The Effects of Polychlorinated Biphenyl on Circulating Leptin and Thyroid Hormone Status in Sprague-Dawley Rats, *Rattus norvegicus*[1]

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Abstract. **Polychlorinated biphenyls (PCB) are persistent environmental chemicals that are known thyroid hormone disrupters. Frequently the disruption of one endocrine axis and the timing of the disruption have an impact on other interdependent hormonal responses. Although the mechanisms for the interdependency of thyroid hormones and leptin have not been fully characterized, both are linked to development and regulation of metabolism. Furthermore, PCB accumulation in depot fat could potentially alter leptin production. In the present study 15-and 30-day-old Sprague-Dawley rats were exposed gestationally and lactationally to 1.25 ppm of Aroclor 1254®, a mixture of 52 PCB congeners, via maternal diet, to determine the effect on leptin and thyroid hormones. Additionally, young adult female rats were fed 1.25 PCB for 21 days and the same hormones were assessed. Serum leptin concentrations were determined by a sensitive murine leptin ELISA (DSL, Inc., Webster, TX). Serum thyroid hormone levels were determined by RIA kits (MP Biomedicals, Carson, CA). Leptin concentrations were significantly depressed in 15-day-old animals exposed to PCB when compared to same-aged control animals, while thyroid hormones were similar in control and experimental animals. Thirty-day-old PCB treated rats displayed significantly elevated leptin levels and depressed triiodothyronine concentrations. Young adult rats exposed to PCB for 21 days displayed significantly depressed leptin concentrations, however PCB had no effect on thyroid hormones in this group. In summary, exposure to dietary PCB, at relatively low concentrations, is leading to measurable alterations in serum leptin levels. We speculate that the accumulation of fat-soluble PCB in adipocytes may be sufficient to cause these alterations. Further investigation into the mechanism causing leptin alteration and long-term effects of such alterations is warranted.**

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INTRODUCTION

Polychlorinated biphenyls (PCB) are endocrine disrupters that were widely used in industry until banned from production more than 25 years ago. The thermal and chemical stability and biological persistence of these aromatic hydrocarbons causes them to remain a public health concern. However, as a temporary measure to protect the public, the United States Food and Drug Administration (FDA) has determined tolerable limits of PCB in food. Exposure to concentrations similar to FDA tolerable limits and concentrations found in nature has been shown to alter endocrine function (Schantz and others 1997; Desaulniers and others 1997; Pritts 1996). Transcutaneous and digestive absorption of PCB leads to bioaccumulation, and passage to offspring occurs primarily from breast with limited exposure through the placenta. Perinatal exposure to PCB either directly or through maternal exposure causes alterations in concentrations of growth-related hormones and growth in birds (Gould and others 1997), hippocampal information processing and neurochemical status in rats (Provost and others 1999), auditory and hormonal deficits in rats (Goldey and others 1995), and depressed immunocompetence (Tryphonas and others 1998) and decreased reproductive success in rats (Donahue and others 2002; Tryphonas and others 1998).

The direct alteration of hormonal status leads to many of the other PCB-induced physiological alterations linked to PCB exposure. However, no previous studies have investigated the impact of PCB on the circulating concentrations of leptin, a hormone produced in adipose tissue, the primary site of PCB accumulation. During development, alterations in the production of leptin by the bioaccumulation of PCB in adipose tissue could cause long-term problems. In developing animals leptin plays a role in growth (Proulx and others 2002) and reproductive system development (Chehab and others 1997), as well as the establishment of brain circuitry involved in eating behavior (Bouret and others 2004). Disruption of circulating leptin concentrations during this time could have long-term implications. In young adult animals leptin is one of several hormones responsible for maintaining body weight and preventing obesity. Alterations in leptin concentrations in young adult animals could contribute to the depressed body weight and reproductive abnormalities caused by PCB exposure.

