Embryotoxic Effects of Polychlorinated Biphenyls (PBB) in Rats
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Pregnant Sprague Dawley rats were given 0, 0.25, 0.5, 1, 5, and 10 mg of a commercial polychlorinated biphenyl, FireMaster BP-6 (PBB), in olive oil by gavage each day from days 7 through 15 of pregnancy. Laboratory chow and water were given ad libitum. Treatment with PBB had no significant effect on body weight gain, food and water consumption, and urine production. The mothers were killed on day 20, and the only significant effect observed was an increased liver weight of those given 1, 5, and 10 mg PBB. Spleen, kidney, ovarian, gravid uterine, and perirenal fat pad weights were similar to those of control mothers. PBB had no significant effect on number of live/dead fetuses, crown-rump length or fetal weight. No grossly malformed fetuses were observed in PBB-treated mothers.

The effects of PBB transfer from mothers to nursing pups was studied by reciprocal exchange of pups between control mothers and mothers treated with 10 mg PBB. When the pups were 21 days old, they were weaned and fed control chow. The following four combinations of prenatal-postnatal exposure were studied: control-control (C:C); control-PBB (C:PBB); PBB-control (PBB:C); and PBB-PBB. Although the birth weights of pups from PBB-treated mothers were similar to those of the controls, body weights of 60-day-old males exposed prenatally and postnatally (PBB:C, C:PBB, and PBB:PBB) were less ($p < 0.05$) than those of the controls (C:C). The weights of the perirenal fat pads of male and female pups exposed to PBB were less ($p < 0.05$) than the control. Liver weights, on a body weight basis, were higher in male and female pups exposed to PBB. Vaginal openings were delayed; the percentages of 36-day-old pups with open vaginas were 50 (C:C), 38 (PBB:C), 28 (C:PBB), and 30 (PBB:PBB).

In 1973, livestock in Michigan were severely poisoned when feed was accidentally mixed with FireMaster BP-6, a technical mixture of polychlorinated biphenyls used as an industrial flame retardant. FireMaster BP-6 (Michigan Chemical Corp.) is a mixture of brominated biphenyls with an average bromine content of 75%, equivalent to about six bromine atoms per biphenyl molecule. This product is a mixture of the following brominated biphenyls: tetrabromobiphenyl, 2.0% pentabromobiphenyl, 10.6%; hexabromobiphenyl, 62.8%; heptabromobiphenyl, 13.8%; other bromobiphenyls, 11.4% (2).

Cows fed the contaminated feed had decreased feed intake, weight loss, and decreased milk production; those in very early stages of pregnancy returned to estrus, and those in later stages of pregnancy gave birth to stillborn calves (1, 3). Until the time FireMaster (PBB) was identified as the causative agent of the poisoning, little research had been done on the effects of this product on animal systems. Results in recent studies have shown PBB to be nonteratogenic in rats (4, 5), teratogenic in rats (6), nonteratogenic in mice (7), or mildly teratogenic in mice (4). Large doses of PBB (67 mg/kg body weight) have been shown to cause heifers to abort, whereas lower doses (0.65 and 0.00065 mg/kg) had no effect (8).

There is a growing concern that PBB may be hazardous to humans ingesting contaminated milk during their neonatal period of development (9). Little information is available on the effects PBB may have on parturition and neonatal development. Sleight and Sanger (10) reported that rat pups nursing dams fed 10 ppm PBB had microscopic and ultrastructural hepatic lesions. Dent et al. (11) also reported the induction of microsomal enzymes in neonatal liver. The present study was undertaken to determine the effects of PBB on embryonic development, parturition, and neonatal and postnatal development of rat pups nursing mothers exposed to PBB during gestation.

