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POLYBROMINATED BIPHENYL TOXICOSIS IN SWINE: EFFECTS
ON SOME ASPECTS OF THE IMMUNE SYSTEM IN
LACTATING SOWS AND THEIR OFFSPRING

By
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ABSTRACT

POLYBROMINATED BIPHENYL TOXICOSIS IN SWINE: EFFECTS ON SOME ASPECTS OF THE IMMUNE SYSTEM IN LACTATING SOWS AND THEIR OFFSPRING

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Sows were fed diets containing 0, 100, or 200 ppm of Firemaster BP-6, a mixture of polybrominated biphenyls (PBB), during the last half of gestation and the following 4 weeks of lactation. Mitogen stimulation (phytohemagglutinin, lipopolysaccharide, pokeweed mitogen) of peripheral blood lymphocytes was studied. Lymphocytes from sows fed PBB for 12 weeks showed significantly decreased mitogen responses. Mitogen responses of lymphocytes from piglets of sows fed PBB were normal at birth, but responses were significantly decreased when the piglets were 4 weeks of age. Hematological parameters were normal. Bactericidal activity was assayed in whole blood from the sows once during gestation and twice during lactation, and from the piglets at 2 and 4 weeks of age. No significant changes in bactericidal activity occurred in sows fed PBB, or in their nursing piglets.

Dedicated with love to my husband, Don,
and my daughter, Terri

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INTRODUCTION

The lack of knowledge of the metabolism, toxicity, and interactions of common industrial compounds decreases our ability to cope with the unexpected chemical contamination of the environment. The accidental mixing of polybrominated biphenyls (PBB) into livestock feed in Michigan in 1973, and the resulting contamination of the food chain, brought about a difficult crisis and was made worse by the lack of information regarding these chemicals. More than 34,000 cattle and 1.5 million chickens were destroyed, as well as many tons of milk, eggs, cheese, and other farm products. There is continuing anxiety and concern about the unknown effects on the health of man and livestock exposed to PBB.

During 1973 and 1974, an estimated 10,000 Michigan residents, mostly farm families and their neighbors, were exposed to high levels of PBB, and another unknown number of persons were exposed to lower concentrations. Numerous reports of ill health among persons and farm animals exposed to PBB stimulated research into the toxicity of these chemicals. Investigations into the kinetics, metabolism, and environmental distribution of these chemicals resulted in valuable information for dealing with this accidental contamination of the environment. However, many questions regarding the effects of exposure to PBB on health still remain unanswered, and research continues. The objectives of this thesis were to

investigate the effects of the ingestion of PBB upon some aspects of the immune system of swine during gestation and lactation, and to determine the toxicity of PBB to the developing immune system in piglets exposed to PBB by placental transfer and consumption of breast milk.

LITERATURE REVIEW

The Polybrominated Biphenyls (PBB)

The following is a general review of relevant information about PBB, with emphasis on the literature available regarding the transfer of PBB to young animals via the placenta or breast milk, and the influence of PBB on immune functions.

Chemistry

The flame retardant that was substituted for magnesium oxide in a livestock feed supplement was produced by Michigan Chemical Company under the trade name "Firemaster BP-6." It is a mixture of polybrominated biphenyls (Figure 1), the major fraction (60-70%) being a 2,2',4,4',5,5'-hexabromobiphenyl. This mixture of brominated biphenyls is insoluble in water but highly soluble in organic solvents and fats. Calcium polysilicate was added to the mixture as an anticaking agent, but there are few other impurities. Very small amounts of hexa-, penta-, and tetrabromonaphthalenes have been identified, but there has been no evidence of the presence of any highly toxic substances such as the dibenzofurans (19,22,27).

Kinetics

Polybrominated biphenyls are apparently poorly absorbed from the intestine and much of that ingested is excreted in the feces (59). Some components of the mixture may be more effectively

