



Polybrominated Diphenyl Ether Exposure and Thyroid Function Tests in North American Adults

**Colleen M. Makey, Michael D. McClean, Lewis E. Braverman,
Elizabeth N. Pearce, Xue-Mei He, Andreas Sjödín,
Janice M. Weinberg, and Thomas F. Webster**

<http://dx.doi.org/10.1289/ehp.1509755>

Received: 23 January 2015

Accepted: 11 September 2015

Advance Publication: 15 September 2015

This article will be available in a 508-conformant form upon final publication. If you require a 508-conformant version before then, please contact ehp508@niehs.nih.gov. Our staff will work with you to assess and meet your accessibility needs within 3 working days.



Polybrominated Diphenyl Ether Exposure and Thyroid Function Tests in North American Adults

Colleen M. Makey¹, Michael D. McClean¹, Lewis E. Braverman², Elizabeth N. Pearce², Xue-Mei He², Andreas Sjödin³, Janice M. Weinberg⁴, and Thomas F. Webster¹

¹Department of Environmental Health, Boston University School of Public Health, Boston, Massachusetts, USA; ²Section of Endocrinology, Diabetes, and Nutrition, Boston University School of Medicine, Boston, Massachusetts, USA; ³National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; ⁴Department of Biostatistics, Boston University School of Public Health, Boston, Massachusetts, USA

Address correspondence to Colleen Makey, Department of Environmental Health, Boston University School of Public Health, 715 Albany Street, T4W, Boston, MA 02118 USA.

Telephone: 617-640-0095. E-mail: cmakey@bu.edu

Running title: PBDEs and thyroid function tests

Acknowledgments: We thank J. Ames, K. Burke, C. Carignan, E. Collins, A. Miller, S. Nicholson, and B. Weldon for their contributions and the participants of the FlaRE Study.

This study was supported by grants from the National Institute of Environmental Health Sciences (R01ES015829 and T32ES014562).

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention

Competing financial interests: The authors declare they have no competing financial interests.

Abstract

Background: Polybrominated diphenyl ethers (PBDEs) are flame retardant chemicals that were added to many consumer products. Multiple animal studies have shown PBDEs to be thyroid hormone (TH) disruptors. Epidemiologic evidence of PBDE exposure associated with TH disruption has been inconclusive.

Objectives: We used repeated measures to estimate associations between serum PBDE concentrations and THs in a North American adult cohort.

Methods: From 2010-11, we collected ≤ 3 serum samples at approximately 6-month intervals from 52 healthy adult office workers from Boston, Massachusetts for analysis of PBDE congeners and THs.

Results: Geometric mean sum concentrations of the most prevalent PBDE congeners (BDE 28, 47, 99, 100, 153) were 22 ng/g lipid in Winter 2010, 23 ng/g lipid in Summer 2010, and 19 ng/g lipid in Winter 2011. BDE-47 was the predominant congener. Based on a multivariable mixed regression model, we estimated that, on average, a 1-ng/g serum increase in BDE-47 was associated with a 2.6 $\mu\text{g/dL}$ decrease in total T_4 (95% CI: $-4.7, -0.35$). Total T_4 was inversely associated with each PBDE congener. Serum concentrations of PBDEs were not strongly associated with total T_3 , free T_4 , or TSH.

Conclusion: These results are consistent with those from animal studies showing exposure to PBDEs is associated with a decrease in serum T_4 . Because the other TH concentrations did not appear to be associated with BDE exposures, our findings do not support effects on the pituitary/thyroid axis. Taken together, our findings suggest that PBDE exposure might decrease the binding of T_4 to the serum T_4 binding proteins.

Introduction

Polybrominated diphenyl ethers (PBDEs) have been used since the 1970's as additive flame retardants. The technical formulation Pentabromodiphenyl ether (PentaBDE), composed of PBDE congeners containing three to six bromines, was added primarily to products containing polyurethane foam. Due to their persistence in the environment, ability to bioaccumulate, and potential adverse health effects, PentaBDEs were added to the list of persistent organic pollutants in the Stockholm Convention in 2004 (Stockholm Convention, 2004). US manufacturers of PentaBDE and OctaBDE voluntarily halted production in 2004. Nevertheless, PBDEs are still found in food products (Schechter et al. 2010) and indoor microenvironments (e.g. homes, offices, and vehicles) (Watkins et al. 2012). Therefore, it is likely that people in the US continue to be exposed to PBDEs through exposures to indoor dust and diet (Fraser et al. 2009; Watkins et al. 2012).

Animal studies have established that the PentaBDEs are endocrine disrupting chemicals, which can modify thyroid hormone (TH) levels (Dishaw et al. 2014; Fowles et al. 1994; Stoker et al. 2004; Zhou et al. 2002). The PentaBDEs and their CYP 450-mediated metabolites, hydroxylated PentaBDEs (OH-PBDEs), are structurally similar to thyroxine (T_4) and triiodothyronine (T_3) (Meerts et al. 2000). Multiple experimental studies in rodents have shown that exposure to these PentaBDE congeners leads to hypothyroxinemia, characterized by a decrease in serum total T_4 (TT_4) concentrations (Fowles et al. 1994; Stoker et al. 2004; Zhou et al. 2002).

In humans, THs are essential for proper growth and development *in utero* and infancy, as well as maintenance of many organ systems and metabolism throughout life (Cooper and Bondi,

2012). While toxicological evidence of PBDEs causing thyroid disruption is robust, the epidemiologic evidence has been inconsistent, with positive, negative, and null associations reported between PBDE exposures and THs (Abdelouahab et al. 2013; Bloom et al. 2008; Chevrier et al. 2011; Stapleton et al. 2011; Turyk et al. 2008; Zota et al. 2011).

Our study uses repeated serum measures to assess the association between PBDE exposure and thyroid function in a longitudinal cohort of healthy adults. Our primary aim was to use these data to examine the association between the PBDEs and thyroid function tests (TFTs): TT₄, free thyroxine (fT₄), total T₃ (TT₃), and thyroid stimulating hormone (TSH). We also evaluated confounding and effect modification of these associations in our cohort.