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Materials and Methods

Timed pregnant adult Sprague-Dawley rats, weighing approximately 250 g, were housed individually in wire cages in a room controlled at 21°C and with a 14 hr light/10 hr dark cycle. Olive oil solutions with varying amounts of PBB were prepared by dissolving FireMaster BP-6 in benzene, adding the appropriate amount of olive oil, and removing the benzene by bubbling nitrogen through the olive oil solution. The PBB was given by gavage in 0.5 ml olive oil each day for 9 days (days 7 through 15 of pregnancy). The same amount of olive oil was given by gavage to control rats. Food and water consumption, urine production, and body weight were measured daily throughout pregnancy. Three experiments were conducted to (1) determine the embryotoxicity of PBB, (2) determine the growth of pups of mothers treated with PBB during pregnancy and (3) differentiate the effects of prenatal exposure to PBB through placental transfer and postnatal exposure to PBB during lactation.

Experiment 1

Pregnant rats were given 0, 0.25, 0.50, 1.0, 5.0, and 10.0 mg PBB/day on days 7 through 15 of gestation. Commercial laboratory chow and tap water were provided ad libitum. The rats were killed by exsanguination on day 20 of gestation, 1 day before expected parturition. The uterine horns and ovaries were exteriorized through a midline incision in the abdominal wall, and the number and position of live, dead, and resorbed fetuses were noted. The gravid uterus was weighed, fetuses were weighed, fetal crown-rump length measured with a vernier caliper and examined for gross malformations, and ovaries were weighed and corpora lutea counted.

Experiment 2

Eight pregnant rats per treatment were given 0 and 10.0 mg PBB/day on days 7 through 15 of gestation. After the pups were born, they remained with their mothers until weaning at 21 days. Mothers and pups were fed laboratory chow throughout the experiment. Pups were weighed at 3-day intervals until they were 9 days old and at 5-day intervals thereafter. The mothers and pups were killed by exsanguination at 48 and 60 days, respectively.

Experiment 3

Eight pregnant rats per treatment were given 0 and 10.0 mg PBB/day on days 7 through 15 of gestation. All the PBB-treated rats were fed laboratory chow ad libitum, and the food intake of each was measured daily. Each control was fed an amount equal to that consumed the previous 24 hr by its treated partner until weaning. After littering, all rats were fed laboratory chow ad libitum. At the time of littering, 50% of the pups from each control mother were exchanged with 50% of the pups from a PBB-treated mother; each mother nursed 7–8 pups. This exchange resulted in four combinations of prenatal and postnatal treatments: control mothers nursing their own pups (C:C); control mothers nursing pups from mothers treated with PBB during pregnancy (C:PBB); PBB-treated mothers nursing pups from control mothers (PBB:C); and PBB-treated mothers nursing pups from mothers treated with PBB during pregnancy (PBB:PBB). The parameters studied were the same as those in experiment 2.

In this paper, single-factor analysis of variance and Dunnett’s test for multiple comparisons were used to determine statistical significance.

Results and Discussion

The administration of a range of multiple doses from 0.25 mg to 10 mg PBB/day during pregnancy did not significantly affect food and water consumption or urine production. Body weight gain of pregnant rats was significantly affected by only one dose level; multiple doses of 0.25 mg PBB significantly (p < 0.025) lowered body weight gain (Table 1). The 14 g reduction in body weight was due to a 17 g reduction in the gravid uterine weight (Table 2).

Liver weight of the pregnant rats was significantly increased by the daily administration of 1, 5, and 10 mg PBB (Table 2). If we assume an average daily food consumption of 20 g, the doses of 1, 5, and 10 mg would be equivalent to the rats consuming diets containing 50, 250, and 500 ppm PBB from days 7 through 15 of pregnancy. This finding is in agreement with Corbett et al. (4), who reported increased liver weight in mice fed 1000 ppm PBB during pregnancy. Increased liver weight has also been reported in male rats fed 1 to 500 ppm PBB for 30 days (10, 12); induction of hepatic microsomal enzymes have been reported in rats fed 4.7 to 300 ppm PBB for 14 days (13).

