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TOXICOPATHOLOGIC EFFECTS OF POLYBROMINATED BIPHENYLS (PBB) ON LACTATING GUINEA PIGS AND THEIR NEONATES

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TOXICOPATHOLOGIC EFFECTS OF POLYBROMINATED BIPHENYLS (PBB) ON LACTATING GUINEA PIGS AND THEIR NEONATES

By

Alexander Dale Hall

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TOXICOPATHOLOGIC EFFECTS OF POLYBROMINATED BIPHENYLS (PBB) ON LACTATING GUINEA PIGS AND THEIR NEONATES

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Twenty pregnant guinea pigs were randomly placed into 5 equal groups and fed diets containing 0, 1 or 10 ppm PBB (Firemaster BP-6) during gestation. Two groups also received PBB in their diets during lactation. At all ages, the piglets from the sows ingesting PBB were smaller and their livers weighed less than those of the controls. Conversely, the livers of treated sows were larger than those of control sows.

Tissue levels of PBB increased with increased dietary levels of PBB. The livers of neonates contained higher levels of PBB than the livers of their dams. The highest tissue levels of PBB were found in 3-week-old piglets whose dams were fed diets containing 10 ppm PBB throughout gestation and lactation.

Blood urea nitrogen levels were elevated in newborn piglets whose dams had ingested PBB. Serum sorbitol dehydrogenase and hydroxybutyric dehydrogenase were not altered by PBB. Gross and histopathologic tissue changes were inconsistent.

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INTRODUCTION

Like a tiny pebble falling into the middle of a quiet farm pond as the mist rises in the early morning haze, so started an environmental contamination that the people of Michigan will long remember. Inadvertently dropped by a passing bird, this stone sets up a series of ever-expanding ripples which shatter the peaceful reflections on the surface of the water. Similarly, the Michigan livestock industry was rocked as polybrominated biphenyls, or "PBB", insidiously found its way into mills and grain elevators throughout this state.

The ripples on the pond turn into waves, and every shore will eventually be affected by their far-reaching infiltration. And so it was that in the nine months from the summer of 1973 to the spring of 1974 the cross-contamination of livestock feeds expanded the spread of PBB to nearly every shore of Michigan and throughout much of the land in between. The Michigan food chain had been penetrated by this xenobiotic and many men, women and children would ingest various amounts of PBB.

Several papers have been published on the series of events that led to the contamination of the Michigan food chain with the flame-retardant chemical known as Firemaster BP-6 (4,13,18,26,51). As a result of this contamination, thousands of cattle, chickens and swine were condemned and slaughtered. Likewise, tons of meat, milk, eggs and dairy products had to be destroyed. But this was just the first

small step in the attempted decontamination process. Millions of dollars were spent in legal reimbursements to farmers. Millions more in loans were made available to the agricultural community to assist the farmers' recovery and to enable them to begin again.

Now six years later, with much of the pain and near panic behind us, the Michigan agricultural community has essentially rid itself of PBB and the stigma that was associated with this contamination. Yet what are the real effects of PBB in humans and what will they be five, ten or twenty years from now?

To date, much of the research dealing with PBB has been accomplished through the use of livestock species (cattle and swine) and laboratory animals (rats and mice). Limited studies have been performed with poultry, dogs, mink, and non-human primates. The use of the guinea pig as an experimental model for PBB toxicosis has been very slight. Therefore, the objectives of this experiment were to study the pathologic effects of PBB on pregnant and lactating guinea pigs and their neonates. Correlations between dietary concentrations of PBB and tissue concentrations were made with respect to tissue changes in the sow, the newborn, and the weanling piglet.

ultraviolet light can degrade PBB to lesser brominated biphenyls (12). Brominated naphthalenes and brominated dibenzofurans are possible contaminants of this mixture that may cause at least some of the clinical signs or lesions associated with PBB toxicosis (28).

Kinetics

Willett and Durst (54) found measurable levels of PBB in plasma within 4 hours of oral administration of PBB to cattle. With continuous oral exposure to PBB, these same researchers concluded that plasma steady-state levels were reached in 15 days. Approximately 50% of the daily intake was excreted in the feces, yet no measurable amounts could be detected in the urine. The feces, then, is the major route of excretion in the nonlactating (or non-egg laying) animal.

Polybrominated biphenyls are lipophilic compounds, and thus it would be expected that those tissues with the highest fat content would also have the highest concentrations of PBB. However, brain tissue, which is a lipid-rich tissue, generally has one of the lowest levels of PBB of any tissue of the body. This is probably due to a combination of 2 factors: 1) the effectiveness of the blood-brain barrier, and 2) the different type of lipids present in the brain, i.e., phospholipids (17). Due to the propensity for adipose tissue to accumulate PBB (49), the steady-state levels in fat are reached much more slowly than plasma (17). There appears to be more resistance to PBB crossing biologic membranes as the number of bromine atoms on the biphenyl ring increases; similarly, this increased bromination leads to slower metabolism of this compound. However, Dannan et al. (8) also concluded that the arrangement of the bromine atom affected the resistance of the PBB compound to microsomal metabolism. They stated

that bromination of both para positions of the biphenyl ring increased the resistance to metabolism, regardless of the number of bromine atoms.

Another major form of excretion of PBB is lactation. Since mammary tissue and milk both have a high fat content, PBB tend to accumulate in them. Willett and Irving (55) were able to detect PBB in milk within 13 hours after oral administration. Polybrominated biphenyl levels peaked in the milk in approximately 60 hours, at which point 23% of the daily ingested dose was being excreted via the milk (55). Fries (14) reported that as long as the feed contained PBB, the levels of PBB were higher in the milk than in the body fat. In general, the time to reach steady state in milk is inversely related to the ease with which the compound enters the body fat (16). Upon ceasing the oral administration of PBB, a steady-state concentration ratio was reached between bovine milk fat and body fat of 0.42:1 (15). When PBB contaminated feed is no longer available to the lactating cow, the concentration of PBB in the milk decreases in a 2-phase pattern with a rapid decrease in the first 10 to 15 days followed by a more gradual decline. Fries (15) found that after 60 days the PBB milk fat concentration had dropped by 60 to 70%. This decline was controlled by 3 factors: 1) the level of milk production, 2) total amount of body fat, and 3) changes in body fat concentration.

Another method of elimination of PBB is transplacental transfer. Fries et al. (17) and Rickert et al. (46), studying cattle and rats respectively, demonstrated that the fetal tissue levels of PBB were about 1/3 those of the dam. Even though the tissue levels were less in the fetus, the distribution throughout the body was nearly identical to the dam (54). Rickert et al. (46) also showed that the rat pups

received more PBB via the milk than transplacentally. Another significant finding of this same study was that the PBB concentrations in the liver of the nursing rat pup were higher than the liver levels of its dam. Thus, while the excretion of PBB via the milk was beneficial to the dam, it jeopardized the health of the nursing neonate.