Methods

Study Design and Population. We recruited 26 male and 26 female adult office workers living in the Boston Metropolitan area and collected serum samples at approximately six-month intervals from January 2010 to May 2011. This study is part of an extensive study of the exposure and health effects of flame retardants to office workers. Eligible subjects had to be non-smoking adults over the age of 18, self-described as generally healthy, and planning to reside in the Boston Metropolitan area for the study duration. Participants were excluded for having a prior diagnosis of thyroid disease or if they were pregnant. Characteristics and descriptions of the FlaRE (Flame Retardant Exposure Study) population have been presented elsewhere (Makey et al. 2014). Forty-one participants completed all three study visits, nine completed two study visits and two completed only one study visit (total of 143 serum samples). There were four participants (total of six serum samples) with serum samples excluded from analysis. The reasons were as follows: use of a thyroid-affecting medication (one participant, three serum

samples), pregnancy at third sampling round (one serum sample), inadequate serum volume at first sampling round (one serum sample), or suspected field contamination at third sampling round (one serum sample). The contamination of one sample in the field was suspected because the concentration of hexaBDEs was 10 times higher than in the other two samples collected from the same participant, while lower brominated congeners were comparable among the three samples, suggesting sample contamination with residential dust containing the octaBDE technical mixture. The present study thus utilized 137 PBDE and hormone measures in serum collected from 51 participants. We obtained informed consent prior to participation and the Boston University Medical Center Institutional Review Board approved the study protocol. The involvement of the Centers for Disease Control and Prevention (CDC) did not constitute engagement in human subjects research.

Blood Samples. A trained phlebotomist collected 30 mL of blood from non-fasting participants at each sampling round. Serum samples were analyzed for 11 PBDE congeners (BDE-17, BDE-28, BDE-47, BDE-66, BDE-85, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, BDE-209) at the CDC using established methods (Sjödin et al. 2004). The coefficient of variation (CV) of included quality control (QC) samples was less than 10% (data not shown). The limits of detection of the PBDE congeners ranges from 0.2 to 0.8 ng/g lipid. Serum samples were analyzed for total triglycerides (Triglycerides, GPO-PAP) and total cholesterol (Cholesterol, CHOD-PAP) using text kits from Roche Diagnostics at the CDC. Final determinations were made on a Hitachi Modular P Chemistry Analyzer. The total lipids concentration was calculated by summation of the individual lipid components (Phillips et al., 1989).

Serum TFTs were measured at the Boston University School of Medicine, Section of Endocrinology, Diabetes, and Nutrition. Enzyme-linked immunosorbent assays were used to measure serum TSH (normal range: 0.4-4.2 mIU/L), fT₄ (normal range: 0.8–2.0 ng/dL), TT₃ (normal range 0.52-1.85 ng/mL), and TT₄ (normal range 4.4-10.8 µg/dL in men and 4.8-11.6 µg/dL in women) (Immuno-Biological Laboratories, Inc.). Thyroid peroxidase antibodies (TPO antibody) were measured by immunometric enzyme immunoassay (Orgentec Diagnostika) with reference range: normal <50 IU/mL; borderline 50-75 IU/mL, and elevated >75 IU/mL.

Urine Samples. We collected 90 mL of urine from participants at each sampling round. Urinary specific gravity was measured using a refractometer. We measured levels of urinary iodide, urinary perchlorate, and urinary thiocyanate by ion chromatography–mass spectrometry using established methods (Valentin-Blasini et al. 2005) at the Boston University School of Medicine, Section of Endocrinology, Diabetes, and Nutrition. The limit of detection (LOD) and CV were 0.05 µg/L (CV = 2.2% to 5.9%), 10 µg/L (CV < 5%), and 0.05 mcg/L (CV <5%) for perchlorate, thiocyanate, and iodide, respectively.

Statistical Analysis. For PBDE measurements below the LOD, we substituted ½ LOD. ΣPBDE was defined as the sum of the five most prevalent PentaBDE congeners: BDE-28, -47, -99, -100, and -153. We assessed normality of PBDEs, TFTs, and covariates using histograms and Shapiro-Wilks tests. TSH, fT₄, and TT₃ were log-normally distributed and, therefore, were natural log-transformed for regression analysis. TT₄ was normally distributed and modeled without transformation (see Supplemental Material, Figure S1). We used Spearman’s correlation coefficient to assess associations between PBDE congeners. All statistical analyses were

conducted using SAS statistical software version 9.3 (SAS Institute) and statistical significance is reported at the 0.05 level.

We used a general linear model with a random intercept to assess the association between PBDEs and TFTs, which in its simplest form is

$$Y_{ij} = \beta_o + \beta_l \text{PBDE}_{ij} + b_i + \varepsilon_{ij} \quad [1]$$

where Y_{ij} represents the thyroid hormone level of the i th participant at the j th sampling round. β_o is the fixed effect intercept, β_l is an estimate of the mean difference in the TFT (or ln-TFT) with a one-unit change in PBDE concentration, b_i is the random intercept of the i th individual, and ε_{ij} is the random error. The following covariates were added to equation (1): Time (indicator variables for sampling round), total lipids (mg/dL), urinary iodine concentration (UIC) ($\mu\text{g/L}$), age (years), sex (male/female), BMI (mg/kg^2), TPO antibody (yes/no), oral contraceptive (yes/no), perchlorate ($\mu\text{g/L}$), thiocyanate ($\mu\text{g/L}$), and specific gravity (SG). We selected potential confounding variables based on *a priori* expectation that they could be associated with the exposure and outcome but not on the causal pathway. We assessed confounding using a change of 10% or greater in the PBDE beta coefficient as a guide. We ran regression models on datasets excluding women on oral contraceptives ($n=7$) and participants who were TPO antibody positive ($n=3$) to assess their potential to affect regression estimates. We assessed effect measure modification by stratification on the following variables: sex and UIC ($<100 \mu\text{g/L}$ vs. $\geq 100 \mu\text{g/L}$). For the continuous covariates, we assessed possible effect modification by including a cross product of PBDE level and covariate (total lipids, iodine, age, BMI).

