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Effect of Polybrominated Biphenyls (PBB) on the Pituitary–Thyroid Axis of the Rat (41099)[1,2]

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Abstract. The effects of polybrominated biphenyls (PBB) on the pituitary–thyroid axis were investigated in male rats. PBB was administered by gavage for 20 days at 1, 3, or 6 mg/kg/day; controls were gavaged with vehicle alone. PBB treatment had no effect on body weight. There was a definite time- and dose-dependent reduction in plasma thyroxine (T_4) levels after 10 and 20 days of PBB administration. Reduced plasma T_4 levels were correlated with increased plasma TSH levels at 20 days. In rats treated with 6 mg/kg PBB, an increase in 5-hr thyroidal ^{131}I uptake was seen, with a decrease in ^{131}I incorporation into monoiodotyrosine and an increase in intrathyroidal iodine. In contrast to propylthiouracil, acute administration of PBB had little effect on intrathyroidal radioiodine accumulation and incorporation into iodoamino acids. Liver and thyroid weights remained elevated at 2 and 5 months after the last 1 and 3 mg/kg PBB administration, respectively, and plasma T_4 levels were significantly lower than controls in both groups. PBB was detected in thyroid tissue after 20 days of 3 mg/kg treatment and was retained in thyroid and liver for 5 months post-treatment. Exposure to PBB resulted in a disruption of the normal homeostasis of the pituitary–thyroid axis and was associated with accumulation and retention of PBB in thyroid and liver.

Accidental introduction of polybrominated biphenyls (PBB) into the food chain occurred in Michigan during 1973. The recent results of a random survey of PBB levels in breast milk of lactating women in Michigan revealed detectable body burdens of PBB (1) and point to the biostability of this compound. Earlier reports demonstrated that PBB induced hepatic microsomal mixed-function oxygenases, e.g., arylhydrocarbon hydroxylase (2–4) and UDP-glucuronyltransferase (5, 6), in the livers of exposed laboratory animals. Since these enzymes are important in the normal metabolism of many hormones, the findings suggest that chronic low-level exposure to PBB could produce deleterious effects upon the endocrine system. In fact, there have been reports of compromised adrenal (7, 8) and reproductive (8–10) function in various animal species. Sleight *et al.* (7) and Kasza *et al.* (11) have reported increases in thyroid weight and changes in thyroid histology of animals fed a PBB-contaminated diet; these findings suggest the development of hypothyroidism (7–11). These authors also reported thyroidal ultrastructural changes which suggest PBB-induced alterations in the synthesis and/or secretion of thyroid hormones. The present study reports on the effects of oral administration of PBB on the pituitary–thyroid axis in the adult male rat. The persistent effects of PBB upon thyroid function following the cessation of PBB administration were also examined.

Materials and Methods. Adult male Sprague–Dawley rats weighing 300–400 g were housed individually in a quarantined biohazard laboratory and maintained on a standard 12-hr-dark–12-hr-light schedule with Purina rat chow and water available *ad libitum*. Experiments consisted of compa-

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rable numbers of animals receiving 1, 3, or 6 mg/kg PBB per day (obtained from the National Institute of Environmental Health Science, Lot No. FF-1312-FT, Batch 3) incorporated into lecithin liposomes. Control rats received gavages of lecithin liposomes alone (12). PBB was administered Monday through Friday between 0830 and 1000 hr for a total of 20 treatment days. After 10 days of PBB or vehicle administration, blood was withdrawn from the tail vein for plasma thyroxine (T_4) analysis. Following the last PBB administration, the animals treated with 1 and 3 mg/kg and four of the animals treated with 6 mg/kg and their corresponding controls were decapitated and blood was collected for plasma T_4 and TSH analysis. The thyroids and livers were removed and weighed. Thyroid tissues from animals treated with 3 mg/kg were stored at $-20°$ and representative samples were analyzed for PBB content. PBB- ($n = 9$) and vehicle-treated ($n = 9$) animals receiving 6 mg/kg were injected (ip) with 5 μCi of carrier-free ^{131}I. Five hours later the animals were decapitated and blood was collected for plasma T_4 analysis. The liver and thyroid weights were recorded. After being weighed, the thyroids were placed in Tris buffer (pH 8.5) containing 0.05 *M* methyl mercaptoimidazole and stored in an ice bath until the glands were counted for uptake of ^{131}I. After measurement of ^{131}I incorporation, the glands were individually homogenized and digested, the digests were chromatographed (5:1:2, butanol:ethanol:ammonia), and the chromatograms were subsequently scanned for the radiolabeled iodoamino acids (13). This procedure allowed for quantification of the intrathyroidal incorporation of ^{131}I into tyrosines and thyronines compared with that remaining as free iodide.

