

## Thyroid Hormone Disruption by PBDEs in Adults Sport Fish Consumers: A First Look

Mary Turyk<sup>1</sup>, Victoria Persky<sup>1</sup>, Pamela Imm<sup>2</sup>, Lynda Knobeloch<sup>2</sup>, Robert Chatterton Jr<sup>3</sup>, Yu-cai Lu<sup>3</sup>, John Mathew<sup>4</sup>, Carol Buelow<sup>4</sup>, and Henry Anderson<sup>2</sup>

<sup>1</sup>Division of Epidemiology and Biostatistics, School of Public Health, University of Illinois at Chicago, 1601 W. Taylor Street, Chicago, IL 60612, <sup>2</sup>Wisconsin Division of Public Health, Bureau of Environmental Health, 1 W. Wilson Street, Room 150, Madison, WI, 53703. <sup>3</sup>Departments of Ob/Gyn and Physiology, Feinberg School of Medicine, Northwestern University, 710 N. Fairbanks Court, Chicago, IL 60611, <sup>4</sup>Wisconsin State Laboratory of Hygiene, 2601 Agriculture Drive, Madison, WI 53718.

### Introduction

Polybrominated biphenyl ethers (PBDE) are persistent environmental chemicals that are increasing in prevalence in humans, particularly in North America. PBDEs are similar in chemical structure to polychlorinated biphenyls (PCBs) and dioxins, which have been shown to affect thyroid hormones in humans (Persky et al. 2001). Health effects related to PBDE exposure have not been well studied, but animals investigations suggest that thyroid hormones may be affected (Hallgren et al., 2001).

### Materials and Methods

A cohort of 4,206 frequent and infrequent consumers of Great Lakes fish established during the early 1990s was re-contacted during 2004-5 and invited to participate in a follow-up study. Complete data was available for 215 men and 84 women, after exclusion of 100 participants who had thyroid disease, diabetes, or were taking selected medications known to affect thyroid function (steroid hormones, melatonin, furosamide, carbidopa, and lithium). Associations between hormone and PBDEs were modeled separately for males and females using Spearman correlation coefficients, adjusted for total serum lipids, age, BMI, and medication use (betablockers, antilipemics, NSAIDs).

Serum samples were extracted three times with a hexane/ethyl ether mix. The extract was then concentrated to approximately 2 ml, fractionated using Florisil and silica-gel columns, and concentrated to 0.5 ml. For PCBs the concentrated sample was injected onto a gas chromatograph equipped with an electron-capture detector. PCBs were analyzed on a 60 meter DB-5 capillary column, with confirmation on a DB-1 column. PCB congeners included in this method are quantitated using the "Mullin 1994" mixture of Aroclors. For PBDEs the concentrated sample was injected onto a gas chromatograph-mass spectrometer operating in the negative ion mode. PBDE congeners were analyzed on a 30-meter DB-5HT capillary column.

Thyroid stimulating hormone (TSH) and thyroid binding globulin (TBG) were measured in the Immulite System (Diagnostic Products Corporation, Inc., Los Angeles, CA). The assay for TSH is a third generation procedure with a sensitivity of 0.002  $\mu$ IU/ml. TBG is measured with a sensitivity of 1.1  $\mu$ g/ml. Interassay CVs were 12.7% for TSH and 10.2% for TBG. Total thyroxine ( $T_4$ ) total triiodothyronine ( $T_3$ ) and the free  $T_4$  were measured with kits obtained from Diagnostic Products Corporation. Specificity was greater than 99%. Inter and intra assay coefficients of variation (CVs) were 2.5% and 3.3% for total  $T_3$ , 3.9% and 5% for total  $T_4$ , 6.8% and 4.3% for free  $T_4$ . The distribution of thyroxine binding in plasma was conducted by radioelectrophoresis (Leopold et al., 1987, Borst et al.,