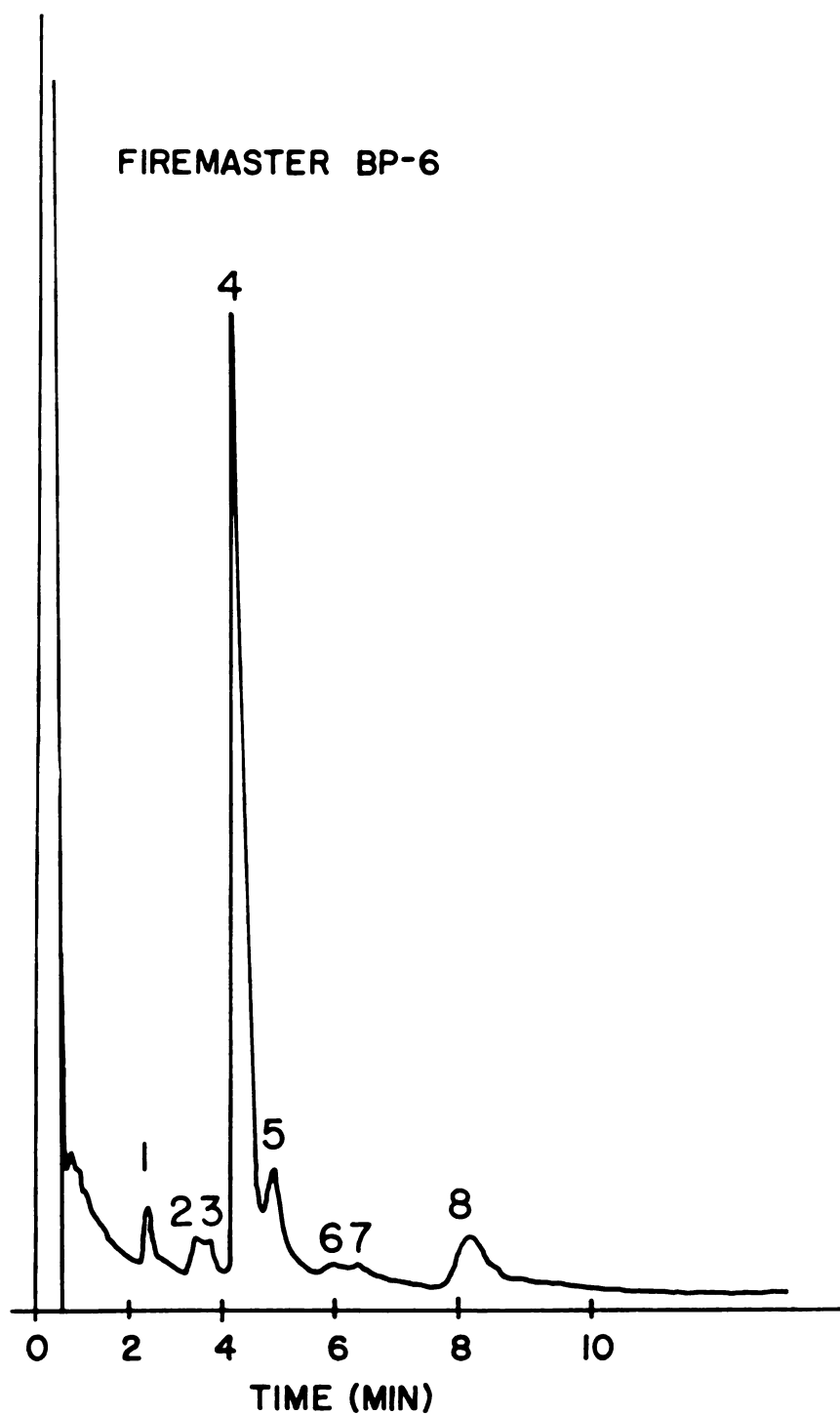


Figure 1. Gas chromatogram of Firemaster BP-6 on an OV-1 column, using an electron capture detector (Varion 3700). The major component (peak 4) is a 2,2',4,4',5,5'- hexabromobiphenyl.

absorbed than others, as residues in tissues have a higher concentration of hexabromo fractions and a markedly lower concentration of the heptabromo fraction relative to the standard Firemaster mixture (18). The rate of metabolism of PBB in the body, like that of the polychlorinated biphenyls (PCB), is very low. Also similar to PCB, the distribution of residues in the tissues is closely correlated to the fat content of the tissues. Exceptions are the liver, in which concentrations are higher than would be expected, and the brain, in which residue concentrations are low despite the high lipid content of the tissue. It has been suggested that the low concentration of residues in the brain is due either to the effectiveness of the "blood-brain barrier" or to the presence of different forms of lipids in the brain (17,30,43).

Storage of PBB residues in adipose tissue results in a body fat concentration to blood concentration ratio of approximately 1000:1 (17). In lactating animals and laying chickens, milk and eggs are the major source of elimination of PBB from the body. The ratio of milk fat concentration to body fat concentration is approximately 0.42:1 in cattle (17,18). Fries et al. (18) did extensive studies on the kinetics of PBB and found that milk fat concentration in cattle reached a steady state at about 4 times the diet concentration after 20 to 30 days of feeding. Milk fat residues have almost 25 times more hexabromo- than hepta- and octabromo-isomers, suggesting that the less halogenated components of Firemaster BP-6 are more easily transferred across biological membranes (17,18).

In cattle and rats, PBB crosses the placental barrier in the blood, accumulating in the fetal tissues and blood at a concentration of about 1/3 that of the dam (17,43). Exposure of the suckling

young to breast milk of a dam fed PBB raises the concentration of the residues in tissues and blood to equal or greater than those of the dam. Because milk fat concentrations are so much higher than blood concentrations, PBB transferred through the milk is considered a more important source of exposure than placental transfer for offspring of animals consuming PBB (43).

Fries et al. (13,17) have reported that when exposure to PBB is stopped, the residue concentration in milk fat declines in a two-phase, first order pattern like that of PCB. The rapid initial drop is followed by a more gradual but continued decline, resulting in a half-life of approximately 60 days in cattle. The rate of elimination is influenced by several factors: level of milk production, total amount of body fat, and changes in body fat concentration. Some limited studies have been made of the influence of different compounds on the elimination of PBB from the body. Cook et al. (9) reported that administration of activated carbon, sodium phenobarbital or vitamins A, D and E did not affect the rate of excretion in milk or feces, or the half-life of residues in body fat.

Toxicosis

Before the accidental contamination of the environment with polybrominated biphenyls in Michigan in 1973, little work had been done on the toxicity of these chemicals. In investigating the suitability of some of the PBB isomers as flame retardants, octabromobiphenyls were tested for chloracne-type activity and there was no response other than a slight reddening of the skin (40). Aftosmis et al. (1) also found low acute skin toxicity with octabromobiphenyl and hexabromobiphenyl, but reported enlarged livers

in animals exposed to hexabromobiphenyl. After exposure of farm animals to very high levels of PBB in contaminated feeds, there were many reports of adverse health effects in these animals. These included decreased milk production, loss of weight, increased urination and lacrimation, hematomas, abscesses, poor wound healing, and problems associated with gestation (19,23,27,35). It has been estimated that these animals were exposed to concentrations of PBB as high as 13,000 ppm. Experimental exposure of animals to lower concentrations (1 to 500 ppm) has generally resulted in a much lower level of toxicity and the failure to demonstrate many of the severe clinical symptoms reported in the heavily contaminated farm animals. Sleight and Sanger (48) reported that rats fed concentrations of up to 100 ppm PBB for 60 days had no overt clinical signs of disease. Similar results for other species have been reported (19,27,35), and surveys of the health status of cattle exposed to low concentrations of PBB failed to uncover any consistent clinical signs (35). However, toxicity may vary among species, as guinea pigs apparently have a greater sensitivity to low levels of PBB (19).

Less overt signs of the toxicity of PBB have been reported in various species, even at low concentrations. Pathologic effects on the liver have been consistently observed (19,48). Changes appear to be dose dependent and include an increased liver to body weight ratio and swelling, hyperplasia, and vacuolation of the hepatocytes. Some changes in serum alkaline phosphatase, blood urea nitrogen, and serum glutamic oxalocetic transaminase have been reported in animals exposed to dietary concentrations of 100 to 500 ppm PBB (14,19,48). Several investigators have reported that PBB, like PCB, is a potent stimulator of the hepatic microsomal enzyme system

(11,37,45,47). An important result of this induction of the mixed-function oxidase system is its effect on the hepatic metabolism of drugs and other xenobiotics (7).