Thyroid hormones and leptin both contribute to development and cellular metabolism. Since thyroid hormones are depressed by PCB exposure (French and others 2001; Juárez de Ku 1992) these hormones were measured to determine potential leptin-thyroid relationships during contaminant exposure. Depression or elevation in serum thyroid hormone and leptin levels has age-dependent ramifications. Endocrine disruption in developing animals can have age-dependent, life altering effects. Alterations in the endocrine status of adults are often times reversible, with the effects being far more transient than those during development. If environmental contaminants are causing endocrine disruption in animals in the wild there could be previously unidentified changes in ecosystems. Therefore, the age-dependent differences in leptin and thyroid hormone function and the regulatory relationship between the hormones provide an important rationale for investigating exposure to PCB in developing and young adult animals.

MATERIALS AND METHODS

Aroclor® 1254 is a mixture of about 52 PCB congeners that was produced by Monsanto Co. (St. Louis, MO) and commonly used in industry because of the thermal stability. The Aroclor® 1254 used in this study was purchased from AccuStandard (New Haven, CT) and was hand mixed with ground rat chow from Harlan Teklad (Madison, WI) to achieve 0.0 or 1.25 ppm

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(0.0, or 1.25 mg Aroclor® 1254/Kg diet). This diet was fed to Sprague-Dawley rats obtained from the animal care facility at Bowling Green State University, Bowling Green, OH. Food and distilled water were provided ad libitum and a 12-hour light dark cycle was maintained. Rats were housed in plastic shoebox cages with metal lids in a climate-controlled room. Dust covers were used to provide protection from cross contamination. All animal procedures for this study were approved by the Bowling Green State University Institutional Animal Care and Use Committee.

To investigate the effect of PCB during development thirty-two female Sprague-Dawley rats were mated to males of the same strain. The pregnant females ($n = 32$) were randomly assigned to the treatment group or the control group upon conception. On the day of conception, determined by a sperm positive vaginal washing, females were fed diets containing 0.0 or 1.25 ppm of Aroclor® 1254. Treatment was provided through maternal diet during gestation and lactation to determine the effects of exposure during development in 15- and 30-day-old animals. The United States Environmental Protection Agency (USEPA) has established reference doses of 5.0 mg/kg of body weight/day of Aroclor® 1254 based on the lowest level of exposure with observable effects (USEPA 1999). In the present study the mean dose of Aroclor® 1254 for adult females was 0.6 mg/kg of body weight/day during gestation and lactation. Pups maintained to 30 days also received some PCB through direct dietary ingestion. Offspring remained in the maternal cage until decapitation. Litters were standardized at 8 pups (4 males and 4 females when possible) on day 3 after birth. Food was weighed daily to monitor food consumption and to determine PCB intake.

Young adult animals were exposed to PCB to determine the difference between exposure during early development and during later life. Young adult rats were fed diets containing 0.0 or 1.25 ppm of Aroclor® 1254 for 21 days. Animals were fed the diet beginning when they first weighed at least 85 g. This resulted in a beginning age range of 30-35 days and a termination age of 51-56 days. Food was weighed daily to monitor food consumption.

At 15, 30, or 51-56 days of age trunk blood was collected and allowed to clot before centrifugation for 15 min to separate serum. Serum was stored at -20° C until assays were conducted. Serum leptin concentrations were determined using commercially available enzyme-linked immunosorbant assay (ELISA) kits (Diagnostic Systems Laboratories, Webster, TX). Each unknown was tested in duplicate using 25 µl of serum per well. The percent variability between wells for each sample was <10%. The supplier reported the minimal detectable level to be 0.08 ng/ml.

