Bull. Environ. Contam. Toxicol. (1992) 48:715–720


Environmental Contamination and Toxicology

# Influence of Maternal Ingestion of Aroclor 1254® (PCB) or FireMaster BP-6® (PBB) on Unstimulated and Stimulated Corticosterone Levels in Young Rats

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The organohalides polychlorinated biphenyl (PCB) and polybrominated biphenyl (PBB) remain troublesome environmental pollutants. For example, the percentage of the population in which PCB is detectable in adipose tissue remains high (95.3%; Robinson *et al.* 1990). These compounds are of particular interest to residents of the North Central United States, especially in regions surrounding the Great Lakes where contaminated fish may be a regular component of the diet. Additionally, PBB was mistakenly fed to cattle and chickens in Michigan during the early 1970s, products of which were ingested by humans (Lilis *et al.* 1978).

Among the physiological effects of ingestion of PCB or PBB is the depression of thyroid status, which has been reported in adult humans (Bahn *et al.* 1980), in adult experimental animals (Allen-Rowlands *et al.* 1981), and in the offspring of these animals (Collins and Capen 1980). In adult rats, circulating levels of thyroid hormones are inversely proportional to dose of PCB or PBB in the diet (Byrne *et al.* 1987). On the other hand,reports of effects of these organohalides on adrenocortical function remain equivocal, describing both PCB- and PBB-induced depression (Byrne *et al.* 1988), and absence of effect in rats (Dunn *et al.* 1983) and monkeys (Loo *et al.* 1989).

Despite the possible consequences of maternal ingestion of PCB or PBB on future generations, little work has been done previously to determine whether consumption of these materials by pregnant and lactating animals confers hypothyroidism on their offspring, and/or influences other mechanisms of endocrine control in the young. Since early studies showed that hypothyroidism induced by feeding pregnant rats the goitrogen thiouracil altered the functional capabilities in their young of the hypothalamus-pituitary-adrenal (HPA) axis, as revealed by circulating corticosterone levels (Meserve and Leathem 1973, 1981), the present study was done to determine whether ingestion of either PCB (Aroclor 1254®) or PBB (FireMaster BP-6®) by pregnant and lactating rats resulted in depressed thyroid status and/or modified HPA axis function in their 15 day old young.

## MATERIALS AND METHODS

Female Sprague-Dawley rats weighing approximately 200 g were mated to males of the same strain. From the first day of pregnancy, as determined by sperm in

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vaginal washings, rats were caged singly and fed either Wayne Lab Blox mash (Premier Laboratory Diets, Inc., Bartonville, IL) or mash with the addition of PBB (FireMaster BP-6®, 50 ppm by weight) or PCB (Aroclor 1254®, 250 ppm by weight). The PBB was a generous donation of Ivan Egry, USFDA. An anonymous donor provided the PCB, which was originally produced by the Monsanto Company (St. Louis, MO). Mother rats were continued on the appropriate diet from conception to completion of the experiment, when pups were 15 days old. At 3 days of age, litters were standardized to 8 pups, 4 males and 4 females. Each dietary group consisted of at least 10 litters of rats. The functional capability of the HPA axis was tested in rat pups at 15 days of age. From each litter one male and one female served as unstimulated control animals, one pair received stimulation of the entire HPA axis through exposure to ether fumes, one pair received an injection of corticotropin-releasing factor (CRF) to by-pass the hypothalamic portion of the axis, and the remaining pair received an injection of adrenocorticotropic hormone (ACTH) to by-pass both hypothalamic and pituitary components. Unstimulated pups were removed from the home cage and rapidly decapitated. Ether-stressed rats were placed in a jar containing ether fumes for 1 min, and were decapitated 15 min later. Rats receiving CRF were injected ip (10 ng/g b wt, CRF-rat/human; Bachem, Inc., Torrance, CA), and were decapitated 15 min later. Animals receiving ACTH were injected subcu (4 IU/rat, Acthar Gel; Armour Pharmaceuticals, Phoenix AZ), and were decapitated 15 min later. Since novel manipulation readily activates the HPA axis, mock injections were administered for three days prior to CRF or ACTH injection to minimize anomalous stimulation (Walker *et al.* 1986). Upon decapitation, trunk blood was collected and serum removed for future determination of thyroid hormone content and corticosterone concentration. Additionally, body weights were determined to the nearest 0.1 g, and livers were weighed to the nearest 0.01 g. Gross effects of the halogenated biphenyls on endocrine glands were determined by weighing adrenal glands and thyroid glands to the nearest 0.1 mg. Serum concentrations of the thyroid hormones thyroxine (tetraiodothyronine, T4) and triiodothyronine (T3), and of corticosterone, were determined by using commercially available radioimmunoassay (RIA) kits. Thyroid hormone RIA kits were obtained from ICN Biomedicals, Inc.(Carson, CA). Kits for corticosterone were initially obtained from Cambridge Medical Diagnostics (Billerica, MA), but most were supplied by ICN Biomedicals when the initial supplier discontinued production. Corticosterone values for the same sample determined with each kit varied by less than 5%. Data were statistically evaluated by analysis of variance using a split plot design with repeated measures (Zar 1984). Significance was ascribed to $p<0.05$.

