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Larissa Pardo Date

Assessing exposure to polybrominated diphenyl ethers (PBDEs) and thyroid function as measured by thyroid-stimulating hormone (TSH) in children in the metropolitan Atlanta, Georgia area

By

Larissa Pardo
Master of Science in Public Health

Environmental Health-Epidemiology

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Lyndsey Darrow, PhD
Committee Chair

_____

Paige, Tolbert, PhD
Committee Member

Assessing exposure to polybrominated diphenyl ethers (PBDEs) and thyroid function as measured by thyroid-stimulating hormone (TSH) in children in the metropolitan Atlanta, Georgia area

By

Larissa Pardo

B.A.
George Washington University
2010

Thesis Committee Chair: Lyndsey Darrow, PhD

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Abstract

Assessing exposure to polybrominated diphenyl ethers (PBDEs) and thyroid function as measured by thyroid-stimulating hormone (TSH) in children in the metropolitan Atlanta, Georgia area

By Larissa Pardo

Background: Polybrominated diphenyl ethers (PBDEs), which are synthetic flame-retardants used in a variety of consumer products, are widely detected in the blood serum levels of U.S. residents and have been suggested to disrupt thyroid function due to their structural resemblance of T_4. Some evidence suggests that children have higher levels of exposure to PBDEs than do adults because of high uptake during breast-feeding and ingestion of household dust during hand-to-mouth activity. However, mechanistic effects, if there are effects, are poorly understood as few studies have investigated the relationship between thyroid function disruption and PBDEs in developing humans.

Methods: The authors investigated the association between PBDE (BDE-47, -99, -100, -153) exposure and thyroid function as measured by thyroid stimulating hormone (TSH) in a pilot, cross-sectional study conducted at Children's Healthcare of Atlanta, Georgia between 2011 and 2012. The study enrolled 89 children who were undergoing common childhood surgeries. Blood was drawn from each participant while under anesthesia. Parents of children answered a questionnaire regarding demographic information, family health history, and child behavior. Multiple linear regression was used to estimate the relationship between PBDE exposure and TSH levels.

Results: In the analysis, BDE-100 was the only congener that was associated with TSH ($p<0.05$). It was also the only congener that had a significant linear trend across quartiles of exposure ($p=0.01$). However, there was some suggestion of increased TSH levels with higher exposure to PBDEs, after adjusting for gender, age, time of blood draw, family history of thyroid disease, race/ethnicity, breastfeeding history and duration, socioeconomic status, and smoking status.

Conclusion: Our findings in this pilot cross-sectional study did not show strong evidence of an association between PBDEs and TSH, however there was some suggestion that PBDEs, at the highest levels, are associated with higher TSH levels in children. These preliminary results are consistent with the only other study conducted in children, and therefore support further investigation in a larger study.

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Larissa Pardo

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Table of Contents

Introduction

Polybrominated diphenyl ethers (PBDEs) are synthetic flame-retardants used in a variety of consumer products ranging from electronics to plastics to carpets to foam. PBDEs are widely detected in the blood serum levels of all U.S. residents and have been suggested to disrupt thyroid function due to their structural resemblance of other thyroid hormones. PBDEs leach from consumer products into the environment, bioaccumulate, and can persist in the human body anywhere from a few days to 3 to 6 years, depending on the congener (Geyer et al., 2004). Exposure to PBDEs can occur through different routes such as inhalation, ingestion, or dermal exposure (Talsness, 2008). There has been evidence to suggest that children ages two to five have much higher levels of exposure to PBDEs than adults (Eskenazi et al., 2011; Lunder et al., 2010; Sjodin et al., 2008; Toms et al. 2009; Lorber et al., 2007). Children may be exposed to higher levels of PBDE through routes of exposure that are thought to be different from adult exposure, including high uptake during breast-feeding, due to their lipophilicity, as well as ingestion and inhalation of household dust during hand-to-mouth activity (Toms et al. 2009; Lorber et al., 2008; Sjodin et al., 2008).