1982). Albumin-bound and thyroid binding globulin (TBG)-bound  $^{125}\text{I-T}_4$  were separated on agarose gels after incubation of  $^{125}\text{I-T}_4$  with the serum for 2 hr at 37 °C. Transthyretin is clearly separated from TBG in this system. The gels were stained with bromthymol blue to identify albumin in the samples. Standards of TBG and transthyretin were run in parallel to determine the location relative to albumin on the gel. The areas corresponding to  $\text{T}_4$  and albumin were cut out of the gel, counted in a gamma counter, and the percentage of the total  $^{125}\text{I}$ -thyroxine in each of the fraction was determined. Interassay CVs were 3.5% for TBG-bound  $\text{T}_4$  and 13.6% for albumin-bound  $\text{T}_4$ .

## Results and Discussion

The study participants were adults with mean age in the 50s; 63% of the women were postmenopausal (Table 1). PCBs and years of sport fish consumption, but not PBDEs were higher in men than women. TBG and total  $\text{T}_4$  were higher in women.

**Table 1: Participant Characteristics Mean or Percentage of Category**

Characteristic	Men	Women	P-value for Gender
Number of Participants	215	84	-
Age, years	58.4	52.7	0.0001
Serum Lipids, mg/dL	726	699	0.48
Body mass index (BMI) , kg/m <sup>2</sup>	29.6	28.0	0.0001
Post menopausal, %	NA	63%	-
Sum PBDEs, ng/g	0.47	0.53	0.44
BDE 47, ng/g	0.22	0.27	0.70
Sum PCBs, ng/g	3.98	2.27	0.0001
Sport fish consumption, years	37.8	26.6	0.0001
Antilipemic medication, %	32%	18%	0.02
Beta blocker medication, %	14%	8%	0.18
NSAID medication, %	51%	42%	0.11
Total $\text{T}_3$ , ng/dL	99.3	100.2	0.73
Total $\text{T}_4$ , ug/dL	7.2	7.6	0.03
Free $\text{T}_4$ , pg/mL	1.2	1.2	0.52
TSH, uIU/mL	1.8	2.0	0.84
TBG, ug/mL	18.8	21.4	0.0001
$\text{T}_4$ -bound TBG, %	77.8	78.9	0.18
$\text{T}_4$ -bound albumin, %	17.5	16.4	0.15

Associations of hormones with total PBDEs and BDE 47 in men are shown in Table 2. TBG, the major carrier protein for  $\text{T}_4$  in the blood, was negatively associated with both total PBDEs and BDE 47. TBG-bound  $\text{T}_4$  was negatively and albumin-bound  $\text{T}_4$  was positively associated with BDE 47, and similar associations were seen with total PBDEs, although they did not reach significance. Total  $\text{T}_4$  and free  $\text{T}_4$  however were not associated with PBDEs. TSH was negatively associated with BDE 47 in men, but this association was of borderline significance (Table 2).

**Table 2: Associations of PBDEs with Hormones in 215 Men: Partial Correlation Coefficients adjusted for lipids, age, BMI, and medication use**

Hormone	BDE 47	Total PBDEs	Total PCBs	Years Sport Fish Consumption
Total T <sub>3</sub>	-0.10	-0.11	<b>-0.13*</b>	-0.05
Total T <sub>4</sub>	-0.01	0.03	-0.05	<b>-0.19**</b>
Free T <sub>4</sub>	0.03	0.07	-0.05	<b>-0.12*</b>
TSH	<b>-0.13*</b>	-0.11	<b>0.13*</b>	0.03
TBG	<b>-0.19**</b>	<b>-0.19**</b>	-0.04	-0.10
T <sub>4</sub> -bound TBG, %	<b>-0.15**</b>	-0.11	0.06	-0.01
T <sub>4</sub> -bound albumin, %	<b>0.15**</b>	<b>0.12*</b>	-0.07	-0.01

\*0.05<p<0.10 \*\*p<0.05

We found a positive association, of borderline significance, for TBG with BDE 47 and total PBDEs in postmenopausal, but not premenopausal women (Table 3). Total T<sub>4</sub> was positively, but not significantly associated with BDE 47 and total PBDEs in postmenopausal women, suggesting a potential slight increase in total T<sub>4</sub> in response to increased TBG with exposure to PBDEs. The small number of women in the current sample limits our ability to interpret these results. However, these results are preliminary, as testing for PBDEs is currently incomplete for 200 male and female study participants with measured hormone levels.