In two additional experiments, groups ($n = 8$) of animals were administered either 1 or 3 mg PBB/kg as described above, and controls ($n = 16$) received lecithin liposomes suspended in saline. After the 20th day of treatment all the animals were placed on a restricted food intake to decrease and maintain their body weight at 80% of that measured at the end of the PBB treatment in order to use these animals in operant conditioning studies (12). In the 1 mg/kg group, the PBB-treated and control animals were killed 2 months after the last PBB administration. Rats treated with 3 mg/kg PBB were killed 5 months after the last PBB administration. All the animals were killed as described above, and the blood was collected for analysis of plasma T_4. Liver and thyroid weights were recorded, and the tissues from animals receiving 3 mg/kg were stored at $-20°$. Selected liver and thyroid samples were analyzed for PBB content. PBB was undetectable in liver and thyroid from two gavaged control animals.

To evaluate the acute effects of PBB compared with propylthiouracil (PTU) upon thyroidal ^{131}I uptake and incorporation into the amino acids of thyroglobulin, additional groups of rats were gavaged with different doses of PBB (1, 10, or 100 mg/rat) or PTU (5 or 50 mg/rat) ($n = 3$ rats per each group). Three control rats were gavaged with the vehicle (corn oil) alone. Forty-five minutes later, all animals were injected (ip) with 10 μCi carrier-free ^{131}I. Four hours later, the animals were decapitated and the thyroids were removed, weighed, and placed in Tris buffer. The uptake of radioactivity by the thyroids was determined, and the thyroids were subjected to the same digestion and chromatographic procedures as described above.

Radioimmunoassays (*1*) T_4. Plasma T_4 was measured by radioimmunoassay with antisera and ^{125}I-T_4 obtained from Nuclear Medical Laboratories (Dallas, Tex.). The lower limit of detectability was 1.2 μg/dl. All samples from each experiment were assayed in duplicate. At a mean concentration of 3.0 μg/dl, intraassay and interassay coefficients of variation were 5% ($n = 5$) and 12% ($n = 10$), respectively.

(*2*) *TSH*. Plasma TSH was measured according to the RIA kit and procedure for rat TSH provided by the Rat Pituitary Hormone Distribution Program, NIAMDD (14). The lower limit of detectability was 10 μU/ml. All samples from the rats treated with 3 and 6 mg/kg PBB for 20 days were assayed in duplicate; the intra- and interassay coefficients of variation were both 15%.

PBB analysis. PBB was extracted from weighed tissue samples as described by

TABLE I. EFFECT OF 20 DAYS OF PBB ADMINISTRATION ON BODY, LIVER, AND THYROID ORGAN WEIGHTS IN MALE RATS

Treatment	Body weight (g)	Liver weight (g)	Thyroid weight (mg)
Control $N = 8$	315 ± 10[a]	12.3 ± 0.6	—
PBB 1 mg/kg $N = 8$	308 ± 10	16.5 ± 0.3*	—
Control $N = 8$	433 ± 11	14.5 ± 1.1	21.7 ± 1.3
PBB 3 mg/kg $N = 8$	432 ± 14	21.4 ± 1.3**	35.0 ± 2.9**
Control $N = 11$	483 ± 7	16.6 ± 0.3	22.6 ± 0.9
PBB 6 mg/kg $N = 11$	473 ± 8	24.3 ± 0.8*	27.0 ± 1.0***

[a] Mean ± SEM.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.025$.

Willett *et al.* (15). Samples were analyzed for free PBB residues by gas–liquid chromatography with electron capture detection. The 3% OV-17 column was maintained at 270° with a 5% methane in argon flow rate of 65 ml/min. The injection port and electron capture detector temperatures were 280 and 350°, respectively. Quantification was achieved with the use of an external standard method based on the area of the major peak, a hexabromobiphenyl, in the PBB mixture. Recovery of PBB added to blank tissue samples and then extracted averaged 96 ± 2.5% (mean ± SD). Results are presented as micrograms of PBB per gram of wet weight of tissue and are not corrected for recovery.