Polybrominated biphenyl is known to be transported across the placenta to the fetus (17,43). These chemicals do not appear to be potent teratogens in rats or mice, but reports of the severity of embryotoxicity and teratogenicity are somewhat contradictory. Cattle exposed to contaminated feed were reported to suffer increased calf losses, abortions, and malformed fetuses (23,27,35). With dietary concentrations of 1000 ppm, some laboratory animals have decreased fetal weight and decreased numbers of live offspring, but no severe teratogenic effects. When using lower concentrations of PBB (100 $\mu\text{g/kg}$ body weight), other investigators found no embryotoxic or teratogenic effects (19).

Human Exposure

An unknown quantity of PBB-contaminated dairy products and meat entered the food chain in Michigan before the contaminated farms were quarantined. An estimated 10,000 Michigan farm residents were exposed to high concentrations of PBB during this period. Fries et al. (17), having worked extensively with the kinetics of halogenated hydrocarbons, estimated that the most highly exposed people consumed from 5 to 15 grams of PBB over a 230-day period through consumption of contaminated milk alone. At the time the quarantine was imposed in 1974, some of the farm residents had blood concentrations of PBB as high as 2.3 ppm (27). An unknown number of persons was exposed to lower concentrations of PBB in contaminated food products. A Michigan Department of Public Health study (5)

found detectable levels of PBB in 96% of human breast milk samples tested in the Lower Peninsula and in 43% of those from the Upper Peninsula. From these data, it is estimated that 8 million of Michigan's 9.1 million residents have detectable body burdens of PBB.

Many of the persons exposed to high levels of PBB complained of health problems. These complaints, including easy fatigability, muscle and joint pain, nervousness, irritability, skin rashes, headache, and decreased resistance to infections, are similar to the symptoms observed in persons accidentally exposed to high concentrations of PCB (29,34). Several studies of the PBB-exposed population were conducted by government agencies. The Michigan Department of Public Health did a short-term health study of persons on quarantined farms in 1975 and a more extensive study in 1976. Intensive medical examinations of exposed persons were made by research teams from the Center for Disease Control and Michigan's medical schools. In these studies there were no identifiable increases in the frequency or severity of health problems in persons exposed to PBB. It was concluded that there was no set of symptoms or physical or biochemical abnormalities which could be associated with exposure to PBB (6,36). In 1978, the Michigan Department of Public Health began a long-term study of the human health effects of PBB under a Health, Education, and Welfare grant.

Effects on the Immune System

In areas of environmental contamination with PBB, the potential toxic immunosuppression by these chemicals is of great importance to the assessment of human health status and to the

animal industries. The reports of immunosuppressive effects of the closely related compounds, polychlorinated biphenyls (PCB), give reason to suspect that PBB may also have toxic effects on the immune system. Some mixtures of PCB cause a reduction in thymic and splenic weights, depletion of lymphocytes in lymphoid tissues, a severe reduction in humoral immunity and delayed-type hypersensitivity, and a decreased resistance to infection and endotoxins (29,31,55,56).

Data on the effects of PBB on the immune system are contradictory. This may be due to the different species involved, variability of age and immune development, or difference in length and amount of exposure to the chemicals. Luster et al. (32), working at the National Institutes of Environmental Health Sciences, reported decreased mitogen responses, immunoglobulin levels and antibody production in rats and mice given oral doses of PBB. These animals were exposed to 3 and 30 mg/kg body weight/day for 30 days. In a study of PBB-exposed Michigan dairy farm residents conducted by a research team from the Mount Sinai School of Medicine, Bekesi et al. (4) reported decreased mitogen and mixed-lymphocyte responses and alterations of lymphocyte membrane markers in 60% of those tested. Since the tests were conducted four years after the exposure, the results suggest an ongoing immunosuppression or a failure to correct acute toxic injury to the immune system. Fraker (16) found that exposure of mice to dietary levels as low as 10 ppm for 30 days reduces humoral immune response to about 1/3 that of normal mice. There was no apparent effect on delayed hypersensitivity. In contrast, Kately and Bazzell (24) found no long-term effects on the immune system of cattle with body burdens

of up to 30 ppm PBB in the body fat. They reported no significant changes in mitogen responses, immunoglobulin levels, surface markers or *in vivo* antibody production. In a collaborative study conducted by the Michigan Department of Public Health and the University of Michigan in 1977 (26), lymphocyte function was assessed in persons with high and low blood serum concentrations of PBB compared to persons with no detectable blood serum concentrations. As a group, those with measurable blood serum levels of PBB did not have any alterations of absolute T and B lymphocyte counts and lymphocyte responses to mitogens were not depressed. However, 15% of these individuals did have some depression of *in vitro* lymphocyte responses.

Assays of Immune Function

Following is a brief overview of the methods used to assess the function of phagocytic cells, and the use of mitogen stimulation of lymphocytes to monitor their ability to respond to antigenic stimulation.

Assessment of Phagocytic Cell Function

Due to early reports of increased numbers of infections and slow-healing skin lesions in farm animals consuming PBB-contaminated feeds, there was an interest in determining the effect of exposure to PBB upon phagocytic cell function. Many *in vitro* systems have been developed to assess phagocytic cell activities, such as motility, adherence, ingestion, and the destruction of ingested material. Abnormalities may occur in any of these processes. The Boyden chamber has been widely used to assess *in vitro* response of neutrophils to chemotactic factors. It is known that complement and antibody play important roles in phagocyte adherence and, thus, the

presence of complement and antibody Fc receptors on neutrophils has been related to normal phagocytic adherence. Measurements used to assess ingestion include direct counting of particles, estimation of cell-bound radioactivity after ingestion of radio-labeled particles, and the spectrophotometric measurement of ingested lipid particles (50,52).

