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Endocrine Disruptive Effects of Polychlorinated Biphenyls on the Thyroid Gland in Female Rats

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Kiliç, N., Sandal, S., Çolakoğlu, N., Kutlu, S., Seyran, A. and Yilmaz, B. *Endocrine Disruptive Effects of Polychlorinated Biphenyls on the Thyroid Gland in Female Rats.* Tohoku J. Exp. Med., 2005, **206** (4), 327-332 —— Polychlorinated biphenyls (PCBs) are persistent environmental pollutants. Aroclor 1221 (A1221) and Aroclor 1254 (A1254) are commercial PCB mixtures with low and high number of chlorination, respectively. We have comparatively investigated effects of A1221 and A1254 on serum levels of thyroid hormones and thyroid gland histology in adult female Wistar rats. Animals were subcutaneously injected with A1221 (10 mg/kg) or A1254 (10 mg/kg) for six weeks. One group of animals served as control. At the end, all animals were decapitated and trunk blood collected. Serum levels of triiodothyronine (T3) and thyroxine (T4) were measured by the electrochemiluminescence immunoassay method. Thyroid glands were removed for histopathological examination under light microscopy. Serum total T4 levels were significantly increased in A1221- and A1254-treated rats ($p < 0.05$). Serum free T4 levels were significantly increased in the A1254-treated rats ($p < 0.01$), but not in the A1221-treated rats. In contrast, the treatment with A1221 caused a significant increase in serum free T3 concentrations ($p < 0.05$) but not with A1254. Notably, either A1221 or A1254 caused distinct histopathological changes, such as formation of many microfollicles in the thyroid gland, which mimic the changes seen in thyrotoxicosis. In conclusion, both PCB mixtures induce toxic effects in the thyroid gland regardless of their degree of chlorination. We suggest that these environmental contaminants may disrupt thyroid hormone homeostasis in exposed individuals and thus pose a threat to human health. ———— PCBs; thyroxine; triiodothyronine; Aroclor and thyroid histology
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Polychlorinated biphenyls (PCBs) are a major class of environmental contaminants (Hansen 1999; ATSDR 2000). Because of their exceptional chemical properties and stability, they were widely used in transformers and capacitators as dielectrics, in hydraulic systems, in pesticides, and as additives in paints, copying paper, adhesives, sealants and plastics (Carpenter 1998; Hansen 1999). In the United States, PCBs were manufactured as complex mixtures under the trade name Aroclor with a designation indicating the extent of chlorination (Karcher et al. 2004). Production and use of PCBs were banned or limited in late 1970s, however, they are still in use in

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closed system applications. It is estimated that approximately 33% of the PCBs ever produced are still environmentally available (Tanabe 1988; Kimbrough 1995). They are lipophilic and therefore tend to accumulate in fatty tissues (Safe 1994). Contamination with these persistent toxic substances occurs primarily by ingestion of contaminated food (Carpenter 1998). PCBs have been reported to elicit various adverse effects on human health, including carcinogenicity, endocrine disruption, neural, immune, developmental and reproductive toxicity (Robertson and Hansen 2001; Nakai et al. 2004; Ulbrich and Stahlmann 2004). Relative toxic potencies of congeneric PCBs and/or their commercial mixtures depend largely on the degree of chlorination and pattern of chlorine substitution (Carpenter 1998; Karcher et al. 2004).

Thyroid hormones are of importance for regulation of metabolism, and interference with thyroid function has serious consequences at all ages, but particularly during development (Porterfield and Hendrich 1993). The thyroid gland stores several weeks supply of triiodothyronine (T3) and thyroxine (T4), which are released at amounts of approximately 7% and 93%, respectively. These hormones in the circulation are largely bound to thyroid hormone-binding globulin, transthyretin or albumin. T4 is converted to T3 primarily in the liver and kidney, and T3 is the active form of thyroid hormones in the target tissues (Griffin 2000).

