DiCarlo et al., 1978).

The PBB are lipophilic compounds. Once absorbed they accumulate preferentially in adipose tissue and in fat-containing tissues. Relatively little PBB accumulate in the brain, either because of failure of PBB to cross the blood-brain barrier or because of an inability of PBB to accumulate in the phospho-, glyco-, and sulfolipids of nervous tissues (Willett and Durst, 1978). Studies have shown that PBB accumulate in the liver in concentrations higher than what would be expected (Willett and Durst, 1978). They also accumulate to a higher extent in mammary tissue of non-lactating pregnant rats (Rickert et al., 1978).

Matthews and co-workers (1977) studied the kinetics of 2,4,5,2',4',5'-hexabromobiphenyl in rats after oral or intravenous administration. They found that initially, muscle retained about 40% of the compound, the liver retained about 10%, and the adipose tissue retained about

25%. After 7 days, the percentages of retention were 7%, 2%, and 60%, respectively. The concentrations of PBB in the adipose tissue increased even more later in the experiment.

Polybrominated biphenyls can cross the placental barrier and are absorbed by the developing fetus of rats and cows (Aftosmis et al., 1972a; Detering et al., 1975; Fries et al., 1978; Harris et al., 1978; Rickert et al., 1978). The concentration of PBB in the tissues of the fetus was about 1/3 of that in the dam (Fries et al., 1978; Rickert et al., 1978). There is a lack of information on changes in the proportions of the different isomers of PBB as they cross the placenta. According to Fries and Marrow (1975), brominated biphenyls containing 7 or more atoms of bromine per molecule have a greater difficulty moving across biological membranes than less brominated biphenyls.

It was assumed that hexa- and the more highly brominated biphenyls would be absorbed poorly and would not be metabolized (Willett and Durst, 1978; Fries, 1978). For PCB it is generally agreed that metabolism and thus excretion are inversely proportional to the degree of chlorination so long as there are 2 adjacent nonchlorinated carbon atoms in the biphenyl molecule (DHEW, 1978). There is evidence that these assumptions may not all be true. The more highly brominated PBB are indeed metabolized, only at a slower rate than the more metabolically active, less brominated biphenyl isomers (Safe et al., 1978). Moreover,

it appears that the distribution of the halogen atoms in the biphenyl ring, rather than the degree of halogenation, is the determining factor in the metabolism of halogenated biphenyls. The presence of one nonhalogenated carbon at the *para* position renders the halogenated biphenyl susceptible to metabolism (Dannan et al., 1978). From the 12 major isomers present in PBB, only peak 1 (2,4,5,2',5'-pentabromobiphenyl) and peak 3 (a hexabromobiphenyl of unknown structure) were metabolized *in vitro* by isolated rat liver microsomes (Dannan et al., 1978). The gas chromatograms of tissues from Michigan farmers (Wolff and Aubrey, 1978) mimic the gas chromatograms of tissues from rats injected intraperitoneally with PBB (Dannan et al., 1978). Dannan and co-workers hypothesized that the less highly brominated biphenyls could yield metabolites with carcinogenic activity.

The half-life of PBB in the body of lactating cows averaged 60 days (Fries et al., 1978). Three factors affect the half-life of PBB: the total amount of fat in the body, changes in the amount of fat, and the amount of milk production (Fries, 1978). In the nonlactating animal, PBB remains in the body for a long period. For rats, it was stated that less than 10% of the total absorbed dose of PBB would ever be excreted (Matthews et al., 1977).

The PBB are eliminated through fat-containing products, i.e., milk and eggs (Fries, 1978). During continuous feeding of a diet containing 10 ppm of PBB to lactating cows, the concentration of PBB in the milk reached a stable

concentration after 20 days. At that point, the proportion of PBB excreted daily in the milk was approximately 18% of the amount ingested daily (Fries and Marrow, 1975). The concentration of PBB in the milk fat was closely related to the concentration of PBB in the major body fat stores when cows were no longer consuming PBB (Fries, 1978). For hens, the concentration of PBB in the eggs was approximately the same as the concentration of PBB in the diet (Fries et al., 1976). Excretion in eggs accounted for about 50% of the daily intake (Fries, 1978).

Feces are an important route of excretion of PBB only while the contaminated feed is being cleared from the digestive tract (Willett and Irving, 1976). Apparently, fecal excretion during exposure reflects mostly nonabsorbed PBB (Getty et al., 1977). Urine was considered a minor route of excretion, since free PBB were either not detectable or in a concentration too low to quantitate in the urine of cows given PBB (Willett and Durst, 1978). A pig excreted only 1% of a single intraperitoneal dose of PBB in urine and feces in 7 days (Kohli and Safe, 1976).

Polybrominated Biphenyl Toxicosis

History

In July 1973, in Michigan, an estimated 250 to 500 kg of PBB were accidentally substituted for magnesium oxide in the preparation of a feed supplement for lactating cows (Getty et al., 1977). In some instances, the contaminated feed contained 4,000 to 13,000 ppm of PBB (Kay, 1977).

Later, it was discovered that other feeds manufactured in the same feed mills also became contaminated. As a result, PBB-contaminated feed was fed to thousands of cattle, other livestock, and poultry (Dunkel, 1975).

The PBB were identified in the contaminated feed nearly a year after the first contamination took place. In the meantime, several dairy herds in Michigan began to have excessive health problems, decreased milk production, and loss of weight (Getty et al., 1977). Samples of the suspected feed were sent to a USDA laboratory, where the contaminant was identified as PBB (Jackson and Halbert, 1974).

Soon after the identification of PBB in animal feed, the FDA established tolerance levels for PBB contamination at 0.3 ppm in the fat of milk, meat and poultry. The Michigan Department of Agriculture quarantined 500 farms and killed thousands of cattle, swine, and sheep and about 1.5 million chickens. Hundreds of tons of feed and animal products were destroyed because they exceeded the FDA tolerance levels (Carter, 1976). In July 1977, the Michigan Legislature voted to lower the Michigan tolerance guidelines for PBB in cattle to 0.02 ppm in the fat of meat, and to 0.005 for milk.

Approximately 10,000 Michigan residents, principally farm families and their neighbors, were exposed to relatively high amounts of PBB in 1973 and 1974 (Cordle et al., 1978). In October 1976, 96% of nursing mothers from Michigan's lower peninsula had at least trace amounts of

PBB in their milk (Michigan Department of Public Health, 1978). Unverified reports of health problems began to appear in newspapers, and several studies were undertaken to assess the health effects of PBB exposure in people (Cordle et al., 1978). Many studies are still being conducted (DHEW, 1978). In October 1977, the Michigan State Agriculture Experiment Station sponsored a workshop on PBB, and results of the most recent research on PBB were presented. The proceedings were published in an issue of Environmental Health Perspectives (1978).

Clinical Signs

Jackson and Halbert (1974), a practicing veterinarian and a farm owner, respectively, described the clinical signs and lesions of cattle accidentally exposed to PBB. The affected cows had anorexia, decreased milk production, frequent micturition, and excessive lacrimation. Some cows were lame, and abnormal growth of hooves was observed. There were areas of matting of the hair, areas of alopecia, and the skin was thickened. There was increased calf mortality and many calves were born dead. Hydrops amnii developed in 4 cows.

Some of the aforementioned signs were reproduced by Moorhead and co-workers (1977) by giving PBB orally to pregnant heifers at the rate of 25 g/day. The treated heifers had anorexia, excessive lacrimation and salivation, and diarrhea. They became emaciated and some aborted. These authors suggested that many of the signs described

by Jackson and Halbert and observed by other Michigan farmers resulted from mismanagement, nutritional deficiencies, or indigenous microbial and parasitic infections.

Decreased feed intake has been reported as a consequence of PBB toxicosis in rats (Sleight and Sanger, 1976). Growing pigs had decreased weight gains as a consequence of reduced feed intake when fed diets containing 20 to 200 ppm of PBB. However, the feed conversion to weight gain was better for pigs fed the diets containing PBB (Ku et al., 1978).

Pathologic Changes

The liver appears to be an important target for PBB. Pathologic changes in the liver have been consistently observed. The changes appear to be dose-related and include an increase in liver weight to body weight ratio (Sleight and Sanger, 1976; Sleight et al., 1978; Ku et al., 1978). Increase in liver weight was also observed in rats inhaling fumes of octabromobiphenyl heated to 290 C (Aftosmis et al., 1972b) and in rats fed diets containing octabromobiphenyl (Norris et al., 1974). Cows that died from accidental exposure to PBB apparently had enlarged livers (Jackson and Halbert, 1974), but data were not provided on liver weight for cows. Microscopically, fatty change, swelling and vacuolation of hepatocytes, and necrosis were among the lesions observed in livers of animals exposed to PBB (Jackson and Halbert, 1974; Sleight and Sanger, 1976; Sleight et al., 1978). Hyperplasia of

hepatocytes has been described. Kimbrough and co-workers (1977) described hyperplasia of hepatocytes and neoplastic nodules in the liver of female rats 10 months after a single oral dose of 1 g of PBB/kg of body weight.

Some authors described renal lesions in rats fed diets containing 0.1% and 1% of octabromobiphenyl (Aftosmis et al., 1972a; Norris et al., 1974). The lesions consisted of enlargement, petechial hemorrhages, and hyaline degeneration. These lesions were not seen when rats were fed diets containing up to 500 ppm of PBB (Sleight and Sanger, 1976). McCormack and co-workers (1978), studying the effects of PBB on kidney function in rats, concluded that PBB are not potent nephrotoxic agents.

Thyroid hyperplasia was observed in rats that had received 0.1 and 1% of octabromobiphenyl in their diets (Norris et al., 1974). Similar effects were observed in rats fed diets containing 10 and 100 ppm of PBB (Sleight et al., 1978) and in chicks fed diets containing 200 ppm of PBB (Ringer and Polin, 1977).

Skin changes (hyperkeratosis) were observed in cows fed diets contaminated with PBB (Jackson and Halbert, 1974; Moorhead et al., 1977). Chloracne, an eruptive skin lesion resembling adolescent acne, is a typical finding in PCB toxicosis (Kuratsune et al., 1972). Norris and co-workers (1974) tested octabromobiphenyl for chloracne activity and observed only slight erythematous and edematous changes in the ear of rabbits where the compound

was applied locally for 24 hours. Rhesus monkeys given PBB had pathological changes in the skin, but typical lesions of chloracne were not observed (Lambrecht et al., 1978; Allen et al., 1978).

The PBB are teratogenic only when administered in extremely high doses to pregnant animals. Aftosmis and co-workers (1972) reported the occurrence of gastroschisis and anasarca in fetuses when pregnant rats were fed diets containing 0.1 and 1% of octabromobiphenyl. The PBB were weakly teratogenic to mice, inducing exencephaly, cleft palate, and hydronephrosis in fetuses of dams fed diets containing 1,000 ppm of PBB (Corbett et al., 1978). Harris and co-workers (1978) observed no effects on embryonic development when pregnant rats were force-fed 10 mg of PBB in oil from day 7 through day 15 of pregnancy.

Species Differences

Certain animal species are more sensitive to PBB toxicosis than others. This is particularly evident in mink (Aulerich and Ringer, 1979). Reproduction and kit survival were reduced drastically when PBB were added to the diets in concentrations as low as 1 ppm. Ninety percent of the adult mink died when fed diets containing 6.25 ppm of PBB. The LD_{50} for mink was calculated to be 112 mg/kg. Guinea pigs are also sensitive to PBB toxicosis. Deaths, apparently from feed refusal, occurred in 4 of 6 guinea pigs on diets containing 100 ppm of PBB, and in all 6 fed diets containing 500 ppm of PBB. In the

same experiment, rats were fed diets containing 100 ppm of PBB and did not have any signs or clinicopathologic evidence of PBB toxicosis (Sleight and Sanger, 1976). Chikens also are more sensitive than rats to PBB toxicosis (Ringer and Polin, 1977).

Biochemical Pharmacology

Several investigators have reported that PBB, like PCB, are potent inducers of hepatic drug metabolizing enzymes, also called mixed-function oxidases (MFO). The PBB induced MFO in the liver of rats (Moore et al., 1976; Sleight and Sanger, 1976; Dent et al., 1977), Japanese quail (Cecil et al., 1975; Babish et al., 1975), dogs (Farber et al., 1976), and mammary gland and kidneys of rats (Dent et al., 1977). Moore and co-workers (1976) demonstrated induction of MFO in the liver of rats nursing dams fed diets containing up to 10 ppm of PBB. These authors observed that the pups appeared to be more sensitive to the effects of PBB than their mothers. However, the authors did not report the concentrations of PBB in the dams' milk. An important function of MFO is the metabolism of drugs and other xenobiotics. The increased MFO activity increases the total metabolic capacity of the liver (Dent et al., 1976a).

The PBB increase the microsomal content of cytochrome P450 and P448 and induce the activity of MFO. Because the induction of MFO by PBB is similar to the induction caused by phenobarbital (PB) or by 3-methylcholanthrene

(3-MC), the PBB are considered a mixed-type inducer (Dent et al., 1976b). Since the product that contaminated livestock feed is a mixture of polybrominated biphenyls, it may be possible that one or several of the congeners in the mixture are responsible for the PB-like action and others for the 3-MC-like action (Dent et al., 1976b).

Induction of hepatic microsomal enzymes may affect the toxicity of other agents (Dannan et al., 1978b). Depending on the nature of these agents, previous exposure to PBB may increase, decrease, or leave unchanged the biological response (Getty et al., 1977). Additionally, PBB affect sex hormones in cockerels, estrogens in hens, the estrogen-progesterone balance in cows, and the catabolism of thyroxine (DiCarlo et al., 1978). The PBB may affect the metabolism of vitamin A (Sleight et al., 1978).

Toxicity of Chlorinated Dibenzofurans and Chlorinated Naphthalenes

It is known that most of the commercial mixtures of PCB contain trace amounts of chlorinated dibenzofurans (DHEW, 1978). These compounds, and the chlorinated dioxins, are highly potent inducers of hepatic MFO enzymes (Dent et al., 1976), being about 170 times more potent than PCB (DHEW, 1978). A single dose of 1 μg of tri- or tetrachlorodibenzofuran given orally to rabbits caused severe and often fatal liver necrosis. Applications of the same compound to the ear of rabbits caused severe hyperkeratosis at the application site. The single oral LD_{50} for

tetra-chlorodibenzofuran for guinea pigs is between 5 and 10 µg/kg (Kimbrough et al., 1978a).