PBDEs are suggested to act as potential endocrine disruptors of the thyroid system. The thyroid is a self-regulating system that works in congruence with the hypothalamus and the pituitary gland to make thyroid-stimulating hormone (TSH), which triggers the thyroid to release thyroixine (T_4) from stored iodine. As the availability of circulating T_4 increases, this triggers a feedback loop resulting in a decrease of the production of TSH until the next meaningful drop in T_4. T_4 circulates until reaching target cells where it is transformed from T_4 to Triiodothyronine (T_3). This system can be

easily disrupted by environmental contaminants like PBDEs (Brown, 2003). PBDEs are thought to disrupt the system and its feedback loop by mimicking thyroxine (T_4) due to its structural resemblance to the thyroid hormone (Boas et al., 2006; ATSDR, 2010). Such disruption is suggested to result in greater susceptibility for early life development windows, as it is known that adverse effects on brain development can result from abnormal levels of thyroid hormone (Haddow et al., 1999; Darnerud, 2008).

Concerns regarding thyroid disruption by PBDEs first occurred from a number of studies on animals. A reduction in T_4 is well depicted in rodent studies; however, the feedback response of thyroid-stimulating hormone (TSH) is still not completely clear (Fowles et al., 1994; Hallgren et al., 2001; Zhou et al., 2001, 2002; Ellis-Hutchings et al., 2006; Darnerud et al., 2007; Kuriyama et al., 2007). There has also been evidence of an association between hyperthyroidism in cats and exposure to PBDEs in indoor dust (Mensching et al. 2012; Dye et al., 2007).

While there is substantial evidence for thyroid hormone disruption in the animal literature, few epidemiologic studies have investigated the association between PBDE exposure and thyroid function in humans. Even among these, there has been little consistency. Studies among e-waste workers occupationally exposed to PBDEs and adult male sport fisherman found statistically significant inverse associations between PBDE exposure and TSH levels (Wang et al., 2010; Turyk et al., 2008; Yuan et al., 2008). The Turyk study followed 308 frequent and infrequent adult male consumers of Great Lake fish and found that PBDE levels were positively related to measures of T_4 and reverse T_3 and inversely related to total T_3 and TSH. Other studies, however, have found no association between PBDEs and TSH (Bloom et al., 2008; Julander et al., 2005). Reasons

for no observed association might be attributable to different sample sizes, study designs, or geographic location. The Julander study, for example, had a small sample size (n=11).

To date, most studies have focused on the association between PBDE exposure and thyroid function in animals or adult humans; very few studies investigated this association in children. To our knowledge, only two studies have analyzed post-natal exposure of PBDEs and thyroid function in children. One study estimated the effects of pre- and post-natal exposure to PBDEs in Spanish children, finding a weak positive relationship between PBDE concentrations and TSH at 4 years of age (Gascon et al., 2011). This, however, was not statistically significant. The low exposure levels and smaller sample size contributed to various limitations in the Gascon study including the inability to assess individual congeners and limited variation above the limit of detection. The second study assessed concentrations of PBDEs in breast milk up to 30 days after delivery and TSH levels 3 days after delivery in Norwegian infants. Investigators found no association between PBDEs and TSH levels (Eggesbo et al., 2012). This study, however, did not take into consideration childhood exposure, only exposure from pregnancy and the first few days of breastfeeding. It also had low levels of exposure and problems with temporality. Both studies, also, only examined PBDE concentrations in children of European nations, which although industrialized, do not have comparable PBDE levels to the United States, which has human body burdens that are about 10 times higher (Sjodin et al., 2008; Birnbaum and Cohen, 2006).