Biochemical Pharmacology

Polybrominated biphenyls have been shown repeatedly to be inducers of the mixed function oxidase system (MFO). This system is responsible for the metabolism of many xenobiotics as well as some endogenous compounds. The MFO system is located within the endoplasmic reticulum of individual cells of various tissues. There are 2 major, distinct types of inducing agents: 1) the phenobarbital (PB) type inducers, and 2) the 3-methylcholanthrene (3MC) type inducers. The PB-type agents induce NADPH-cytochrome P-450 reductase, epoxide hydratase, and aminopyrine demethylation, while 3MC agents induce aryl hydrocarbon hydroxylase (AHH), UDP-glucuronyltransferase, and benzo[a]pyrene hydroxylation (9). Several investigators have shown that PBB are mixed-type inducers, i.e., they have properties of both PB-type and 3MC-type induction (48).

Recent studies (43) using 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a potent AHH inducer, have elucidated the presence of a hepatic cytosolic-binding protein that acts as a receptor for TCDD and other stereospecific compounds. The combination of TCDD with this receptor apparently acts as a stimulus for the production of AHH. There also appears to be a high degree of correlation between the binding affinity of these compounds for the receptor protein and their toxic potencies.

Moore and Aust (35) have identified at least 8 of the 14 congeners found in Firemaster BP-6. Of these 8, the enzyme induction patterns of the following have been characterized: peak 6 - 2,3',4,4',5,5'-hexabromobiphenyl - PB type and 3MC type; peak 4 - 2,2',4,4',5,5'-hexabromobiphenyl; peak 8 - 2,2',3,4,4',5,5'-heptabromobiphenyl; and peak 12 - 2,2',3,3',4,4',5,5'-octabromobiphenyl. Peaks 4, 8 and 12 are all PB-type inducers (3,9,10,35,36,38,39).

What is the significance of these enzymatic inductions? Aryl hydrocarbon hydroxylase (AHH), which is induced by 3MC-type agents, catalyzes the formation of very reactive arene oxides from inert aromatic compounds. Epoxide hydratase (EH), a PB-induced enzyme, metabolizes arene oxides to less toxic dihydrodiols. In the liver, both of these enzymes are increased by PBB administration (11). However, McCormack et al. (34) found increased AHH levels with decreased EH levels in the kidney of rats treated with PBB, and Dent et al. (11) found similar enzyme activities in rat mammary tissues. Thus, with increased AHH and concomitant decreased EH activity, the kidney (and mammary tissue) could accumulate excess arene oxides and thus predispose these tissues to toxicities by other compounds. This was supported by Kluwe et al. (29), who observed that mice that had been pretreated with PBB were more susceptible to renal and hepatic toxicosis caused by chlorinated hydrocarbons.

Dent (10) demonstrated that PBB are effective enzyme-inducing agents in rat fetuses because of their ability to cross the placental barrier. Dent also stated that developing rats are apparently more sensitive to the 3MC-type agents. Moore et al. (36) found that the ability of PBB to cause a mixed-type induction was passed through the milk of lactating rats. Moore discovered that the nursing rat pups were actually affected more than their mothers. He speculated that

this increased effect might be due to the milk concentrations of PBB congeners being different from the original mixture given to the dam or to the fact that the neonatal liver might be more sensitive to PBB.

Clinical Signs and Tissue Changes

The first description of clinical signs in dairy cattle fed a PBB-tainted ration during the original contamination in 1973 came from Jackson and Halbert (23). They noted anorexia, decreased milk production, frequent urination, excessive lacrimation, sporadic lameness, and abnormal hoof growth. Jackson also mentioned an increase in calf mortality. Moorehead et al. (40) observed many similar clinical signs in pregnant heifers that had been given 25 g PBB/day. Depression, dehydration, diarrhea, emaciation, and abortions were also seen in this experiment. (Other heifers that were given 0.25 mg and 250 mg had no clinical signs of illness that could be associated with PBB.) Gross lesions at the time of necropsy included: dehydration, subcutaneous emphysema and hemorrhage, enlarged liver and kidneys, abomasal edema, mucoid enteritis, fetal death, pneumonia, and thymic atrophy (40,41,49). All these lesions were again confined to the group given 25 g PBB/day, with no visible lesions in the heifers given the lower dosages. The following histologic changes were observed in the animals dosed with 25 g PBB/day: fatty degeneration and glycogen depletion of the liver; dilatation of renal tubules and collecting ducts with epithelial degeneration, hyperplasia and dilatation of mucous glands in the gallbladder; mucosal edema and hemorrhage of the terminal colon; and hyperkeratosis of the skin (40,41).

From the PBB toxicity studies which have been completed in pigs, several interesting conclusions have been made. Ku et al. (30)

demonstrated a dose-related decrease in weight gain over time with pigs fed a diet containing PBB. However, the pigs given the highest level of PBB (200 ppm) posted the most efficient feed conversion to weight gain. In a study utilizing pregnant and lactating sows and their neonates, Werner (52) could not find any significant differences in the weight gains of the nursing piglets up to 4 weeks of age. Werner did note a dose-related increase in liver weight to body weight ratio in the piglets at 4 weeks of age. These same piglets had centrolobular hepatocellular necrosis and diffusely swollen hepatocytes as opposed to no histologic lesions in their newborn littermates. Werner (52) also found increased levels of serum alkaline phosphatase (SAP) and serum glutamic pyruvic transaminase (SGPT) in the piglets nursing dams on a ration containing 10 ppm PBB, while those piglets nursing dams with 100 ppm and 200 ppm PBB in the diet had decreased levels of these same enzymes. Newborn piglets farrowed by dams given a diet containing 200 ppm PBB had increased levels of blood urea nitrogen (BUN).

In the area of food animal research, PBB toxicosis in poultry has also received some emphasis. Ringer (47) found that various dietary levels of PBB produced decreased appetite with resulting loss of body weight. Specific organs, such as the testes, spleen and bursa of Fabricius, also decreased in size. In contrast, the weights of the liver and thyroid gland from PBB-fed birds were increased. Premature regression of the bursa and thymus with a depletion of lymphocytes was seen histologically. Hydropericardium and ascites were consistent gross findings. Polin and Ringer (44) studied the effects of PBB on laying hens. Their research concluded that 45 ppm PBB or higher in the diet resulted in decreased production,

hatchability and viability of offspring. Feed intake was decreased at 125 ppm and inanition became marked at 625 ppm and higher. All production eventually returned to normal at various lengths of time following the cessation of PBB intake with the diet. Although there was a complete loss of egg production by those birds fed levels of 625 and 3125 ppm PBB within 2 weeks of the onset of PBB intake, their production did reach precontamination levels 5 to 6 weeks following the removal of PBB from the diet. Even though production returned to normal in the birds fed the higher doses, hatchability remained low.

One animal that apparently is very susceptible to the effects of PBB is the mink. Aulerich and Ringer (2) found that daily diets including 6.25 ppm PBB became lethal to adult mink in 10 months. Likewise, 1 to 2.5 ppm over 9 months resulted in decreased litter size, decreased kit weight at birth, and decreased kit survival. They found that the body fat residue of PBB was about 60 times greater than dietary levels.