By analogy, polybrominated dibenzofurans are possible contaminants of the PBB mixture. Although there have been no reports so far of the findings of such compounds in commercial mixtures of PBB (Pomerantz et al., 1978), they are formed in minute amounts (<1 ppm) after pyrolysis of PBB at 380 to 400 C for 20 minutes (O'Keefe, 1978). When daily doses of 4 µg of 2,3,7,8-tetrabromodibenzofuran were applied to the ear of rabbits for 5 days, in a total dose of 20 µg/rabbit, the rabbit developed hyperkeratosis at the treated site and hepatic necrosis (Kimbrough et al., 1978b).

Chlorinated naphthalene poisoning in cattle is known as bovine hyperkeratosis or X-disease (Smith et al., 1972). The most striking manifestation of the disease is a generalized hyperkeratosis of the skin and epithelial metaplasia. There is evidence of impaired metabolism of vitamin A, although experimental deficiency of vitamin A does not reproduce all the lesions of chlorinated naphthalene poisoning. Cattle are apparently more sensitive to chlorinated naphthalene poisoning than other species.

The commercial mixture of PBB contains approximately 220 ppm of brominated naphthalenes, but at these concentrations they apparently are not toxic and are not potent inducers of MFO enzymes (Dannan et al., 1978a).

MATERIALS AND METHODS

Experimental Design

Twelve pregnant sows weighing approximately 200 kg each from the Michigan State University swine herd were used and were fed a standard ration for pregnant and lactating sows.[a] The experimental design is shown in Table 1.

Table 1. The experimental design, number of piglets, average litter size and number of piglets necropsied

Concentration of PBB in the diet (ppm)	Number of sows	Number of piglets	Average litter size	Number of piglets necropsied	
				at birth	at 4 weeks
0	4	45	11 ± 2	15	24
10	2	22	11 ± 4	7	12
100	4	47	12 ± 3	14	19
200	2	21	11 ± 4	7	8
Totals	12	135	11 ± 3	37	63

[a]Michigan State University Swine Farm.

During the last half of gestation and during lactation, for a total of 12 weeks, a commercial mixture of PBB[b] was added to the diet to attain a concentration of 0, 10, 100 or 200 ppm of PBB. The sows were fed 2.5 kg of the ration/sow/day. The sows were given 0, 25, 250 or 500 mg of PBB/sow/day, the equivalent of approximately 0, 0.125, 1.25 or 2.50 mg of PBB/kg body weight, respectively. The same type and amount of ration were supplied during the whole experiment. Water was supplied *ad libitum* in steel troughs. The piglets' diet consisted of sow's milk, and they did not have access to the sow's feed.

Feces and wet and dirty bedding were removed daily and incinerated. Special precautions were employed to prevent cross contamination of sows.

One week before parturition the sows were placed in steel farrowing crates. Each farrowing was closely monitored, and help was provided when necessary. Immediately after birth the piglets were weighed and ear-notched for identification. The piglets were weighed weekly thereafter. Approximately 1/3 of each litter, randomly selected, was killed and necropsied immediately after birth. Any piglet born dead or which died during the experiment was necropsied. Four weeks after farrowing, the sows and remaining piglets were killed and necropsied.

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[b]FireMaster BP-6, Michigan Chemical Co., St. Louis, MI.

Parameters Evaluated

The following parameters were evaluated during the experiment:

1. Clinical signs
2. Weight gain of piglets
3. Gross and microscopic lesions
4. Absolute and relative weights of liver, thymus, thyroid gland, adrenal glands, and brain
5. Hematology
   - leukocyte and erythrocyte morphology
   - total and differential leukocyte counts
   - erythrocyte counts
   - packed cell volume
   - hemoglobin concentration
6. Clinical chemistry
   - serum cholesterol
   - blood urea nitrogen (BUN)
   - serum alkaline phosphatase (SAP)
   - serum glutamic pyruvic transaminase (SGPT)
   - serum ornithine carbamyl transferase (OCT)
7. Electrophoresis
   - serum protein
   - serum LDH isoenzymes
   - serum lipoproteins
8. Polybrominated biphenyl analysis of milk and of liver, kidney, brain, and adipose tissue of sows and piglets
9. Induction of hepatic and renal microsomal enzymes

10. Vitamin A analysis in the liver of piglets
11. Thyroid hormone analysis of the serum of sows and piglets.

Collection of Samples

Blood Samples

Blood from the sows was collected from the marginal ear vein. Blood from the piglets was collected from either the right brachiocephalic vein or the cranial vena cava through a 21-gauge needle.

Blood samples for hematologic examination were collected into tubes containing ethylenediamine tetraacetic acid. Blood collected without anticoagulant was allowed to clot, and the serum was separated and used for clinical chemistry. A portion of the serum was frozen at -24 C and later used for OCT and thyroid hormone analysis.

Milk Samples

Colostrum was collected during parturition, and milk samples were collected weekly thereafter. Ejection of the milk was induced by injecting 2.0 USP units of oxytocin[C] intravenously. Milk was collected into 20 ml test tubes and frozen at -24 C until PBB analysis.

Necropsy

The piglets were killed by electrocution. The sows were immobilized with succinylcholine and then electrocuted.

[C]Oxytocin, D-M Pharmaceutical Inc., Rockville, MD.

After the body weight was recorded, the liver, thymus, thyroid gland, spleen, and brain were removed and each was weighed on a top-loading balance.[d]

Samples of thyroid gland, thymus, trachea, lungs, heart, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, liver, gallbladder, pancreas, spleen, kidney, urinary bladder, adrenal gland, mediastinal and medial iliac lymph nodes, cerebrum, cerebellum, brain stem, medulla oblongata, pituitary gland, bone and bone marrow, skeletal muscle, eye, eyelid, and skin were fixed in 10% neutral buffered formalin for histologic examination.

Samples of liver, fat, kidney, and brain were wrapped in aluminum foil and frozen at -24 C for PBB analysis.

Carcasses and the remainder of organs and tissues were incinerated.

Examination of Samples

Hematology

Blood smears were stained with Wright's stain in an automatic slide stainer.[e] The morphology of erythrocytes and leukocytes was evaluated, and differential leukocyte counts were made. Packed cell volume was determined using microhematocrit techniques. Hemoglobin concentration was

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

[e]Hema-Tek slide stainer, Ames Co., Elkhart, IN.

determined by using a cyanmethemoglobin standard and a spectrophotometer.[f]

Clinical Chemistry

Serum concentrations of BUN, GPT, AP, and LDH were measured using commercial reagents[g] and an automatic analyzer.[h] Serum concentrations of cholesterol were measured by using cholesterol reagents and an automatic analyzer.[i] Serum concentrations of OCT were measured by using commercial reagents[j] and a spectrophotometer.[f]

The values were expressed in mg/dl for BUN and cholesterol; International Units/liter (IU/l) for SGPT, SAP and LDH; in Sigma Units/ml for OCT; and in g/dl for serum protein.

Serum Electrophoresis

For serum protein determination, serum was applied to cellulose acetate plates[k] and the serum proteins were

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[f]Perkin Elmer Coleman 44, Coleman Instrument Division, Oak Brook, IL.

[g]Spin Chem reagents, Smith Kline Instruments, Inc., Sunnyvale, CA.

[h]Gemsaec analyzer, Electro-Nucleonics, Inc., Fairfield, NJ.

[i]Gemini analyzer, Electro-Nucleonics, Inc., Fairfield, NJ.

[j]Sigma Chemical Co., St. Louis, MO.

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

separated by electrophoresis at 180 V for 15 min. Two applications were made for sera with extremely low protein concentration. The plates were stained with Ponceau stain[l] and were scanned in a densitometer.[m]

For lipoprotein determination, serum was applied to cellulose acetate plates[n] and the lipoprotein fractions were separated by electrophoresis at 165 V for 20 min. The plates were stained with oil red O[o] and were scanned in a densitometer.[m]

For LDH isoenzymes determination, serum was applied to cellulose acetate plates[p] and the LDH isoenzymes were separated by electrophoresis at 300 V for 10 min. The LDH isoenzymes were stained indirectly by reaction with LDH substrates, according to the manufacturer's instructions. The plates were then scanned in a densitometer[m] using a 570 nm filter.

Polybrominated Biphenyl Analysis

Tissue Samples

Approximately 0.5 g of tissue was ground with washed sand[q] in a stainless steel beaker by using a stainless

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[l]Helena Laboratories, Beaumont, TX.

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

[n]Titan III Lipo, Helena Laboratories, Beaumont, TX.

[o]Oil Red Om, Helena Laboratories, Beaumont, TX.

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

[q]J. T. Baker Chemical Co., Phillipsburg, NJ.

steel pestle. The sample was then dehydrated by adding 10 to 20 g of granular anhydrous sodium sulfate.[r] Twenty to twenty-five milliliters of glass-distilled hexane[s] were added to the cup and brought to a boil over a heated aluminum plate. The contents of the cup were filtered and the filtrate was collected in a 100 ml volumetric flask. The addition of hexane and filtration were repeated 3 times. The volume of the liquid in the volumetric flask was raised to 100 with glass-distilled hexane.[s] Two aliquots of 20 ml were separated and each one was condensed to approximately 0.5 ml by evaporation.[t] The first aliquot was used for PBB determination after being eluted in a magnesium silicate column. The second aliquot was transferred to a previously weighed aluminum container and allowed to dry by evaporation. The aluminum container was weighed again and the lipid weight was recorded.

Milk Samples

Five milliliters of milk were placed in a disposable 20 x 150 mm test tube. Five milliliters of methanol and 5 ml of a 1:1 mixture of ethyl ether and glass-distilled hexane were added to the test tube. The test tube was then agitated for 20 minutes and centrifuged at 1,500 rpm

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

[s]Burdick & Jackson Laboratories, Inc., Muskegon, MI.

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

for 5 minutes. The supernatant layer was removed and transferred to another test tube. The extraction steps were repeated 3 times. The combined extracts were condensed to approximately 0.5 ml by evaporation.[t] The condensed extract was transferred to a previously weighed aluminum container and allowed to dry by evaporation. The aluminum container was weighed again, and the lipid content was recorded. The lipid was redissolved with glass distilled hexane and transferred to a 100 ml volumetric flask. The volume in the flask was raised to 100 ml with glass-distilled hexane and a 10 ml aliquot was separated. The aliquot was condensed to approximately 0.5 ml by evaporation and was used for PBB determination after being eluted in a magnesium silicate column.

Elution of the Samples

The columns were prepared by packing 1.6 g of activated magnesium silicate[u] into a 50 ml thistle tube measuring 200 x 7 mm. A small amount of glass wool was placed at the tapered end of the tube to hold the magnesium silicate. A small amount of granular anhydrous sodium sulfate[v] was added to the top of the column. The column

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[u]Florisil, 60-100 Mesh, Fisher Scientific Co., Fair Lawn, NJ.

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

was washed with 5 ml of glass-distilled hexane and the washing was discarded. The previously condensed sample was transferred into the column and was eluted with 13 ml of glass-distilled hexane. The eluate was collected in a 15 ml graduated centrifuge tube and was evaporated to approximately 0.5 ml.

Gas Chromatography

The eluted samples were dissolved qs to 2 to 10 ml, depending on the expected concentration of PBB in the sample, with glass-distilled iso-octane.[w] Two microliters of the samples were injected into the gas chromatograph.[x] The column temperature was 250 C, the detector temperature was 310 C, and nitrogen was the carrier gas at a flow rate of 30 ml/min. Results were compared to standard samples containing 0.05 µg of PBB/ml and to control calf liver tissue or to store milk that had been processed by the same procedures. Results were expressed on ppm of PBB in a fat basis and on a whole weight basis.

Vitamin A Analysis[y]

Vitamin A was extracted from liver by the same procedures as for PBB extraction. Vitamin A was quantitated

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[w]Burdick & Jackson Laboratories, Inc., Muskegon, MI.

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

[y]Dr. Howard Stowe, Department of Pathology, Michigan State University, East Lansing, MI.

by high pressure liquid chromatography according to Dennison and Kirk (1977).

Thyroid Hormone Analysis[z]

Concentrations of triiodothyronine and thyroxine in the serum of sows and piglets were determined by radioimmunoassay methods according to Chopra and co-workers (1971, 1972).

Histologic Preparation

Tissues for light microscopic examination were fixed in 10% neutral buffered formalin, processed in an automatic processor,[aa] embedded in paraffin and sectioned at 5 to 6 μm. Tissue sections were stained with hematoxylin-eosin. Frozen sections of liver were stained with oil red O for lipid identification. Samples of liver fixed in Carnoy's fixative were embedded in paraffin, sectioned at 5 to 6 μm and stained by the periodic acid-Schiff reaction for glycogen identification. Sections of bone marrow were stained with Giemsa's stain.

Hepatic Microsomal Enzymes[bb]

The hepatic microsomal enzymes measured were: ethylmorphine-N-demethylase (Anders and Mannering, 1966);

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[z]Dr. Raymond Nachreiner, Department of Large Animal Surgery and Medicine, Michigan State University, East Lansing, MI.

[aa]Autotechnicon, The Technicon Co., Chauncey, NY.

[bb]Dr. Lee Shull, Department of Dairy Science, Michigan State University, East Lansing, MI.

cytochrome-C-reductase (Pederson et al., 1973); ethoxycoumarin deethylase (Ulrich and Weber, 1972: cytochrome P_{450} and cytochrome b_5 (Omura and Sato, 1964a,b); and hexabarbital hydroxylase (Kupfer and Rosenfeld, 1973).

Renal Microsomal Enzymes[cc]

The activity of arylhydrocarbon hydroxylase in samples of kidney was measured according to the technique described by Nerbert and Gelboin (1968) as modified by Oesch (1976).

Statistical Analysis

Data were statistically analyzed using the Statistical Package for Social Sciences (SPSS-Northern University) at the Michigan State University Computer Center. One-way analysis of variance followed by comparison of the means by Duncan's multiple range tests were performed.

[cc]Dr. Kevin McCormack, Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI.

RESULTS

Clinical Signs

At parturition, sows and newborn piglets were clinically normal. All sows lost considerable weight during lactation. There was significantly higher mortality among piglets nursing sows fed diets containing PBB (Table 2). Although some of the piglets that died had acute

Table 2. Death losses from birth to 4 weeks of age of piglets from sows fed diets containing different concentrations of PBB during last half of gestation and during lactation

PBB in diet (ppm)	Piglets born[a]	Stillborn piglets	Piglets killed at birth	Deaths during lactation	
				Total	Percent
0	45 (4)	5	15	1	4.0
10	22 (2)	2	7	1	7.7
100	47 (4)	8[b]	14	6	24.0
200	21 (2)	1	7	5	38.5

[a]Numbers in parentheses represent number of litters.