We conducted regression analysis between PBDEs and TFTs with (51 participants, 137 serum samples) and without a participant (50 participants, 134 serum samples) with very high PBDE exposure that was identified as influential in this analysis with a Cook's distance of 13.

We estimated intraclass correlation coefficients (ICCs) to assess the stability of TFTs and UIC using an intercept-only general linear model with a random intercept. The ICCs were estimated by dividing the between-subject variance by the total variance.

Results

The final study population for the thyroid analysis consisted of 26 males and 25 females. Participation rate by sampling round was: 96% in round 1 (Winter 2010), 96% in round 2 (Summer 2011), and 81% in round 3 (Winter 2011). The median age was 37 years old, 98% had a college degree, and 63% had BMI < 25 kg/m² (Table 1). Forty-four participants identified as white non-Hispanic, three as Hispanic/Latino and three as Asian. Table 2 presents the round-specific geometric means (GM), geometric standard deviations (GSD), detection frequency and range for PBDE congeners that were detected in >50% of serum samples: BDE-28, BDE-47, BDE-99, BDE-100, BDE-153. Detection rates for other PBDE congeners were low and results were not further analyzed (Makey et al. 2014). GM concentrations and ranges of Σ PBDE in sampling Round 1, 2 and 3 were 22 (2.3 to 290) ng/g lipid, 23 (2.7 to 290) ng/g lipid, and 19 (2.4 to 210) ng/g lipid, respectively. BDE-47 was the predominant serum congener. BDE-28, BDE-47, BDE-99, and BDE-100 were highly and significantly correlated ($r \geq 0.85$ for concentrations measured at the first visit), whereas BDE-153 was moderately correlated with the lower brominated PBDE congeners ($r = 0.29-0.47$) (Supplemental Material, Table S1)

Table 2 presents the measures of central tendency and ranges of the TFTs: TT₄, TSH, fT₄, TT₃ and TPO. TFTs were predominantly within normal ranges (data not shown). Three female participants (ages = 37, 54 and 57 years old) had elevated TPO antibodies, which are markers for thyroid autoimmunity, but their TFT values were within normal ranges (data not shown). Sensitivity analysis excluding these three participants did not alter results (data not shown). The median UICs by sampling round were all above 100 µg/L, indicative of iodine sufficiency (WHO 2013).

Significant, inverse associations between PBDEs and serum TT₄ were estimated in this cohort of North American office workers (Table 3). Adjusting for sampling round, blood lipid level, age, sex, BMI, UIC, urinary specific gravity, urinary perchlorate, and urinary thiocyanate (Model C), we found that for every one unit increase in BDE-47 there was a 2.6 µg/dL (95% CI: -4.7, -0.35) decrease in TT₄ (p=0.02). Additionally, we found that for every unit increase in BDE-100 there was a 7.8 µg/dL (95% CI: -14, -1.6) decrease in TT₄ (p =0.01). Estimates from the crude (Model A) and lipid-adjusted (Model B) models also indicated inverse associations between PBDEs and TT₄ (Table 3). Associations between TT₄ and BDE-28, BDE-99, and BDE-153 also were inverse, but only some of the associations were statistically significant (Table 3). Unadjusted cross-sectional associations between PBDEs and TT₄ at each sampling round were consistently negative (data not shown). Supplemental material, Figure S2A-B presents the unadjusted, cross-sectional negative association between BDE-47 and TT₄ in Round 1.

We identified a possible influential point in the relationship between PBDEs and TT₄. The participant was a 64 year-old, white, non-Hispanic male with high exposure levels of BDE-47 (e.g approximately 13-15 times the FlaRE BDE-47 population sampling round geometric

means). When the point was omitted in regression analysis, the inverse relationships between PBDEs and TT₄ were larger (data not shown).

Table 3 also presents the associations between the TFTs: TSH, fT₄ and TT₃ and PBDEs. These three outcomes were non-normally distributed and natural ln-transformed for regression analysis; see Supplemental Material, Figure S1. The associations of PBDEs with TSH were positive, but small and not statistically significant. We did not find any important associations between PBDEs and fT₄ and TT₃. We estimated an inverse association between BDE-153 and ln(TT₃) (95% CI: -0.54, 0.20). We report a positive, significant association between BDE-153 and ln(fT₄) (95% CI: 0.03, 0.67). However after removal of the influential participant (three serum samples) the positive association between BDE-153 and fT₄ was attenuated ($\beta=0.03$, 95% CI: -0.08, 0.13). There were no consistent associations between the lower brominated PBDEs and fT₄.

For the primary models described above (Model A, B, C) we included PBDEs on a wet weight basis (ng/g serum), treating lipids as a covariate in our regression models, which allows us to assess the independent effects of PBDEs and serum lipids. In almost all cases, adjusting for serum lipids affected the beta coefficients (greater than 10% change). On the other hand, adjusting for demographic and other covariates (e.g. age, sex, BMI, urinary iodine, urinary specific gravity, urinary perchlorate and urinary thiocyanate) did not affect our results as strongly. We ran regression models standardizing PBDEs to lipids and the inverse relationships between PBDEs and TT₄ were persistent; see Supplemental Material, Table S2.

Our results were not confounded (e.g no confounding if <10% change in the point estimate) by urinary iodide (data not shown). When we stratified our cohort by sex, and ran

regression models on datasets that excluded women taking oral contraceptives (seven women), or those participants that were TPO antibody positive (three women), we observed an inverse association between PBDEs and TT₄ (data not shown). We did not find statistically significant interaction terms for PBDE level with age, sex, BMI, or total lipids (data not shown). Inverse relationships between PBDEs and TT₄ were persistent and similar in groups dichotomized by iodide status at round one: UIC <100 µg/L vs. UIC ≥ 100 µg/L (data not shown).

The ICCs for the TFTs were: TSH=0.72, 95% CI: 0.56, 0.78; TT₄=0.80, 95% CI: 0.68, 0.85); fT₄=0.69, 95% CI: 0.53, 0.77; TT₃=0.56, 95% CI: 0.39, 0.67; see Supplemental Material, Table S3. The ICCs estimated in our cohort were similar to those from an earlier study of monthly variation in TFTs among 15 healthy, Caucasian men (Anderson et al. 2002).