## RESULTS AND DISCUSSION

The concentrations of dietary PBB and PCB used in the present study (50 ppm and 250 ppm, respectively) could be interpreted as being unrealistically high, especially since Byrne *et al.* (1987) found chronic administration of PBB or PCB at doses between 1 and 50 ppm to significantly depress thyroid status. However, these doses were chosen for two reasons. Firstly, it was felt that it would be necessary to provide a sizeable dose to the pregnant and lactating mother so that sufficient material would transfer to the young to cause a measurable effect, particularly since the rat appears to require fairly high doses of PCB to cause physiological deficits such as of neurological alterations (Tilson *et al.* 1990). Secondly, the time of exposure of the young, from conception to termination, would be a maximum of 37 days (22 days prenatal, 15 days postnatal), a length of time causing minimal thyroid

depression in adult rats. The relatively minor effect on the average number of live births per litter (Control, 12 pups; PBB, 10 pups; PCB, 12 pups) provides evidence that the dosages used in the present study did not severely alter pregnancy.

By 15 days of age, maternal ingestion of either FireMaster BP-6®or Aroclor 1254® resulted in young with significantly subnormal body weight and markedly elevated liver mass (Table 1), as has been previously reported (Overman *et al.* 1987). Adrenal weights were not significantly influenced by PBB, but were depressed by PCB (84% of control). Both PBB (Allen-Rowlands *et al.* 1981) and PCB

Table 1. Effect of PBB or PCB on body and organ weights, and on circulating thyroid hormone levels, in 15 day old rats.

| | Control | PBB (FireMaster BP-6) | PCB (Aroclor 1254) |
|---|---|---|---|
| Body Wt. (g) | 31.4 ±0.8 | 26.1* ±0.3 | 22.4* ±1.0 |
| Liver Wt. (g/100 g b wt) | 3.20 ±0.03 | 5.22* ±0.11 | 5.85* ±0.27 |
| Adrenal Wt. (mg/100 g b wt) | 31.3 ±1.9 | 36.0 ±1.7 | 26.2* ±1.4 |
| Thyroid Wt. (mg/100 g b wt) | 19.4 ±1.1 | 23.4 ±2.2 | 17.5 ±1.3 |
| Thyroid Hormones | | | |
| $T_4$ (µg/dl) | 5.5 ±0.2 | 3.9* ±2.2 | 0.4* ±0.1 |
| $T_3$ (ng/dl) | 82.3 ±5.4 | 85.9 ±5.6 | 64.3* ±4.2 |

Organohalides were fed to pregnant and lactating dams (PBB, 50 ppm; PCB, 250 ppm). Values represent mean ± SEM of 10 litters of rats, 8 pups per litter.
*Significantly different from mean of corresponding control ($p<0.05$).

(Bastomsky 1976) have been reported to cause enlargement of thyroid glands in adult rats, but such was not the case in the young animals used in the present study (Table 1). Despite the normal thyroid mass, circulating $T_4$ levels were depressed significantly in rat pups by PBB (71% of normal) and even more pronouncedly by PCB (7% of normal). The maintenance of normal thyroid mass in these animals in the face of subnormal $T_4$ levels may be the result of the developmental status of the hypothalamus-pituitary-thyroid axis at this age, or the relatively minor effect of the organohalides on circulating concentrations of $T_3$ (Table 1), the purported biologically active thyroid hormone. In any event, maintenance of normal thyroid

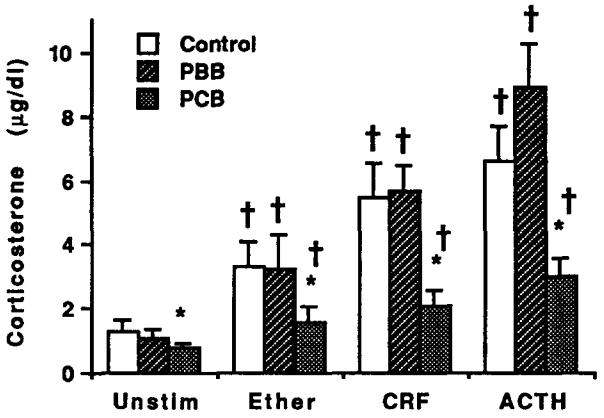


Figure 1. Effect of PBB (FireMaster BP-6®, 50 ppm) or PCB (Aroclor 1254®, 250 ppm) in the maternal diet on circulating corticosterone levels in 15 day old rats before or after stimulation of the hypothalamus pituitary adrenal axis. Unstim: decapitated upon removal from cage. Ether: 1 min exposure to ether fumes, decapitated 15 min later. CRF: injected ip with 10 ng/g b wt, decapitated 15 min later. ACTH: injected subcu with 4 IU/rat, decapitated 15 min later. Bars represent mean ± SEM for ten litters of rats, 2 pups per litter. *Significantly different from control ($p<0.05$). †Significantly different from unstimulated ($p<0.05$).

mass does not preclude damage to the thyroid gland, as previously reported by Collins and Capen (1980) using PCB.