[b]Six piglets of a litter of 14 died from umbilical hemorrhage shortly after birth.

suppurative pneumonia, no clinical sign could be directly attributed to PBB toxicosis.

Two piglets nursing a sow fed a diet containing 200 ppm of PBB had incoordination, occasional tremors and decreased proprioceptor reflexes. The clinical signs appeared during the second week of age and persisted for approximately 1 week. These piglets acted normally otherwise and did not weigh less than their littermates.

Six piglets of a litter of 14 from a sow fed a diet containing 100 ppm of PBB died from umbilical hemorrhage shortly after birth. Their plasma had abnormally low concentration of fibrinogen (80±10 mg/dl). The normal value for fibrinogen is 150-300 mg/dl (Duncan and Prasse, 1977). Other blood clotting factors were not evaluated.

A few piglets from every treatment group had arthritis. *Micrococcus* sp., *Mycoplasma* sp., and coagulase negative *Staphylococcus* sp. were isolated from their joints. The incidence of arthritis decreased after thorough disinfection of the pens.

Body Weight

The body weights of piglets from birth to 4 weeks of age are presented in Table 3. Piglets nursing sows fed diets containing 100 ppm of PBB weighed less than any other treatment group. However, the difference in body weight was statistically significant only at the 4th week between the piglets nursing sows fed diets containing 100 ppm of PBB and those nursing sows fed diets containing 200 ppm of PBB.

Table 3. Mean body weights of piglets from birth to 4 weeks of age. Dams were fed diets containing different concentrations of PBB during last half of gestation and during lactation.

Concentration of PBB in diet of sows (ppm)	Number of litters	Body weight (kg)				
		Birth	1st week	2nd week	3rd week	4th week
0	4	1.42±0.09	2.63±0.22	4.04±0.21	5.82±0.41	7.19±0.20
10	2	1.34±0.09	2.37±0.09	3.95±0.63	5.36±0.49	7.54±0.53
100	4	1.30±0.07	1.97±0.24	3.27±0.18	4.68±0.19	6.50±0.29
200	2	1.51±0.14	2.77±0.70	3.97±0.75	6.23±1.21	8.13±0.75[a]

Values are expressed as means ± SEM.

[a]Different ($p<0.05$) from 100 ppm group.

The PBB did not adversely affect the rate of weight gain of piglets (Figure 1). The slopes of the curves for body weights are almost identical for all groups.

Organ Weights

Liver

The liver weight and the liver weight to body weight ratios of sows were not affected by PBB.

The liver weight and liver weight to body weight ratios of piglets at birth and at 4 weeks of age are shown in Table 4. Absolute and relative liver weights were increased in dose-related response in piglets nursing sows fed diets containing PBB. The differences were more pronounced between control piglets and piglets from sows fed diets containing 100 or 200 ppm of PBB.

The interaction between time and concentration of PBB on the liver weight to body weight ratios of piglets is shown in Figure 2. Except for a slight interaction between time and dosage in the 100 and 200 ppm groups, PBB affected the liver weight of piglets at birth as much as at 4 weeks of age. Data on liver weight were also plotted as a ratio to brain weight (Figure 3). The results were similar to the results for absolute liver weights (Figure 4).

Thyroid Gland

Thyroid weight to body weight ratios of sows and piglets at birth and at 4 weeks of age are shown in Table 5. At birth the ratios were significantly higher in

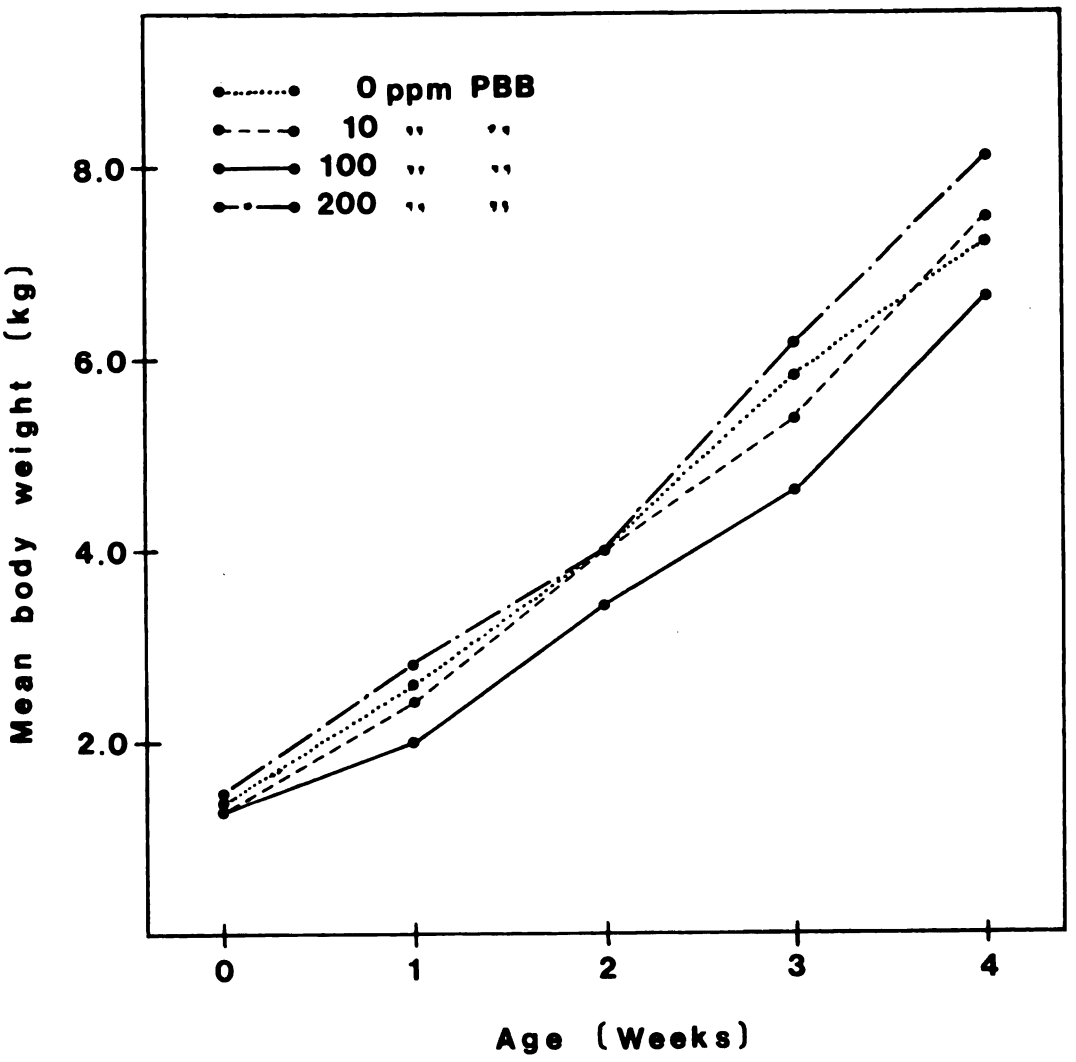

Figure 1. Effects of PBB and time on the body weight of piglets from birth to 4 weeks of age. Dams were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

Table 4. Mean liver weights (absolute and as percentage of body weight) of piglets at birth and at 4 weeks of age. Dams were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

PBB in diet (ppm)	Number of litters	Liver weight (g)		Liver weight (% of bw)	
		at birth	at 4 weeks	at birth	at 4 weeks
0	4	29.81± 3.52	168.42± 13.33	2.40± 0.12	2.31± 0.12
10	2	31.16± 0.98	185.75± 17.50	2.55± 0.22	2.44± 0.08
100	4	34.30± 2.55	184.00± 6.00	2.73± 0.23	2.71± 0.08[a]
200	2	41.34± 4.61	224.06± 11.92[a]	2.75± 0.01	2.68± 0.20[a]

Values represent mean ± SEM.

[a]Different ($p<0.05$) from control group.

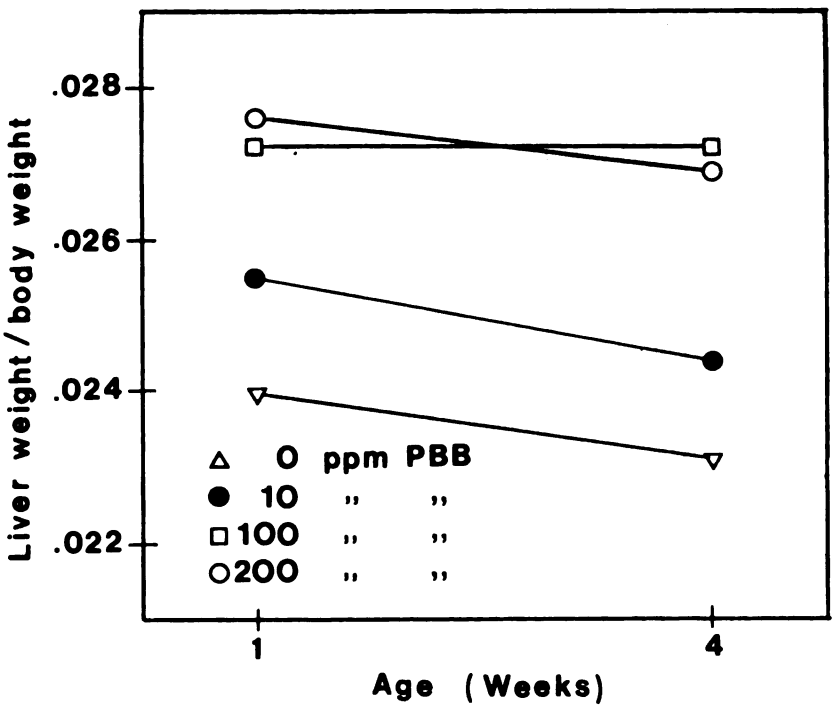

Figure 2. Effects of PBB and time on the liver weight to body weight ratios of piglets at birth and at 4 weeks of age. Dams were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

Figure 3. Effects of PBB on liver weight to brain weight ratios of piglets at birth and at 4 weeks of age. Dams were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

Figure 4. Effects of PBB on liver weight of piglets at birth and at 4 weeks of age. Dams were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

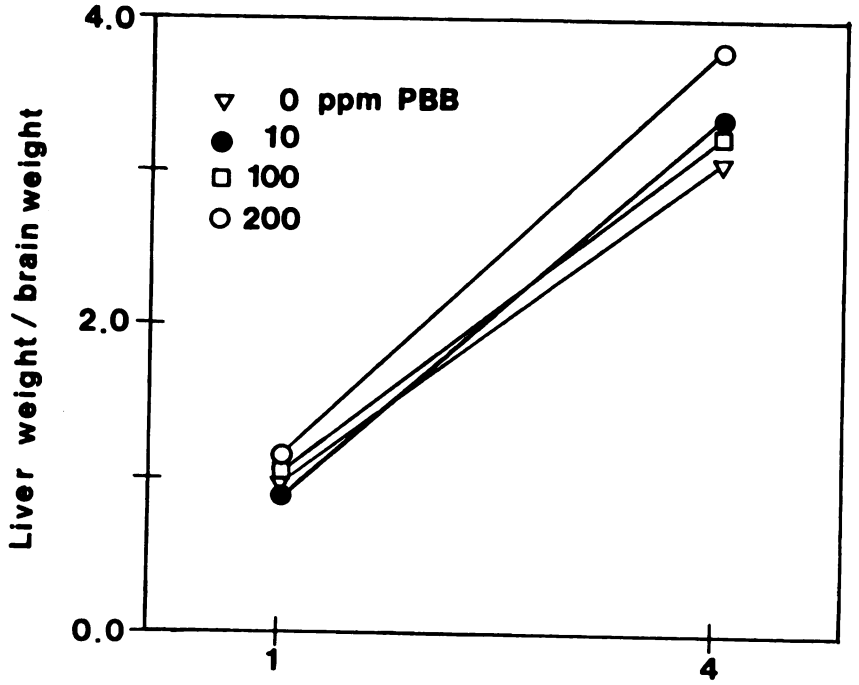

Figure 3

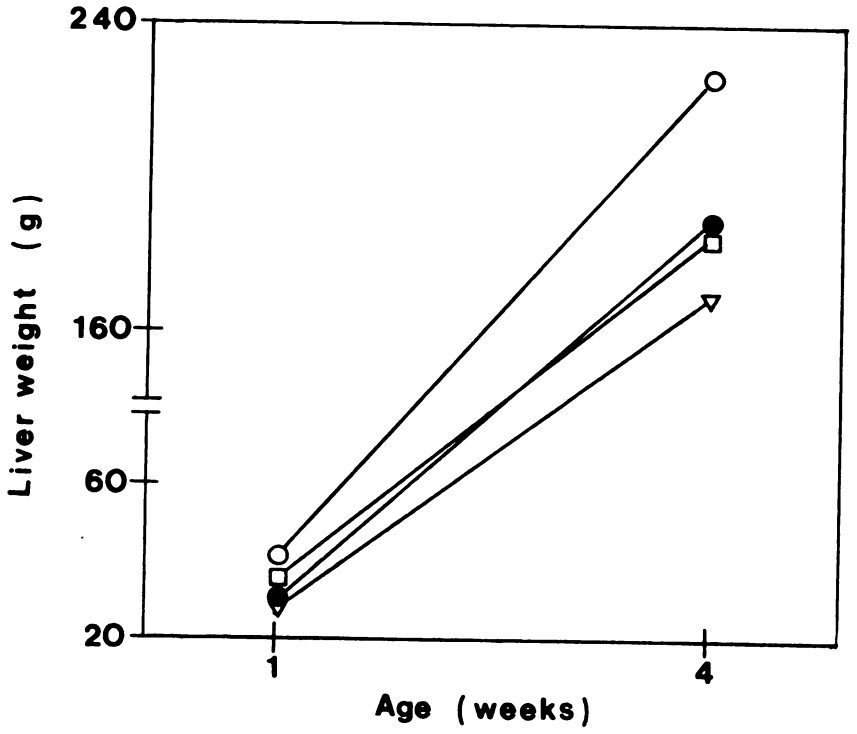

Figure 4

Table 5. Thyroid weight to body weight ratios of sows and their piglets at birth and at 4 weeks of age. The sows were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

PBB in diet (ppm)	Number of litters	Thyroid weight (g/kg body weight)		
		Sows	Newborn	4-week-old
0	4	0.12±0.02	0.17±0.03	0.10±0.01
10	2	0.11±0.02	0.14±0.01	0.15±0.05
100	4	0.11±0.00	0.23±0.02[a]	0.12±0.02
200	2	0.10±0.03	0.15±0.05	0.11±0.00

Values represent mean ± SEM.