Allen, Lambrecht, and Barsotti (1) studied the effect of PBB on rhesus monkeys. The following is a list of their findings as they are related to clinical signs and lesions: anorexia, weight loss, joint swelling, dry skin, decreased immunoglobulins and altered T-cell function, liver enlargement with improved liver function, and hyperplastic, ulcerative gastroenteritis (1). Lambrecht (32) also reported that 0.3 ppm PBB given to female monkeys over a 15-month period prolonged the menstrual cycle and resulted in smaller newborn that exhibited a slower rate of growth.

In one of the earlier studies involving the pathologic changes associated with PBB toxicosis in laboratory animals, Sleight and Sanger (50) found that a diet containing 500 ppm PBB caused a decreased

weight gain and feed efficiency in growing rats. The livers of these rats were approximately twice the weight of the livers of control animals. The hepatocytes were swollen and vacuolated, and there was an increased amount of smooth endoplasmic reticulum (SER), enlarged hepatic mitochondria, and the presence of myelin bodies within the cytoplasm.

Ultrastructural changes produced by PBB toxicosis in mice were further studied by Corbett et al. (6). These findings included: decreased amounts of rough endoplasmic reticulum, increased smooth endoplasmic reticulum, mitochondrial degeneration with loss of cristae, increased numbers of lysosomes, and enlarged nuclei with increased numbers of nucleoli and the occurrence of intranuclear pseudoinclusions.

Gupta and Moore (20) orally administered PBB to male and female rats and found the females to be apparently more susceptible to lower doses of PBB than the males. They calculated the LD_{50} as 149 mg/kg/day for males as opposed to 65 mg/kg/day for females. The female rats also showed signs of excess porphyrin accumulation in the teeth, bones, and liver.

In a study utilizing pregnant rats, Harris et al. (21) administered 0.5, 1, 5, and 10 mg PBB daily from day 7 to day 15 of gestation. These researchers found no significant effect on fetal mortality, length of fetuses, or weight of fetuses. No malformed fetuses were observed. Wertz and Ficsor (53) concluded that PBB does not cause chromosome aberrations in the developing fetus. Corbett et al. (5) fed PBB to pregnant rats and mice at 100 ppm and 1,000 ppm levels. From this experiment they discovered that the higher levels did cause a decreased mean fetal weight. They also observed a few occurrences

of exencephaly, cleft palate and hydronephrosis and thus concluded that PBB was "weakly teratogenic."

Sleight and Sanger (50) also performed a pilot study on PBB toxicosis in guinea pigs. With 500 ppm PBB in the diet, the guinea pigs completely refused their food and all 6 animals died within 15 days. At a dietary level of 100 ppm, anorexia was also noted and 4 of the 6 pigs had died by day 30. At 1 and 10 ppm PBB there were no signs of clinical toxicosis in the guinea pigs and there were no consistent liver changes. Microscopically, there was some hepatocellular swelling with the presence of large vacuoles.

Kasza (24) performed clinicopathologic studies on Beagles that had received PBB in their daily diet for 61 days. At 4 mg/kg/day Kasza noted a decreased total number of hematopoietic cells, an increased M:E ratio, focal necrosis of the bone marrow, and proliferation of the reticuloendothelial cells of the bone marrow. The spleen contained marked extramedullary hematopoiesis. From tests conducted on mice fed 3 and 30 ppm PBB daily for 30 days, Luster et al. (33) concluded that PBB did suppress the cell-mediated immunity. Kately (25), on the other hand, reported that from his studies with cattle PBB does not alter or interfere with lymphocyte surface antigens or the biological events required for antibody formation and cell-mediated immune reactions.

To briefly summarize this section, chronic toxicity with PBB may result in: decreased fertility in mink and avians, decreased hatchability in chickens, decreased survival rate of newborn calves, chicks and mink, abortion in cattle, suppression of cellular immunity in mice and rats, and weak teratogenicity in mice.

Yusho

The PBB contamination of the Michigan food chain in 1973 was not the first time that an industrial chemical had accidentally polluted the environment, nor, unfortunately, will it probably be the last. Since the mid-1930s, polychlorinated biphenyls have been insidiously leaking into the environment. These PCBs, as they are more commonly called, were widely used in many industries as heat exchangers. In 1968 an unknown quantity of PCB, under the trade name of Kanechlor 400, found its way into rice oil being manufactured in the Japanese village of Yusho. It was later discovered that the concentration of PCB in this tainted oil was over 2,500 ppm. Hundreds of Japanese people ingested PCB in alarmingly high levels because of the common practice of using rice oil in daily meals. The syndrome of clinical signs and illnesses that resulted from this toxicant became known as "Yusho disease." Further tests elucidated the presence of chlorinated dibenzofurans (PCDF) within the rice oil as a contaminant of the PCB.

The clinical signs of Yusho disease are: severe chloracne and increased skin pigmentation, excessive eye discharge, transient visual disturbances, weakness, numbness in limbs, headaches, and disturbances of liver function (7). Babies that were born to PCB-affected women were small and slow growing (31). The regression of signs and symptoms of Yusho disease was very slow, and even today many people still are plagued by the consequences of this accident.

Polychlorinated biphenyls, like PBB, will bioaccumulate, and as much as 2,000 mg of PCB were found within the bodies of some Japanese people (7). Polychlorinated biphenyls also cause induction of hepatic enzymes and have been shown to produce hyperplasia (or neoplastic nodules) in the liver of affected mice and rats (22,27). Polychlorinated

biphenyls appear to be more slowly metabolized and excreted from the body, since they remain at higher concentrations in the liver for a longer period of time than PBB (22). Milk from mammals will contain higher levels of PCB when fed at equal concentrations. So, while there are comparisons that can be drawn between PCB and PBB, there are also many dissimilarities.

MATERIALS AND METHODS

Experimental Design

Twenty bred English guinea pig sows were randomly placed into 5 experimental groups. Table 1 enumerates these 5 groups and the number of offspring in each. Since these sows were pen-bred prior to their arrival on campus, there were no known breeding dates. The length of time between the initiation of the experiment and parturition varied from 5 weeks to 9 weeks (the average gestation period for a guinea pig being 10 weeks). Therefore, this trial did lack uniformity in the length of time that individual guinea pigs were exposed to PBB.

The sows were fed a commercial pelleted guinea pig diet[a] which had been ground to a powdery consistency to allow thorough mixing and equal distribution of the PBB additive.[b] This diet was offered to the sows *ad libitum* along with fresh drinking water. Four sows in each group were fed diets containing 0, 1, and 10 ppm PBB during gestation only. For these 3 groups, the lactational diet contained no PBB. Two other groups of 4 sows each had 1 and 10 ppm PBB, respectively, during their gestation and lactation.

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[a]Purina Guinea Pig Chow, Ralston-Purina Company, Checkerboard Square, St. Louis, MO.

[b]Firemaster BP-6, Michigan Chemical Company, St. Louis, MI.