The final stage of phagocytosis, the destruction of the engulfed material, is dependent upon normal motility, recognition and ingestion, as well as several intracellular metabolic systems. The nitroblue tetrazolium dye reduction test and chemiluminescence monitor metabolic processes presumably involved in this final stage of phagocytosis, whereas the bactericidal assay directly measures intracellular killing of engulfed organisms. By varying the test organisms, the bactericidal assay can be used to assess a variety of bactericidal activities. The assay may be done with isolated neutrophils, but this approach ignores several physiologic factors that may contribute to normal *in vivo* phagocyte function (8,39). The use of whole blood allows the investigation of phagocytic function in the presence of natural opsonins and takes into account the importance of the interaction of neutrophils with other leukocytes and blood components. By maintaining the phagocyte/bacteria ratio in the range of those of naturally occurring bacteremias, a closer approximation to an *in vivo* system is achieved. The smaller volume of blood required allows the use of the assay for small animals and for repeated measures.

Keusch et al. (28) developed a simplified whole blood bactericidal assay using serum resistant strains of *Escherichia coli* and *Staphylococcus aureus*. Designed to help in the diagnosis of chronic

granulomatous disease, the assay is also sensitive enough to detect the non-symptomatic heterozygote carrier. Thus, this assay seems appropriate to investigate any PBB-induced toxic changes in phagocytes severe enough to contribute to the reported increase in incidence of infections.

Assessment of Lymphocyte Response to Mitogens

Numerous environmental contaminants, from pesticides to heavy metals, have been reported to have deleterious effects on the immune system (56). The mechanism of immunosuppression of these toxic chemicals is unknown, but in most cases it appears to be a functional alteration of the cells involved in the immune process. The toxic effects may be principally in the humoral or in the cell mediated immune functions, or in both systems. The mode of action may be direct alteration of the lymphocytes or an indirect effect through adrenal glucocorticoids or changes in metabolic patterns.

Stimulation of isolated lymphocytes with antigens or non-specific activators is an *in vitro* technique used to assess cellular immune function. It measures the functional capability of the lymphocytes to respond to an antigenic stimulus by undergoing blastogenesis and proliferation. A number of plant lectins and other substances have been used as non-specific stimulants to lymphocytes, requiring no previous sensitization of the cells to the antigen. There is evidence that B and T lymphocytes of some species are somewhat selectively stimulated by these mitogens (51). Phytohemagglutinin (PHA), a plant mitogen made from the kidney bean *Phaseolus vulgaris*, appears to be predominantly a T cell mitogen, only secondarily stimulating B cells. Pokeweed mitogen (PWM), made

from *Phytolacca americana*, may activate principally the B cells of some species, while apparently stimulating both B and T cells in other species. Lipopolysaccharides (LPS) have been reported to be B cell mitogens in some species. Due to lymphocyte subpopulation interactions, this apparent selectivity of activation by mitogens is by no means complete, and there is considerable species variation.

Although responses and conditions for culture vary, plant mitogens have been widely used to assess cellular immune response in many species. Symons et al. (54) reported successful stimulation of porcine peripheral blood lymphocytes with PHA, PWM, LPS, and anti-immunoglobulin serum. However, they found considerable individual variability in magnitude of response to these mitogens even with optimal assay conditions. They did not attempt to identify subpopulations responding to the different mitogens.

Blastogenesis and proliferation of lymphocytes following mitogen stimulation is most often assessed by monitoring DNA synthesis. DNA synthesis begins within 24 hours after PHA exposure in human lymphocytes and reaches a peak between 48 and 72 hours (41). The incorporation of tritiated thymidine into the culture media allows the quantitation of DNA synthesis in the cells by liquid scintillation. Although this assay must be rigidly standardized for cell density, mitogen concentration, and incubation conditions, it is more reliable and less laborious than the morphological determination of blastogenesis. Mitogen stimulation of peripheral blood lymphocytes provides a sensitive and practical technique to monitor functional capability of the lymphocytes to respond to antigenic stimulation.

MATERIALS AND METHODS

Experimental Design

Sows were fed 2.5 kg/day of a standard ration containing 0, 100, or 200 ppm Firemaster BP-6 (Michigan Chemical Company) during the last half of gestation and the following 4 weeks of lactation (a total of 12 weeks). These dietary concentrations of PBB are equivalent to approximately 1.25 and 2.5 mg/kg body weight per day. Firemaster BP-6 was pulverized and mixed with a ground standard Michigan State University swine ration. Two sows were fed a ration containing no PBB, 3 were fed a ration containing 100 ppm PBB, and 2 were fed a ration containing 200 ppm PBB.

At parturition, one-third of each litter was randomly selected and necropsied immediately (colostrum deprived). The remainder of the litter and the dam were necropsied at 4 weeks postpartum. Blood and tissue samples were taken at that time for evaluation of various parameters. A total of 21 piglets from the control sows, 24 piglets from the sows fed 100 ppm, and 15 piglets from the sows fed 200 ppm were evaluated.

Mitogen Stimulation of Lymphocytes

At necropsy of piglets and sows, blood was collected into preservative-free heparin (10 units/ml, Sherwood Medical Industries) by cardiac puncture of piglets and from the jugular vein of sows. Fifteen milliliters of blood was mixed with an equal volume of

phosphate buffered saline (PBS) and layered onto 10 ml ficoll/hypaque (24 parts of 9% ficoll, Sigma Chemical Co., to 10 parts of 33.9% hypaque, Winthrop Laboratories) in a 50 ml plastic centrifuge tube (Corning) and centrifuged for 40 minutes at 400 x g at 18 to 20 C. The mononuclear cell layer was removed to a sterile plastic tube and washed 3 times with 10 ml sterile PBS, centrifuging for 10 minutes at 150 x g at 18 to 20 C. Washed cells were suspended in 2 ml of cell culture media (RPMI 1640, Flow Laboratories) containing 10% fetal calf sera, 10,000 Units penicillin, 10,000 µg streptomycin, and 200 mM glutamine (Grand Island Biological Company). A hemacytometer was used to count the cells and the cell suspensions were diluted as necessary in RPMI to obtain a final concentration of 1 to 2 x 10⁶ cells/ml. Viability was assessed by trypan blue exclusion and ranged from 90 to 99%. Cell suspensions were greater than 98% mononuclear cells.