[a]Different ($p<0.01$) from other piglets of the same age.

piglets from sows fed diets containing 100 ppm of PBB. For sows and for piglets at 4 weeks of age, the ratios did not differ significantly. However, the ratios were higher for piglets nursing sows fed diets containing 10 ppm of PBB. This effect is more evident in Figure 5, which shows the interaction between time and concentration of PBB on the thyroid weight to body weight ratios of piglets at birth and at 4 weeks of age. Apparently, low doses of PBB had a stimulatory effect on the thyroid weight of the piglet.

Other Organs

The thymus of the piglet extends from the anterior mediastinum to the parotid gland. The complete removal of the thymus was nearly impossible, and in some instances the data on the weight of the thymus were unreliable. Histopathologic examination indicated that there were no structural changes in the thymus of piglets at birth and at 4 weeks of age.

The weight of adrenal glands, brain, and spleen of sows and piglets and the weight of the stomach of sows were not affected by PBB.

Hematology

The PBB did not affect the morphology of erythrocytes and leukocytes, blood cell counts, differential and total leukocyte counts, packed cell volume, or hemoglobin concentrations.

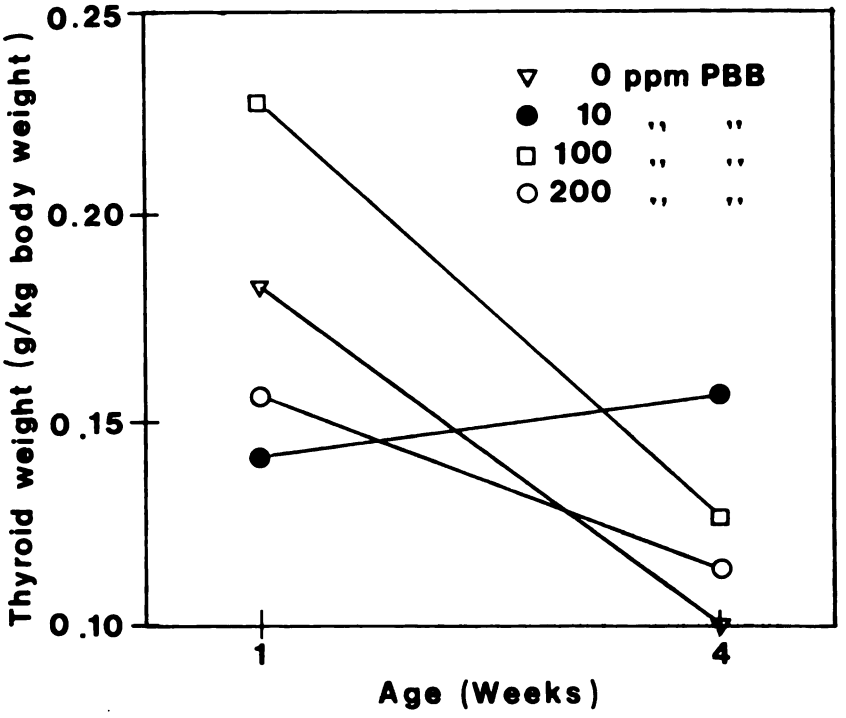

Figure 5. The effects of PBB and time and their interaction on thyroid weight to body weight ratios in piglets at birth and at 4 weeks of age. Dams were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

Clinical Chemistry

Blood Urea Nitrogen

Serum concentrations of BUN were significantly higher ($p<0.05$) in 4-week-old piglets nursing sows fed diets containing 100 or 200 ppm of PBB. Those piglets had BUN values of 10.1±0.2 and 10.5±0.9 mg/dl, respectively, whereas control piglets had BUN values of 8.5±1.0 mg/dl. Serum concentrations of BUN for newborn piglets and for sows were not affected by PBB.

Serum Alkaline Phosphatase

The concentrations of SAP for piglets at birth and at 4 weeks of age are shown in Table 6. Newborn piglets from sows fed diets containing 100 or 200 ppm of PBB had lower concentrations of SAP than piglets from control sows. The effect of PBB on the concentrations of SAP was dose-related. Concentrations of SAP for sows and for 4-week-old piglets were not affected by PBB.

The interaction between time and the concentration of PBB on concentration of SAP for piglets is shown in Figure 6. There was no interaction, except for piglets from sows fed diets containing 10 ppm of PBB. At 4 weeks of age, PBB at a concentration of 10 ppm had a slight stimulatory effect on the concentrations of SAP.

Serum Glutamic Pyruvic Transaminase

The concentrations of SGPT for piglets at birth and at 4 weeks of age are presented in Table 6. Data on SGPT

Table 6. Concentrations of serum alkaline phosphatase (SAP) and serum glutamic pyruvic transaminase (SGPT) from piglets at birth and at 4 weeks of age. Dams were fed diets containing different concentrations of PBB during the last half of gestation and during parturition.

PBB conc. (ppm)	Number of litters	SAP (IU/1)		SGPT (IU/1)	
		Birth	4 weeks	Birth	4 weeks
0	4	1812± 189	461± 39	24.4± 6.8	24.2± 2.8
10	2	1558± 660	582± 354	14.4± 5.9	31.0± 0.5[b]
100	4	1080± 110[a]	336± 40	19.6± 5.2	21.9± 0.9
200	2	544± 27[a]	317± 79	16.0± 5.7	16.0± 0.0[b]

Values represent mean ± SEM.

[a]Different ($p<0.03$) from other piglets of the same age.

[b]Different ($p<0.01$) from other piglets of the same age.

Figure 6. The effects of PBB and time on the concentrations of serum alkaline phosphatase (SAP) in piglets at birth and at 4 weeks of age. Dams were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

Figure 7. The effects of PBB and time on the concentrations of serum glutamic pyruvic transaminase in piglets at birth and at 4 weeks of age. Dams were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

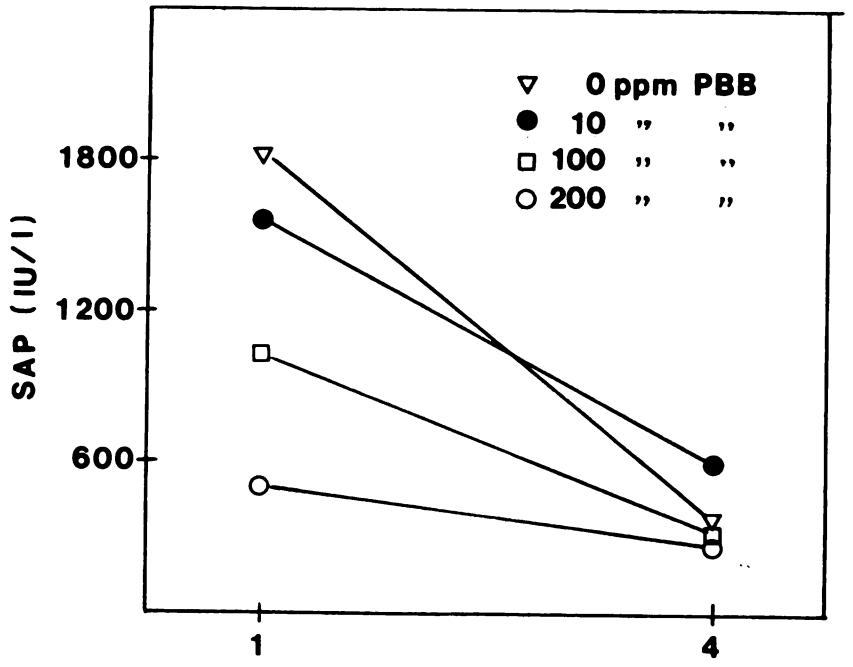

Figure 6

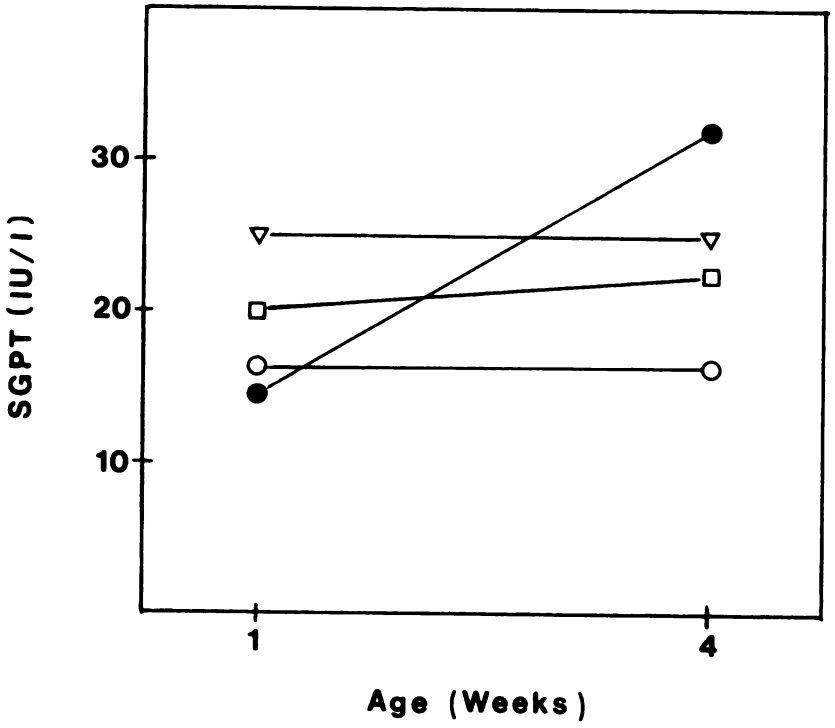

Figure 7

at birth were variable and the differences were not significant. At 4 weeks of age, piglets nursing control sows had higher concentrations of SGPT than piglets nursing sows fed diets containing 100 or 200 ppm of PBB. The concentrations of SGPT in 4-week-old piglets nursing sows fed diets containing 10 ppm of PBB were significantly higher than the concentrations in piglets nursing control sows.

The interaction between time and dosage of PBB on the concentration of SGPT for piglets is shown in Figure 7. There was no interaction except for piglets nursing sows fed diets containing 10 ppm of PBB. In those piglets, PBB had a marked stimulatory effect on the concentrations of SGPT at the end of 4 weeks.

Ornithine Carbamyl Transferase

The serum concentration of OCT for sows and for piglets at birth and at 4 weeks of age are shown in Table 7. The concentrations of OCT in the serum of sows were not significantly increased by PBB. For piglets at birth, there was a dose-related response, although the differences were not significant. At 4 weeks of age, the concentrations of OCT in the serum of piglets nursing sows fed diets containing PBB were significantly increased in a dose-related response.

There was no interaction between time and dosage of PBB on the serum concentrations of OCT for piglets (Figure 8). The concentrations of OCT were equally increased at

Table 7. Concentration of ornithine carbamyl transferase (OCT) in the serum of sows and their piglets at birth and at 4 weeks of age. The sows were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

PBB in diet (ppm)	Number of litters	OCT (Sigma Units/ml)		
		Sows	Newborn	4-week-old
0	4	150.0±21.3	125.4±21.3	48.4± 8.4
10	2	140.0±25.1	a	78.8± 8.3
100	4	250.0±84.7	146.6±33.6	89.2±16.8[c]
200	2	182.2±27.6	172.6±52.6	121.9±21.9[b]

Values represent mean ± SEM.

[a]Not examined.

[b]Different ($p<0.01$) from control group.

[c]Different ($p<0.05$) from control group.

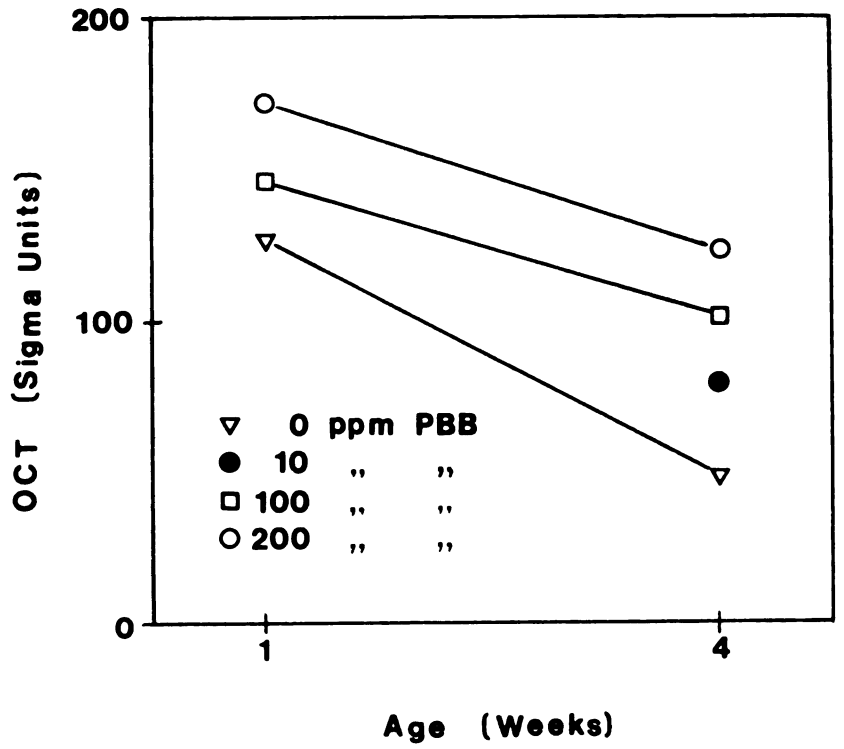

Figure 8. The effects of time and PBB on the concentrations of serum ornithine carbamyl transferase (OCT) in piglets at birth and at 4 weeks of age. Dams were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

birth and at 4 weeks of age by the different concentrations of PBB. For piglets nursing sows fed diets containing 10 ppm of PBB the absence of interaction was only assumed, since the concentrations of OCT were not measured at birth.

Serum Cholesterol

The concentrations of serum cholesterol for sows at the end of lactation and for their piglets at birth and at 4 weeks of age are shown in the appendix. The concentrations of serum cholesterol for piglets were markedly variable, even within the same litter. The values were not significantly different between treatments within any age group.

Thyroid Hormone Analysis

The concentrations of triiodothyronine (T_3) and thyroxine (T_4) in the serum of sows and their piglets are shown in Table 8. The concentrations of T_3 and T_4 were significantly lower in the serum of sows fed diets containing 200 ppm of PBB and in the serum of newborn and 4-week-old piglets from those sows. Concentrations of T_4, but not of T_3, were significantly lower in the serum of piglets nursing sows fed diets containing 100 ppm of PBB than in the serum of piglets nursing control sows. With the exception of piglets nursing sows fed diets containing 10 ppm of PBB, the concentrations of T_3 and T_4 in the serum of piglets decreased proportionally to the concentrations of PBB in the sows' diets. There were no interactions

Table 8. Concentrations of triiodothyronine (T_3) and thyroxine (T_4) in the serum of sows and their piglets at birth and at 4 weeks of age. The sows were fed diets containing different concentrations of PBB (ppm) during gestation and during lactation.