Table 1. Experimental design

Concentration of PBB in Sows' Diet (ppm)	No. of Sows	Total No. of Piglets	No. of Piglets Necropsied At	
			1 day old	3 weeks old
0	4	11[b]	4	7
1 (gestation only)	4	14	4	10
10 (gestation only)	3[a,c]	7	2	5
1 (gestation and lactation)	4[d]	8	3	5
10 (gestation and lactation)	4	11	4	7
Total	19	51	17	34

[a]One sow died as a result of dystocia.

[b]Two additional piglets were stillborn (1 each in 2 separate litters).

[c]One sow delivered 3 mummified fetuses.

[d]One sow delivered 4 stillborn piglets.

The sows were kept in standard guinea pig cages with 2 sows per cage. The sows were weighed weekly during their gestation and lactation and on the day of parturition. The piglets were weighed on their day of birth and once weekly thereafter, until 3 weeks of age.

One piglet from each litter was euthanatized and necropsied at 1 day of age. The sows and remaining piglets were euthanatized and necropsied at 3 weeks after the birth date.

Experimental Evaluations

The following is a list of the observations and testing procedures employed in this experiment:

1. Body weight changes
2. Clinical signs
3. Gross and light microscopic tissue examination
4. Absolute liver weight as well as liver weights relative to total body weight
5. Hematology
    a. Complete blood count (CBC)
    b. Differential blood count
    c. Packed cell volume (PCV)
6. Serum chemistries
    a. Blood urea nitrogen (BUN) (mg/dl)
    b. Sorbitol dehydrogenase (SDH) (IU/l)
    c. Hydroxybutyric dehydrogenase (HBD) (IU/l)
7. Liver and adipose tissue analysis of polybrominated biphenyl levels
8. Analysis of sows' milk for PBB levels

Sample Collection

Milk Samples

Milk samples from lactating guinea pigs were collected by hand and with the aid of a "guinea pig milking machine" as described by Gupta (19). The nursing piglets were separated from their dam for 4 to 6 hours prior to milking. The sample was collected in a 10 ml test tube and stored at -20 C until processed for PBB content.

Necropsy - Hematologic Samples - Tissue Samples

All guinea pigs were euthanatized by a lethal intraperitoneal injection of sodium pentobarbital. After loss of consciousness, but prior to cardiac arrest, blood samples were obtained by cardiac puncture with a 20-gauge, 1-1/2 inch needle and 10 ml syringe. A portion of this sample was placed into a tube containing the anti-coagulant ethylenediaminetetraacetic acid (EDTA), and the remainder of the sample was allowed to clot and the serum removed following centrifugation.

A postmortem examination of each animal was performed and the liver was weighed on a top loading balance.[C] Brain, lung, heart, liver, kidney, stomach, ileum, and spleen were removed and portions of these tissues placed into 10% neutral buffered formalin for future histologic examination. Samples of liver and body fat were frozen until processed for PBB analysis.

[C]Mettler Series P, Model 163, Mettler Instrument Corporation, Hightstown, NY.

Hematologic Evaluation

The blood cell counts and PCV were determined by the use of an electronic counter.[d] The differential blood cell evaluation was made from Wright's/Giemsa-stained blood smears. The levels of BUN, SDH and HBD within the serum samples were obtained by using a centripetal autoanalyzer.[e]

Histologic Preparation

The formalin-fixed tissues were trimmed to appropriate size, automatically processed,[f] and paraffin embedded for sectioning at 5 to 7 μm. Tissue sections were then stained with hematoxylin-eosin.

Selected frozen liver sections were stained with oil red O for relative lipid content.

Polybrominated Biphenyl Analysis

Quantitative PBB tissue analysis was performed according to a standardized procedure employed by the clinical laboratory within the Department of Pathology at Michigan State University. Briefly, this procedure entailed the grinding of 0.5 g of tissue (either fat or liver) with prewashed sand[g] in a stainless steel beaker. Ten to twenty grams of granular anhydrous sodium sulfate[h] was added to dehydrate the sample. After the further addition of 25 ml distilled-in-glass hexane,[i] the entire mixture was brought to a boil and then

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[d]Coulter Counter SsR, Coulter Electronics, Inc., Hialeah, FL.

[e]Gemsac Analyzer, Electro-Nucleonics, Inc., Fairfield, NJ.

[f]Histomatic, Fisher Scientific Co., Pittsburgh, PA.

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

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

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

filtered into a 100 ml volumetric flask. Three repeated hexane washes and subsequent filtrations were performed on the original sample. The combined filtrates were then brought to 100 ml by the addition of glass-distilled hexane. Two 20-ml aliquots were removed and each was condensed down to 0.5 ml by evaporation.[j] One aliquot was used later for PBB quantitation. The remaining aliquot was allowed to completely evaporate in a preweighed aluminum pan to determine the lipid weight.

Milk Samples

The method normally employed by our laboratory for the PBB analysis of milk called for the use of 5 ml of milk. However, due to the small size of the experimental animal, it was impossible to obtain this amount at one milking. Therefore, the amounts used varied from 0.25 ml to 2 ml. Five milliliters of methanol and 5 ml of a 1:1 mixture of ethyl ether and glass-distilled hexane were added to the sample in a 20 x 150 mm test tube. This mixture was agitated for 20 minutes, then centrifuged at 1,500 rpm for 5 minutes, and the supernatant layer was drawn off. This process was repeated 2 more times, and the combined supernatants were condensed to approximately 0.5 ml. This fluid was then completely evaporated in a preweighed aluminum pan to determine the lipid weight. Glass-distilled hexane was then added to redissolve the lipid, and the volume was raised to 100 ml by additional amounts of hexane. A 10 ml aliquot was removed and condensed by evaporation to approximately 0.5 ml.

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

Columnmatic Sample Elution

Columns for elution were prepared by packing granular anhydrous sodium sulfate[k] over 1.6 g of activated magnesium silicate[l] within a 50 ml thistle tube measuring 200 x 7 mm. A small plug of glass wool prevented the column from passing through the open end. Five milliliters of distilled-in-glass hexane was used to wash the column and was then discarded. The 0.5 ml condensed sample was passed through the column with 13 ml of glass-distilled hexane. The eluate was then evaporated to approximately 0.5 ml.

Gas Chromatography

Glass-distilled iso-octane[m] was added to the eluates to bring their volume to 2 ml or 10 ml, depending on their expected PBB concentration. The gas chromatograph[n] was injected with 2 µl of this sample. The column temperature was maintained at 250 C, while the detector temperature was 310 C. Gaseous nitrogen acted as the carrier at a flow rate of 30 ml/minute. Sample results were compared with standards containing 0.05 µg PBB/ml. Control samples of calf liver and raw goat milk were also used for comparisons.

Tissue levels of PBB were expressed in ppm on both a whole weight basis and a fat basis.

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

[l] Florisil, 60-100 mesh, Fisher Scientific Co., Fairlawn, NJ.

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

[n] G. C. Model 3700, Varian Instrument Division, Palo Alto, CA.