Differences between mean values for the measured parameters for the vehicle (control) and PBB-treated groups were analyzed for significance with Student's *t*-test. A *P* value of 0.05 or less was considered as a statistically significant difference.

Results. *Effect of 20 days of PBB administration on thyroid, liver, and body weights.* The effects of PBB on organ weight as compared with vehicle-treated controls are shown in Table I. There was no significant effect of PBB administration on mean body weights compared with the vehicle-treated controls in any of these studies. At all three doses of PBB, the mean

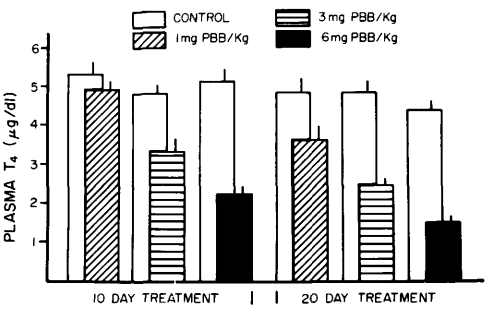

FIG. 1. Dose and time response to PBB treatment of plasma thyroxine (T_4) levels. Values are the mean ± SEM of 8 to 11 rats. Plasma T_4 levels were significantly depressed by all treatments ($P < 0.05$) except the 10-day 1 mg PBB/kg regimen ($P < 0.1$). Plasma T_4 levels in controls corresponding to any PBB dose level for any time interval were not significantly different from each other. In rats treated with 1 mg/kg PBB there was a significant decline in T_4 levels from Days 10 to 20 ($P < 0.02$). In the 3 and 6 mg/kg treated rats there was also a significant decline over this same time period ($P < 0.05$). In addition, a significant decline in T_4 level was seen with increasing PBB dose at both 10 and 20 days after the initiation of treatment. T_4 levels in the 3 mg/kg treated animals were significantly less at 10 days ($P < 0.01$) than those of the 1 mg/kg treated animals. Similarly in animals treated with 6 mg/kg PBB there was a significant decline from levels seen in the 3 mg/kg treated animals at 10 days ($P < 0.01$). In the 1 mg/kg treated rats T_4 levels at 20 days were significantly greater than those in the 3 mg/kg treated rats ($P < 0.02$) and in the 6 mg/kg treated rats there is a further significant decline in T_4 levels when compared with 3 mg/kg treated rats ($P < 0.01$).

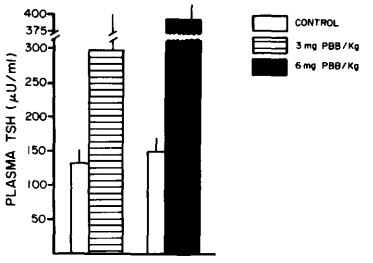

FIG. 2. Dose response to PBB treatment of TSH levels. Values are the means ± SEM of 8 to 11 animals. Plasma TSH levels were elevated following 3 mg/kg ($P < 0.1$) and 6 mg/kg PBB ($P < 0.01$) treatment for 20 days.

liver weights of the treated rats were greater than those of the vehicle-treated controls. PBB administration produced an increase in mean thyroid weight following both the 3 and 6 mg/kg doses of PBB. Thyroid weights were not obtained following the 1 mg/kg PBB treatment.

Effect of 10 and 20 days of PBB administration on plasma T_4 levels. The effects of PBB on plasma T_4 levels are shown in Fig. 1. Both the 3 and 6 mg/kg dose produced significant depressions of the mean T_4 levels after 10 days of PBB administration (Fig. 1). Following 20 days of treatment, there were significant depressions of the mean plasma T_4 levels at all three doses of PBB (Fig. 1). A significant time- and dose-dependent effect is seen in these results (Fig. 1).

Effect of 20 days of PBB administration on plasma TSH levels. Following 20 days of PBB administration at both the 3 and 6 mg/kg doses, plasma TSH levels were elevated compared with the control levels (Fig. 2); however, only the 6 mg/kg PBB dose produced a statistically significant elevation.

Thyroidal ^{131}I uptake and incorporation into the amino acids of thyroglobulin following 20 days of PBB administration. PBB administration at 6 mg/kg produced an increase in the 5-hr thyroid uptake of ^{131}I (Table II). The relative intrathyroidal distribution of radioiodinated amino acids showed a significant depression in the incorporation of ^{131}I into monoiodotyrosine (MIT) without any apparent effect upon the incorporation of radioiodine into diiodotyrosine (DIT), triiodothyronine (T_3) or T_4. However, PBB treatment did cause a significant increase in the amount of intrathyroidal iodide (I^-) compared to the vehicle-treated controls.