PBB in diet	Sows		Newborn		4-week-old	
	T_3	T_4	T_3	T_4	T_3	T_4
0	0.63± 0.13	27.0± 3.9	1.19± 0.12	52.5± 1.5	1.52± 0.07	49.1± 1.4
10	0.46± 0.03	24.0± 1.6	1.28± 0.25	54.5± 4.1	1.70± 0.16	51.0± 2.8
100	0.53± 0.15	28.7± 4.3	0.88± 0.14	48.9± 2.0	1.32± 0.11[a]	43.6± 1.5[b]
200	0.15± 0.10[b]	15.8± 0.0[b]	0.68± 0.10[b]	42.4± 3.1[b]	1.01± 0.08[b]	39.4± 1.6[b]

Values represent mean ± SEM in ng/ml.

[a]Different ($p<0.05$) from 10 ppm group.

[b]Different ($p<0.05$) from control and 10 ppm groups.

between time and concentrations of PBB on the serum concentrations of T_3 (Figure 9) and T_4 (Figure 10).

Vitamin A Analysis

The PBB did not affect the concentrations of vitamin A in the liver of piglets at birth and at 4 weeks of age. The concentrations of vitamin A in the liver of piglets are shown in the appendix.

Serum Electrophoresis

Serum Proteins

The concentrations of albumin and the albumin to globulin ratios (A/G) in the serum of sows and in the serum of piglets at birth and at 4 weeks of age are shown in Table 9. There was a significant increase in the concentrations of albumin and in the A/G ratios in the serum of newborn piglets from sows fed diets containing 200 ppm of PBB. The PBB did not induce changes in the concentrations of albumin or in the A/G ratios in the serum of sows or piglets at 4 weeks of age. The concentrations of the different protein fractions in the serum of sows and piglets are shown in the appendix.

Lactic Dehydrogenase Isoenzymes

The percentages of LDH-4 were higher ($p<0.05$) in the serum of sows fed diets containing 200 ppm of PBB (22.6± 2.7%) than in the serum of control sows (13.9±0.4%). The concentrations of LDH-4 were not affected by any other treatment in any age group. The concentrations of LDH-5,

Figure 9. The effects of PBB and time on the serum concentrations of triiodothyronine (T_3) in piglets at birth and at 4 weeks of age. Dams were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

Figure 10. The effects of PBB and time on the serum concentrations of thyroxine (T4) in piglets at birth and at 4 weeks of age. Dams were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

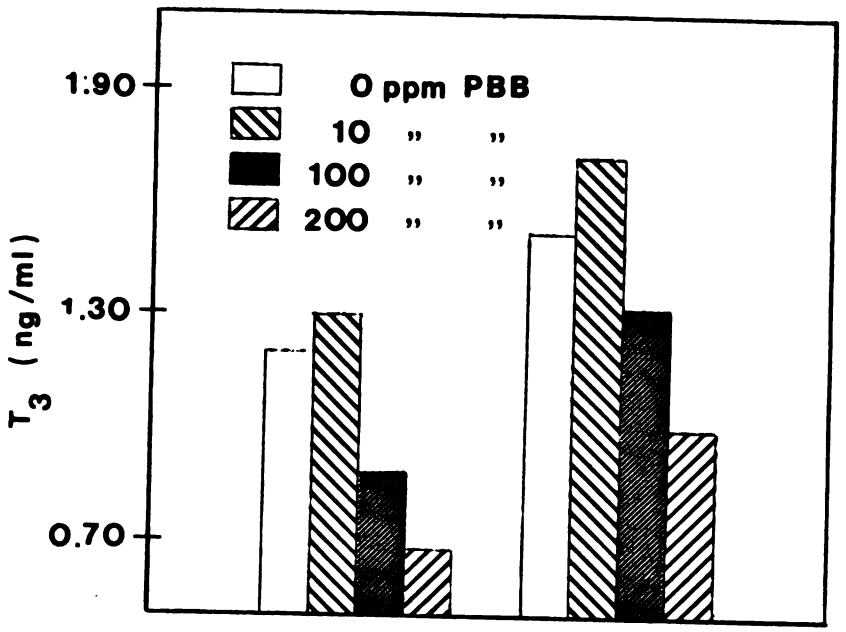

Figure 9

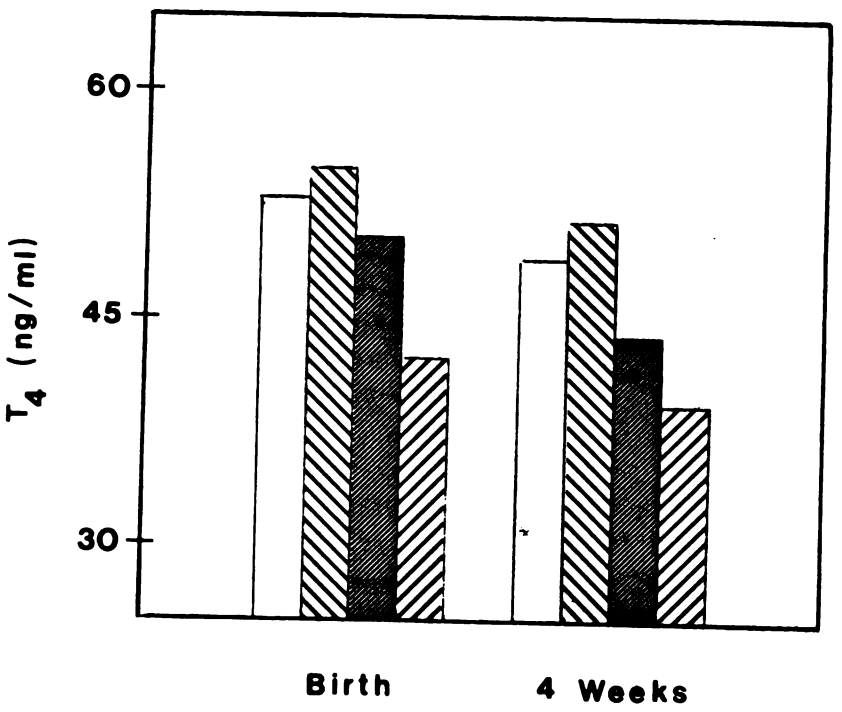

Figure 10

Table 9. Concentrations of albumin (g/dl) and albumin/globulin (A/G) ratios in the serum of sows and their piglets at birth and at 4 weeks of age. The sows were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

PBB in diet (ppm)	Sows		Newborn		4-week-old	
	Albumin	A/G	Albumin	A/G	Albumin	A/G
0	3.60± 0.14	0.90± 0.13	0.36± 0.04	0.17± 0.02	2.85± 0.08	1.20± 0.03
10	3.75± 0.15	0.91± 0.24	0.27± 0.07	0.10± 0.02	2.99± 0.10	1.32± 0.09
100	3.38± 0.15	0.77± 0.06	0.45± 0.05	0.17± 0.02	3.01± 0.07	1.17± 0.02
200	3.30± 0.10	0.85± 0.01	0.48± 0.06[a]	0.24± 0.03[b]	3.04± 0.16	1.20± 0.07

The values represent mean ± SEM.

[a]Different ($p<0.05$) from 10 ppm group.

[b]Different ($p<0.05$) from 0, 10 and 100 ppm groups.

LDH-3, LDH-2, and LDH-1 in the serum of sows and piglets were not affected by PBB. The concentrations of the lactic dehydrogenase isoenzymes for sows and piglets are shown in the appendix.

Lipoprotein Fractions

The percentage of the lipoprotein fractions in the serum of sows is shown in Table 10. The pre-beta lipoprotein

Table 10. Lipoprotein fractions in the serum of sows fed diets containing different concentrations of PBB during the last half of gestation and during lactation

PBB in diet (ppm)	Alpha (%)	Pre-Beta (%)	Beta (%)
0	50.3±2.6	3.1±0.5	45.5±2.6
10	45.8±3.2	8.4±0.8	45.9±3.8
100	44.6±2.8	8.0±2.3	47.5±4.3
200	44.2±1.4	12.1±0.3[a]	48.8±6.1

Values are expressed as mean ± SEM.

[a]Different ($p<0.05$) from other treatment groups.

fraction was significantly elevated in the serum of sows fed diets containing 200 ppm of PBB. Other fractions were not significantly affected. In the serum of piglets, the pre-beta fraction could not be accurately separated from the beta fraction. When these 2 fractions were considered

together, there were no differences between the concentrations of pre-beta/beta and alpha fractions.

Polybrominated Biphenyl Analysis

The gas-chromatographic profiles of PBB in the sows' diet, in the adipose tissue of a sow fed a diet containing 200 ppm of PBB, in her milk, and in the adipose tissue of her offspring (pooled sample) at 4 weeks of age are presented in Figure 11.

Tissue concentrations of PBB for sows and for piglets at birth and at 4 weeks of age are presented in Tables 11 and 12. For sows and for piglets, the concentrations of PBB in the tissues were proportional to the concentrations of PBB in the sows' diet. The concentrations of PBB (fat basis) were the highest in the liver, followed by the adipose tissue, kidney, and brain, in decreasing order.

The concentrations of PBB in the sows' milk are presented in Table 13. In general, the concentrations of PBB in the milk fat correlated with the concentration of PBB in the sows' diet. Highest concentrations of PBB were observed in the colostrum. The concentrations of PBB in the milk decreased gradually during lactation (Figure 12).

The concentrations (area percent) of the different isomers (peaks) of PBB in the liver and adipose tissue of sows fed diets containing 200 ppm of PBB and in the liver and in the adipose tissue of their piglets at birth and at 4 weeks of age are presented in Table 14. The concentrations of PBB congeners in the milk of those sows, from parturition to 4 weeks postpartum, are presented in

Figure 11. Gas-chromatographic profiles of PBB in the sows' diet (FireMaster BP-6), in the sows' adipose tissue, in the sows' milk, and in the adipose tissue of 4-week-old nursing piglets. The sow was fed a diet containing PBB during the last half of gestation and during lactation. Notice the changes in height of the peaks. Each numbered peak represents a different PBB congener, as follows:

Peak number 1: 2,4,5,2',5'-pentabromobiphenyl

2: 2,3,5,3',4'-pentabromobiphenyl

3: Hexabromobiphenyl (unknown structure)

4: 2,4,5,2',4',5'-hexabromobiphenyl

5: 2,3,4,2',4',5'-hexabromobiphenyl

6: 2,4,5,3',4',5'-hexabromobiphenyl

7: Heptabromobiphenyl (unknown structure)

8: 2,3,4,5,2',4',5'-heptabromobiphenyl

(Moore and Aust, 1978)

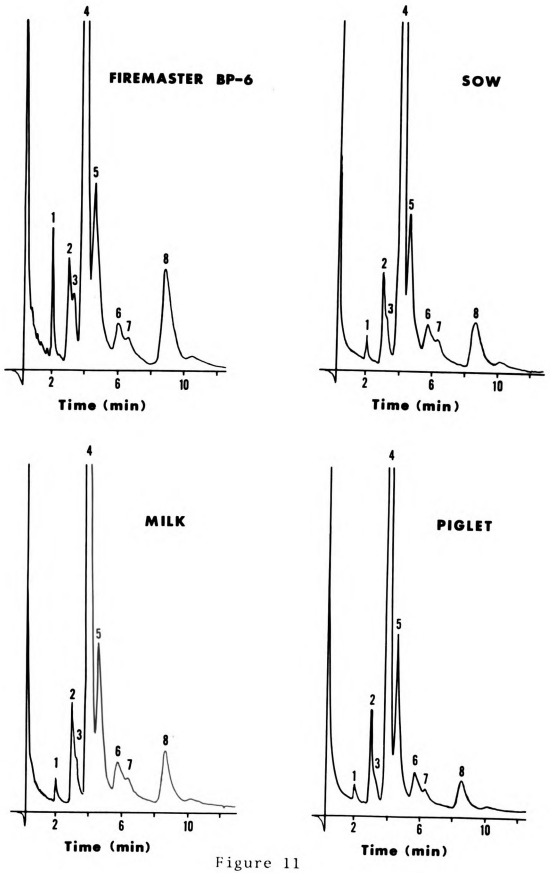
FIREMASTER BP-6
SOW
MILK
PIGLET
Time (min)
Time (min)
Time (min)
Time (min)

Figure 11

Table 11. Mean concentrations of PBB (ppm) in the liver and in the adipose tissue of sows, newborn, and 4-week-old piglets. The sows were fed diets containing PBB during the last half of gestation and during lactation.

PBB conc. in sows' diet (ppm)	Liver		Adipose tissue	
	Whole weight basis	Fat basis	Whole weight basis	Fat basis
Sows				
0	[a]	[a]	0.1± 0.1	0.2± 0.1
10	1.0± 0.5	28.1± 8.0	15.2± 5.9	28.0± 12.4
100	45.8± 7.8	1674.6±235.1	96.3±24.0	147.8± 27.9
200	92.6±34.3	2384.8±371.9	194.2±32.4	258.5± 36.9
Piglets at birth				
0	[a]	[a]	0.0± 0.0	1.6± 0.9
10	1.0± 0.1	35.6± 6.5	0.4± 0.1	39.0± 9.2
100	11.5± 1.0	439.6± 16.7	4.9± 1.4	432.2±129.2
200	24.2±10.3	1646.0±652.2	40.3±21.8	2454.7±734.3
Piglets at 4 weeks				
0	[a]	[a]	0.2± 0.2	0.6± 0.4
10	2.4± 0.8	116.0± 28.9	14.8± 0.3	41.8± 4.7
100	30.2± 2.2	1174.7±103.0	96.7± 7.9	226.8± 19.9
200	41.3±10.1	2174.9± 32.9	225.2±49.1	552.2±100.7

[a]Technical problems invalidated data.

Table 12. Mean concentrations of PBB (ppm) in the kidneys and brain of sows, newborn, and 4-week-old piglets. The sows were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

PBB conc. in sows' diet (ppm)	Kidneys		Brain	
	Whole weight basis	Fat basis	Whole weight basis	Fat basis
Sows				
0	0.0±0.0	2.1± 1.2	0.0±0.0	0.1± 0.0
10	0.6±0.2	16.5± 4.8	0.2±0.0	2.4± 0.2
100	2.3±0.5	167.3±34.7	1.7±0.1	27.0± 4.6
200	3.7±0.5	182.9±23.0	2.7±0.6	44.7± 0.7
Piglets at birth				
0	0.0±0.0	2.1± 0.9	0.0±0.0	0.5± 0.1
10	a	a	a	a
100	a	a	a	a
200	1.5±0.0	228.0±12.0	1.8±0.6	52.2±11.0
Piglets at 4 weeks				
0	0.0±0.0	0.5± 0.3	0.0±0.0	0.4± 0.1
10	a	a	a	a
100	a	a	a	a
200	4.1±0.9	298.2±83.8	4.2±1.0	90.8± 1.8

[a]Not analyzed.