Few previous studies have assessed exposure to PBDE specifically during early-life when exposure is highest and the thyroid is still developing. Previous studies, when examining childhood exposures, only considered prenatal exposure or pre-and post-natal

exposures together without separating out the effects of exposure time. However, post-natal exposure is crucial to distinguish, as the first few years of life have a peak in PBDE levels due to breastfeeding and hand-to-mouth activity and are a critical period for development and potential dysfunction of the thyroid, leading to neurological impairment (Brucker-Davis, 1998; Fisher et al., 2000). Thus, in our study, we will investigate the association between childhood exposure to PBDEs and TSH in children (age 15 months to 5 years). We hope to bolster what is currently known in the literature on early-life exposure to PBDEs and their effect on TSH during this critical period of development. Another critical issue for other prior studies has been the study country of origin since exposure levels across countries are drastically different. In our study, we will focus on U.S. children, who are expected to have the highest exposures to PBDEs, by enrolling children born in the U.S. and currently residing in Georgia.

The aim of our study is to investigate an association of exposure to polybrominated diphenyl ethers (PBDEs) in blood with thyroid function as measured by thyroid-stimulating hormone (TSH) levels in a pilot, cross-sectional study of 89 children ages 15 months to 5 years undergoing common childhood surgeries (myringotomy, adenoidectomy, tonsillectomy, bronchoscopy, or mastoidectomy) at Children's Healthcare of Atlanta.

Methods

Study population

The BEAT (Brominated flame retardant Exposure And Thyroid) Study is a cross-sectional study assessing PBDE exposure and thyroid function in children in Atlanta,

Georgia. Participants were recruited from a pediatric Ear Nose and Throat (ENT) clinic population at Children's Healthcare of Atlanta (CHOA), who were undergoing myringotomies, adenoidectomies, tonsillectomies, bronchoscopies, or mastoidectomies. Participants ranged in age from 15 months to 5 years and were sampled with the intent of having the same ratio of males to females and similar distributions of race and ethnicities. To be eligible, participants must have been born in the United States, have no current illness or underlying health condition, and not currently taking the following medications: gluccorticoids, beta blockers, anti-arrhythmic, estrogens, tamoxifen, androgens, anticonvulsants, furosemide, high dose salicylates, lithium, or iodine. The types of surgeries chosen to sample from were not thought to affect study outcomes and are common surgeries among young children who are otherwise considered healthy. The purpose of choosing pediatric anesthesia patients was to gain consent for blood draws on children with ease. The consent rate to agree to join the study was well over 90%, with 89 children enrolled.

Data Collection

Upon arrival for surgery, study administrators determined eligibility, obtained consent and a HIPPA waiver, and asked parents to fill out a short survey answering questions related to demographic information, health history, and child behavior. Questions included age, race and ethnicity, family history of thyroid or other endocrine or auto-immune diagnoses, breastfeeding history and duration, height and weight, birth order, hours per day spent inside the home, time at the current residential address, medications, occupation of parents, and current smoking status of parent. If the survey indicated a family history of thyroid disease and the diagnosis was unknown, an

additional thyroid disease survey was administered. The study nurse also recorded participant information including: insurance status, height and weight of child, birth date, date and time of blood draw, surgery type, and any additional notes.

Once under anesthesia, up to 15 mL of blood was drawn from each child. Blood was collected in two red-top Vacutainer tubes and transported on the day of collection to the Analytical Exposure Science & Environmental Health laboratory at Emory University's Rollins School of Public Health. Samples were processed upon arrival, manually inverted, allowed to clot for no longer than 60 minutes, and centrifuged (IEC Medispin, Thermo Scientific®) for 30 minutes at 3000 RPM to separate the serum from the cellular fraction. The resulting serum was then aliquoted by sterile transfer pipette into two 5 mL cryovials. One vial was intended for PBDE analysis, while the other for thyroid hormone analysis. The serum aliquots were frozen at -20°C and stored for later analysis. The Emory University and Children's Healthcare of Atlanta Institutional Review Boards approved the protocol of the study.