Residual effects of PBB administration on liver, thyroid, and body weights and plasma T_4 levels. The liver and thyroid weights were significantly elevated 2 months following the last 1 mg/kg administration of PBB (Table III). Plasma T_4 levels in the 1 mg PBB/kg-treated animals remained significantly depressed compared to controls (Fig. 3). Five months after administration of the final 3 mg/kg dose of PBB, the thyroid and liver weights were significantly elevated over the control values (Table III). Plasma T_4 levels in the 3 mg/kg post-treatment group remained significantly depressed compared to the vehicle controls (Fig. 3).

Thyroidal ^{131}I uptake and incorporation into thyroglobulin following the acute administration of either PBB or PTU. Acute (45-min) administration of PTU, but not PBB, produced a significant depression of the 4-hr thyroid uptake of ^{131}I (Table IV). PTU administration inhibits thyroidal peroxidase activity (16), thereby blocking the organification of iodide. Acute administration of PTU produced a decrease of DIT and MIT, and the iodothyronines (T_3 and T_4) were not detectable. There was no change in the relative distribution of ^{131}I incorporation into the amino acid residues of thyroglobulin as a result of the acute administration of PBB.

Analysis of PBB in liver and thyroid tissues. Figure 4 shows the results of gas chromatographic analysis of a control and a PBB-exposed thyroid. The thyroid from the exposed animal contains substantial amounts of PBB, whereas that of the control animal has none. For comparison, the chromatographic profile of the NIEHS PBB used in these studies is also shown (Fig. 4, inset). The "on column" amount of PBB obtained from this particular thyroid is 638 pg, which corresponds to 20.13 μg/g. Thyroid and liver tissue obtained from control animals did not contain detectable amounts of PBB. Substantial amounts of

PBB were found in the thyroid glands after 20 days of PBB treatment (3 mg/kg) and in thyroids and livers 5 months after the last PBB administration (Fig. 5). There was no significant depression in the mean PBB tissue concentration in the thyroids 5 months post-treatment compared with the level at the cessation of treatment.

Discussion. These results clearly indicate that exposure of rats to PBB exerts both immediate and residual effects on thyroid function. The degree of plasma T_4 reduction resulting from PBB ingestion was both dose and time dependent. Rats given the lowest total cumulative PBB dose, approximately 9.0 mg, had a significant reduction in plasma T_4 levels which was maintained for at least 2 months following the end of PBB administration. The effect of PBB on plasma T_4 levels produced the expected increase in plasma TSH levels, which was more pronounced as the PBB dosage was increased.

Polybrominated biphenyls are potent inducers of microsomal enzymes, particularly in the liver, with proliferation of hepatocyte smooth endoplasmic reticulum (2, 4, 17). In the present studies, the smallest cumulative dose (9.0 mg) of PBB resulted in a significant increase in liver weight as compared with that of controls. This condition persisted up to 2 months following the final dose of PBB. Increased liver weight in male rats that had received PBB in their feed was associated with an increase in liver microsomal UDP-glucuronyltransferase activity (5). The same PBB-induced alterations in hepatic function in pups obtained from PBB-treated dams have also been reported (4). These findings are particularly relevant to the present study since UDP-glucuronyltransferase is responsible for the glucuronidation of T_4 and triiodothyronine prior to their biliary excretion (16). This ability of PBB to induce UDP-glucuronyltransferase activity in rats may result in accelerated metabolism of peripheral thyroid hormone and thus contribute to reduced T_4 levels seen in this study. Another contributing factor may be a reduced binding of T_4 to plasma proteins which may accelerate metabolism, an effect reported for polychlorinated biphenyls (PCB) (18).