One milliliter of the standardized cell suspension was pipetted into each of 12 sterile plastic tubes with Morton closures (Falcon Plastics). The first 3 tubes were used as unstimulated control cultures. To each 3 of the remaining tubes, 0.1 ml of the appropriate mitogen was added: phytohemagglutinin, 25 µg/ml (Burroughs Wellcome), pokeweed mitogen, final dilution of 1:40 (Grand Island Biological Company), and *Escherichia coli* lipopolysaccharide, 10 µg/ml (Difco Laboratories). Tubes were loosely capped and incubated at 35 to 37 C in 5% CO₂ for 60 hours. One µCi of [³H]thymidine in aqueous solution (Sp Act 6.7 Ci/mol, New England Nuclear) was added to each culture tube, and the tubes were incubated for an additional 12 hours.

Cultures were terminated by suspension in cold PBS and resedimentation by centrifugation at 400 x g for 10 minutes. After 2 washes with PBS, 1 drop of 2% bovine serum albumin and 1 ml of 6% trichloroacetic acid (TCA) were added to each tube. After centrifugation at 1000 x g for 2 minutes, and removal of the supernatant, the precipitate was resolubilized in 1N NaOH. Precipitation and solubilization were repeated twice more, and the final precipitate dissolved in 0.2 ml tissue solubilizer (Unisol, IsoLab Inc.). One milliliter of glass distilled absolute methanol was added to each tube and the contents mixed and transferred to a scintillation vial containing 10 ml scintillation fluid (Complement, IsoLab Inc.). Liquid scintillation counting was done with a Beckman LS-230 counter. Data were expressed as mean counts per minute of the 3 replicate cultures $\pm$ the standard error of the mean. Counts were normalized by subtracting the counts per minute of control cultures from those of stimulated cultures.

Bactericidal Assay

Blood samples were collected into heparinized syringes from the ear vein of the sows and the superior vena cava of the piglets. Samples were obtained from the sows after 4, 10, and 12 weeks of feeding the experimental diets, and from the piglets at 2 and 4 weeks of age. Total and differential white blood cell counts were performed by standard methods.

Whole blood was assayed for bactericidal activity according to the method of Keusch et al. (28). *Escherichia coli* 286 and *Staphylococcus aureus* 986663 were determined to be resistant to porcine serum bactericidal activity by standard viability studies.

For use in the bactericidal assay, bacteria were grown overnight in trypticase soy broth, sedimented by centrifugation at 100 x g, washed 3 times in PBS, and resuspended in PBS to an optical density of 0.6 at 620 nm (PE Coleman 44). This standardized suspension was diluted 1:5000 in PBS, resulting in a concentration of approximately 10^5 organisms/ml.

For the assay, 0.1 ml of the diluted bacterial suspension was added to 0.9 ml heparinized whole blood in a capped sterile glass tube. After thorough mixing, a 0.1 ml (zero-time) sample was removed and added to 9.9 ml sterile distilled water. After agitation on a vortex mixer, aliquots were added to molten trypticase soy agar, mixed, and poured into petri dishes, yielding dilutions of 10^{-2} and 10^{-3} . Plates were incubated 18 to 24 hours at 37 C, and then colonies were counted to determine the number of viable bacteria.

After removal of the zero-time sample, the blood/bacteria mixture was immediately incubated on a tilting aliquot mixer (Lab-Tek) at 37 C. After 1 and 2 hours of incubation, samples were removed and pour plates prepared as described above. At the 2-hour sampling, a 10^{-1} dilution was also prepared.

As a bacterial viability control, a second tube with whole blood and bacteria was prepared and polyanethol sulfonate added at a final concentration of 0.25% to inhibit phagocytosis and intracellular killing. One control for *Staphylococcus* and one for *E. coli* was prepared each time the assay was performed.

Cholesterol

Blood samples were taken for cholesterol assay at necropsy of sows and piglets. Serum was separated from the cells as soon as possible. Kinetic assays were performed on the Gemani Mini Centrifugal Analyzer (Electron Nucleonics Inc.) at the laboratory of the Michigan State University Veterinary Clinical Center.

Statistical Evaluation

Data are presented as means $\pm$ standard error of the mean (SEM). Statistical evaluation of data by analysis of variance, followed by comparison of means by Duncan multiple range tests, was performed by computer using the Statistical Package for Social Science (SPSS, Northwestern University). Differences were considered significant when at the level $p < 0.05$.

RESULTS

Mitogen Stimulation of Lymphocytes

Results of the stimulation of peripheral blood lymphocytes with mitogens are recorded in Table 1. The responses to pokeweed mitogen (PWM) and phytohemagglutinin (PHA) of the lymphocytes of sows fed PBB for 12 weeks were significantly decreased ($p < 0.05$). Lymphocytes from sows fed 200 ppm had the greatest decrease in mitogen response. Lymphocyte stimulation by *E. coli* lipopolysaccharide was minimal, and there was no significant difference in lymphocyte response between sows fed PBB and those fed no PBB. Total leukocyte counts and absolute numbers of lymphocytes (Table 2) were similar in all sows and all were within normal ranges.

Peripheral blood lymphocyte responses from piglets of all sows to PWM and PHA were similar at birth. However, after 4 weeks of lactation, lymphocyte responses to PWM in piglets of sows fed PBB were significantly decreased ($p < 0.002$). No significant differences in response to PHA were detected. Lymphocyte responses to PWM were the most depressed in piglets of sows fed 200 ppm PBB. No lymphocyte stimulation by *E. coli* lipopolysaccharide was observed with any of the piglets at either birth or at 4 weeks of age.

Bactericidal Assay

There was no statistically significant difference between the bactericidal activity of blood from sows fed PBB and those not fed

Table 1. Lymphocyte responses to mitogens in sows after 12 weeks of feeding experimental diets

PBB in ration (ppm)	Number of sows	Unstimulated counts ^a	Stimulated counts ^a		
			Phytohemagglutinin	Lipopolysaccharide	Pokeweed Mitogen
0	2	7205 ± 761	252,000 ± 26,000	11,300 ± 1,500	338,000 ± 26,000
100	3	7222 ± 583	218,000 ± 5,000 ^b	6,400 ± 1,800 ^c	223,000 ± 26,000 ^d
200	2	8093 ± 370	182,000 ± 2,000 ^b	7,900 ± 300 ^c	209,000 ± 6,500 ^d

^aAll counts recorded as cpm ± SEM. Stimulated counts normalized by subtraction of unstimulated counts.