Discussion

Our study population of 51 adults living in the Greater Boston Metropolitan area was iodide sufficient and did not have overt thyroid dysfunction. Using repeated serum measures in our prospective cohort, we found an inverse association between PentaBDE congeners and TT₄. The inverse association between PBDEs and TT₄ was persistent regardless of method of lipid-adjustment and in regression models adjusted for potential confounders. Our result is consistent with animal studies which show that exposure to PentaBDEs often causes a reduction in TT₄ (Dishaw et al., 2014). TSH, free T₄, and TT₃ concentrations were not clearly associated with PBDE exposures in our study population of healthy adults, which suggests that associations with TT₄ were not a consequence of effects on the hypothalamic-pituitary-thyroid axis (HPT axis).

PBDEs and their hydroxylated metabolites (OH-PBDEs) are structurally similar to the thyroid hormones T₄ and T₃ (Meerts et al. 2000). Therefore PBDEs have been extensively

researched as possible TH disruptors in animal studies. Many *in vivo* toxicological studies in rodents have reported a reduction in TT₄ following exposures to commercial PBDE (e.g., DE-71) or individual congeners (Blanco et al. 2013; Ernest et al. 2012; Fowles et al. 1994; He et al. 2011; Kodavanti et al. 2010; Stoker et al. 2004; van der Ven et al. 2008; Zhou et al. 2001; Zhou et al. 2002), except for one study showing an increase in TT₄ (Blake et al. 2011). A decrease in TT₄ has been observed in rodents using multiple exposure protocols (acute, sub-acute, chronic) and at various developmental time points (prenatal, perinatal, postnatal, adolescent, adult). Some animal exposure experiments have shown decreases in TT₃ (Blanco et al. 2013; Bondy et al. 2013; Stoker et al. 2004; Zhou et al. 2001); T₃ (total or free) was not always measured in *in vivo* rodent studies (Ernest et al. 2012; Fowles et al. 1994;) or the decrease in T₃ was not significant (He et al. 2011; Van der Ven et al. 2008; Zhou et al. 2002). The effects of PBDEs on TSH have been less consistent. Two *in vivo* rodent studies reported an increase in TSH in response to PBDE exposure (Stoker et al. 2004; Ellis-Hutchings et al. 2006), which would be indicative of PBDEs having an effect on the HPT axis. Four *in vivo* rodent studies have reported a decrease in TT₄ without an increase in TSH (Ernest et al. 2012; He et al. 2011; Zhou et al. 2001; Zhou et al. 2002).

A decrease of peripheral THs (TT₄ or TT₃) in animals in response to PBDE exposures has prompted investigations on the possible underlying mechanisms for this phenomenon. *In vitro* experiments have shown that some OH-PBDEs interact with the serum thyroid hormone binding protein transthyretin (TTR), possibly displacing T₄ from TTR (Marchesini et al. 2008; Meerts et al. 2000). Additionally, BDE-47 may alter T₄ transport by affecting hepatic TTR mRNA expression, decreasing the amount of binding protein available (Richardson et al. 2008). *In vivo* experiments demonstrate that exposure leads to an induction of uridine diphosphate

glucuronosyltransferase, which may increase T₄-glucuronidation and deplete circulating T₄, leading to decreased TT₄ levels (Stoker et al. 2004; Zhou et al. 2002). The mechanism(s) underlying TH disruption by PBDEs in animals has not been completely elucidated and is likely multifactorial. Nevertheless, toxicological studies to date have consistently shown that exposure leads to a decrease in serum TT₄ concentrations.

T₄ exists in two forms: approximately 99.97% of circulating T₄ is bound to plasma proteins (thyroxine binding globulin [TBG], TTR, and albumin), with the remaining T₄ (fT₄) being unbound and available for deiodination in the outer phenolic ring to generate the bioactive hormone, T₃ (Braverman and Cooper, 2013). In humans, measurements of fT₄ have mostly replaced TT₄ (which is predominately bound hormone) as a measure of thyroid status in clinical practice (Garber et al. 2012). Abnormally high or low TT₄ is affected by factors involving TH serum transport proteins and is not necessarily indicative of thyroid dysfunction (Garber et al. 2012). Typically TT₄ is decreased if substances are present that can displace TH from protein binding sites (Stockigt and Lim, 2009) or if there is a decrease in the TH transport proteins, mainly TBG (De Groot et al. 2012). We report that serum PBDE concentrations were inversely associated with serum TT₄ in our study population of healthy adults. Two possible hypotheses are that PBDEs displace THs from their transport proteins or that PBDEs decrease the amount of plasma binding proteins, resulting in a decrease in TT₄. However, it should be emphasized that comparisons of T₄ binding to plasma proteins in rodents and humans may be challenging since the major binding protein in rodents is TTR and in humans TBG (Choksi et al. 2003).

Epidemiological studies have reported associations between PBDEs and TFTs, but the results have been inconsistent. While our results showing inverse associations in TT₄ are

consistent with those typically observed in animal experiments and some human studies (Abdelouahab et al. 2013; Herbstman et al. 2008; Lin et al. 2011), they differ from other studies which have reported positive associations with TT₄. Turyk et al., reported that serum PBDEs were positively associated with serum TT₄ and urinary T₄ in a cohort of 405 adult men that consumed sport fish (Turyk et al. 2008). However, they also reported that PBDEs were related to the percentage of TBG bound to T₄, and an increase in the percentage of TT₄ bound to albumin, indicating PBDEs may affect serum binding patterns (Turyk et al. 2008). Two studies of pregnant women reported that PBDE exposure was inversely associated with TSH, including a study of 207 women with measurements at approximately 27 weeks of gestation (Chevrier et al. 2010) and a study of 25 women with samples collected during the second trimester (Zota et al. 2011). A study of 140 women with samples collected after 34 weeks of gestation reported a positive association with TT₄ and fT₄ (Stapleton et al. 2011). It is difficult to compare the results in pregnant women to those in our study because TT₄ is increased by up to 50% in the first trimester of pregnancy because of estrogen-induced elevations of serum TBG (Stagnaro-Green et al. 2011), which can increase TT₄ levels.