Table 13. Concentrations of PBB (ppm) in the colostrum and in the milk of sows during lactation. The sows were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

PBB in diet (ppm)	Colostrum		1st week		2nd week		3rd week		4th week	
	Whole weight basis	Fat basis	Whole weight basis	Fat basis	Whole weight basis	Fat basis	Whole weight basis	Fat basis	Whole weight basis	Fat basis
0	0	0(4)	0	0(6)	0	0(6)	0	0(6)	0	0(5)
10	1	30(4)	2	22(9)	1	11(9)	1	11(7)	1	7(9)
100	26	471(6)	41	593(7)	19	292(7)	9	163(6)	12	167(7)
200	19	737(3)	30	426(7)	26	371(7)	22	308(7)	22	364(6)

Number in parentheses represents the percentage of fat in the colostrum and in the milk.

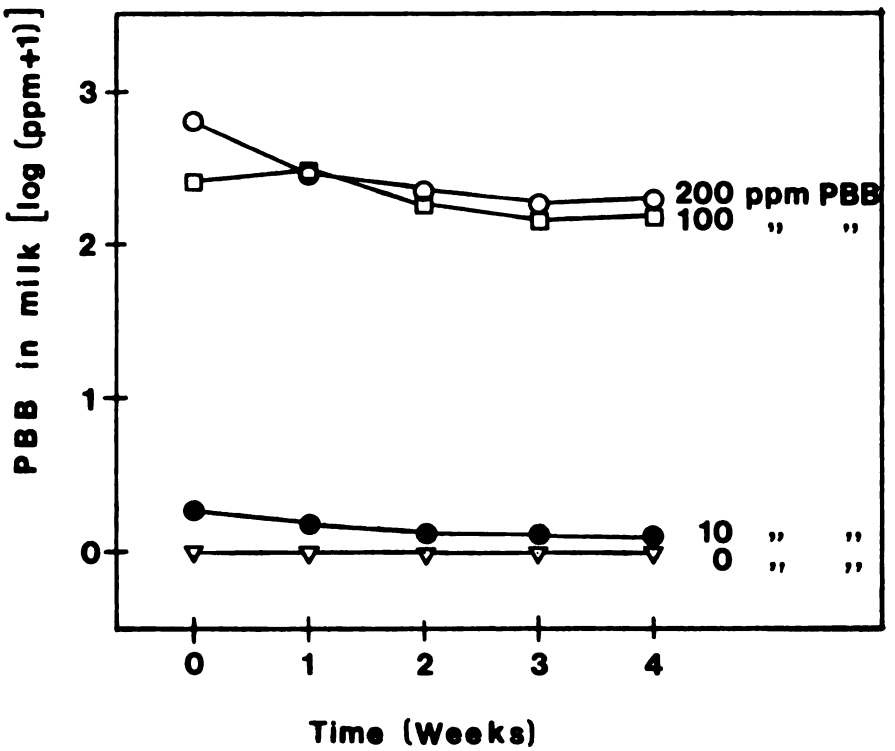

Figure 12. Changes in concentrations of PBB in the milk fat of sows during lactation. The sows were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

Table 14. Concentrations (area percent) of PBB congeners (peaks) in the diet of sows and in the liver and adipose tissue of sows and their piglets at birth and at 4 weeks of age. The sows were fed diets containing 200 ppm of PBB during the last half of gestation and during lactation.

		Sow		Newborn		4-week-old	
Peak	Sows' diet	Liver	Adipose tissue	Liver	Adipose tissue	Liver	Adipose tissue
1	2.6	a	0.5	a	0.4	a	0.4
2-3[b]	5.0	0.9	3.7	a	4.0	1.8	3.8
4	63.8	71.4	66.5	89.2	72.8	75.2	70.0
5	13.1	14.2	19.6	10.1	11.6	10.5	20.5
6-7[b]	5.1	5.2	4.8	1.2	5.0	8.1	3.0
8	10.3	8.4	5.1	a	6.2	4.5	2.7

[a]Too low to calculate.

[b]Could not be separated.

Table 15. Peaks 2 and 3 and peaks 6 and 7 were considered together because they could not be separated.

Table 15. Concentrations (area percent) of PBB congeners (peaks) in the diet of sows and in the colostrum and milk of sows during the lactation period. The sows were fed a diet containing 200 ppm of PBB during the last half of gestation and during lactation.

Peak	Sows' diet	Colostrum	1st week	2nd week	3rd week	4th week	Avg.
1	2.6	0.3	0.4	0.6	0.6	0.5	0.5
2-3[a]	5.0	3.4	4.2	4.2	5.3	5.5	4.5
4	63.8	66.9	69.0	66.7	65.1	67.2	67.0
5	13.1	15.0	16.5	18.1	17.0	16.3	16.6
6-7[a]	5.1	4.4	4.3	4.3	5.5	4.9	4.7
8	10.3	9.4	5.2	5.0	5.5	5.5	6.1

[a]Could not be separated.

The relative concentrations of peaks 1, 2-3, and 8 were comparatively lower in the tissues of sows and piglets than in the PBB in the diet. The area of peak 1 was too low to calculate in the gas chromatograms resulting from PBB analysis of livers of sows and piglets. In the liver of newborn piglets, only peaks 4, 5 and 6-7 were present in measurable amounts. The concentration of peak 6-7 in the adipose tissue and in the liver of sows and in the adipose tissue of newborn piglets was similar to the concentration in the PBB standard. The concentration of

peak 6-7 was comparatively lower in the liver of the newborn piglet and comparatively higher in the liver of the 4-week-old piglet. The concentration of peak 8 was somewhat lower than the standard in tissues of sows and markedly decreased in tissues of 4-week-old piglets.

The concentrations (area percent) of the PBB congeners (peaks) in the milk of sows remained approximately the same throughout the lactation period. The percentage of the congeners in the milk was similar to the percentage in the sows' adipose tissue.

The changes in the percentage of the PBB congeners as they move from the diet, to the sows' adipose tissue, to the milk and to the adipose tissue of the nursing piglet are shown in Figure 13. Except for peak 1, which was almost completely eliminated as it moved from the diet to the sows' adipose tissue, the changes in the percentage of the peaks between the diet and the sows' adipose tissue were similar to the change in the percentage between the milk and the piglets' adipose tissue.

Histopathology

Liver

The liver of sows fed diets containing 0 or 10 ppm of PBB and the liver of their piglets were essentially normal. Irregular cords of hepatocytes separated by prominent sinusoids radiated from the central vein to the periphery of the hepatic lobule (Figure 14). Glycogen, as demonstrated by PAS stain, was present in large amounts

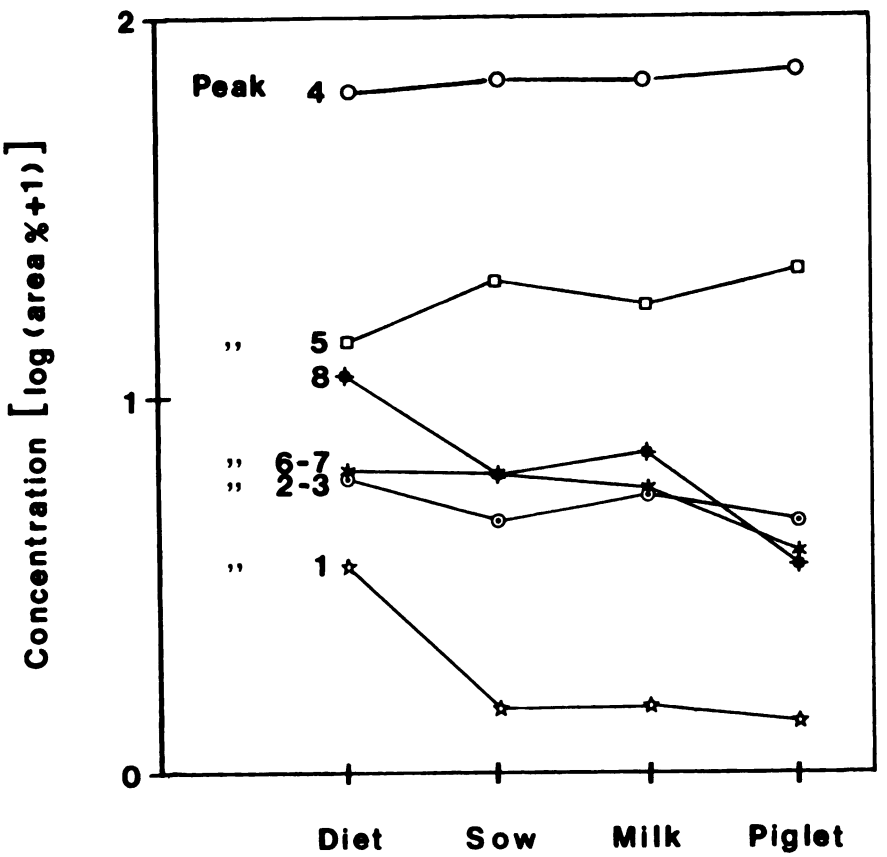

Figure 13. Changes in concentration (area percent) of the congeners (peaks) of PBB in the sows' diet, in the sows' adipose tissue, in the sows' milk, and in the adipose tissue of the nursing piglet. The sows were fed a diet containing 200 ppm of PBB during the last half of gestation and during lactation.

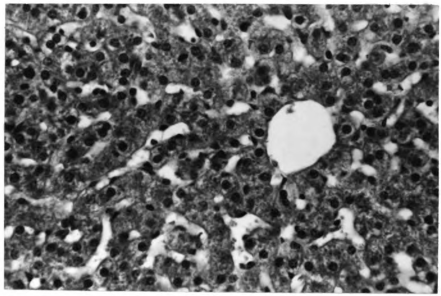

Figure 14. Liver section from a piglet that nursed a control sow. Notice normal appearance of hepatocytes around central vein. H&E stain; X400.

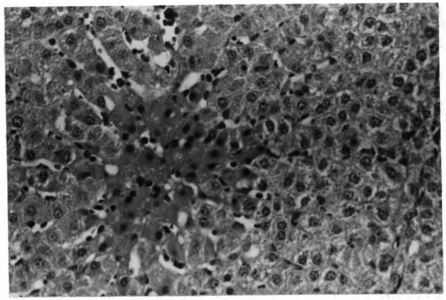

Figure 15. Liver section from 4-week-old piglet that nursed a sow fed a diet containing 200 ppm of PBB during the last half of gestation and during lactation. Notice necrosis of hepatocytes in centrolobular area. H&E stain; X400.

within hepatocytes, especially in the newborn piglet. Fat, as demonstrated in frozen sections stained with oil red O, was present in negligible amounts within hepatocytes. The hepatocytes of sows contained more fat than the hepatocytes of piglets. There were no histopathologic changes in the liver of newborn piglets. The liver of sows fed diets containing 100 or 200 ppm of PBB was affected more intensely than the liver of piglets nursing those sows. The lesions observed in the liver of sows and 4-week-old piglets consisted basically of necrosis and fatty change. The necrotic changes were strictly centrolobular in the liver of piglets and involved only a few layers of hepatocytes (Figure 15). In the sows, the necrosis was sometimes panlobular, but was usually more intense in the centrolobular area (Figure 16). In many instances, the hepatocytes of sows fed diets containing 100 or 200 ppm of PBB were swollen and the cytoplasm was homogeneous. This change was seen in individual as well as in groups of hepatocytes. Occasionally, mitotic figures were also seen. In other instances, there was proliferation of Kupffer cells and focal collections of inflammatory cells, mostly lymphocytes. Occasionally, individual hepatocytes with eosinophilic cytoplasm and a pyknotic nucleus were seen. The liver of 1 sow fed a diet containing 100 ppm of PBB had several areas of focal hyperplasia (Figure 17). The hyperplastic areas were clearly demarcated from the adjacent normal hepatic tissue by a layer of flattened hepatocytes.

Figure 16. Liver section of a sow fed a diet containing 200 ppm of PBB during the last half of gestation and during lactation. Notice severe necrosis and hemorrhage in centrolobular area. H&E stain; X64.

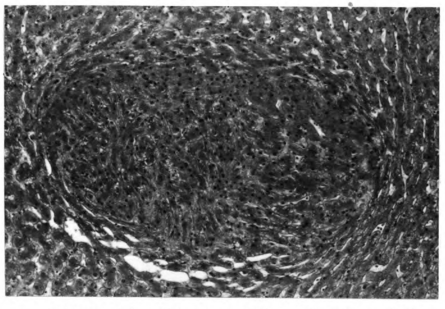

Figure 17. Liver section of a sow fed a diet containing 100 ppm of PBB during the last half of gestation and during lactation. Notice well demarcated area of focal hyperplasia. H&E stain; X160.

Thyroid Gland

There were no histopathologic changes in the thyroid gland of sows and 4-week-old piglets. The histologic section of the thyroid gland of a newborn piglet from a control sow is shown in Figure 18. The thyroid gland of newborn piglets from sows fed diets containing 100 and 200 ppm of PBB had increased cellularity, and the colloid was scant and vacuolated and stained faintly with eosin (Figure 19). An occasional follicle had short papillary projections and was lined by columnar cells (Figure 20).

Other Organs

The lungs of sows in the experiment and their 4-week-old piglets had chronic interstitial pneumonia. The lesions were not severe, and many lobules were spared. There was thickening of alveolar walls and hypertrophy of smooth muscle. Inflammatory cells were not numerous and consisted mostly of lymphocytes and macrophages.

Some of the piglets that died during the experiment had acute suppurative pneumonia. Their thymuses were smaller and the cortical lymphocytes were decreased in number. No histopathologic changes were observed in other organs.

Microsomal Enzymes

The concentration of cytochrome P_{450} and the activity of hexobarbital hydroxylase, ethylmorphine demethylase, and ethoxycoumarin deethylase were increased in a dose-related manner in the liver of sows fed diets containing

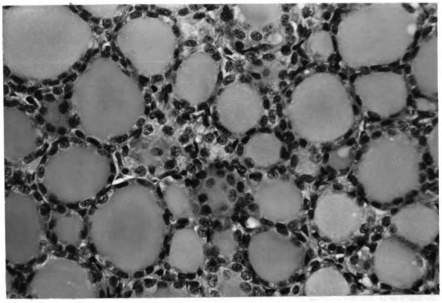

Figure 18. Thyroid follicles of a newborn piglet from a control sow. The follicles are lined by low cuboidal cells and are filled with homogeneous colloid. H&E stain; X400.