^bp=.050

^cp=.203

^dp=.043

Table 2. White blood cell counts in sows fed PBB and in their piglets

	PBB in ration fed sows (ppm)	Number of animals	Time on feed or age	Total leukocytes (mm ³) ^a	Lymphocytes (mm ³) ^a
Sows	0	2	12 weeks	12,400 ± 2,300	7,201 ± 737
	100	3	12 weeks	15,333 ± 1,910	7,777 ± 576
	200	2	12 weeks	12,325 ± 500	6,271 ± 115
Piglets	0	8	newborn	6,563 ± 611	1,489 ± 179
	100	10	newborn	5,646 ± 928	1,420 ± 188
	200	5	newborn	7,035 ± 965	2,061 ± 438
	0	12	4 weeks	7,798 ± 593	5,392 ± 476
	100	10	4 weeks	8,233 ± 1,111	6,246 ± 1,136
	200	8	4 weeks	9,138 ± 952	6,151 ± 894

^aMean ± SEM.

Table 3. Lymphocyte responses to mitogens at birth and 4 weeks of age in piglets of sows fed PBB

PBB in ration fed to sows (ppm)	Number of piglets	Age	Unstimulated counts ^a	Stimulated counts ^a	
				Phytohemagglutinin	Pokeweed Mitogen
0	4	newborn	1786 ± 193	252,900 ± 5,600	341,700 ± 6,100
100	4	newborn	1553 ± 190	249,079 ± 5,600	342,400 ± 7,700
200	6	newborn	2078 ± 283	257,200 ± 6,400	333,200 ± 6,000
0	12	4 weeks	6681 ± 250	248,000 ± 9,800	301,000 ± 13,000
100	9	4 weeks	6259 ± 271	245,000 ± 10,400 ^b	286,000 ± 13,500 ^c
200	8	4 weeks	7118 ± 280	227,000 ± 11,900 ^b	227,000 ± 13,700 ^c

^aAll counts recorded as cpm ± SEM. Stimulated counts normalized by subtraction of unstimulated counts.

^bp=.351

^cp=.002

PBB at any of the sampling periods during feeding. In some cases (Figure 2), data suggest a decreased bactericidal activity after 1 hour of incubation, especially in sows fed 100 ppm, but these differences are not significant at the 5% level. Results of the bactericidal assay at 3 periods during the feeding are given in Table 1A in the Appendix.

Whole blood bactericidal activity of all piglets at 2 and 4 weeks of age was generally lower than that of the sows. There were no significant differences between the bactericidal activity of blood from piglets from sows fed PBB and that of piglets from sows fed no PBB (Table 2A, Appendix).

Cholesterol

Serum cholesterol values for sows after 12 weeks of feeding and for piglets at birth and at 4 weeks of age are given in Table 4. Serum cholesterol values for piglets were quite variable, even within the same litter. Although values were slightly lower in the sows fed 100 ppm and slightly higher in the newborns of sows fed 200 ppm, there was no significant overall treatment effect.

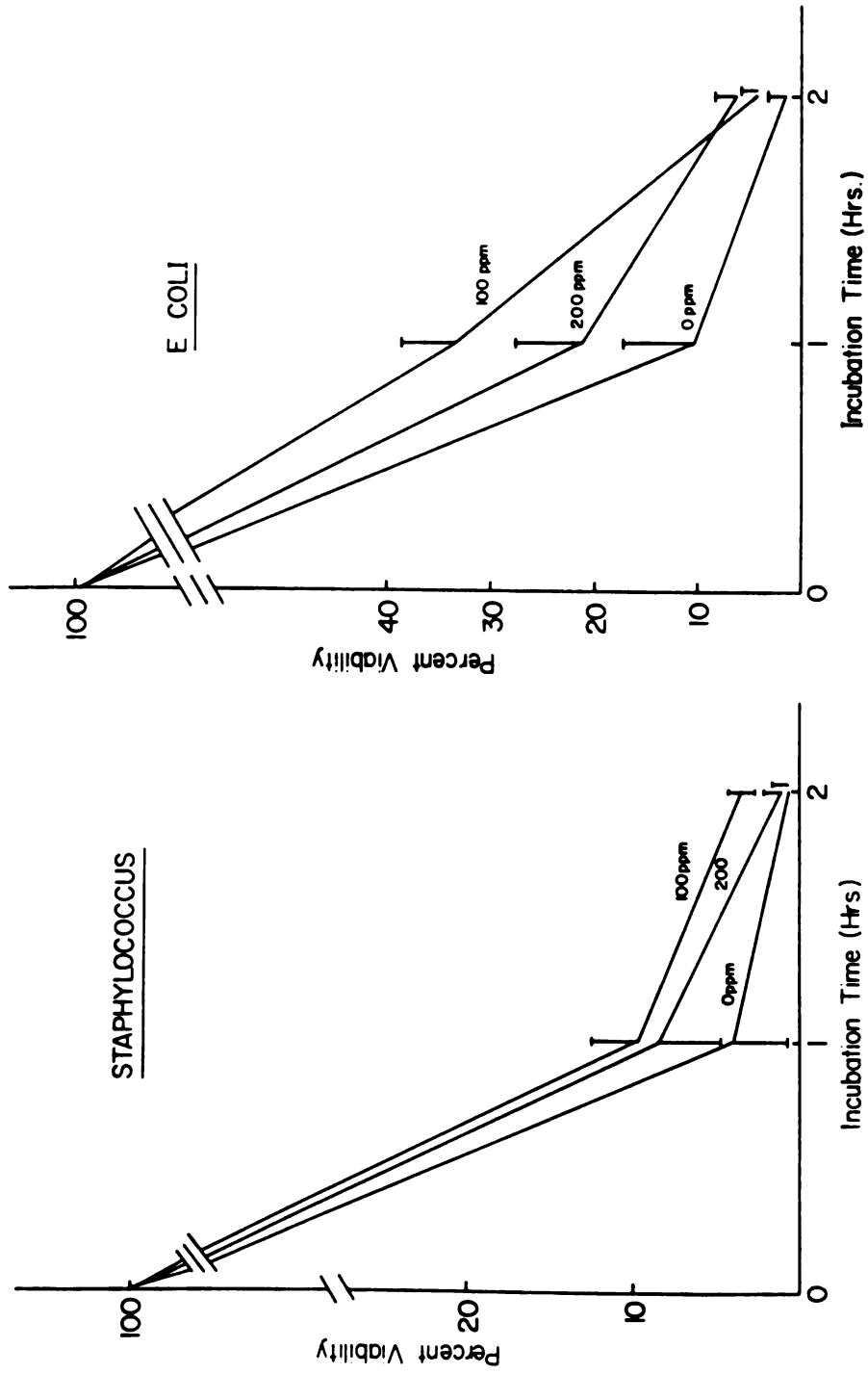


Figure 2. Bactericidal activity in whole blood of sows after 12 weeks of feeding experimental diets. Viability is expressed as mean percent $\pm$ SEM.