Although both PBB and PCB depress thyroid status in young animals (Table 1), there appears to be a difference between the effects of FireMaster BP-6® and Aroclor 1254® with regard to HPA axis function (Fig. 1). Specifically, the PBB formulation did not modify pre-stimulation levels of corticosterone in circulation, nor did it prevent an increase in corticosterone after either the general axis stimulus of ether fumes, the direct stimulation of the pituitary with CRF, or the direct stimulation of the adrenals by ACTH. Indeed, the mean value of corticosterone was greater than normal in PBB fed rats injected with ACTH. On the other hand, the mixture of PCB significantly depressed pre-stimulation corticosterone levels, and resulted in subnormal (but statistically significant) elevations of corticosterone in response to all stimuli. This effect may be the result of PCB-induced hypothyroidism, since previous studies have found that hypothyroidism induced with thiouracil blunts the HPA axis response to ether in young rats (Meserve and Leathem 1973, 1981). However, the effect of PCB differs from that of thiouracil in that thiouracil did not decrease the adrenal response to direct stimulation by ACTH as did PCB. It is interesting to note that the adrenal output of corticosterone, as reflected by circulating levels, is greater the more closely to the adrenals the stimulation of the HPA axis was applied. That is, the response to

ACTH > CRF > ether, suggesting that PCB has additive detrimental effects on each component of the axis which become apparent as the consecutive components are called into play.

There are a number of possibilities why PBB did not influence the HPA axis as did PCB (Fig. 1), the simplest being that the dosage of FireMaster BP-6® was 20% of that at which Aroclor 1254® was administered, and that its effect was less in proportion to the dose. Supportive of this alternative is the lesser depression of thyroid status caused by PBB as compared to PCB (Table 1). However, the rationale for incorporating PBB into the maternal diet at a lesser concentration than PCB was that the study by Byrne *et al.* (1987) with adult animals demonstrated PBB to be about five times as potent in modifying thyroid status. Additionally, in the present study PCB at 250 ppm did not alter number of live births per litter, whereas PBB at 50 ppm depressed it slightly (by two pups per litter, see above). Thus, a higher dosage of PBB may have severely influenced litter size. A second possibility is that PCB and PBB may differentially influence the adrenal gland, with PCB having the more detrimental effect. Evidence from the present study for such a possibility is the pre-stimulation depression of corticosterone level caused by PCB, which does not occur with PBB (Fig. 1). Recent studies have found that PCB localizes in the zona fasciculata of the adrenal cortex (Durham and Brouwer 1990), the primary site of adrenal production of corticosterone, and alters the activity of enzymes involved in adrenal steroid production (Goldman and Yawetz 1990). Similar localization studies have not been done with PBB. A third possibility is that PBB and PCB cause differential toxicity to liver and kidney, thus resulting in a different rate of clearance of circulating corticosterone from circulation. Both PBB and PCB are known to damage liver and kidney (Kluew *et al.* 1979), but studies of relative toxicity have not been done. The influence of PBB on the number of live births per litter may have resulted from effects on fetal liver and kidney function, which might have had the effect in newborns of decreasing the rate of corticosterone clearance. Such an effect in the present study may have resulted in corticosterone levels which appeared normal, even though adrenal production of the hormone was subnormal. Because of the acute nature of the corticosterone response to stimulation, this possibile reason for a difference between PBB and PCB is the least likely of those suggested.

In summary, PBB (50 ppm) and PCB (250 ppm) in the maternal diet of rats depresses thyroid status of 15 day old offspring, with the effect on T4 being more pronounced than that on T3. The effect on HPA axis function is minimal with PBB at this dose, but PCB caused subnormal corticosterone levels before and after stimulation of axis function. The reason for the difference between PBB and PCB effects awaits further investigation.

**Acknowledgements.** The authors thank Phyllis Luke of ICN Biomedicals for the gift of RIA kits. Thanks are also due Laura Juarez de Ku, Ed Russ, and Lillian Shaffer for reading early drafts of the paper, and to Lorraine DeVenney for word processing advice. Supported by a Research Challenge Grant from the Ohio Board of Regents and Bowling Green State University.

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Received September 13, 1991; accepted November 13, 1991.