The effect on adrenal weight was inconsistent. Only the 0.50 mg PBB dose significantly (p < 0.05) increased adrenal weight. Treatment with PBB had no significant effect on spleen, kidney, and ovarian weights of pregnant rats (Table 2).

Treatment with PBB did not interfere with implantation, and the number of live fetuses on day 20 of pregnancy was comparable with the number in the control; no grossly malformed fetuses were observed (Table 3). Only the 0.25 mg PBB dose sig-
Table 1. Mean body weight, food consumption, water consumption and urine production of pregnant rats given 0–10 mg PBB/day from day 7 through day 15 of pregnancy.

| Dose level, mg/day | n  | Body weight at various times of pregnancy, g | Weight gain at day 20, g | Food consumption, g/rat/day | Water consumption, g/rat/day | Urine production, ml/rat/day |
|-------------------|----|---------------------------------------------|--------------------------|-----------------------------|----------------------------|-----------------------------|
| 0                 | 30 | 213, 227, 243, 259, 304                    | 93                      | 19.3                        | 34.8                        | 7.8                         |
| 0.25              | 6  | 212, 221, 236, 256, 293                    | 79c                     | 18.2                        | 32.8                        | 7.7                         |
| 0.50              | 8  | 209, 226, 243, 266, 304                    | 105                     | 20.5                        | 37.0                        | 9.1                         |
| 1.0               | 7  | 192, 210, 232, 242, 293                    | 110                     | 20.6                        | 36.0                        | 7.6                         |
| 5.0               | 6  | 214, 229, 242, 268, 309                    | 90                      | 20.2                        | 34.2                        | 6.0                         |
| 10.0              | 7  | 223, 232, 236, 245, 297                    | 81                      | 19.3                        | 36.1                        | 8.2                         |

* First day of treatment = day 7 of pregnancy.

Table 2. Mean organ weights on day 20 of pregnancy of rats given 0–10 mg PBB/day from day 7 through day 15 of pregnancy.

| Dose level, mg/day | n  | Liver, g | Spleen, g | Kidneys, g | Adrenals, mg | Ovaries, mg | Gravid uterus, g | Perirenal fat pads, g |
|-------------------|----|----------|-----------|------------|--------------|-------------|------------------|-----------------------|
| 0                 | 30 | 10.3     | 0.55      | 1.50       | 65.3         | 99.8        | 61.4             | 1.10                  |
| 0.25              | 6  | 10.6     | 0.34      | 1.63       | 68.7         | 110.8       | 43.8             | 1.59                  |
| 0.50              | 8  | 11.8     | 0.62      | 1.63       | 80.2         | 100.7       | 57.0             | 1.19                  |
| 1.0               | 7  | 12.2     | 0.54      | 1.48       | 62.5         | 95.1        | 57.6             | 1.27                  |
| 5.0               | 6  | 12.1     | 0.56      | 1.57       | 68.6         | 88.8        | 59.2             | 1.14                  |
| 10.0              | 7  | 12.4     | 0.51      | 1.55       | 59.8         | 90.0        | 58.9             | 0.79                  |

* p < 0.025, treated vs. control.

Table 3. Fetal development on day 20 of pregnancy of rats given 0–10 mg PBB/day from day 7 through day 15 of pregnancy.

| Dose level, mg/day | Pregnant live fetuses | Dead fetuses | Resorption sites | Corpore lutea | Corpore lutea/rat | Live fetuses/rat | Crown/rump length, cm | Weight, g | Grossly malformed, number |
|-------------------|-----------------------|--------------|------------------|--------------|------------------|------------------|-----------------------|----------|------------------------|
| 0                 | 30                    | 311, 4       | 6, 371           | 12.7         | 10.4             | 3.79             | 3.84                  | 1        | 0                      |
| 0.25              | 6                     | 68, 0        | 4, 85            | 12.1         | 9.7              | 3.48             | 2.58                  | 0        | 0                      |
| 0.50              | 8                     | 74, 5b       | 1b, 112          | 14.0         | 10.6             | 3.47             | 3.95                  | 0        | 0                      |
| 1.0               | 7                     | 60, 0        | 0, 69            | 10.1         | 9.3              | 3.84             | 4.08                  | 0        | 0                      |
| 5.0               | 6                     | 66, 0        | 0, 73            | 12.2         | 11.0             | 4.11             | 4.98                  | 0        | 0                      |
| 10.0              | 7                     | 64, 0        | 1, 101           | 12.2         | 9.0              | 4.11             | 4.98                  | 0        | 0                      |