Exposure analysis

PBDEs in serum were analyzed using gas chromatography-tandem mass spectrometry (GS-MS/MS; Agilent Technologies; 7000 GC/MS Triple Quad) at Emory University's Rollins School of Public Health's Environmental Toxicology and Exposure Assessment Laboratory. The following PBDE congeners were analyzed: BDE-47, -85, -99, -100, -153, and -154. The method developed for PBDE analysis was based on three previous methods in the literature, but further refined by the Environmental Toxicology and Exposure Assessment Laboratory to optimize extraction recovery and analytic precision (Hovander et al., 2000; Sandau et al., 2003; Zhang et al., 2011). From serum

samples, PBDEs were extracted using a solid-phase extraction (SPE) method. A clean-up method was also utilized in order to remove lipids and other biogenic materials. For quality control, each set of samples included a blank serum sample as well as low (QCL) and high concentration (QCH) quality control serum samples. The standard concentration for QCL was 100 ng/mL of BDE-209 and 10 ng/mL of BDE-47, -85, -99, -100, -153, and -154 in methanol, whereas the standard concentration for QCH included 250 ng/mL of BDE-209 and 100 ng/mL of BDE-47, -85, -99, -100, -153, and -154 in methanol. The limit of detection (LOD) by congener using this method and instrumentation were as follows: 0.0005 ng/mL for BDE-47, 0.003 ng/mL for BDE-85, 0.001 ng/mL for BDE-99, 0.002 ng/mL for BDE-100, 0.02 ng/mL for BDE-153, and 0.006 ng/mL for BDE-154. Where the measurement was below the LOD, a serum value was imputed by dividing the congener-specific LOD by the $\sqrt{2}$. All serum values were then lipid-adjusted. Total serum lipid content was calculated for each sample and PBDE concentrations were expressed on a ng/g lipid basis.

Hormone analysis

Blood samples were analyzed for TSH concentration by immunoassay using a commercially prepared kit by Alpco Immunoassays at the Biomarkers Core Laboratory at Emory University's Yerkes National Primate Research Center. Samples were analyzed for other markers of thyroid function including free and total thyroxine (T_4); free, total and reverse triiodothyronine (T_3); thyroid-stimulating hormone (TSH); T_3 uptake (an indirect measure of thyroid binding globulin (TBG), albumin-bound T_4 and TBG-bound T_4); anti-thyroid peroxidase (TPO); anti-thyroglobulin; and thyroid stimulating immunoglobulins (TSI).

Statistical methods

Eighty-nine children were initially enrolled in the study. Nine children were ultimately excluded from the analysis for the following reasons: child was too dehydrated to draw sufficient blood (n=5), the parent did not return the survey to the research nurse (n=2), the child did not meet the eligibility criteria for age (n=1), or there was not sufficient serum volume for measuring TSH (n=1). Complete information on exposure and hormone levels as well as questionnaire data was available for 80 children.

PBDEs were summed over all measured congeners to get a total PBDE serum concentration, as well as kept separate to examine individual congeners (BDE-47, -99, -100, -153). All PBDE serum concentrations were highly skewed and therefore log transformed. The exposure variables were kept as continuous variables, but also categorized into quartiles. Thyroid-stimulating hormone (TSH) was log-transformed as it was also highly skewed.

For Body Mass Index (BMI), a z-score was calculated for all children older than 2 years. For those within 3 months of 2 years, ages were rounded to 2 years just for the calculation of BMI z-scores. Z-scores for BMI were batch calculated using the "zanthro" package in STATA, version 13.1 (StataCorp LP, College Station, Texas), which standardizes BMI measures in children using the LMS method and reference data from the 2000 CDC Growth Reference (Vidmar et al., 2004). Only those children that were within 3 months of 2 years and above could be analyzed in the models that included BMI as a covariate (n=72).

A variable was created to combine the survey questions regarding if the mother breastfed, and if so, the duration of breastfeeding. If the parent reported no breastfeeding,

the participant was coded as a 0; if the parent reported breastfeeding, but breastfed for less than 6 months, then the participant was coded as a 1; else they were coded as a 2. A variable was created, combining the survey data on race and ethnicity. A new variable was categorically coded as 0 if non-Hispanic black; 1 if non-Hispanic white; and 2 if other (including Hispanic, Asian, Pacific Islander, or multiracial). If a question was left missing or not reported for family history of thyroid disease (n=3) or smoking status (n=11), we assumed participants did not have these attributes and were coded as such. For study participants that did not have time of blood draw listed, that value was assigned as an hour after signing the consent form (n=1).