It is generally believed that PCB exposure decreases serum thyroxine (T4) concentrations in both adult and neonatal rodents (McKinney and Waller 1994; Ulbrich and Stahlmann 2004). In an early study, Collins and Capen (1980) reported that perinatal rats exposed in utero and by the milk to PCBs showed structural changes and reduction in serum thyroid hormone levels. Similar observations have been reported by a number of other investigators (Anbalagan et al. 2003; Khan and Hansen 2003). However, increased levels of thyroid hormones as an immediate response to PCB treatment have also been documented (Guengerich 1989). It has been suggested that there is structural similarity between thyroid hormone (T4) and some PCB congeners (Chauhan et al. 2000). As a consequence, these contaminants may interfere with thyroid function by binding to the thyroid transporting proteins, reducing production or increasing metabolism of T4 or reducing conversion of T4 to T3 (McKinney and Waller 1994). It may thus result in a functional hypothyroidism, most likely due to displacement of the endogenous hormone from the binding proteins which promote excretion of the thyroid hormone.

Aroclor 1221 (A1221) and Aroclor 1254 (A1254) are two commercial PCB mixtures with low and high number of chlorination, respectively. There is limited literature on the use of A1221 in animal studies. This study was undertaken to comparatively investigate the effects of A1221 or A1254 on serum levels of thyroid hormones and histology of the thyroid gland in adult female rats.

Materials and Methods

Adult female Wistar rats (200-220 g) were used in this study. They were divided into three groups each consisting of eight animals. Animals were subcutaneously injected with A1221 (10 mg/kg) or A1254 (10 mg/kg) every other day for a period of six weeks. These moderate doses and treatment period for both PCB mixtures were chosen on the basis of a recent review (Ulbrich and Stahlmann 2004) and a report by Anbalagan et al. (2003). One group of animals served as control and subcutaneously received vehicle dimethyl sulfoxide (DMSO, 4%; Amresco, OH, USA) alone. Aroclor mixtures (lot numbers: A1221: 083-166 and A1254: 124-191-B) were obtained from AccuStandard Inc. (New Haven, CT, USA). All experiments were performed in compliance with institutional and international guidelines for laboratory animal care.

At the end of the experiments, all animals were decapitated and trunk blood collected. Serum samples were obtained after centrifugation of blood samples at 3,000 rpm (4°C) for 10 min. Serum levels of thyroid hormones (free T3: fT3, free T4: fT4, total T3: tT3 and total T4: tT4) were measured with a Hitachi E170 (Elecsys module) immunoassay analyzer by using electrochemiluminescence immunoassay method (Roche Diagnostics GmbH, Mannheim, Germany). Determination of T3 and T4 was achieved by using polyclonal antibodies specifically directed against T3 and T4 in a competitive test. Endogenous T3 and T4, released by the action of 8-anilino-naphthalene sulfonic acid,

compete with the added biotinylated T3 and T4 derivatives for the binding sites on the antibodies labeled with a ruthenium complex. In the Elecsys tests, determination of fT3 and fT4 is made with the aid of specific anti T3 and T4 antibodies labeled with the ruthenium complex.

Thyroid glands were removed for histopathological examination under light microscopy. They were fixed in 10% formaldehyde solution, processed for light microscopy and then embedded in paraffin blocks. Tissue sections (5 μm) were cut and stained with Hematoxylin Eosin (HE). They were examined in an Olympus BH2 microscope and photographed.

The hormone findings were statistically analyzed by using One-Way ANOVA followed by post-hoc Borferroni correction test. The results were expressed as mean ± S.E.M. $P < 0.05$ was considered to be statistically significant.

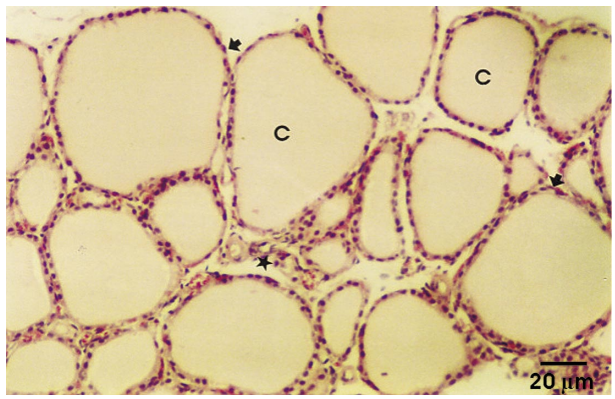


Fig. 1. Follicles, colloid and interfollicular area appeared to be in normal structure in the thyroid tissue of the control group. Arrow: follicle, C: intrafollicular colloid and *: interfollicular area. (HE ×120).