* p < 0.05, treated vs. control.

In experiment 2, the rats were allowed to litter, and delivery appeared normal; litter size and birth weight of pups from PBB-treated mothers were similar to those of control mothers. However, the pups born and nursed by PBB-treated mothers did not gain weight as well as pups born and nursed by control mothers. The initial reduction in body weight was observed at 3 days of age. At 1, 3, 6, 9, and 15 days of age, the body weights of PBB pups/control pups were: 6.1/6.6, 7.4/9.8, 10.0/14.0, 13.0/17.7, and 22.6/23.6 g, respectively. During the first 21 days, mortality was 14.3% in the pups of PBB-treated mothers and 1.5% in the pups of control mothers. Although the pups were fed control chow after weaning at 21 days, body weights of pups born and nursed by PBB-treated mothers continued to be lower than body weights of pups from control mothers (Fig. 1). The body weight gain was inhibited more in male pups than female pups nursing PBB-treated mothers; at 60 days of age the body significantly (p < 0.05) reduced fetal weight and crown-rump length. Corbett et al. (4) reported a slight decrease in fetal weight after pregnant rats were exposed to 1000 ppm PBB in their diet.
weight differences between treated and control pups were 50 g for male pups and 18 g for female pups. Food consumption of the pups was not measured.

In an effort to differentiate prenatal from postnatal nursing effects, pups were switched at the time of birth, and control mothers nursed pups from PBB-treated mothers and PBB-treated mothers nursed pups from control mothers (experiment 3, Tables 4 and 5). The foster male pups from the control–PBB and PBB–control treatments weighed less than control pups which had never been exposed to PBB (control–control) (Table 4). However, these reductions in body weights were not as great as those of male pups borne and nursed by PBB-treated mothers (PBB:PBB). A reduction in the weight of the perirenal fat pad was associated with body weight reductions in male pups. PBB also caused a reduction in the perirenal fat pad weight of female pups although body weights were not reduced significantly (experiment 3, Table 5). Mortality for each group of pups was: 11% (C:C); 24% (PBB:C); 16% (C:PBB); and 12% (PBB:PBB).

The absolute organ weights of 60 day old male (Table 4) and female pups (Table 5) were not affected by PBB. However, on a body weight basis, the liver and spleen weights of pups were increased.

Vaginal openings were delayed by either prenatal or postnatal nursing exposure to PBB (Table 6). The foster female pups from the control–PBB and PBB–control treatments had a delay in vaginal openings comparable with that of female pups borne and nursed by PBB-treated mothers (PBB:PBB).

In these experiments, the amount of milk secreted and the amount of PBB in the milk were not measured. Cows accidently contaminated with PBB had decreased milk production concomitant with decreased feed consumption (1); the decrease in feed consumption of the cow, rather than PBB, may have caused the reported decrease in milk production. In our studies, feed consumption was not reduced in mothers given PBB during pregnancy. However we did find a preweaning reduction in body weight of pups borne by control mothers and

![Figure 1. Mean body weights of pups from birth until 60 days of age. PBB pups were borne and nursed by rats given 10 mg PBB/day by gavage from days 7 through 15 of pregnancy. After weaning, all pups were fed control chow. Number of pups weighed were: male pups with control mother, 41; male pups with PBB-treated mother, 39; female pups with control mother, 23; and female pups with PBB-treated mother, 21.](image)