We created a variable for socioeconomic status (SES) by combining parent occupation reported in the survey and insurance status noted by the study nurse. Socioeconomic status was defined as three levels—low, medium, and high. To estimate annual salary, we used occupation title listed and found respective salaries from the Bureau of Labors Statistics' (BLS) Occupational Employment Statistics (OES), specifically the Occupational Employment and Wage Estimates for May 2012 for Georgia (Bureau of Labor Statistics, 2013). Where the occupation was specific to a company and title (n=3), the salary was taken from the database of salaries listed on the Glassdoor website (Glassdoor, 2014).

Once salary was obtained, a cutoff of $29,438 per year for a family of four, or 125% above the federal poverty line, was implemented, as this is the Georgia Department of Community Health's Office of Health Planning's definition of an indigent patient in Georgia for 2013 (Georgia Department of Community Health, 2013). Those above the cutoff were coded as above the poverty line, whereas those at or below were coded as

below the poverty line. We then verified this classification with insurance status listed for each child, as those with public insurance, or Medicaid, were placed in the low category, and those with private insurance were placed in the higher category. Where occupation was not missing, most insurance and salary cutoffs matched. However, in the cases that they did not match (n=34, often due to missing occupation on the survey), we applied the following rules. If occupation title was missing, meaning salary was missing, but insurance status was Medicaid, we coded these as the low category based on the rationale that these individuals were likely unemployed. If occupation title was missing, meaning salary was missing, but insurance status was selected as private, these individuals were assigned to the middle SES category. If occupation corresponded to an income above $100,000 per year, regardless of insurance, we coded these individuals in the high category. If individuals were above the poverty line cutoff for Georgia, but had private insurance selected, we coded these individuals as the middle SES category. There were no study participants that had low income, but private insurance.

For sensitivity analyses, the continuous covariates were categorized. The time of blood draw was documented and categorized into one hour and fifty minute intervals—early morning (7:30 am-9:20 am), late morning (9:20 am-11:10 am), early afternoon (11:10 am-1:00 pm), and late afternoon (1:00 pm-3:00 pm). Age in months was categorized into approximately equal groups: 1.3-2 years, 2-4 years, and 4+ years.

The associations between thyroid stimulating hormone and either individual PBDE congeners or total PBDE serum concentration were analyzed using linear regression. Bivariate associations for every categorized covariate were assessed using the median value of each exposure variable and the median value of TSH. Collinearity was assessed

using the VIF function in SAS; no evidence of collinearity was observed. It was not feasible to test for effect measure modification since power to detect interaction was low due to the small sample size. To perform confounding assessment, all covariates were added to the model, and taken out one-by-one to determine if any covariate meaningfully changed the estimate more than 10% when removed. Several variables changed the parameter estimate more than 10%. However, since the estimates were so small, a 10% change did not meaningfully change conclusions. The only variable that did meaningfully change the estimate was the time that blood samples were collected. Since there is no harm to the precision of the estimate by keeping in other covariates, all were kept, with the exception of BMI z-score and type of surgery, which were dropped for reasons of retaining a larger sample size. The full model included the following covariates: gender, age in months, time of blood draw, family history of thyroid disease, race/ethnicity, breastfeeding history and duration, socioeconomic status, and smoking status.

Sensitivity analyses were also conducted. The first analysis included the full model plus BMI, which limited our analysis to 72 children. We also investigated the effect of categorizing the two continuous covariates—age and the time of blood draw. For the final sensitivity analysis, we excluded participants with exposure values below the limit of detection (LOD) and kept our analysis to only those study participants with exposure values above the LOD. All analysis was conducted using SAS 9.3, Cary, NC.