Commercially available radioimmunoassay (RIA) kits (MP Biomedical, Irvine, CA) were used to determine serum thyroxine (T_4) and triiodothyronine (T_3). To determine serum T_4 concentrations 25 µl of serum was mixed with radioactively labeled antigen in antibody-coated tubes. Each sample was tested in duplicate and the percent variability between tubes for each sample was <10%. As reported by the supplier, the percent cross-reactivity by weight with L-thyroxine was reported to be 100%, D-thyroxine 30.9%, and 3,3',5-triiodo-L-thyronine 1.0%. The detection limits as set by the standards were 2.0-20 mg/dl. To determine serum T_3 concentrations 100 µl of serum was mixed with radioactively labeled antigen in antibody-coated tubes. Each sample was tested in duplicate and the percent variability between tubes for each sample was <10%. Reported cross reactivity with L-triiodothyronine, D-triiodothyronine was 100% and with other iodinated thyronines was <1.0%. The detection limits as set by the standards were 25.0-800 ng/dl.

Data were compared statistically by analysis of variance (ANOVA) with multiple comparisons of means performed by Tukey HSD Test to determine differences from control. SPSS software (SPSS Inc., Chicago, IL) was used to perform the analyses. Statistical significance was ascribed at $p < 0.05$. Pearson correlation coefficient was calculated to measure the relationship between changes in serum leptin concentrations and changes in serum thyroid hormone concentrations.

RESULTS

Body weights of rats exposed to PCB gestationally, lactationally, or in early adulthood revealed no differences in body weight when compared with same aged control animals (Table 1). Food consumption of young adult animals exposed to PCB was not significantly different from same-aged controls throughout the study (Table 1).

TABLE 1

*Mean Body Weight and Food Intake of Rats. The body weights for 1-, 7-, and 15-day-old animals represent mean ± SD for 16 litters of 8 rats (*n = *16). Data for 30-day-old animals represent mean ± SD for 8 litters of 8 rats (*n = *8). Body weight data for young adult rats represent mean ± SD for individual rats (*n = *7). Food intake data for young adult rats represent mean g of ground rodent chow consumed per rat each day ± g.*

	Control (g ± SD)	PCB (g ± SD)
Rat Pup Body Weight		
Day 1 ($n = 128$)	6.9 ± 0.4	6.8 ± 0.4
Day 7 ($n = 128$)	18.4 ± 1.3	17.6 ± 3.1
Day 15 ($n = 128$)	41.7 ± 3.9	42.3 ± 3.1
Day 30 ($n = 64$)	110.2 ± 6.7	116.0 ± 8.8
Young Adult Rats		
Beginning Weight ($n = 7$)	96.2	95.7
Final Weight ($n = 7$)	179.5	175.3
Day 1 Food Consumption ($n = 7$)	11.5 g ± 1.2 g	10.8 g ± 0.9 g
Day 20 Food Consumption ($n = 7$)	18.3 g ± 1.5 g	18.0 g ± 1.7 g

Exposure of rats to PCB from conception through early development resulted in a significant depression of leptin at 15 days of age (0.57 ng/ml below control values), but a pronounced elevation by 30 days of age (0.49 ng/ml above control) (Fig. 1). Mean serum leptin concentrations in 15-day-old control animals were 1.5 ng/dl and 0.9 ng/dl in PCB exposed animals, with standard error of the mean (SEM) 0.03 and 0.02, respectively. Thirty-day-old control animals had serum leptin concentrations of 0.34 ng/ml ± 0.01 SEM while leptin levels in PCB exposed animals, of

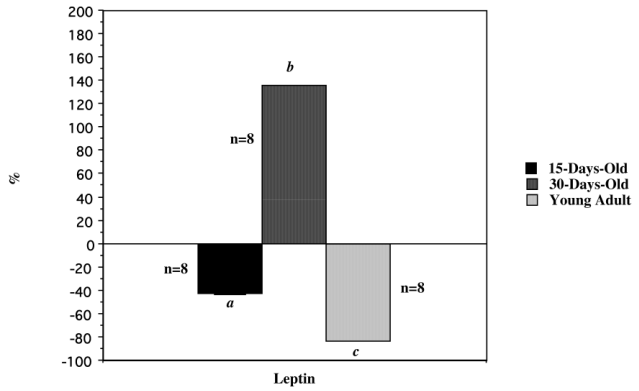

FIGURE 1. Percent difference in leptin concentrations in PCB exposed animals compared with controls. *a* = 15-day-old animals exposed to PCB had significantly depressed serum leptin concentrations when compared with same aged controls (p <0.05); *b* = 30-day-old animals exposed to PCB had significantly elevated serum leptin concentrations when compared with same aged control animals (p <0.05); *c* = young adult animals exposed to PCB had significantly depressed serum leptin concentrations when compared with same aged control animals (p <0.05). Bars represent grand mean ± SEM. Variability was sufficiently small that error bars are nearly invisible.