Statistical Analysis

Data were analyzed statistically by using the Statistical Package for Social Sciences (SPSS-Northern University) at Michigan State University's Computer Center. This program produced a one-way analysis of variance for unbalanced data. Mean effects of PBB were evaluated by independent contrasts, using F-ratios. Specifically, controls were compared with animals given PBB, and the effects of lactation, dose, and their interaction were examined separately in adult females and newborn and 3-week-old piglets.

RESULTS

Clinical Signs

At no time during this experiment did any of the guinea pigs demonstrate clinical signs of illness that could be related to the presence of PBB in their diet or in their tissues. Nor could the stillbirth of 6 piglets be directly attributable to any specific level of PBB in the sows' diets. The 1 sow fed a diet containing 10 ppm PBB that died during dystocia appeared normal and active on the day prior to her death.

There was no apparent refusal of feed due to the presence of PBB in the diet. Nor was there any observable decreased activity between the animals in the different groups.

Body Weight

Table 2 contains the mean body weights of the piglets from birth through 3 weeks of age. The growth patterns of these piglets can be more readily visualized in Figure 1. There was a statistically significant difference ($p<0.05$) in body weight in the control group, at both 1 day of age and at 3 weeks of age, when compared with the PBB-treated groups. However, there was no significant difference in body weights between the groups fed 1 ppm PBB and 10 ppm PBB. Nor did the interaction of lactation and dose result in a significant change in body weight at 3 weeks of age. Likewise, the presence of

Table 2. Mean body weights of piglets from birth to 3 weeks of age

Concentration of PBB in Sows' Diet (ppm)	Time of Administration	No. of Litters	Body Weight (grams) 1 day	1 week	2 weeks	3 weeks
0	gestation and lactation	4	105±9[a]	175±17	243±16	310±28[a]
1	gestation	4	85±6	117±11	174±14	215±16
10	gestation	2	90±4	137± 7	173± 6	205± 7
1	gestation and lactation	3	80±6	143± 9	205± 7	237±10
10	gestation and lactation	4	80±6	135± 3	194± 4	214± 4

Values are expressed as means ± SEM.

[a]Different ($p<0.05$) from treated groups. (The mean values of all treated animals were grouped together to make the comparison of control vs treated animals.)

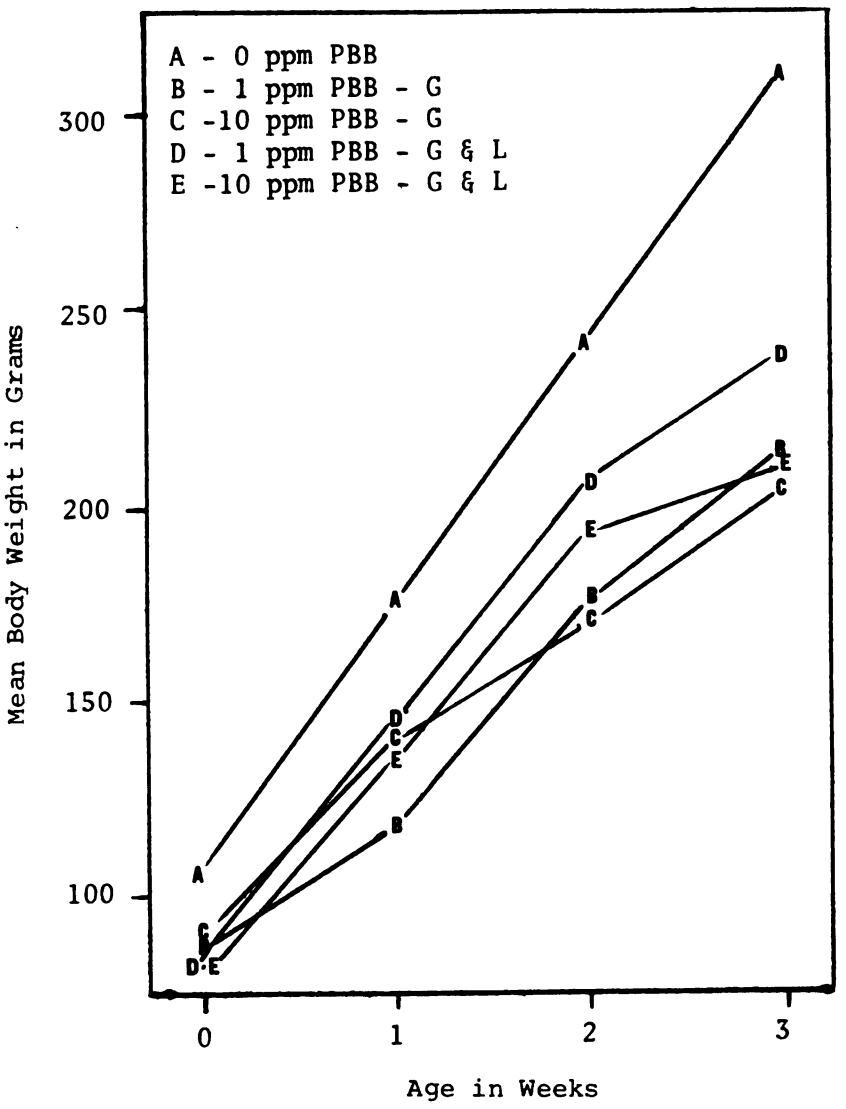

Figure 1. Effects of PBB and time on the body weight of piglets from birth through 3 weeks of age. Varying levels of PBB were present in the diets of the sows during gestation and lactation.

PBB in the diet through the lactational period did not have any significant adverse effect on the body weights of nursing piglets.

Liver Weight

The mean liver weights of the guinea pigs in this experiment are listed in Table 3. Examination of these figures reveals a discrepancy between the sows and their neonates. The PBB-treated sows all had significantly higher mean liver weights ($p<0.05$) than did the control sows. However, the piglets were exactly opposite, with their mean liver weights being statistically higher in the control groups ($p<0.05$) at both ages than in the PBB-treated groups. (Statistical analysis showed a $p<0.01$ difference for the 3-week-old group.) Neither the dosage levels of PBB nor the continuation of PBB in the diet during the lactational period had any effect on the gross liver weights among those piglets exposed to PBB (Figure 2).

When the liver weights are represented as a percentage of the body weights, further significant data can be obtained (Table 4). By using this liver weight to body weight ratio, we found no significant difference between the control groups versus the PBB-treated groups. However, there was a very significant change ($p<0.05$) in this ratio between the 2 dose groups at all ages (sows, newborns, and 3-week-olds). The 3-week-old piglets in the 10 ppm groups had a much higher ratio ($p<0.005$) than those in the 1 ppm groups. Neither the continuation of PBB in the diet during lactation nor the interaction of lactation and dose produced any significant changes in this ratio.