There is current debate regarding how (or whether) to adjust for serum lipids—lipids as a covariate, lipid-standardization—when studying the health effects of lipophilic chemicals (Schisterman et al. 2005). As expected, serum PBDE concentrations are positively associated with serum lipid concentrations in our population (Makey et al. 2014). Additionally, thyroid hormones maintain lipid homeostasis by affecting gene expression in adipose tissue and the liver, affecting lipolysis and clearance (Pearce 2012), making serum lipids dependent on the thyroid hormones. The causal structure between PBDEs, serum lipids, and THs is currently unclear.

Therefore as recommended elsewhere, we compared multiple methods for lipid adjustment (Chevrier 2013; Schisterman et al. 2005). We found that the results obtained from our crude and lipid-adjusted models were generally consistent in the direction of the association but the magnitude of the β -coefficients were altered by more than 10%. Direction of associations between PBDEs and TT₄ remained between the lipid-adjusted and lipid-standardized models. Comparison between these models is difficult to make as the denominator for the β -coefficients are different.

Iodide, an essential component of the THs, has been reported to be an effect measure modifier in epidemiologic studies, such that subgroups with less iodine intake appear to be more vulnerable to xenobiotic effects on TH levels than iodine-sufficient subgroups (Blount et al. 2006). Iodide did not appear to modify associations between PBDEs and TT₄ in our iodine-sufficient study population.

A major strength of our study is the use of three serum samples from a longitudinal cohort free of overt thyroid dysfunction. We collected demographic and medical information to assess confounding and effect measure modification. Our study, however, also has some limitations. We did not measure the OH-PBDE metabolites, which have been shown to have binding affinities to TBG and TTR (Marchesini et al. 2008). We did not have three serum samples from all participants. Selected samples were excluded based on *a priori* criteria (medication use, pregnancy, inadequate samples) and some samples were missing because participants completed only one (n=2) or two (n=9) of the three (n=41) study visits, leaving a total of 137 serum samples from 51 participants. We believe that the likelihood that an individual sample was missing would have been unrelated to the actual TFT value of the missing sample,

which would, on average, result in data that are missing completely at random (MCAR). If this assumption is correct for our population, missing data should not have biased associations because general linear models are robust to missing data that are MCAR (Little and Rubin, 2002). Our study sample size was relatively small; we used a convenience sample of office workers in the Boston area, who were 85% white and highly educated and we cannot be certain our results can be generalized to the general US adult population. Furthermore, our small sample size limited our ability to evaluate effect measure modification.

Conclusion

The results from our repeated measures cohort study suggest that environmental exposure to PBDEs was associated with lower TT₄ levels. The lack of clear associations with other thyroid function parameters suggests that the negative association with TT₄ might be a consequence of decreased serum binding of T₄. This finding is consistent with the toxicological literature and some human studies. Our conclusions were robust to potential confounders and various methods of data analysis. Future prospective studies are needed to further understand how PBDEs and their metabolites may affect TH homeostasis in healthy adults.

References

- Abdelouahab N, Langlois MF, Lavoie L, Corbin F, Pasquier JC, Takser L. 2013. Maternal and cord-blood thyroid hormone levels and exposure to polybrominated diphenyl ethers and polychlorinated biphenyls during early pregnancy. *Am J Epidemiol* 178(5):701-713.
- Andersen S, Pedersen KM, Bruun NH, Laurberg P. 2002. Narrow individual variations in serum T4 and T3 in normal subjects: a clue to the understanding of subclinical thyroid disease. *J Clin Endocrinol Metab* 87:1068–72.
- Blake CA, McCoy GL, Hui YY, LaVoie HA. 2011. Perinatal exposure to low-dose DE-71 increases serum thyroid hormones and gonadal osteopontin gene expression. *Exp Biol Med* 236(4):445-455.
- Blanco J, Mulero M, Heredia L, Pujol A, Domingo JL, Sanchez DJ. 2013. Perinatal exposure to BDE-99 causes learning disorders and decreases serum thyroid hormone levels and BDNF gene expression in hippocampus in rat offspring. *Toxicology* 308:122-128.
- Bloom M, Spliethoff H, Vena J, Shaver S, Addink R, Eadon G. 2008. Environmental exposure to PBDEs and thyroid function among New York anglers. *Environ Toxicol Pharmacol* 25(3):386-392.
- Blount BC, Pirkle JL, Osterloh JD, Valentin-Blasini L, Caldwell KL. 2006. Urinary perchlorate and thyroid hormone levels in adolescent and adult men and women living in the United States. *Environ Health Perspect* 114:1865-1871.
- Braverman LE, Cooper GS. 2013. Peripheral thyroid hormone binding and metabolism. In: Werner & Ingbar's *The Thyroid: A Fundamental and Clinical Text*, 10th Edition. Philadelphia: Lippincott Williams and Wilkins, 93-126.
- Chevrier J. 2013 Invited commentary: maternal plasma polybrominated diphenyl ethers and thyroid hormones – challenges and opportunities. *Am J Epidemiology* 178(5):714-19.
- Chevrier J, Harley KG, Bradman A, Gharbi M, Sjodin A, Eskenazi B. 2010. Polybrominated diphenyl ether (PBDE) flame retardants and thyroid hormone during pregnancy. *Environ Health Perspect* 118(10):1444-1449.
- Chevrier J, Harley KG, Bradman A, Sjodin A, Eskenazi B. 2011. Prenatal exposure to polybrominated diphenyl ether flame retardants and neonatal thyroid-stimulating hormone levels in the CHAMACOS study. *Am J Epidemiol* 174(10):1166-1174.

Choksi NY, Jahnke GD, St. Hilaire C, Shelby M. 2003. Role of thyroid hormones in human and laboratory animal reproductive health. *Birth Defects Res (Part B)* 68:479-491.

Cooper DS, Biondi R. 2012. Subclinical thyroid disease. *Lancet* 379(9821):1142-54.