the same age, were 0.84 ng/ml ± 0.02 SEM. These differences resulted from a decrease in serum leptin concentration of 1.09 ng/ml or 71% in control animals between 15 and 30 days of age, while leptin in PCB exposed animals remained relatively stable. Young adult animals exposed to PCB for 21 days had serum leptin concentrations of 0.26 ng/ml ± 0.01 SEM, a significant depression when compared to control animal concentrations of 1.44 ng/dl ± 0.05 SEM (Fig. 1).

Although mean serum T_4 values varied above and below those of controls, the concentrations were not significantly altered in any animals exposed to PCB (Fig. 2). The mean serum T_4 concentrations in 15-day-old control animals was 5.8 μg/dl ± 0.26 SEM, while T_4 levels in 30-day-old rats was 3.4 μg/dl ± 0.2 SEM. Exposure to PCB caused a 16% greater decline in T_4 between 15 (6.6 μg/dl ± 0.3 SEM) and 30 days of age (2.8 μg/dl ± 0.26 SEM) when compared with the change in control animals, but this modification did not reach significance.

Serum T_3 levels were not significantly altered by PCB exposure in 15-day-old animals (Control = 148.5 ng/dl ± 7.6; PCB = 163.6 ± 8.1). However, serum T_3 concentrations were

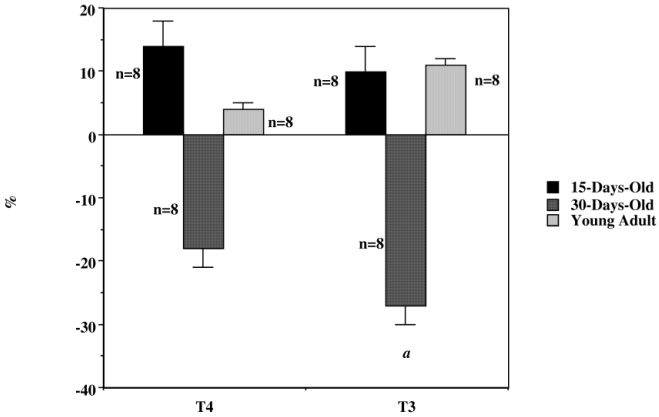

FIGURE 2. Percent difference in thyroid hormone concentrations in PCB exposed animals. *a* = serum T_3 concentrations were significantly depressed in 30-day-old PCB exposed animals when compared with same aged controls (p <0.05). Bars represent grand mean ± SEM.

significantly depressed in 30-day-old animals exposed to PCB (98.7 ng/dl ± 7.7 SEM) when compared with control animals (135.2 ng/dl ± 7.5 SEM) (Fig 2). Triiodothyronine declined 37% more in PCB exposed animals than control animals between 15 and 30 days of age.

Serum thyroid hormone concentrations, in young adult animals, were not significantly affected by exposure to this concentration of PCB. Serum T_4 concentrations were 4.6 μg/dl ± 0.23 SEM in control animals and 4.8 μg/dl ± 0.3 SEM with PCB exposure. Serum T_3 concentrations in control young adult animals were 173.16 ng/dl ± 3.7 SEM and 192.56 ng/dl ± 3.9 SEM in same-aged PCB exposed animals.