Hematology

No apparent abnormal effects were produced on the hematopoietic system by the presence of PBB in the diet. White blood cell counts,

Table 3. Mean liver weights of sows and piglets (grams)

Concentration of PBB in Sows' Diet (ppm)	Time of Administration	No. of Sows	Sows	No. of Piglets	Piglets	
					1 day	3 weeks
0	gestation and lactation	4	26.18±0.12[a]	4	4.20±0.26[b]	15.63±1.59[c]
1	gestation	4	29.91±1.41	4	3.35±0.48	9.76±0.67
10	gestation	3	35.45±2.24	2	3.95±0.06	10.04±0.81
1	gestation and lactation	4	31.98±2.03	3	2.27±0.18	10.80±0.31
10	gestation and lactation	4	34.98±0.83	4	3.07±0.39	11.68±0.29

Values are expressed as means ± SEM.

[a]Different ($p<0.05$) from treated groups

[b]Different ($p<0.05$) from treated groups

[c]Different ($p<0.01$) from treated groups

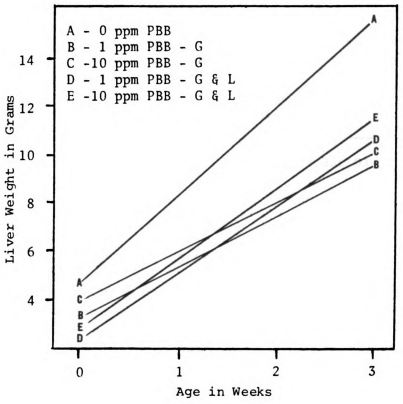

Figure 2. Effects of PBB and time on the liver weight of piglets from birth through 3 weeks of age. Varying levels of PBB were present in the diets of the sows during gestation and lactation.

Table 4. Mean liver weight as a percent of body weight in sows and piglets (%)

Concentration of PBB in Sows' Diet (ppm)	Time of Administration	No. of Sows	Sows	No. of Piglets	Piglets	
					1 day	3 weeks
0	gestation and lactation	4	4.92±.17	4	4.33±.03	4.98±.17
1	gestation	4	4.53±.19	4	4.15±.33	4.55±.05
10	gestation	3	5.65±.70[a]	2*	4.80±.00[b]	5.28±.21[c]
1	gestation and lactation	4	4.85±.29	3†	3.55±.07	4.45±.16
10	gestation and lactation	4	5.60±.24[a]	4	4.63±.44[b]	5.35±.10[c]

Values are expressed as means ± SEM.

[a]Combined values for sows given 10 ppm of PBB different ($p<0.05$) from sows given 1 ppm.

[b]Combined values for newborn piglets whose dams were given 10 ppm of PBB different ($p<0.05$) from piglets whose dams were given 1 ppm.

[c]Combined values for 3-week-old piglets whose dams were given 10 ppm of PBB different ($p<0.005$) from piglets whose dams were given 1 ppm.

*One less litter because of mummification of fetuses.

†One less litter because of stillbirth of fetuses.

red blood cell counts, hematocrits, and differential cell proportions were all within normal ranges in all of the groups of animals in this project.

Serum Chemistries

Table 5 contains the mean BUN values for this experiment. Although there are no large differences in values, statistically there are 2 areas of difference. In the adult sows there was a significant ($p<0.05$) dose-related elevation in BUN in the 10 ppm group over the 1 ppm group. Secondly, statistical analysis of BUN levels in the newborn piglet revealed a difference ($p<0.05$) between the control group and the PBB-treated group, with the latter having consistently higher values.

In the analysis of the values reported for both SDH and HBD, no significant variations could be detected. In this experiment the values for SDH in the control animals ranged from 17 to 79 IU/l, with a mean of 40 IU/l. The HBD control data revealed a range of 60 to 214 IU/l, with a mean of 172 IU/l. The serum levels of these 2 enzymes in the treated animals all occurred within their respective normal ranges.

Gross and Histologic Lesions

Except for variations in liver size, no gross tissue changes were noted at the time of necropsy. Even the enlarged livers were not extraordinarily swollen, nor were there changes in color or consistency.

Histologic examination of the tissues revealed sporadic lesions of a mild interstitial lymphocytic pneumonia with some focal atelectasis in the lungs of the sows. At least 1 or 2 sows from each group

Table 5. Concentrations of blood urea nitrogen in the serum of the sows and their neonates

Concentration of PBB in Sows' Diet (ppm)	Time of Administration	No. of Sows	Sows	No. of Piglets	Piglets	
					1 day	3 weeks
0	gestation and lactation	4	22.25±0.63	4	21.00±2.86[b]	22.00±1.78
1	gestation	4	19.75±2.46	4	33.00±2.00	22.25±3.22
10	gestation	3	25.50±2.06[a]	2	22.25±1.89	27.00±3.24
1	gestation and lactation	4	27.33±1.53	3	23.66±1.76	21.75±0.75
10	gestation and lactation	4	33.00±5.57[a]	4	27.75±1.25	23.50±1.25

Values are expressed as means ± SEM.

[a]Combined values for sows given 10 ppm of PBB different ($p<0.05$) from sows given 1 ppm.

[b]Different ($p<0.05$) from piglets in treated groups.

harbored these pulmonic lesions. Another consistent finding that was present in all groups, both control and PBB-treated, was the observation of marked extramedullary hematopoiesis within the spleens of the 3-week-old piglets. One sow from the control group had evidence of a mild focal interstitial lymphocytic nephritis. No significant microscopic changes were seen in the heart, brain, stomach, or intestine of any guinea pig.

The microscopic changes present in the liver tissue were "consistently inconsistent" in all 5 experimental groups and at all ages. There was never any evidence of distinct hepatic necrosis. Instead, the hepatic lesions varied from very mild hepatocellular vacuolization to severe hepatocellular vacuolization. The distribution of these vacuolar changes also varied from diffuse to centrolobular in a few cases. The most severely affected liver belonged to a control sow, whereas all of the livers from PBB-treated sows had only mild to moderate vacuolar changes. Figures 3 through 8 depict some of the inconsistent hepatocellular changes.

Although more of the 1-day-old piglets had severe hepatocellular vacuolization, each of the 5 experimental groups had equal numbers of moderately and severely affected livers.

In the 3-week-old category, no livers were diagnosed as being "severely" vacuolated. Again, each experimental group in this age bracket had equal distribution of liver vacuolization changes.

Oil red O stains confirmed the presence of fat droplets in only those livers that had been classified as severely vacuolated. In the remaining livers that had this stain applied, only occasional lipid droplets could be seen.

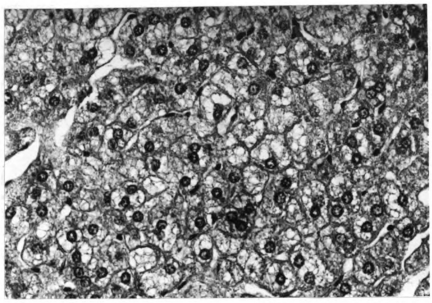

Figure 3. Liver tissue from an adult sow on a control diet (0 ppm PBB). Classified as severe hepatocellular vacuolization. H&E stain, 300X.