Table 4. Effects of PBB administration during pregnancy on body and paired organ weights of 60-day-old-male offspring.

| Expt | Pup exposure | n | Body weight g | Liver | Spleen | Kidneys | Adrenals | Testes | Seminal vesicles | Perirenal fat pads |
|------|--------------|---|---------------|-------|--------|---------|----------|--------|-----------------|------------------|
| 2    | C:C          | 22 | 285           | 11.8  | 0.66   | 2.40    | 0.044    | 2.74   | 0.62           | 1.24             |
|      |              |   |               | (4.1) | (0.23) | (0.83)  | (0.015)  | (0.96) | (0.22)         | (0.32)           |
| 2    | PBB:PBB      | 28 | 235b          | 10.0  | 0.65   | 2.00    | 0.038    | 2.28   | 0.42           | 0.68b            |
|      |              |   |               | (4.3) | (0.28)b| (0.86)  | (0.017)  | (0.99) | (0.19)         | (0.28)           |
| 3    | C:C          | 14 | 299           | 12.7  | 0.73   | 2.45    | 0.047    | 2.87   | 0.37           | 1.88             |
|      |              |   |               | (4.3) | (0.25) | (0.82)  | (0.016)  | (0.90) | (0.12)         | (0.60)           |
| 3    | C:PBB        | 13 | 264b          | 12.0  | 0.68   | 2.27    | 0.042    | 2.69   | 0.31           | 1.07b            |
|      |              |   |               | (4.6)b| (0.26) | (0.85)  | (0.016)  | (1.02) | (0.11)         | (0.27)b          |
| 3    | PBB:C        | 16 | 258b          | 10.3  | 0.77   | 2.17    | 0.042    | 2.57   | 0.30           | 1.03b            |
|      |              |   |               | (4.2) | (0.30)b| (0.85)  | (0.017)  | (1.00) | (0.12)         | (0.40)b          |
| 3    | PBB:PBB      | 8  | 239b          | 11.3  | 0.70   | 2.01    | 0.038    | 2.50   | 0.26           | 1.08             |
|      |              |   |               | (4.7)b| (0.30)b| (0.84)  | (0.016)  | (1.08) | (0.11)         | (0.43)b          |

* Pregnant rats were given 0 and 10 mg PBB by gavage on days 7 through 15 of pregnancy. Pup exposure to PBB was through placental transfer (prenatal) and/or mother’s milk during nursing (postnatal).

b $p < 0.05$ compared with C:C within same experiment.
nursed by PBB-treated mothers (C:PBB); this finding indicated an effect of PBB on the quality or quantity of the milk, or on pup appetite.

The secretion of PBB into cow's milk has been extensively studied (14). In the lactating cow, the concentration of PBB in the milk fat is approximately four times that in the total diet. Also, after withdrawal of PBB from the cow's diet, PBB continues to be drawn from body stores and secreted in the milk. The long biological half-life of PBB suggests that PBB will continue to be secreted into the milk for a considerable period of time and thus presents a contamination hazard to suckling young.

In our experiments, pregnant rats were dosed with 10 mg PBB/day for 9 days and no PBB was given after day 16 of pregnancy. At weaning, the mothers' abdominal fat contained 207 μg PBB/g fat; thus a considerable store of maternal PBB remained after lactation. At 21 and 48 days postpartum, body and organ weights of the PBB-treated mothers were comparable with those of control mothers (Table 7). The fact that the enlarged livers returned to normal weights may indicate a normal adaptive response of the liver to the increased metabolic load during PBB ingestion with subsequent recovery.