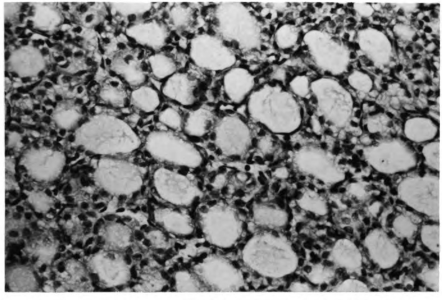

Figure 19. Thyroid follicles of a newborn piglet from a sow fed a diet containing 200 ppm of PBB during the last half of gestation. There is increased cellularity. The follicles are small, and the colloid is scant and vacuolated. H&E stain; X400.

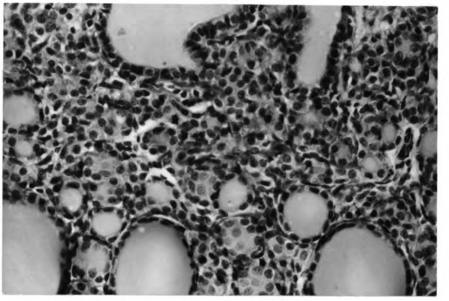

Figure 20. Thyroid follicles of a newborn piglet from a sow fed a diet containing 100 ppm of PBB during the last half of gestation. There is slight hyperplasia with an occasional follicle with papillary projections lined by columnar cells. H&E stain; X400.

PBB and in the liver of piglets nursing those sows. Aryl hydrocarbon hydroxylase (AHH) activity was not measured in the liver. In the kidney, AHH activity was increased in a dose-related manner in piglets nursing sows fed diets containing PBB. The activity of other microsomal enzymes was not measured in the kidney of piglets.

Microsomal protein also was increased in a dose-related manner in the liver of sows fed diets containing PBB and in the liver of piglets nursing those sows.

DISCUSSION

The ability of PBB to accumulate in the body for long periods, to be transferred to the fetus, and to be eliminated through the milk is of extreme public health importance. Sows were considered an excellent model to study the toxicity and kinetics of PBB in the pregnant or lactating animal as well as in the fetus and in the nursing young.

The PBB were demonstrated in the present experiment to be toxic to the sow, to the fetus, and to the nursing piglet. As anticipated, the severity of the pathologic changes was greatest when the sows were fed the highest concentrations of PBB in the diet. However, at 10 ppm of PBB the effects of PBB were strikingly different from what would be expected. For example, the weight of the thyroid gland (Table 5), the serum concentrations of thyroid hormones (Table 8), and the concentrations of SAP and SGPT (Table 6) were elevated in piglets nursing sows fed diets containing 10 ppm of PBB, whereas these values were decresaed in piglets nursing sows fed diets containing 100 or 200 ppm of PBB. The stimulatory effect of low doses of PBB, in contrast to the inhibitory effect of higher doses of PBB, was probably related to the fact that

PBB are a mixture of various congeners. Possibly, at low doses of PBB in the diet the effects of the congeners present in higher concentration in the PBB mixture were dominant. With an increased amount of PBB in the diet, the effects of other congeners present in lower concentrations, but perhaps of higher toxicity, became evident. Studies using purified PBB-congeners are needed to explain the mechanism of these effects.

The sows and newborn piglets were clinically unaffected. The hemorrhage observed in piglets from a sow fed a diet containing 100 ppm of PBB probably was not related to PBB.

There was significantly higher mortality among piglets nursing sows fed diets containing PBB. Some of the piglets died from acute suppurative pneumonia, which did not occur in control piglets. Piglets nursing sows fed diets containing PBB had a decreased lymphocytic response to mitogen stimulation (Howard, 1979). Whether the pneumonia occurred because of decreased immune protection is not known. Jackson and Halbert (1974) reported increased susceptibility to infection in dairy cows exposed to PBB, but their observations have been contested (Moorhead et al., 1977). Reports of immunosuppressive effects of polychlorinated biphenyls give reason to suspect that PBB may also have a toxic effect on the immune system (Kimbrough et al., 1978a). Some of the piglets that died from pneumonia had a smaller thymus with decreased numbers of lymphocytes in the cortical area. The changes in the

thymus could have resulted from protein-caloric malnutrition (Law et al., 1973), since the sick piglets stopped nursing. But the question of why only piglets nursing sows fed diets containing PBB had pneumonia remains unanswered.

The incidence of arthritis was not associated with PBB since arthritis occurred in control piglets also. The nervous signs observed in piglets nursing a sow fed a diet containing 200 ppm of PBB could have resulted from many causes. Hypovitaminosis A is a possibility, since decreased concentrations of vitamin A in the liver have been reported in rats given PBB (Pratt and Sleight, 1979; Mangkoewidjojo, 1979). However, in the present experiment PBB did not affect the liver concentrations of vitamin A of sows or piglets. The possibility of a viral infection or a manifestation of PBB toxicity cannot be excluded.

Body Weight

The weight of animals is usually adversely affected by PBB. Rat pups from dams given PBB during gestation weighed less than control pups at weaning age (Harris et al., 1978). Growing pigs fed diets containing 20 or 200 ppm of PBB for 16 weeks weighed less than control pigs (Ku et al., 1978). In contrast to what would be expected, piglets nursing sows fed diets containing 200 ppm of PBB weighed more than piglets nursing other sows (Figure 1). The litters of sows fed diets containing 200 ppm of PBB were smaller because of the higher mortality among those

piglets. Uneven litter sizes could induce false conclusions on the effects of PBB on the weight of piglets. Two other factors can be pointed out. Pigs given PBB in the work of Ku and co-authors weighed less than control pigs only after the 4th week on the experimental diet. Secondly, in the work of Ku and co-authors the pigs were fed *ad libitum*, and PBB are known to decrease feed intake (Jackson and Halbert, 1974; Sleight and Sanger, 1976; Ringer and Polin, 1977). Milk consumption of the piglets was not recorded because it would involve a nearly impossible task of weighing the piglets before and after every suckling period. It was impossible then to correlate weight gain with milk consumption.

Laboratory Results

None of the hematologic parameters evaluated in the present study was affected by PBB. Ku and co-workers (1978) reported decreased values for hemoglobin concentration and hematocrit of growing pigs only after 16 weeks of continuous feeding of diets containing 200 ppm of PBB. Dietary exposure of chickens to PBB also caused a decrease in hemoglobin concentration and hematocrit (Ringer and Polin, 1977).

Concentrations of BUN were significantly increased, but still within normal limits, in piglets nursing sows fed diets containing 100 or 200 ppm of PBB. The increase in BUN most likely represented increased protein catabolism since no lesions were observed in the kidneys. Impaired

kidney function would result in increased BUN only after 75% of the nephrons were nonfunctional (Duncan and Prasse, 1977). Sleight and Sanger (1976) also reported increased BUN without concurrent kidney lesions in rats fed diets containing 500 ppm of PBB. Moorhead and co-workers (1977) observed kidney lesions in pregnant heifers dosed orally with 25 g of PBB/day for 33 to 66 days. In that case, the lesions in the kidney may have been related to the extremely high dose of PBB used by the authors (67 mg/kg bw).

The decrease in SAP and SGPT concentrations in piglets from sows fed diets containing 100 or 200 ppm of PBB was unexpected. Decreased concentration of SAP associated with PBB toxicosis was also observed in growing pigs (Ku et al., 1978) and in rats (Mangkoewidjojo, 1979). Other authors did not measure the concentrations of SGPT. The reasons for the decrease in the serum concentrations of SAP and SGPT are unclear but, as stated earlier, may be related to effects of different PBB congeners.

Ornithine carbamyl transferase is found primarily in the liver, and normally only a small amount is present in the serum. Measuring the serum concentration of OCT instead of SGPT has been recommended to assess the degree of hepatocellular damage in animals other than dogs and cats (Duncan and Prasse, 1977). The increase in serum concentrations of OCT is specifically caused by hepatocellular disease. After acute liver necrosis or acute hepatitis, the serum concentration of OCT raises rapidly

and persists for approximately 3 weeks (Wolf et al., 1973). The serum concentrations of OCT were significantly higher in piglets from sows fed diets containing PBB. The increase in serum OCT was dose-related and it was assumed that the degree of elevation represented the degree of liver damage, even though no pathologic changes were observed in the liver of newborn piglets. The concentrations of OCT in the serum of sows varied considerably, but higher values of OCT were apparent in sows fed diets containing 100 or 200 ppm of PBB. Measuring the serum concentrations of OCT should be considered as a specific test for assessment of liver disease in swine.

Serum Electrophoresis

Decreased serum albumin concentrations in association with PBB-induced liver lesions were reported in heifers (Schambacher et al., 1978) and in rats (Sleight et al., 1978). Growing pigs fed diets containing as much as 200 ppm of PBB for 16 weeks did not have alterations in the serum protein profile (Ku et al., 1978). In the present experiment PBB did not induce a decrease in serum protein of sows and piglets. Newborn piglets from sows fed diets containing 200 ppm of PBB had slightly higher serum concentrations of albumin, probably caused by increased liver metabolism.

The serum concentrations of LDH isoenzymes were not good indicators of liver damage for sows and piglets. Only LDH-4 was significantly elevated in serum of sows

fed diets containing 200 ppm of PBB. It is difficult to understand why an elevation of LDH-4 without concomitant elevation of LDH-5 occurred. Although the liver is the most likely source of LDH-4 in the sows, it is not possible to pinpoint the tissue responsible for the increased serum concentration of this particular enzyme (Kachmar and Moss, 1976).

Thyroid Hormone Analysis

Triiodothyronine (T_3) is the functional thyroid hormone. Thyroxine (T_4) is deiodinized at the target cell to form T_3. The concentrations of T_3 and T_4 were decreased in a dose-related manner in the serum of piglets from sows fed diets containing 100 or 200 ppm of PBB. The T_3/T_4 ratios decreased as the concentrations of PBB in the sows' diet increased. Therefore, it appears that the concentrations of T_3 were affected more severely than the concentrations of T_4. At a concentration of 10 ppm in the sows' diet, PBB apparently induced higher concentrations of T_3 and T_4 in the serum of piglets. The different concentrations of PBB isomers in the PBB mixture possibly were responsible for the differences in responses observed with higher or lower doses of PBB. As for SAP and SGPT, studies using purified PBB isomers are needed to clarify these points.

Pathologic Changes in Organs

Liver

The increase in liver weight of piglets was directly proportional to the concentration of PBB in the sows' diet. Liver weights of sows were not increased by PBB. Age probably was a factor in the degree of hepatomegaly, since the most severe liver enlargement has been reported in young and growing animals (Sleight and Sanger, 1976; Sleight et al., 1978; Ku et al., 1978). Jackson and Halbert (1974) reported that dairy cows consuming PBB had enlarged livers, but these authors did not provide data on the liver weights of the cows. Several explanations have been proposed for hepatomegaly in PBB toxicosis. Mangkoewidjojo (1979) observed an increase in lipids and in endoplasmic reticulum in the hepatic cells of rats given PBB. Dent and co-workers (1976) reported an increase in microsomal protein. In the present study, the percentage of lipid content of the liver of treated sows and piglets was not increased, whereas microsomal protein was significantly increased in the liver of sows and piglets consuming PBB. Histologically, many hepatocytes were enlarged in the liver of sows and piglets consuming PBB. It appears, then, that hypertrophy was mainly responsible for the increase in liver weight.

Hepatocellular swelling and centrolobular necrosis were the most prominent histopathologic changes observed in the liver of sows and piglets. Probably the swollen

hepatocytes compressed the sinusoids and impaired the blood flow to the centrolobular hepatocytes. The severity of the lesions in the liver of sows may reflect the increased burden of the liver in being exposed to dietary PBB plus PBB mobilized from fat stores when the sows lost weight during the lactation period.

The focal hyperplastic changes observed in the liver of a sow fed a diet containing 100 ppm of PBB were of interest. Kimbrough and co-workers (1978b) described similar lesions in the liver of female rats given PBB, and the authors considered that these lesions were neoplastic. The lesions observed in the liver of the sow were similar to the lesions observed in the liver of women by Knowles and Wolff (1976). These authors diagnosed those lesions as focal nodular hyperplasia, and hormonal effects were considered as possible causes. In our sow the focal hyperplastic lesions may have been an incidental finding and may not have been a manifestation of PBB toxicosis.

Thyroid Gland

The increase in the weight of the thyroid gland of newborn piglets from sows fed diets containing 100 ppm of PBB was most likely due to hyperplasia. Thyroid hormone analyses indicated a dose-related effect only in piglets from sows fed diets containing 100 or 200 ppm of PBB. Increase in thyroid gland weight was observed only in newborn piglets from sows fed diets containing 100 ppm of

PBB and in 4-week-old piglets from sows fed 10 ppm of PBB. Again, differences in concentration and toxicity of the PBB congeners in the PBB mixture may account for the differences in effects on the thyroid gland of piglets.

There are doubts about the mechanism by which PBB affect the thyroid gland. Norris and co-workers (1974) suggested a physiologic competition between bromine and iodine in the thyroid gland. Mangkoewidjojo (1979) demonstrated interaction between iodine and PBB in the occurrence of thyroid hyperplasia in rats fed diets containing high concentrations of iodine and 100 ppm of PBB. There is evidence that bromine reduces the uptake of ^{131}I by the thyroid gland, and goiter occurred in rats fed diets containing bromine during the first year of life (Underwood, 1977). Ringer and Polin (1977) described thyroid hyperplasia in chickens given PBB and attributed the hyperplasia to enhanced catabolism of thyroxine in the liver. It is also possible that thyroidal effects are secondary to the effect of PBB on the pituitary gland. If so, measuring the serum concentrations of thyroid stimulating hormone should confirm that possibility (Berger and Quinn, 1977). Histologically, no pathologic changes were seen in the pituitary gland of sows and piglets.

Other Organs

The chronic interstitial pneumonia observed in sows and in 4-week-old piglets probably was related to the type

of bedding used. The bedding consisted of wood shavings or coarse sawdust and became extremely dusty. Inhaled dust may have induced the changes observed in the lungs.

Ku and co-workers (1978) reported a hyperplastic appearance on gross examination of the glandular portion of the stomach of pigs fed diets containing 200 ppm of PBB for 16 weeks. In the present experiment, such a change was not observed. The stomach of sows fed diets containing PBB did not weigh more than the stomach of control sows and hyperplastic changes were not observed during gross or microscopic examination of the stomach of sows and piglets.