Thus, leptin was significantly altered in all age groups by exposure to PCB. Triiodothyronine was altered in animals with developmental exposure from conception to 30 days of age. Thyroid hormones in adult animals were unaffected by this concentration of PCB. Statistical analysis of these data revealed no relationship between leptin and thyroid hormone alterations.

DISCUSSION

Optimal circulating concentrations of leptin and thyroid hormones are important for normal development of the nervous and reproductive systems and for overall growth in rats. In the present study, the PCB mixture Aroclor® 1254 in low doses significantly altered leptin concentrations in developing and adult animals depending on duration of exposure but had no statistically significant effect on circulating thyroid hormones. The significant difference in leptin concentrations between control and PCB treated developing animals appears to result from the normal perinatal surge in control animals not occurring in the PCB treated animals. This surge in circulating leptin supports physiological and anatomical development with no decrease in food intake. The hypothalamic feeding circuits fully develop during the second week in mice and depend on the surge in leptin (Bouret and others 2004; Ahima and others 1996; Chehab and others 1997). In the present study there was no interruption or augmentation of linear growth by either hypoleptinemia or hyperleptinemia. The alterations in leptin may not be sufficiently extreme to alter growth or growth may be supported by other mechanisms including the trend in thyroid hormone elevation. However, the reduction in leptin reported here may well contribute to the permanent effects on the brain and reproductive development reported in PCB exposed animals (Juárez de Ku 1992; Pritts 1996; Donahue and others 2002), but such relationships remain to be investigated. Suboptimal leptin concentrations caused by PCB in the first 15 days of development could explain some of the direct effect on development of hypothalamic circuitry. To better understand long-term changes in eating patterns caused by malformation of hypothalamic circuitry, food consumption and food choices must be investigated in animals exposed to PCB during development, and these investigations should be maintained into adulthood.

Adult rats normally respond to a decrease in circulating leptin by increasing food intake and decreasing cellular metabolism with an overall outcome of weight gain (Friedman and Halaas 1998). In the present study, animals exposed to PCB had body weights and food consumption (results not shown) similar to those of controls. Leptin levels may not have been sufficiently depressed to cause an increase in food consumption and weight gain during the duration of the study. Although adult animals appeared to be similar in adiposity, body fat proportion was not

determined and could have contributed to differences in leptin production.

Contrary to previous findings with larger amounts of PCB (Goldey and others 1995; Juárez de Ku 1992), serum thyroid hormones were relatively unaffected by the PCB doses used in the present study. The significant depression in T_3 concentrations with longer exposure and more bioaccumulation supports the idea that significant alterations in thyroid hormone concentrations are dose dependent. However, one would expect to see a similar depression in circulating T_4. The trend toward elevation of thyroid hormones in younger animals is not explainable if the response to this level of PCB is directly dose dependent with gradual increments of change until significance is reached. It appears that PCB could be causing an up-regulation of thyroid hormone production (or release) or a reduction in clearance of the hormones. Clarification of these relationships requires further examination with varying doses of PCB and age groups starting earlier and continuing past 30 days.

Although not all thyroid hormones were altered significantly by exposure to PCB, it is interesting to note that leptin and thyroid hormones were inversely affected by PCB in all test animals when compared to controls. These hormones share some physiological functions during development and in adulthood, and have been shown to have regulatory effects on each other. Serum leptin levels appear to be more sensitive to short-term exposure to small amounts of PCB than do serum thyroid hormones. Accumulation of PCB in adipocytes could cause an interruption in protein synthesis similar to the alterations seen in hepatocytes (Borlakoglu and others 1990). The up-regulation or down-regulation could be caused by a compensatory mechanism to stabilize physiological mechanisms. Further investigation into the effect of PCB on leptin production and storage in adjacent areas of the cell is warranted. The findings in this study identify the importance of continued investigation of the effects of PCB on leptin in doses similar to those found in the environment and considered to be acceptable in the food supply, and how those effects could alter thyroid hormone production and clearance.

LITERATURE CITED

Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS. 1996. Role of leptin in the neuroendocrine response to fasting. Nature 382:250-2.