**Table 3: Associations of PBDEs with Hormones in 53 Postmenopausal and 31 Premenopausal Women: Partial Correlation Coefficients adjusted for lipids, age, BMI, and medication use**

Hormone	Menopause Status	BDE 47	Total PBDEs	Total PCBs	Years Sport Fish Consumption
Total T <sub>3</sub>	Post	0.04	-0.04	-0.21	-0.12
	Pre	-0.01	-0.01	-0.07	0.10
Total T <sub>4</sub>	Post	0.19	0.18	0.01	0.07
	Pre	0.12	0.05	0.17	<b>0.34*</b>
Free T <sub>4</sub>	Post	0.03	0.03	-0.17	-0.19
	Pre	-0.04	-0.10	0.10	0.10
TSH	Post	0.11	0.06	-0.24	0.01
	Pre	0.28	0.19	-0.17	0.19
TBG	Post	<b>0.26*</b>	<b>0.27*</b>	0.06	-0.04
	Pre	-0.05	-0.17	0.32	0.08
T <sub>4</sub> -bound TBG, %	Post	0.12	0.18	0.19	0.18
	Pre	-0.01	-0.11	0.28	-0.14
T <sub>4</sub> -bound albumin, %	Post	-0.05	-0.10	-0.14	-0.13
	Pre	0.04	0.13	<b>-0.36*</b>	-0.01

\*0.05<p<0.10

In conclusion, these results suggest that PBDEs may affect thyroid hormone homeostasis through effects on thyroid binding globulin, the major carrier protein for thyroxine. However, the effects of PBDEs on TBG vary by gender and in women by postmenopausal status. While, in vitro bioassays have shown that PBDEs can displace thyroxine from transthyretin (Hamers et al., 2006), which is a major carrier protein for thyroxine in rats but not humans, the effect of PBDEs on TBG synthesis or binding has not been

studied to our knowledge. TBG was not affected by either PCB exposure or years of sport fish consumption, and the relationship of PBDEs with TBG was independent of total PCBs, DDE or years sport fish consumption. This is the first study to investigate in detail the effects of PBDEs on thyroid hormones in a large cohort of adults.

### **Acknowledgement**

This research was funded by the US Environmental Protection Agency, Grant No RD-83025401-1. The research and researchers were supported in part by the Center for Disease Control and Prevention Training Program Grant #1 T01 CD000189-01.

### **References**

Borst GC, Premachandra BN, Burman KD, Osburne RC, Georges LP, Johnsonbaugh RE. 1982. *Am J Med* 73:283-289.

Leopold B, Wawschinek O, Lind P, Eber O. 1987.. *J Clin Chem Clin Biochem* 25:431-435.

Hallgren S, Sinjari T, Hakasson H, Darnerund PO. 2001. *Arch Toxicol* 75:200-208.

Hamers T, Kamstra JH, Sonneveld E, Murk AJ, Kester MHA, Andersson PL, Legler J, Brouwer A. 2006. *Toxicol Sci* 92:157-173.

Hanrahan L, Falk C, Anderson H, Draheim L, Kanarck M, Olson J. 1999. *Environ Res Sec A* 80:S26-37.

Persky V, Turyk M, Anderson HA, Hanrahan LP, Falk C, Steenport DN, Chatterton R Jr, Freels S., 2001. *Environ Health Perspect* 109:1275-83.