De Groot L, Abalovich M, Alexander EK, Amino N, Barbour L, Cobin RH, et al. 2012. Management of thyroid dysfunction during pregnancy and postpartum: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 97(8):2543-2565.

Dishaw LV, Macaulay LJ, Roberts SC, Stapleton HM. 2014. Exposures, mechanisms, and impacts of endocrine-active flame retardants. *Curr Opin Pharmacol* 19:125-133.

Ernest SR, Wade MG, Lalancette C, Ma YQ, Berger RG, Robaire B, et al. 2012. Effects of chronic exposure to an environmentally relevant mixture of brominated flame retardants on the reproductive and thyroid system in adult male rats. *Toxicol Sci* 127(2):496-507.

Fowles JR, Fairbrother A, Baecher-Steppan L, Kerkvliet NI. 1994. Immunologic and endocrine effects of the flame-retardant pentabromodiphenyl ether (DE-71) in C57BL/6J mice. *Toxicology* 86(1-2):49-61.

Fraser AJ, Webster TF, McClean MD. 2009. Diet contributes significantly to the body burden of PBDEs in the general U.S. population. *Environ Health Perspect* 117:1520-1525.

Garber JR, Cobin RH, Gharib H, Hennessey JV, Klein I, Mechanick JI, et al. 2012. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. *Thyroid* 22(12):1200-1235.

He P, Wang A, Niu Q, Guo L, Xia T, Chen X. 2011. Toxic effect of PBDE-47 on thyroid development, learning, and memory, and the interaction between PBDE-47 and PCB153 that enhances toxicity in rats. *Toxicol Ind Health* 27(3):279-288.

Herbstman JB, Sjodin A, Apelberg BJ, Witter FR, Halden RU, Patterson DG, et al. 2008. Birth delivery mode modifies the associations between prenatal polychlorinated biphenyl (PCB) and polybrominated diphenyl ether (PBDE) and neonatal thyroid hormone levels. *Environ Health Perspect* 116(10):1376-1382.

Kodavanti PR, Coburn CG, Moser VC, MacPhail RC, Fenton SE, Stoker TE, et al. 2010. Developmental exposure to a commercial PBDE mixture, DE-71: neurobehavioral, hormonal, and reproductive effects. *Toxicol Sci* 116(1):297-312.

Lin SM, Chen FA, Huang YF, Hsing LL, Chen LL, Wu LS, et al. 2011. Negative associations between PBDE levels and thyroid hormones in cord blood. *Int J Hyg Environ Health* 214(2):115-120.

Little RJA, Rubin DB. *Statistical analysis with missing data*, 2nd Edition. New York: Wiley. 2002.

Makey CM, McClean MD, Sjobin A, Carignan C, Weinberg J, Webster TF. 2014. Temporal variability of PBDE serum concentrations over one-year. *Environ Sci Tech* doi:10.1021/es5026118.

Marchesini GR, Meimaridou A, Haasnoot W, Meulenberg E, Albertus F, Mizuguchi M, et al. 2008. Biosensor discovery of thyroxine transport disrupting chemicals. *Toxicol Appl Pharmacol* 232(1): 150-160.

Meerts IA, van Zanden JJ, Luijckx EAC, van Leeuwen-Bol I, Marsh G, Jakobsson E, et al. 2000. Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in vitro. *Toxicol Sci* 56(1):95-104.

Pearce EN. 2012. Update in lipid alterations in subclinical hypothyroidism. *J Clin Endocrinol Metab* 97(2):326-333.

Phillips DL, Pirkle JL, Burse VW, Bernert JT, Henderson LO, Needham, LL. 1989. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. *Arch. Environ Contam. Toxicol.* 18: 495-500.

Richardson VM, Staskal DF, Ross DG, Diliberto JJ, DeVito MJ, Birnbaum LS. 2008. Possible mechanisms of thyroid hormone disruption in mice by BDE 47, a major polybrominated diphenyl ether congener. *Toxicol Appl Pharmacol* 226(3):244-250.

Schechter A, Haffner D, Colacino J, Patel K, Papke O, Opel M, et al. 2010. Polybrominated diphenyl ethers (PBDEs) and hexabromocyclodecane (HBCD) in composite U.S. food samples. *Environ Health Perspect* 118:357-362.

Schisterman EF, Whitcomb BW, Louis GM, Louis TA. 2005. Lipid adjustment in the analysis of environmental contaminants and human health risks. *Environ Health Perspect* 113(7):853-857.

Sjödin A, Jones RS, Lapeza CR, Focant JF, McGahee EE, Patterson DG. 2004. Semiautomated high-throughput extraction and cleanup method for the measurement of polybrominated diphenyl ethers, polybrominated biphenyls, and polychlorinated biphenyls in human serum. *Anal Chem* 76:1921-1927.

Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, Negro R, et al. 2011. Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. *Thyroid* 21(10):1081-1125.

Stapleton HM, Eagle S, Anthopolos R, Wolkin A, Miranda ML. 2011. Associations between polybrominated diphenyl ether (PBDE) flame retardants, phenolic metabolites, and thyroid hormones during pregnancy. *Environ Health Perspect* 119(10):1454-1459.

Stockholm Convention. 2004. Decisions and Recommendations: Listing of tetrabromodiphenyl ether and pentabromodiphenyl ether. Available: <http://chm.pops.int/Implementation/NewPOPs/DecisionsRecommendations/tabid/671/Default.aspx> [accessed 14 August 2015].

Stockigt JR, Lim CF. 2009. Medications that distort in vitro tests of thyroid function, with particular reference to estimates of serum free thyroxine. *Best Pract Res Clin Endocrinol Metab* 23(6):753-767.

Stoker TE, Laws SC, Crofton KM, Hedge JM, Ferrell JM, Cooper RL. 2004. Assessment of DE-71, a commercial polybrominated diphenyl ether (PBDE) mixture, in the EDSP male and female pubertal protocols. *Toxicol Sci* 78(1):144-155.

Turyk ME, Persky VW, Imm P, Knobloch L, Chatterton R, Anderson HA. 2008. Hormone disruption by PBDEs in adult male sport fish consumers. *Environ Health Perspect* 116:1635-1641.