Results

Serum levels of thyroid hormones are summarized in Table 1. Although serum tT3 levels were slightly elevated in both PCB-treated groups, the differences were not statistically different. Serum levels of tT4 were significantly increased by A1221 or A1254 ($p < 0.05$). A1221 administration significantly increased serum fT3 concentrations ($p < 0.05$). Treatment with A1254 resulted in significant increases in serum fT4 levels in comparison to the control group values ($p < 0.01$).

Light microscopic examination showed that follicles, colloid and interfollicular area were normal in the thyroid tissue of the control group (Fig. 1). Many microfollicles and reduced colloid content within the follicles were observed in the A1221-treated group (Fig. 2). Perivascular vas-

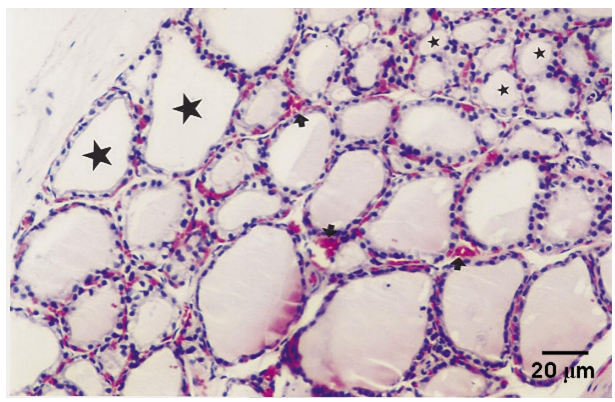
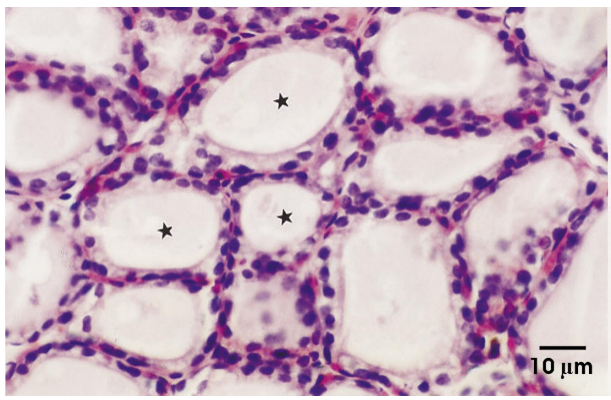


Fig. 2. A1221-treated group. There were several microfollicles in addition to normal thyroid follicles. Colloid content of the follicles were reduced and completely absorbed in some instances. Small *: microfollicles, Big *: reduced intrafollicular colloid volume and Arrow: vascularization in the perifollicular area. (HE × 120).

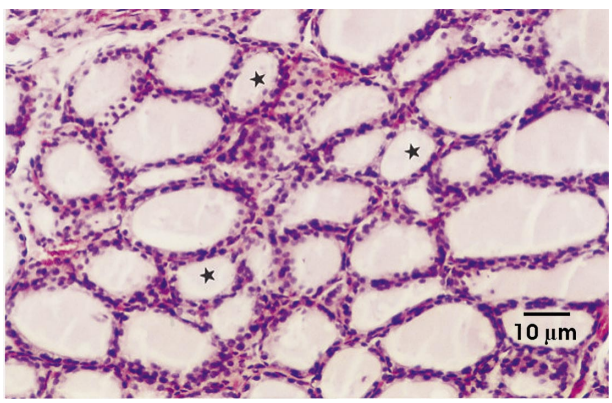
Table 1. *Serum levels of thyroid hormones (mean ± S.E.M.) in female rats following treatment with vehicle (DMSO, 4%), A1221 or A1254 for a period of six weeks.*

Thyroid hormones	Control	Aroclor 1221	Aroclor 1254
tT3 (pg/ml)	195.6 ± 6.4	209.5 ± 5.9	207.0 ± 3.8
fT3 (ng/100 ml)	5.48 ± 0.29	6.31 ± 0.20*	5.97 ± 0.23
tT4 (ng/100 ml)	4.49 ± 0.28	5.32 ± 0.17*	5.46 ± 0.23*
fT4 (μg/100 ml)	3.28 ± 0.23	3.73 ± 0.15	4.09 ± 0.17**

* $p < 0.05$ and ** $p < 0.01$ compared to the control group, using One-Way ANOVA followed by post-hoc Borferroni correction test.