Table 4. Serum cholesterol in sows fed PBB and in their piglets

	PBB in ration fed sows (ppm)	Number of animals	Time on feed or age	Cholesterol (gm/dl)		
				Mean	SEM	Range
Sows	0	4	12 weeks	74 ± 2		70- 78
	100	4	12 weeks	59 ± 2		54- 63
	200	2	12 weeks	75 ± 1		74- 75
Piglets	0	12	newborn	64 ± 8		42- 87
	100	14	newborn	69 ± 10		15- 93
	200	6	newborn	97 ± 14		76-137
	0	18	4 weeks	210 ± 33		93-423
	100	16	4 weeks	235 ± 22		179-433
	200	8	4 weeks	222 ± 25		190-289

DISCUSSION

The effects of exposure to PBB on the immune system are important in assessing the impact on health of environmental contamination with these industrial chemicals. The immunosuppressive activity of similar environmental chemicals and the frequent health complaints among persons exposed to PBB have emphasized the need to further investigate the possible immunotoxicity of PBB. Immune suppression following ingestion of PBB has been reported in rats and mice (16,32) and in man (4). The ingestion of PBB apparently also alters some *in vitro* lymphocyte responses in swine. Lymphocytes of sows fed PBB for 12 weeks had significantly decreased responses to plant mitogens. This depression of *in vitro* cell-mediated response is similar to that reported in rats and mice exposed to PBB for a shorter period of time, and to that of some individuals accidentally exposed to PBB in Michigan. However, normal lymphocyte mitogen responses were reported in studies of environmentally contaminated cattle and in studies of PBB exposed human populations by other investigators (24,26).

The possibility of secondary exposure of animals to PBB by passage of the chemicals through the placenta or breast milk is an important concern. Several researchers have reported that PBB is passed through the placenta and is accumulated in tissues of the fetus (17,43). Because pigs are immunocompetent at birth (42), toxicity to the thymus affecting the ontogeny of the immune system

is most important during fetal development. However, passage of these lipid-soluble chemicals through the breast milk to the suckling young is probably of even greater importance in secondary exposure because the concentration of PBB in breast milk is much higher than that of the blood. Although only the sows were fed PBB, the alteration of lymphocyte responses to mitogens that was observed in sows was also demonstrable in their suckling offspring. Lymphocytes from newborn piglets of sows fed PBB had normal responses to mitogens. However, lymphocytes from piglets that had nursed these sows for 4 weeks had significantly decreased mitogen responses, suggesting the importance of exposure to PBB through consumption of breast milk in pigs.

Alteration of lipid metabolism has also been suggested as a mechanism of immunosuppression. The correlation of altered lipid metabolism and immunodepression in conditions such as obesity, diabetes mellitus, aging, cancer and chemical toxicity suggests a causal relationship (13). Increased cholesterol and low density lipoproteins have been reported as effects of various toxic chemicals and carcinogens (13,25), and it has been shown that these lipids inhibit both lymphocyte responses to mitogens (10,15) and the phagocytic activity of macrophages (12). Exposure to PCB increases cholesterol, phospholipids, and neutral lipids in the liver, and cholesterol in the serum (19,27). In contrast, decreased blood cholesterol levels have been reported in PBB contaminated cattle and in monkeys experimentally exposed to PBB (2,27). Sleight et al. (49) reported an elevation of serum cholesterol and an alteration of serum lipoprotein electropherograms in rats fed 100 ppm PBB for 60 days. In the present study,

no significant changes in serum cholesterol levels were observed in the sows fed PBB or in their piglets.

Reported increased incidences of infections and skin lesions in environmentally contaminated animals warranted an investigation of the effects of PBB on phagocytic cell function. Few studies have reported on the effects of toxic chemicals on phagocytic cells. In animals exposed to organophosphates or carbamate, Street et al. (53) found decreased phagocytic activity in blood neutrophils and in phagocytic cells of the reticuloendothelial system. No data have been reported on the effects of PBB on phagocytic cell function. Results of the present investigation indicate that ingestion of 100 to 200 ppm PBB for 12 weeks has little, if any, effect on the bactericidal function of porcine neutrophils. As the assay used is sensitive enough to detect the symptom-free carriers of genetic defects of phagocytes (28), it seems unlikely that toxic effects of PBB on phagocytes could contribute significantly to any increase in infections after PBB exposure.

The mechanism of the functional alteration of lymphocytes is unknown. Some investigators (32) have proposed an indirect effect of PBB upon immune function through the stimulation of adrenal glucocorticoid production. In many animals, enlargement of the adrenal glands and increased levels of serum glucocorticoids are responses to stress. Glucocorticoids slow release of cells from lymphoid organs to the blood, inhibit lymphoid cell mitosis, and can cause degenerative changes in lymphocytes resulting in lysis (13). Depletion of lymphocytes in lymphoid tissues has been reported in chickens exposed to PBB (44), and Moorhead et al. (38) found thymic atrophy in cattle fed PBB. In rats fed up to 100 ppm

PBB for 60 days, Sleight et al. (49) found no changes in adrenal weight or morphology, and Fraker (16) reported that serum glucocorticoid levels in mice exposed to PBB were not elevated although the mice were markedly immunosuppressed. In sows fed PBB and in their piglets, there was no increase in adrenal/body weight ratio (Table 3A, Appendix), and total lymphocyte counts were not depressed (Table 2). Histopathological examination revealed no premature thymic involution or cellular depletion of lymphoid tissues in sows or piglets (57).