Valentin-Blasini L, Mauldin JP, Maple D, Blount BC. 2005. Analysis of perchlorate in human urine using ion chromatography and electrospray tandem mass spectrometry. *Anal Chem* 77(8):2475-2481.

van der Ven LT, van de Kuil T, Verhoef A, Leonards PE, Slob W, Canton RF, et al. 2008. A 28-day oral dose toxicity study enhanced to detect endocrine effects of a purified technical pentabromodiphenyl ether (pentaBDE) mixture in Wistar rats. *Toxicology* 245(1-2):109-122.

Watkins DJ, McClean MD, Fraser AJ, Weinberg J, Stapleton HM, Sjodin A, et al. 2012. Impact of dust from multiple microenvironments and diet on PentaBDE body burden. *Environ Sci Technol* 46(2):1192-1200.

WHO (World Health Organization). 2013. Urinary iodine concentrations for determining iodine status deficiency in populations. Available: http://apps.who.int/iris/bitstream/10665/85972/1/WHO_NMH_NHD_EPG_13.1_eng.pdf [accessed 11 February 2014].

Zhou T, Ross DG, DeVito MJ, Crofton KM. 2001. Effects of short-term in vivo exposure to polybrominated diphenyl ethers on thyroid hormones and hepatic enzyme activities in weanling rats. *Toxicol Sci* 61(1):76-82.

Zhou T, Taylor MM, DeVito MJ, Crofton KM. 2002. Developmental exposure to brominated diphenyl ethers results in thyroid hormone disruption. *Toxicol Sci* 66(1):105-116.

Zota AR, Park JS, Wang Y, Petreas M, Zoeller RT, Woodruff TJ. 2011. Polybrominated diphenyl ethers, hydroxylated polybrominated diphenyl ethers, and measures of thyroid function in second trimester pregnant women in California. *Environ Sci Technol* 45(18):7896-7905.

Table 1. Baseline Characteristics, Collected in 2010, from the FlaRE Cohort (51 participants)

Characteristic	<i>n</i> (%)
Age	
20-39 years	29 (57)
40-59 years	18 (35)
≥ 60 years	4 (8)
Sex	
female	25 (49)
male	26 (51)
Race/ethnicity	
white	44 (85)
other	6 (15)
Education	
College graduate	50 (98)
< college graduate	1 (2)
BMI (kg/m²)	
<25	33 (63)
25 - 29.9	16 (33)
≥ 30	2 (4)

Table 2. Descriptive statistics of analytes by sampling round (51 participants, 137 serum samples).

Analytes	Round 1 (n=47)			Round 2 (n=49)			Round 3 (n=41)			
	GM (GSD)	(%)	Range	GM (GSD)	(%)	Range	GM (GSD)	(%)	Range	
Serum PBDEs (ng/g lipid)										
ΣPBDE ^a	22 (2.7)	(100)	2.3 - 290	23 (2.4)	(100)	2.7 - 290	19 (2.1)	(100)	2.4 - 210	
BDE - 28 ^a	0.57 (2.3)	(66)	0.20 - 4.3	0.60 (2.2)	(71)	0.20 - 5.1	0.50 (2.2)	(66)	0.20 - 3.6	
BDE - 47 ^a	9.5 (2.9)	(100)	0.60- 150	9.9 (2.7)	(100)	1.3 - 150	7.6 (2.6)	(100)	0.90 - 99	
BDE - 99 ^a	1.8 (3.0)	(94)	0.20 - 44	1.9 (3.0)	(90)	0.20 - 34	1.7 (2.5)	(95)	0.20 - 20	
BDE - 100 ^a	1.8 (3.5)	(87)	0.20 - 42	1.9 (3.3)	(90)	0.20 - 44	1.4 (3.0)	(90)	0.20 - 35	
BDE - 153 ^a	6.4 (3.2)	(100)	0.60 - 97	6.7 (3.1)	(100)	0.70 - 95	5.3 (3.3)	(100)	0.10 - 55	
Serum PBDEs (pg/g serum)										
ΣPBDE ^a	130 (2.7)	(100)	14 - 1900	140 (2.7)	(100)	15 - 2000	120 (2.4)	(100)	15 - 1500	
BDE - 28 ^a	3.5 (2.4)	(66)	1.3 - 29	3.7 (2.4)	(71)	1.3 - 30	3.3 (2.3)	(66)	1.3 - 25	
BDE - 47 ^a	57 (3.0)	(100)	3.9 - 990	59 (2.8)	(100)	7.3 - 1000	48 (2.7)	(100)	5.5 - 710	
BDE - 99 ^a	11 (3.0)	(94)	1.3 - 290	11 (3.2)	(90)	1.3 - 230	10 (2.6)	(95)	1.3 - 140	
BDE- 100 ^a	11 (3.1)	(87)	1.3- 280	11 (3.4)	(90)	1.3 - 300	8 (3.1)	(90)	1.3 - 250	
BDE- 153 ^a	38 (3.6)	(100)	3.9-700	39 (3.1)	(100)	4.5 - 580	36 (2.7)	(100)	5.3 - 400	
Thio. (μg/L) ^a	450 (470)	(100)	72 -2300	570 (360)	(100)	61 - 1500	480 (450)	(100)	16 - 1815	
Perchl. (μg/L) ^a	3.8 (4.3)	(100)	0.4 - 19	2.9 (18)	(100)	0.4 - 130	5.2 (11)	(100)	0.4 - 73	
TT ₄ (μg/dl) ^b	7.3 (1.3)	(100)	3.2 - 9.6	7.3 (1.4)	(100)	3.9 - 10	7.1 (1.3)	(100)	4.7 - 9.2	
fT ₄ (ng/dl) ^a	1.2 (1.2)	(100)	0.9 - 2.1	1.2 (1.2)	(100)	1.0 - 2.2	1.2 (1.2)	(100)	1.0 - 1.6	
TSH (μIU/ml) ^a	0.71 (1.8)	(100)	0.2 - 2.7	0.75 (1.8)	(100)	0.2 - 2.3	0.89 (1.9)	(100)	0.2 - 4.8	
TT ₃ (ng/ml) ^a	1.1 (1.2)	(100)	0.8 - 1.5	1.2 (1.2)	(100)	0.8 - 1.4	1.2 (1.2)	(100)	0.8 - 1.5	
UIC (μg/L) ^a	130 (170)	(100)	27 - 890	140 (100)	(100)	11 - 550	150 (116)	(100)	32 - 660	
Urinary SG ^b	1.02 (0.007)	(100)	1.0 - 1.03	1.02 ^d (0.009)	(100)	1.0 - 1.04	1.02 (0.007)	(100)	1.0 - 1.03	
TPO Ab (IU/mL) ^c	3 n/a	(6)	< 5 - 810	3 n/a	(6)	< 5 - 680	3 n/a	(7)	< 5 - 640	
Chol (mg/dL) ^b	190 (34)	(100)	120 - 290	190 (46)	(100)	110 - 360	190 (44)	(100)	92 - 330	
Trig (mg/dL) ^b	130 (70)	(100)	50 - 340	130 (71)	(100)	42 - 340	140 (66)	(100)	45 - 290	
Lipids (mg/dL) ^{b,e}	620 (110)	(100)	450 - 860	620 (150)	(100)	370 - 1100	630 (140)	(100)	330 - 1000	