A



B

Fig. 3. A1254-treated group. A: Administration of A1254 induced several microfollicles in the thyroid gland. Cellular increase was observed. * microfollicles. HE × 120. B: Microfollicles and reduced intrafollicular colloid volume (*) are evident. HE × 240.

cularization was prominent in this group. Administration of A1254 resulted in cellular increase and formation of microfollicles in the thyroid gland (Fig. 3A). Intrafollicular colloid volume was also reduced in this group (Fig. 3B).

Discussion

The present study has shown that serum levels of thyroid hormones increase following chronic administration of A1221 or A1254. Toxic effects of several individual PCB congeners such as PCBs 28 (Chu et al. 1996) and 118 (Kuriyama et al. 2003) and mixtures such as Aroclor 1242, A1254 and Aroclor 1260 (Mayes et al. 1998; Chauhan et al. 2000) have been examined by a number of groups. A1254 (a high chlorinated PCB) appears to be the most studied PCB mixture with regard to its thyroid-modulating effects. In contrast, actions of a low chlorinated PCB mixture A1221 on the thyroid gland have not been examined. In the present study, effects of A1221 were also tested and compared with those of A1254.

There are number of studies in the literature reporting that PCBs cause a reduction in thyroid hormones, particularly T4, in experimental animal models (McKinney and Waller 1994; Khan et al. 2002; Anbalagan et al. 2003). One of the mechanisms discussed for this decrease may be the ability of PCBs to induce hepatic microsomal enzymes leading to biliary excretion and elimination of the thyroid hormones (Kato et al. 2004; Ulbrich and Stahlmann 2004). It has also been attributed to PCB displacement of T4 and T3 from serum thyroid binding proteins since PCBs show good binding affinity to transthyretin (Chauhan et al. 2000). Results of our study appear to be contradictory to several reports of others.

It has recently been reported that A1254 administration (200 μg/kg body weight) for 15 days induced hypothyroidism in rats (Anbalagan et al. 2003); namely, T3 and T4 levels were reduced in the A1254-treated animals. Administration of A1254 to adult male rats for seven days resulted in significant reductions in serum T3 and T4 concentrations (Hood et al. 1999). The discrepancy between our results and those reports may be explained by the duration of PCB treatment, although a recent study has shown that injection of PCB 77 (20 mg/kg/day) for a period of three months had no significant effect on plasma T4 levels in male rats (Hsu et al. 2004). An alternative mechanism of PCB action on the thyroid homeostasis has recently been suggested. Perinatal exposure to a low dose of PCB 118 permanently disrupted the hypothalamo-pituitary-thyroid axis leading to a significant increase in T4 levels in offspring, as a “thyroid resistance syndrome” (Kuriyama et al. 2003). PCB 118 is one of the most persistent congeners found in human tissues and is regularly detected in human breast milk

(Dagnelie et al. 1992).

Presence of several micro thyroid follicles and reduction of follicular colloid volume after administration of A1221 or A1254 in our study implicate a state of thyrotoxicosis, which seems to be consistent with elevated levels of T4. Our observation is consistent in part with a recent study that A1254 induced an increased rate of thyroid carcinomas in rats (Vansell et al. 2004). A1254 administration brought about an increase in height of the thyroid follicular epithelial cells (Saeed and Hansen 1997). The dose-dependent changes in the histology of thyroid gland after dietary treatment with PCB 28 for 90 days have been shown (Chu et al. 1996). However, no change in thyroid gland morphology was observed following treatment of female rats with PCBs 95 and 101 at 32 mg/kg dose for two consecutive days (Khan and Hansen 2003). Similarly, short-term (seven days) treatment with A1254 had no significant impact on the epithelial, colloid and interstitial areas of the thyroid gland (Hood et al. 1999). Our findings suggest that A1221 or A1254 many induce toxic effects in the thyroid gland after long-term treatment.

In conclusion, both Aroclor mixtures tested increased serum levels of thyroid hormones regardless of their degree of chlorination. These findings indicate that PCBs disrupt thyroid hormone homeostasis in rats. These persistent environmental pollutants may pose a threat to human health in contaminated areas.

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