Polybrominated Biphenyl Analysis

The PBB can cross the placenta and are absorbed by the developing fetus. Fries and Marrow (1975) stated that hexabrominated isomers are transferred more readily across biological membranes than the more highly brominated isomers. However, a heptabrominated isomer (peak 8) was present in the tissues of newborn piglets. The relative concentrations of certain hexabrominated isomers (peaks 4 and 5) in the liver and adipose tissue of sows and piglets increased as the concentrations of other PBB congeners decreased. Dannan and co-workers (1978b) demonstrated that only peaks 1 and 3 would be metabolized at a significant rate. Therefore, lower concentrations of peak 8 in tissues of sows and piglets probably reflect incomplete absorption or a slower movement across biological membranes.

There was an apparent decrease in PBB concentration in the adipose tissue of piglets with age. However, the fat stores increased considerably as the piglet grew older and the PBB were diluted in the fat. The total amount of PBB in the body actually increased manyfold.

Milk was an important route of elimination of PBB for the sow. The elimination of PBB through the milk was increased because the sows lost weight during lactation. According to Fries and co-workers (1978), each unit of milk fat would clear a larger fraction of the body burden of PBB as the amount of body fat is reduced. Much more PBB were transferred to the piglet through the milk than through the placenta. A similar observation was made in pregnant and lactating rats (Rickert et al., 1978). At the end of lactation, the sows fed diets containing 200 ppm of PBB (500 mg PBB/sow/day, or 2.5 mg PBB/kg body weight) were eliminating approximately 63 mg of PBB daily in their milk, or the equivalent of 12.6% of the daily intake. Considering that the sow produced a daily average of 0.7 kg of milk/piglet (Barber et al., 1955), each piglet consumed approximately 15.8 mg of PBB daily, or 2.0 mg PBB/kg body weight. On a body weight basis, this amount is only slightly less than the sows' daily consumption of PBB. Consequently, the concentration of PBB in adipose tissue of nursing piglets was roughly comparable to the concentration of PBB in the adipose tissue of the sow.

On a fat basis, the concentration of PBB was the highest in the colostrum, probably because of the lower fat concentration. Willett and Irving (1976) and Fries (1978) noted a sharp decrease in the concentration of PBB in the milk of cows after the first 5 to 10 days of lactation. Fries suggested that during the dry period the PBB in the mammary gland are in equilibrium with the PBB in the remaining body fat. At the beginning of the lactation period the PBB in the mammary gland are rapidly transferred to the milk. Later, the concentration of PBB in the milk depends on a slower transfer of PBB from the body fat.

Apparently PBB in the milk come directly from the body fat without being metabolized by the liver, since the PBB congeners in the milk were in the same proportion as in the sows' adipose tissue (Figure 13). It is evident also that the piglets consumed a somewhat different mixture of PBB than the mixture given to the sows. More studies on the kinetics and toxicity of purified PBB congeners, probably using radiolabeled components, are needed.

Microsomal Enzymes

The PBB are considered as a mixed-type inducer of microsomal drug metabolizing enzymes. Both PB-type enzymes (hexobarbital hydroxylase, ethylmorphine demethylase) and 3MC-type enzymes (ethoxycoumarin deethylase, aryl hydrocarbon hydroxylase) were induced in sows given PBB and in piglets consuming their milk. It is uncertain

which of the PBB congeners are responsible for the induction of which enzymes. Moore and co-workers (1978, 1979) demonstrated that 2,4,5,2',4',5'-hexa- (peak 4) and 2,3,4,5,2',4',5'-heptabromobiphenyl (peak 8) are stricly PB-type inducers. These authors further stated that components not yet identified, perhaps a contaminant, are responsible for the 3MC-like action. Dannan and co-workers (1978) reported that 2,4,5,3',4',5'-hexabrominated biphenyl (peak 6) induced both PB- and 3MC-type microsomal enzymes.

Increases in microsomal protein and in microsomal enzymes were not observed in the newborn piglet. Most likely, the enzymatic systems of the newborn pig are not fully developed. Studies have indicated that immature animals lack, or possess low activities of, many hepatic microsomal enzymes (Basu et al., 1971; Dickerson and Basu, 1975).

Alterations in microsomal enzyme activity can result in altered susceptibility to other toxic compounds. For example, the sleeping time during pentobarbital anesthesia was decreased, whereas bromobenzene lethality was increased, in rats previously exposed to PBB (McCormack, 1979). The induction of microsomal enzymes enhanced by sixfold the amount of benzo(a)pyrene metabolites binding to DNA (Dannan et al., 1978). Benzo(a)pyrene, a carcinogen found in tobacco smoke and in the polluted atmosphere, is only one of the substrates for aryl hydrocarbon hydroxylase (AHH). This enzyme was induced in kidneys of 4-week-old piglets in the present experiment. Aryl hydrocarbon hydroxylase

activity was not measured in the livers of sows and piglets, but most likely it was increased as well. Some workers have associated the toxicity of certain aromatic compounds to their ability to increase the activity of AHH (Poland and Glover, 1977).

Altered microsomal enzyme activity may alter the metabolism of endogenous compounds such as hormones and vitamins. Rats fed diets containing PBB had thyroid hyperplasia (Aftosmis et al., 1972b; Norris et al., 1974; Sleight et al., 1978) or had decreased hepatic concentrations of vitamin A (Pratt and Sleight, 1979; Mangkoewidjojo, 1979; MacCormack, 1979). There is doubt as to whether the effect on the thyroid hormones reflects enhanced catabolism by the liver or a physiologic competition between bromine and iodine in the thyroid gland. In the present experiment, concentrations of thyroid hormones were significantly decreased in the serum of piglets from sows fed diets containing 100 or 200 ppm of PBB. Hepatic concentrations of vitamin A were not affected by PBB. Species differences may account in part for the differences in results found in this work and in the work done by other authors.

SUMMARY AND CONCLUSIONS

Twelve pregnant sows were fed diets containing 0, 10, 100 or 200 ppm of PBB during the last half of gestation and during lactation. Immediately after birth, and before nursing, approximately 1/3 of each litter was killed and necropsied. The remainder of the litters and the sows were killed and necropsied 4 weeks later.

There was significantly higher mortality among piglets nursing sows fed diets containing PBB. However, no clinical sign could be directly attributed to PBB toxicosis. The weight gain of the surviving piglets was not affected adversely by PBB. Sows and newborn piglets were not affected clinically.

Transplacental passage of PBB to the fetuses occurred, but far more PBB were transferred to the piglets through the milk. On a body weight basis, the piglets consumed PBB in the milk in concentrations similar to the concentrations given to the sows. The PBB accumulated preferentially in the body fat of sows and piglets. On a lipid basis, highest concentrations of PBB were in the liver, followed by the adipose tissue, kidney, and brain, in decreasing order. Analyses of gas chromatograms indicated that piglets consumed a somewhat different PBB mixture than the PBB given to the sows. The PBB present in the

milk apparently came directly from the sows' adipose tissue without being metabolized by the liver, since the proportions of the PBB congeners were nearly identical in the sows' adipose tissue and in the milk.

Some of the changes observed were not proportionally related to the concentrations of PBB in the sows' diet. For example, concentrations of 10 ppm of PBB induced an increase in the concentrations of thyroid hormones, serum alkaline phosphatase (SAP), and serum glutamic pyruvic transaminase (SGPT), whereas a concentration of 100 or 200 ppm of PBB caused a decrease. The different concentrations and toxicity of individual PBB congeners in the mixture of PBB given to the sows may account for that type of response. Studies using purified PBB congeners are needed to clarify this point.

Thyroid weight to body weight ratios were increased in piglets from sows fed diets containing 100 or 200 ppm of PBB. The serum concentrations of triiodothyronine (T_3) and thyroxine (T_4) were decreased in a dose-related manner in newborn and in 4-week-old piglets from sows fed diets containing 100 or 200 ppm of PBB. The concentrations of T_3 and T_4 were increased in piglets from sows fed diets containing 10 ppm of PBB. Apparently the concentrations of T_3 were affected more intensely than the concentrations of T_4, since the ratio of T_3/T_4 decreased proportionally to the increase of the concentrations of PBB in the sows' diet. Microscopically, the thyroid glands of newborn piglets from sows fed diets containing 100 or 200 ppm of PBB

were slightly hyperplastic and the colloid was scanty and vacuolated.

There was an increase in the serum concentrations of blood urea nitrogen (BUN) in newborn piglets from sows fed diets containing 200 ppm of PBB. Since there were no histopathologic changes in the kidneys, the increase in BUN was attributed to an increase in protein catabolism. Hematologic values were not affected in sows or piglets.

Measuring the serum concentrations of ornithine carbamyl transferase was the most effective clinical test in assessing the severity of PBB-induced liver damage in sows and piglets. Hepatic damage was not detected by analysis for serum cholesterol, SAP or SGPT, or by serum electrophoresis of proteins, lipoprotein, and lactic dehydrogenase (LDH) isoenzymes.

The weight of the liver was increased in a dose-related manner in 4-week-old piglets but was not affected in newborn piglets and in sows. Microscopically, the lesions were more severe in the liver of sows than in the liver of 4-week-old piglets. Swelling of hepatocytes and centrolobular necrosis were the most prominent lesions observed. Lesions were not present in the liver of newborn piglets. One sow fed a diet containing 100 ppm of PBB had several hyperplastic nodules in the liver, but this may be only an incidental finding unrelated to PBB toxicosis. The concentrations of vitamin A in the liver of piglets at birth and at 4 weeks of age were not affected by PBB.

Microsomal drug-metabolizing enzymes were induced in a dose-related manner in the liver of sows and 4-week-old piglets. There was no induction of those enzymes in the newborn piglet. In the 4-week-old piglets there was an increase in microsomal protein, cytochrome P_{450}, and the activities of hexobarbital hydroxylase, ethylmorphine demethylase and ethoxycoumarin deethylase. The activity of aryl hydrocarbon hydroxylase (AHH) was measured only in the kidney of 4-week-old piglets, and the activity was increased in a dose-related manner.

APPENDIX

Table A1. Mean concentrations of serum cholesterol of sows and their piglets at birth and at 4 weeks of age. The sows were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

PBB in diet (ppm)	Serum cholesterol (mg/dl)		
	Sow	Newborn	4-week-old
0	73.5±1.8	64.1± 7.5	210.0±33.4
10	81.0±6.0	77.0± 0.0	238.2± 7.8
100	59.3±1.9	66.8± 9.9	235.0±22.1
200	75.0±0.5	92.7±18.3	221.1±24.5

Values represent mean ± SEM.

Table A2. Mean concentrations of vitamin A in the liver of piglets at birth and at 4 weeks of age. Dams were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

PBB in sows' diet (ppm)	Hepatic concentration of vitamin A (µg/100 g)	
	At birth	At 4 weeks of age
0	2260±214	3852±270
10	a	a
100	2170±860	3885±761
200	1800±622	5284±856

Values represent mean ± SEM.

[a]Not analyzed.

Table A3. Mean concentrations (percent) of serum proteins and albumin/globulin (A/G) ratios in sows and their piglets at birth and at 4 weeks of age. The sows were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

PBB in sows' diet (ppm)	Protein fractions (%)				A/G
	Albumin	Alpha	Beta	Gamma	
Sows					
0	46.4±3.5	16.6±1.0	5.4±0.5	31.7±2.8	0.9±0.1
10	46.6±6.3	15.8±1.1	6.5±0.1	31.2±7.3	0.9±0.2
100	43.1±1.7	15.7±0.4	6.9±0.6	34.2±1.3	0.8±0.1
200	46.1±0.2	16.1±0.8	5.6±0.8	32.3±1.4	0.8±0.0
Newborn piglets					
0	14.0±1.3	52.1±2.8	14.2±2.2	20.5±0.8	0.2±0.0
10	[a]	53.5±4.9	13.8±1.5	19.6±0.4	0.1±0.0
100	14.0±1.6	46.0±3.3	13.0±2.3	24.8±2.4	0.2±0.0
200	18.0±1.4	52.2±1.8	10.5±0.7	17.6±3.5	0.2±0.0
4-week-old piglets					
0	54.3±0.7	19.7±0.5	6.1±0.4	20.0±1.0	1.2±0.0
10	56.5±1.8	19.8±0.9	4.1±0.4	18.9±1.4	1.3±0.1
100	53.9±0.5	19.3±0.4	4.9±0.3	21.9±0.6	1.2±0.0
200	54.8±1.2	19.4±0.7	6.4±0.7	19.4±0.9	1.2±0.1

Values represent mean ± SEM.

[a]Not analyzed.

Table A4. Mean concentrations (percent) of lactic dehydrogenase (LDH) isoenzymes in the serum of sows and their piglets at birth and at 4 weeks of age. The sows were fed diets containing different concentrations of PBB during the last half of gestation and during lactation.

PBB in sows' diet (ppm)	LDH isoenzymes (percent)					Total LDH (IU/1)
	LDH-1	LDH-2	LDH-3	LDH-4	LDH-5	
Sows						
0	7.4± 2.7	8.4±0.8	17.1±1.3	14.0±0.4	53.2± 3.8	235± 32
10	5.0± 3.5	8.2±6.7	17.4±6.0	11.2±2.1	58.2±18.3	272± 9
100	10.8± 4.2	11.4±0.8	17.6±1.1	13.2±2.9	47.0± 6.7	303± 47
200	7.5± 3.4	10.4±0.9	18.8±0.8	22.6±2.7[a]	40.8± 4.3	219±171
Newborn piglets						
0	19.4± 2.8	12.5±3.7	16.9±3.8	10.6±1.7	42.8±10.9	391± 45
10	21.2± 1.6	15.4±1.4	22.9±3.3	11.8±4.2	26.8± 4.6	405±162
100	21.6±10.0	17.3±3.5	23.3±5.4	10.6±1.4	27.2± 8.3	322± 82
200	20.9±10.3	20.6±1.0	23.4±0.1	11.9±2.1	23.3± 6.4	271± 54
4-week-old piglets						
0	3.8± 1.3	10.6±1.8	24.7±1.0	22.0±0.7	38.5± 3.5	408± 23
10	9.7± 1.3	13.3±0.7	23.9±1.2	20.0±2.4	32.9± 1.6	493± 61
100	12.4± 1.1	15.4±1.5	25.2±0.7	20.1±1.2	29.0± 3.0	422± 61
200	5.4± 1.5	13.7±1.2	26.7±0.5	21.2±1.2	33.2± 1.9	362± 15

[a]Different ($p<0.05$) from other treatment groups.

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