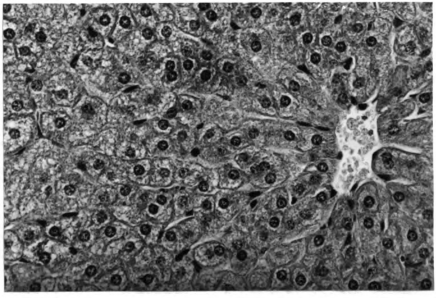

Figure 4. Liver tissue from an adult sow fed a diet containing 10 ppm PBB during pregnancy and throughout lactation. Classified as moderately severe hepatocellular vacuolization. H&E stain, 300X.

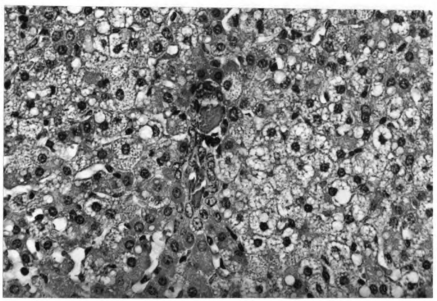

Figure 5. Liver tissue from a newborn piglet whose dam was on a control diet (0 ppm PBB). Classified as severe hepatocellular vacuolization. H&E stain, 300X.

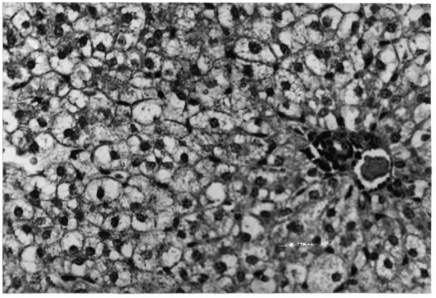

Figure 6. Liver tissue from a newborn piglet whose dam was fed a diet containing 10 ppm PBB. Classified as severe hepatocellular vacuolization. H&E stain, 300X.

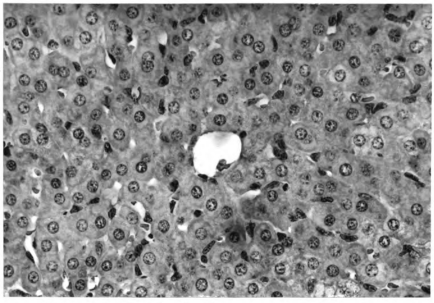

Figure 7. Liver tissue from a 3-week-old piglet whose dam was on a control diet (0 ppm PBB). Classified as mild hepatocellular vacuolization. H&E stain, 300X.

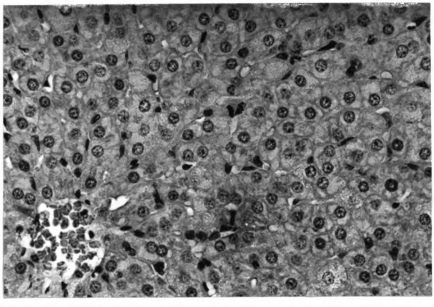

Figure 8. Liver tissue from a 3-week-old piglet whose dam was fed a diet containing 10 ppm PBB during gestation and lactation. Classified as mild hepatocellular vacuolization. H&E stain, 300X.

Polybrominated Biphenyl Analysis

The results of the gas-chromatographic analysis of PBB in the liver and adipose tissues of all 3 age groups are listed in Table 6. The tissue concentrations increased as the diet concentrations increased. In every case, the liver from the newborn piglet had a higher concentration of PBB than the corresponding sow's liver. For the most part, this held true for the liver concentrations of PBB in the 3-week-old piglet as well. The adipose tissue levels were relatively equivalent or lower in the piglets than in the respective sows.

The mean concentrations of PBB in the sows' milk are listed in Table 7. Here, too, the milk concentrations increased with increasing levels of PBB in the diet. Table 6 also shows that the nursing 3-week-old piglets had a 2- to 10-fold increase in PBB tissue levels over their counterparts, whose dams were not receiving PBB in the diet during lactation.

Table 6. Mean concentrations of PBB (ppm) in the liver and in the adipose tissue of sows, newborn, and 3-week-old piglets

Concentration of PBB in Sows' Diet (ppm)	Time of Administration	No. of Sows or Litters	Liver		Adipose Tissue	
			whole weight basis	fat basis	whole weight basis	fat basis
Sows						
0	gestation and lactation	4	0.000	0.000	0.059± 0.02	0.084± 0.03
1	gestation	4	0.056±0.02	2.996± 0.76	11.599± 4.50	15.662± 6.14
10	gestation	3	1.754±0.87	45.237±12.25	84.776±27.25	139.021±37.87
1	gestation and lactation	4	0.099±0.03	9.093± 2.50	4.177± 0.40	5.695± 0.65
10	gestation and lactation	4	0.516±0.11	25.519± 2.95	80.198±11.85	155.860±30.02
Newborn						
0	gestation and lactation	4	0.066±0.01	0.882± 0.01	0.256± 0.01	0.416± 0.01
1	gestation	4	0.955±0.09	9.269± 2.26	4.704± 1.77	7.220± 3.05
10	gestation	2	7.729±1.07	49.066± 7.05	27.751± 1.35	45.569± 3.04
1	gestation and lactation	3	0.443±0.07	9.295± 3.95	1.852± 0.10	3.634± 0.16
10	gestation and lactation	4	6.386±1.12	67.981±15.52	38.367± 2.07	98.573±17.70
3-week-old Piglets						
0	gestation and lactation	4	0.014±0.01	0.742± 0.05	0.462± 0.21	0.855± 0.46
1	gestation	4	0.067±0.02	3.261± 0.93	5.076± 1.17	10.161± 2.62
10	gestation	2	1.167±0.20	45.453± 5.25	69.616± 2.10	121.615± 5.13
1	gestation and lactation	3	0.301±0.04	17.071± 3.14	11.742± 1.13	19.653± 2.38
10	gestation and lactation	4	8.273±4.92	410.842±251.7	89.470± 5.34	186.240±27.92

Values are expressed as means ± SEM.

Table 7. Mean concentrations of PBB (ppm) in sows' milk

Concentration of PBB in Sows' Diet (ppm)	Time of Administration	No. of Samples	Milk	
			Whole Weight Basis	Fat Basis
0	gestation and lactation	1	0.005	0.088
1	gestation	4	1.416±0.96	7.762±4.95
10	gestation	3	0.343±0.11	10.468±3.48
1	gestation and lactation	3	0.083±0.02	1.751±0.08
10	gestation and lactation	3	1.804±0.20	34.639±5.57

Values are expressed as means ± SEM.

DISCUSSION

Ever since the environmental contamination of PBB in 1973, there has been much interest in the possible public health implications of exposure to this xenobiotic. One area of prime concern was that of the combined effects of this chemical on the developing fetus and the nursing infant due to PBB's capability to traverse the placental tissues and to be excreted from the dam via the mammary gland. At the time of initiation of this project, only cattle and laboratory rats had been used to study this aspect of PBB toxicosis. Since initial studies determined that guinea pigs appeared to be more sensitive to PBB than rats (50), it was considered that the guinea pig might prove to be a useful model for pregnancy and lactational studies.