Abbreviations: % (percent detect), Chol (cholesterol), Lipids (total lipids), SG (Specific Gravity), Perchl (Perchlorate), Thio (thiocyanate), TPO Ab (Thyroid peroxidase antibody), Trig (Triglycerides)

^a Geometric mean and geometric standard deviation.

b Mean and standard deviation.

c Number of participants who tested positive for TPO Ab in each round.

d One specific gravity measurement was missing in round 2.

e Total Lipids = [cholesterol (mg/dL)*2.27] + triglycerides (mg/dL) + 62.3

Table 3. Results from general linear regression models evaluating the association between PBDEs (ng/g serum) and the thyroid function tests: TT₄, ln(TSH), ln(fT₄) and ln(TT₃) (51 participants, 137 serum samples).

	BDE-28 β (95% CI)	<i>p</i>	BDE-47 β (95% CI)	<i>p</i>	BDE-99 β (95% CI)	<i>p</i>	BDE-100 β (95% CI)	<i>p</i>	BDE-153 β (95% CI)	<i>p</i>
TT₄ (μg/dL)										
Model A	-51 (-110, 6.2)	0.08	-2.7 (-4.8, -0.61)	0.01	-7.3 (-15, 0.042)	0.05	-8.4 (-14, -2.5)	0.01	-2.8 (-5.5, 0.04)	0.05
Model B	-33 (-95, 29)	0.29	-2.3 (-4.5, -0.18)	0.03	-6.4 (-14, 0.93)	0.09	-7.5 (-14, -1.4)	0.02	-2.4 (-5.2, 0.43)	0.10
Model C	-34 (-97, 28)	0.28	-2.6 (-4.7, -0.35)	0.02	-7.6 (-15, -0.06)	0.05	-7.8 (-14, -1.6)	0.01	-2.3 (-5.2, 0.61)	0.12
TSH ln(μIU/mL)^a										
Model A	0.29 (-28, 28)	0.99	0.32 (-0.71, 1.4)	0.54	1.6 (-2.1, 5.3)	0.39	1.3 (-1.6, 4.2)	0.37	0.97 (-0.34, 2.3)	0.15
Model B	-11 (-41, 18)	0.45	0.10 (-0.95, 1.2)	0.85	1.2 (-2.6, 4.9)	0.54	0.84 (-2.1, 3.8)	0.58	0.83 (-0.49, 2.2)	0.21
Model C	-11 (-42, 20)	0.49	0.17 (-0.92, 1.3)	0.75	1.5 (-2.5, 5.4)	0.46	0.92 (-2.2, 4.0)	0.55	0.81 (-0.58, 2.2)	0.25
fT₄ ln(ng/dL)^a										
Model A	-0.40 (-7.5, 6.7)	0.92	-0.13 (-0.39, 0.1)	0.35	-0.72 (-1.7, 0.23)	0.14	0.27 (-0.47, 1.0)	0.47	0.40 (-0.09, 0.72)	0.01
Model B	1.1 (-6.6, 8.9)	0.77	-0.10 (-0.4, 0.17)	0.46	-0.67 (-1.6, 0.30)	0.17	0.35 (-0.41, 1.1)	0.37	0.42 (0.10, 0.75)	0.01
Model C	0.42 (-7.3, 8.1)	0.91	-0.13 (-0.40, 0.1)	0.34	-0.83 (-1.8, 0.14)	0.09	0.21 (-0.54, 0.95)	0.58	0.35 (0.03, 0.67)	0.04
TT₃ ln(ng/mL)^a										
Model A	0.01 (-0.3, 0.3)	0.99	-0.01 (-0.3, 0.3)	0.94	0.12 (-0.91, 1.1)	0.82	-0.28 (-1.0, 0.47)	0.46	-0.28 (-0.6, 0.05)	0.10
Model B	2.2 (-5.9, 10)	0.60	0.03 (-0.25, 0.3)	0.82	0.23 (-0.81, 1.3)	0.67	-0.20 (-0.98, 0.6)	0.61	-0.27 (-0.6, 0.08)	0.13
Model C	02.2 (-5.9, 10)	0.62	0.04 (-0.24, 0.3)	0.77	0.21 (-0.84, 1.3)	0.69	-0.13 (-0.9, 0.64)	0.73	-0.19 (-0.54, 0.2)	0.28

Abbreviations: β (beta-estimate), CI (confidence interval), fT₄ (free thyroxine), p (p-value), TT₃ (total triiodothyronine), TT₄ (total thyroxine), TSH (thyroid stimulating hormone), SG (Specific Gravity).

^a Dependent variables are natural log-transformed.

Model A: Exposure only, no covariates

Model B: Adjusted for serum lipids only

Model C: Adjusted for serum lipids, age, sex, BMI, urine iodide, urine perchlorate, urine thiocyanate and urine specific gravity. All covariates other than sex were modeled as untransformed continuous variables.