TABLE II. EFFECT OF 20 DAYS OF ADMINISTRATION ON RELATIVE INTRATHYROIDAL DISTRIBUTION OF ^{131}I

Treatment	Relative intrathyroidal distribution of ^{131}I (%)						Thyroidal ^{131}I uptake (5 hr) (%)
	0	DIT[a]	MIT[a]	I^-	T_4	T_3	
Gavage control $N = 9$	4.0 ± 0.2	39.0 ± 0.7	24.1 ± 0.8	5.7 ± 0.5	15.0 ± 0.8	3.2 ± 0.1	7.9 ± 0.4
PBB 6 mg/kg × 20 d $N = 9$	5.3 ± 0.5	37.7 ± 0.6	19.9 ± 0.6*	9.4 ± 0.7*	15.0 ± 0.5	3.4 ± 0.2	16.7 ± 2.3*

[a] DIT, diiodotyrosine; MIT, monoiodotyrosine.
* $P < 0.01$.

TABLE III. BODY, LIVER, AND THYROID WEIGHTS 2 AND 5 MONTHS POST-PBB ADMINISTRATION IN MALE RATS

Treatment	Time post-treatment (months)	Body weight (g)	Liver weight (g)	Thyroid weight (mg)
Control $N = 4$	2	337 ± 12	7.8 ± 0.2	19.2 ± 0.7
1 mg PBB/kg $N = 6$	2	325 ± 0	12.3 ± 0.7*	23.5 ± 3.1**
Control $N = 6$	5	346 ± 6	9.7 ± 0.4	20.1 ± 0.9
3 mg PBB/kg $N = 7$	5	348 ± 9	15.5 ± 0.6*	27.6 ± 1.5*

* $P < 0.01$.
** $P < 0.05$.

Enhanced biliary excretion of T_4 has been reported for PCB-treated rats (19).

A previous report of thyroid ultrastructure following PBB feeding demonstrated increased lysosomal bodies with a concomitant increase in colloid droplet accumulation (11). These observations suggest that PBB might interfere with the normal synthesis and/or secretion of thyroid hormones. In the present study, PBB was shown to accumulate in the thyroid following 20 days of treatment and was still present up to 5 months following the last administration. Five months post-treatment, the thyroid/liver PBB ratio was 4.5- and 5.7-fold greater than the thymus/liver and brain/liver ratios, respectively (M. G. Hamilton, unpublished observations). Preferential sequestration of PBB in the thyroid suggests that PBB may bind to thyroidal macromolecules and may affect the incorporation of radioiodine into intrathyroidal amino acid. PBB administration did produce a significant increase in intrathyroidal iodine and a concomitant decrease in the incorporation of radioiodine into monoiodotyrosine. This suggests that PBB might act in some manner to inhibit the organification of iodide by peroxidase (20). Data from the present studies, comparing the known acute effect of PTU on intrathyroidal peroxidase activity with that of PBB, indicate that PBB does not act acutely to inhibit this enzyme, so the mechanism(s) whereby PBB decreases the utilization of iodide and the ensuing iodination of tyrosine remains obscure. Owing to a prolonged retention of PBB in affected tissues, even short-term administration may result in a persistent exposure, and throws doubt on the validity of terminology such as acute or chronic in the case of such compounds.

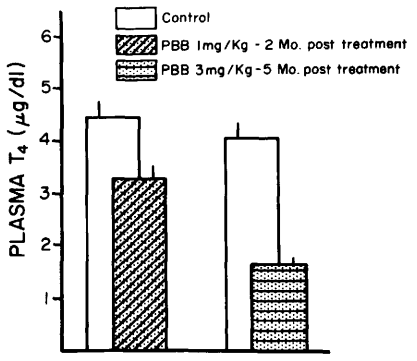

FIG. 3. Persistent effects of PBB on plasma thyroxine (T_4) levels. Values are the mean ± SEM of four to seven animals. Plasma T_4 levels were significantly ($P < 0.01$) depressed in both treatment groups.

The present studies have shown that the PBB-induced reduction in plasma T_4 levels exerts the expected effect upon pituitary secretion of TSH. The significantly elevated plasma TSH levels, associated with significant increases in both thyroid weight and uptake of radioiodine, indicate that PBB treatment resulted in thyroid gland hypertrophy due to decreased negative feedback of thyroxine on the pituitary secretion of TSH. These findings are similar to a recent report showing that rats fed a

TABLE IV. COMPARISON OF ACUTE ADMINISTRATION OF PTU AND PBB ON RELATIVE INTRATHYROIDAL DISTRIBUTION OF ^{131}I[a]