Further studies are needed to investigate the mechanism and permanence of the alteration of lymphocyte responses by ingestion of PBB. Changes in lipid metabolism including alterations in serum lipoprotein fractions and lipid content of lymphocytes should be studied. The effect of serum or serum lipid fractions from animals ingesting PBB upon *in vitro* lymphocyte responses should also be investigated. An assessment of the role of some nutritional factors such as vitamin A, vitamin E, or iron might also be warranted.

To more accurately assess the impact of the immunosuppressive effect of PBB upon the health status of the human population exposed to these chemicals, it will be necessary to determine if this effect of acute toxicity is also present with ingestion of lower concentrations of PBB over a long period of time. However, the use of *in vitro* methods to evaluate low level immunotoxicity is difficult because of the relative insensitivity of most of these assays. Any long-term alteration of *in vivo* lymphocyte function would have importance in two aspects of human health: 1) the ability to resist infection and eliminate foreign materials from

the body, and 2) the efficacy of the immune surveillance system in eliminating inappropriate or malignant clones of cells. Alterations of the ability to resist infectious agents could be investigated by using a known pathogen with an established LD₅₀, and specific pathogen-free laboratory animals chronically exposed to low concentrations of PBB. The immune surveillance system is important in controlling the development of malignant clones, and thus influences the rate of cancer. It is also presumed to be important in eliminating undesirable clones of lymphoid cells which could give rise to autoimmune reactions. The response of PBB exposed animals to certain tumor lines, or the use of allograft rejection, would be helpful in evaluating the effect of PBB on this aspect of immunocompetence. The use of these *in vivo* techniques would yield valuable information needed to assess the long-term effects of ingestion of PBB on human health status.

SUMMARY

The ingestion of polybrominated biphenyls apparently alters *in vitro* lymphocyte responses in swine. This immunotoxic effect can also be demonstrated in the young nourished by milk of dams exposed to PBB. It is not known how permanent these alterations are or if this effect of acute toxicity would also be present in chronic, low-level exposure to these chemicals. Any long-term *in vivo* alteration of the immune response to foreign substances or a suppression of the immune surveillance system would have significant consequences for human health and for the animal industries.

Further studies are needed to investigate the mechanism and permanence of the alteration of lymphocyte responses by PBB, and to determine the effect of these industrial chemicals on the immune surveillance system and the immune response to infectious agents.

APPENDIX

Table 1A. Bactericidal assay in sows fed PBB

PBB in ration (ppm)		Percent viability after 1 hr incubation ^a			Percent viability after 2 hr incubation ^a		
		0	100	200	0	100	200
<u>Escherichia coli</u>							
Time on feed:	4 weeks	4 ± 3	22 ± 15	12 ± 5	0.3 ± 0.1	1.7 ± 0.5	0.7 ± 0.3
	10 weeks	7 ± 3	26 ± 12	14 ± 2	0.3 ± 0.2	3.8 ± 1.2	7.7 ± 6.6
	12 weeks	11 ± 3	24 ± 8	21 ± 1	1.4 ± 1.0	4.4 ± 2.2	6.2 ± 1.5
<u>Staphylococcus</u>							
Time on feed:	4 weeks	3 ± 2	20 ± 8	14 ± 3	1.5 ± 0.9	9.2 ± 7.1	4.0 ± 0.2
	10 weeks	16 ± 4	14 ± 3	8 ± 1	1.5 ± 0.7	2.8 ± 2.0	1.8 ± 0.7
	12 weeks	4 ± 1	10 ± 4	8 ± 1	1.6 ± 0.8	3.5 ± 1.3	1.9 ± 0.2

^aMean ± SEM

Table 2A. Bactericidal assay in piglets of sows fed PBB

PBB in ration fed sows (ppm)	Percent viability after 1 hr incubation ^a			Percent viability after 2 hr incubation ^a		
	0	100	200	0	100	200
<u><i>Escherichia coli</i></u>						
Age: 2 weeks	38 ± 1	26 ± 11	52 ± 10	2.4 ± 1.5	5.8 ± 2.8	6.5 ± 1.1
4 weeks	25 ± 4	44 ± 5	37 ± 27	1.6 ± 0.1	16.5 ± 10.3	3.5 ± 2.4
<u><i>Staphylococcus</i></u>						
Age: 2 weeks	40 ± 3	27 ± 12	36 ± 9	13.0 ± 5.5	12.7 ± 3.5	12.4 ± 2.6
4 weeks	27 ± 15	32 ± 1	45 ± 12	4.5 ± 0.9	14.1 ± 5.3	28.1 ± 6.6

^aMean ± SEM

Table 3A. Ratio of adrenal weight (gm) to body weight (kg) of sows fed PBB and of their piglets

	PBB in ration fed sows (ppm)	Ratio ^a
Sows	0	0.09 ± .01
	100	0.09 ± .00
	200	0.05 ± .00
Piglets		
Newborn	0	0.24 ± .03
	100	0.20 ± .02
	200	0.18 ± .03
4 weeks	0	0.12 ± .01
	100	0.12 ± .01
	200	0.11 ± .01

^aMean ± SEM

Table 4A. Total and differential white cell counts in sows fed PBB and in their piglets

	PBB in ration fed sows (ppm)	Number of animals	Time on feed or age	Total leukocytes (mm ³) ^a	Differential (Mean %)				
					STAB	PMN	LYMP	MONO	EO BASO
Sows	0	2	12 weeks	12,400 ± 2,300	0	32	59	4	5 0
	100	3	12 weeks	15,333 ± 1,910	0	37	53	5	4 1
	200	2	12 weeks	12,325 ± 500	0	40	51	4	5 0
Piglets	0	8	newborn	6,563 ± 611	4	71	24	1	0 0
	100	10	newborn	5,646 ± 928	2	68	29	1	0 0
	200	5	newborn	7,035 ± 965	2	66	31	1	0 0
	0	12	4 weeks	7,798 ± 593	1	27	69	2	1 0
	100	10	4 weeks	8,233 ± 1,111	1	22	74	2	1 0
	200	8	4 weeks	9,138 ± 952	1	30	67	2	0 0

^aMean ± SEM

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