Borlakoglu J, Welch V, Edwards-Webb J, Dils R. 1990. Transport and cellular uptake of polychlorinated biphenyls (PCBs)—II. Changes in vivo in plasma lipoproteins and proteins of pigeons in response to PCBs, and a proposed model; for the transport and cellular uptake of PCBs. Biochem Pharmacol 40:273-81.

Bouret SG, Draper S, Simerly RB. 2004. Trophic action of leptin on hypothalamic neurons that regulate feeding. Sci 304:108-10.

Chehab F, Mounzih K, Lu R, Lim M. 1997. Early onset of reproductive function in normal female mice treated with leptin. Sci 275:88-90.

Donahue DD, Bowen CL, Provost TL, Meserve LA. 2002. Effects of PCB on reproductive success in Sprague-Dawley rats exposed to Aroclor® 1254 for one year. Ohio J Sci 102:102-5.

Desaulniers D, Poon R, Phan W, Leingartner K, Foster WG, Chu I. 1997. Reproductive and thyroid hormone levels in rats following 90-day dietary exposure to PCB 28 (2,4,4'-terichlorobiphenyl) or PCB 77 (3,3'4,4'-tetrachlorobiphenyl). Toxicology and Industrial Health 13:627-38.

French JB, Voltura MB, Tomasi TE. 2001. Effects of pre- and postnatal polychlorinated biphenyl exposure on metabolic rate and thyroid hormones of white-footed mice. Environ Toxicology and Chem 20:1704-8.

Friedman JM, Halaas JL. 1998. Leptin and the regulation of body weight in mammals. Nature 395:763-70.

Goldey ES, Kehn LS, Lau C, Rehnberg GL, Crofton KM. 1995. Developmental exposure to polychlorinated biphenyls (Aroclor 1254) reduces circulating thyroid hormone concentrations and causes hearing deficits in rats. Toxicology and Appl Pharmacol 135:77-88.

Gould JC, Cooper KR, Scanes CG. 1997. Effects of polychlorinated biphenyl mixtures and three specific congeners on growth and circulating growth-related hormones. Gen and Comparative Endocrin 106:221-30.

Juárez de Ku LM. 1992. Effects of polychlorinated biphenyl (PCB) on regulation of thyroid-, growth-, and neurochemically-related developmental processes in young rats [Dissertation]. Bowling Green State Univ, Bowling Green, OH.

Pritts B. 1996. The effects of polychlorinated biphenyl on selected endocrine parameters, and biochemical and behavioral neurological function in Sprague-Dawley rat pups and adults [Dissertation]. Bowling Green State University, Bowling Green, OH.

Provost TL, Juárez de Ku LM, Zender C, Meserve LA. 1999. Dose- and age-dependent alteration in choline acetyltransferase (ChAT) activity, learning and memory, and thyroid hormones in 15- and 30-day old rats exposed to 1.25 or 12.5 PPM polychlorinated biphenyl (PCB) beginning at conception. Prog Neuro-Psychopharmacol and Biol Psychiat 23:915-28.

Proulx K, Richard D, Walker CD. 2002. Leptin regulates appetite-related neuropeptides in the hypothalamus of developing rats without affecting food intake. Endocrin 143:4683-92.

Schantz SL, Seo BW, Moshraghain J, Amin S. 1997. Developmental exposure to polychlorinated biphenyls or dioxin: do changes in thyroid function mediate effects of spatial learning? Amer J Zoology 37:399-408.

Tryphonas H, McGuire P, Fernie S, Miller D, Stapley R, Bryce F, Arnold DL, Fournier M. 1998. Effects of Great Lakes fish consumption on the immune system of Sprague-Dawley rats investigated during a two-generation reproductive study. Regulatory Toxicol and Pharmacol 27:S28-S39.

[USEPA] United States Environmental Protection Agency. 1999. Integrated Information System Chemical File for Aroclor 1254. National Center for Environmental Assessment, Cincinnati, OH.