The lower dosage levels of 1 and 10 ppm PBB were selected because of their known tolerance by guinea pigs (50). Therefore, it was not too surprising that the sows never had clinical signs of toxicosis. Likewise, the piglets never appeared clinically ill. Statistical analysis demonstrated that their birth weights were less than those in the control group, but this apparently had no detrimental effects on their further development, nor did any increased neonatal mortality occur. At 3 weeks of age the PBB-treated piglets were still significantly smaller than their control counterparts. Harris (21) found a similar decreased body weight of weanling rat pups nursing PBB-contaminated dams, even though there had been no difference in birth weights.

Our study also demonstrated that there was very little, if any, difference in the weight gains of the nursing piglets throughout the first 2 weeks of life, even though some of the piglets were receiving higher levels of PBB from the dam's milk. However, those piglets which were nursing dams which in turn were still receiving PBB through the diet during lactation did show a decreased rate of growth between the second and third weeks of life (see Figure 1).

One of the more surprising results of this experiment was the significant *decrease* in total liver weight of the PBB-treated piglets when compared with their control counterparts. This conclusion differs from all other studies. This also explains why there was no increase in the liver to body weight ratio between the control groups and the PBB-treated groups. Several authors have observed increased liver weights and, subsequently, increased liver to body weight ratios in PBB-treated rats (5,11,50). Dent (11) has documented a dose-related increase in liver weight/body weight in PBB-exposed rats. Werner (52) made a similar conclusion while working with young pigs. I can offer no explanation for this curious reversal of PBB effects in the liver, other than to say that histologic examination of the livers was consistent with these results. It is possible that the dietary levels of PBB were not high enough to induce marked liver microsomal activity.

As was stated earlier in this thesis, there were no consistent liver changes seen on microscopic examination. The majority of the livers appeared only mildly vacuolated. Whenever a severely vacuolated liver in a PBB-treated animal was observed, there was one of equal severity in the matching group of control animals. This lack of consistency when using the guinea pig in a study on PBB was also mentioned by Sleight and Sanger (50). Whether this is indeed a peculiarity of

the guinea pig when used in this type of a chemical trial or merely reflects the low dosages of PBB used could not be determined.

One microscopic pattern did seem to be observed, however, and that was the presence of increased vacuolization of the liver in the newborn guinea pig. Of the 3 age groups studied histologically, the 1-day-old piglet had notably more hepatocellular vacuoles diffusely dispersed throughout the hepatic tissue. This occurred in all groups, including the controls, and thus could not be related to the presence of PBB in the tissues.

Hematologic values were apparently unaffected by the ingestion of PBB. All values remained within normal ranges throughout this experiment. This is in contrast to the results reported by Polin and Ringer (44), who found decreased hematocrits and hemoglobin levels in chickens fed PBB-contaminated diets. Ku (30) also noted decreased hemoglobin and hematocrit values, but only after he had fed 200 ppm PBB daily for 16 weeks to young growing pigs.

Blood urea nitrogen was significantly increased in the 1-day-old PBB-treated piglets, even though the values would still be considered within normal limits. The sows ingesting 10 ppm PBB via the diet also had consistently higher BUN levels than the 1 ppm PBB group, but here again, all values were within the normal range. Thus, the increased BUN was not due to renal damage, and histopathologic sections of kidney confirmed this conclusion. Werner (52) found similar BUN elevations in young pigs that were nursing sows given PBB in the diet.

Serum levels of sorbitol dehydrogenase (SDH) and hydroxybutyric dehydrogenase (HBD) were also monitored in this experiment. This is the first time that these two enzymes have been reported in a study of PBB toxicosis. Neither SDH, as an indicator of hepatic damage, nor

HBD, as an indicator of myocardial fiber injury, was affected by the ingestion of PBB.

The newborn piglets had consistently higher liver levels of PBB than did their dams, yet the adipose tissue levels of PBB were opposite, i.e., higher in the dam. It was also interesting to find that the highest tissue level of PBB, both hepatic and fat, was in the 3-week-old piglet whose dam received 10 ppm PBB via her diet during gestation and throughout lactation. In this case, the hepatic levels on a per fat basis were more than 16 times greater in the piglet than in its dam. Similar results were seen in the study performed by Rickert et al. (46) with the feeding of PBB to pregnant and lactating rats.

Our study also demonstrated that the levels of PBB in the milk increased as dietary PBB levels increased. However, no direct correlation between dietary concentrations and milk fat concentrations could be made. This could in part be due to the differing amounts of body fat that individual sows mobilized in the production of their milk. It would stand to reason that the heavier lactating sows would use more of the body fat to produce their milk and by so doing would add at least some of the PBB stored in this body fat to the milk fat. On a per fat basis, a sow's body fat could contain 2 to 10 times more PBB than her milk fat.

Two variables were inherent in the design of this experiment that could not be overcome and that may have influenced the data. First was the different breeding dates of the sows, leading to different lengths of exposure time to PBB. With 20 different breeding dates and consequently 20 different exposure times, it was impossible to attempt to correct the data for length of time exposed. Secondly, due to the

advanced development of the piglet at birth, it was impossible to devise a feeding system that would exclude the piglet. Thus, not only was the piglet exposed to PBB from the mother's milk but also via the feed. This may account for the highest levels of PBB being found in the 3-week-old piglets.

SUMMARY

At the daily dietary levels of 1 and 10 ppm PBB, no clinical signs of illness could be observed in guinea pig sows and their neonates. Small birth weights were observed in the PBB-treated groups, but liver weight to body weight ratios were not altered when treated animals were compared with controls. The animals in all age groups on diets containing 10 ppm PBB did, however, have an increased liver weight to body weight ratio when compared with those animals on a 1 ppm dietary concentration of PBB.

Polybrominated biphenyl-related tissue changes were not observed grossly or histologically. Increased hepatocellular vacuolization, which had been previously described as a consequence of PBB contamination, was not observed in these guinea pigs. There did appear to be some age-related liver changes, with the 1-day-old piglets having the most vacuolar changes. No histopathologic lesions were noted in the kidney, even though the PBB-treated newborn piglets had a significantly increased level of serum BUN when compared with the control newborns. These elevated BUN values were still within a normal range, however.

Tissue levels of PBB rose as a consequence of increased dietary levels of PBB. The neonatal livers had consistently higher levels of PBB than did the livers of their respective dams. The presence of PBB in the diet during gestation and lactation produced the highest

tissue levels of this experiment in a 3-week-old piglet whose dam's diet contained 10 ppm PBB.

Although it appears that the guinea pig may not be a suitable model for PBB toxicosis due to the lack of consistent PBB-related liver changes, this study did reconfirm the dangers of both placental and mammary transfer of PBB from the pregnant and lactating dam to her developing fetus and suckling neonate. The benefits reaped by the mother by her excretion of PBB via the placenta, fetus, and milk only resulted in increased jeopardy to her already vulnerable offspring.

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