Treatment	Relative intrathyroidal distribution of ^{131}I (%)						Thyroid ^{131}I uptake (4 hr) (% dose)
	0	DIT	MIT	I^-	T_4	T_3	
Control $N = 3$	3.9 ± 0.2	38.9 ± 1.6	27.8 ± 1.0	4.5 ± 0.4	14.4 ± 0.6	2.7 ± 0.4	12.3 ± 1.9
1 mg PBB $N = 3$	4.0 ± 0.2	40.6 ± 0.7	26.2 ± 0.9	5.5 ± 0.2	14.8 ± 0.4	3.0 ± 0.3	8.5 ± 1.1
10 mg PBB $N = 3$	3.4 ± 0.1	39.8 ± 0.7	27.0 ± 1.1	6.2 ± 0.2	14.3 ± 1.5	3.2 ± 0.1	11.2 ± 0.9
100 mg PBB $N = 3$	3.7 ± 0.2	42.9 ± 2.2	27.0 ± 1.4	5.3 ± 0.6	13.9 ± 1.1	2.7 ± 0.3	9.5 ± 0.3
5 mg PTU $N = 3$	1.5 ± 0.03*	—	9.3 ± 1.7*	79.8 ± 3.8*	—	—	0.38 ± 0.05*
50 mg PTU $N = 3$	1.3 ± 0.1*	—	2.9 ± 0.6*	88.5 ± 1.8*	—	—	0.38 ± 0.05*

[a] PTU and PBB were administered by gavage; 45 min later rats were injected (ip) with 10 μCi ^{131}I and decapitated 4 hr later.
* $P < 0.01$.

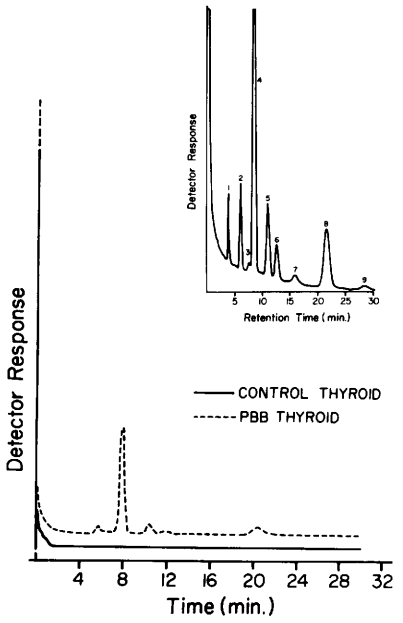

FIG. 4. Gas chromatographic profile of control and PBB-exposed thyroid extract. Note the presence of a large peak with a retention time of 8 min and several minor peaks corresponding to peak numbers 2, 4, 5, 6, and 8 of the PBB mixture used in these studies (upper right). See text for details of gas chromatographic conditions.

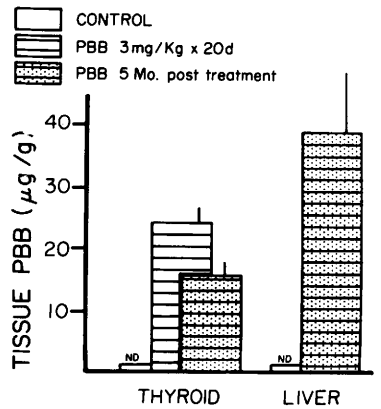

FIG. 5. Thyroid and liver PBB concentration at the end of 20 treatment days of 3 mg PBB/kg and at 5 months post-treatment. Treatment values are mean ± SEM of four to seven animals and control means are from two animals. Thyroid PBB levels were not significantly decreased 5 months posttreatment compared to the mean level obtained at the end of 20 treatment days. No detectable levels of PBB were obtained in control thyroid or liver tissues.

polychlorinated biphenyl diet developed goiters associated with decreased serum T_4, elevated serum TSH, and elevated thyroid uptake of radioiodine (18). TSH was not measured in those rats that were killed at 2 or 5 months after the cessation of PBB administration; however, the persistent hypothyroidism is a strong indication of a persistent elevation of TSH.

In conclusion, the data presented in these studies show that exposure to PBB results in the disruption of the normal homeostasis of the pituitary–thyroid axis. The demonstrated presence of PBB in liver and thyroid tissue and the finding of enlarged thyroids associated with decreased plasma thyroxine levels (up to 5 months after cessation of treatment) clearly demonstrate the persistent and deleterious effects of this bioactive chemical. Further studies to investigate the effect of PBB on other endocrine systems are in progress in our laboratory. The significance of these studies is indicated by the recent report of an increased incidence of hypothyroidism in a group of human males exposed to PBB (21).

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