Results in these studies showed that 10-mg doses of PBB given daily to pregnant rats had no observable effect on embryonic development or parturition. However, transfer of PBB through the placenta and milk decreases the body weight gain of male pups

| Table 5. Effects of PBB administration during pregnancy on body and paired organ weights of 60-day-old female offspring. a |
|---|
| **Expt.** | **Pup exposure, prenatal/nursing** | **n** | **Body weight, g** | **Organ weight, g (organ weight/100 g body weight)** |
| | | | | **Liver** | **Spleen** | **Kidneys** | **Adrenals** | **Ovaries** | **Uterus** | **Perirenal fat pads** |
| 2 | C:C | 20 | 196 | 7.8 (4.0) | 0.45 | 1.67 | 0.054 | 0.082 | 0.34 | 0.73 |
| 2 | PBB:PBB | 18 | 178 b | 7.2 (4.0) | 0.43 | 1.44 | 0.048 | 0.066 | 0.28 | 0.29 b |
| 3 | C:C | 15 | 194 | 7.7 (4.0) | 0.46 | 1.64 | 0.060 | 0.077 | 0.28 | 0.71 |
| 3 | C:PBB | 7 | 189 | 8.2 (4.4 b) | 0.24 | 1.54 | 0.055 | 0.069 | 0.29 | 0.52 b |
| 3 | PBB:C | 7 | 172 | 7.2 (4.2 b) | 0.50 | 1.47 | 0.057 | 0.066 | 0.29 | 0.46 b |
| 3 | PBB:PBB | 12 | 184 | 8.6 (4.7 b) | 0.51 | 1.54 | 0.052 | 0.067 | 0.25 | 0.53 b |

a Pregnant rats were given 0 and 10 mg PBB by gavage on days 7 through 15 of pregnancy. Pup exposure to PBB was through placental transfer (prenatal) and/or mother's milk during nursing (postnatal).

b p < 0.05 compared with C:C within same experiment.

c p < 0.001.

| Table 6. Effect of PBB administration during gestation on vaginal opening time of offspring. a |
|---|
| **Expt.** | **Pup exposure, prenatal/postnatal** | **n** | **Vaginas open, %** |
| | | | **Day 31** | **Day 36** | **Day 41** | **Day 46** | **Day 53** |
| 2 | C:C | 23 | 13 | 48 | 91 | 100 | 100 |
| 2 | PBB:PBB | 17 | 6 | 24 | 82 | 100 | 100 |
| 3 | C:C | 16 | 0 | 50 | 87 | 87 | 100 |
| 3 | C:PBB | 7 | 0 | 28 | 43 | 67 | 83 |
| 3 | PBB:C | 8 | 0 | 38 | 75 | 75 | 75 |
| 3 | PBB:PBB | 10 | 0 | 30 | 54 | 64 | 73 |

a Pregnant rats were given 0 to 10 mg PBB by gavage on days 7 through 15 of pregnancy. Pup exposure to PBB was through placental transfer (prenatal) and/or mother's milk during nursing (postnatal).

| Table 7. Postpartum body and paired organ weights of rats given 0 and 10 mg PBB/day by gavage on days 7 through 15 of pregnancy. |
|---|
| **Expt.** | **Time postpartum, day** | **n** | **Dose, mg/day** | **Body weight, g** | **Liver, g** | **Spleen, g** | **Kidneys, g** | **Adrenals, mg** | **Ovaries, mg** | **Uterus, g** | **Perirenal fat pads, g** |
| 3 | 21 | 8 | 0 | 278 | 14.0 | 0.51 | 2.04 | 64 | 80 | 0.34 | 0.54 |
| 3 | 21 | 8 | 10 | 262 | 14.0 | 0.40 | 1.91 | 63 | 82 | 0.34 | 0.76 |
| 2 | 48 | 8 | 0 | 250 | 8.4 | 0.43 | 1.65 | 56 | 82 | 0.52 | 1.43 |
| 2 | 48 | 8 | 10 | 248 | 9.8 | 0.42 | 1.66 | 64 | 81 | 0.55 | 0.95 |

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and delays vaginal opening in female pups. The long-term biological effects are not known. A second-generation reproduction study could determine whether the delayed ovarian function and maturation in the male were temporary problems which may have been completely restored to normal during later stages of growth.

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