Results

Selected characteristics for study participants are shown in Table 1. Among the 80 children included in the study, who underwent anesthesiology for myringotomies, adenoidectomies, brochoscopies, mastoidectomies, or tonsillectomies at Children's

Healthcare of Atlanta, the average age was 3 years and 4 months, with almost equal proportions of female and male children (females representing 42.5%). Roughly 40% of participants were non-Hispanic Black/African-American, another 40% were non-Hispanic White/Caucasian, while the final 20% identified as other. Most children were breastfed, representing almost 60% of the study population. Of those breastfed, there was an even split between those that were breastfed for less than 6 months, and those breastfed for 6 months or more. Most children reported having Medicaid as their health insurance (63%), and of those who reported occupations, 26.3% had family incomes below $29,438 (125% of the Federal Poverty Line for Georgia). Our socioeconomic status (SES) measure, combining income and insurance status, included 46% in the lowest SES category, 19% in the middle SES category, and 36% in the highest SES category. Roughly 20% of parents of study children reported that at least one adult living in the household smoked (note: 13% failed to respond and were coded as non-smokers), with even more reporting a family history of thyroid disease (32.9%).

There was a substantial range of ∑PBDE exposure (range: 5.7 to 1,079.6 ng/g lipid), with a median of 63.1 ng/g lipid [Table 1]. Not all congeners of PBDE had values above the limit of detection (LOD). The percentages below the LOD for each congener were as follows: 2.5% of BDE-47 values, 1.25% of BDE-99 values, 17.5% of BDE-100, and 33.75% of BDE-153 values. Almost all of the study participants fell within the normal range of TSH (0 to 5 μIU/mL), with the exception of 10 children being in the mildly elevated range (5 to 10 μIU/mL).

As noted in Table 2, we explored how median exposure to PBDEs and TSH level differ by the demographic and health data collected. In terms of exposure, the youngest

children (age: 1.3 to 2 years) tended to have some of the highest levels of PBDE exposure, with the exception of BDE-100. Female children tended to have higher exposures to PBDEs, where the female median level of exposure is, for some congeners, double the male exposure. PBDE exposure levels also appeared to be different across race/ethnicity, as non-Hispanic black children tended to have some of the highest levels of exposure. Of those children that were breastfed, those that breastfed for 6 months or more had higher median levels of PBDEs across all congeners when compared to those that breastfed for less than 6 months.

In terms of TSH, older children and those with parents who smoked tended to have lower levels of TSH, whereas females, children in the lower BMI categories, and those that were breastfed less than 6 months appeared to have higher TSH levels [Table 2]. Children with and without a family history of thyroid disease had roughly the same TSH level. The time that blood samples were collected resulted in different median TSH levels, with the highest levels occurring in the early morning, which is consistent with previous diurnal studies of TSH (Russell et al., 2008). TSH levels gradually declined until late afternoon when they began to rise again.

Linear regression results detailed the association between PBDE levels and TSH including control for all covariates except BMI and surgery type [Table 3]. The reason for leaving out BMI and surgery type was to keep from losing participants in the analysis since the sample size was already small (n=80). While BMI and surgery did occasionally change the parameter estimates more than 10% when removed, neither meaningfully changed conclusions. If kept, we would have lost nine participants, for a total sample size

of 71 children; not every participant had a z-score for BMI (n=8) and one participant had a unique surgery of the five possible surgeries.

For the continuous exposure model, we observed that with increasing PBDE exposure, there was an increase in log-transformed TSH: BDE-47 (β=0.01), BDE-99 (β=0.08), BDE-100 (β=0.14), BDE-153 (β=0.12), ∑PBDE (β=0.12), after controlling for gender, age in months, the time the sample was collected, family history of thyroid disease, race/ethnicity, breastfeeding time, socioeconomic status, and current smoking status. However, only the association between BDE-100 and log-transformed TSH was statistically significant in this pilot study ($p<0.05$). When considering PBDEs as a categorical variable, broken into quartiles, there was a slight general trend of increasing TSH with higher categories of PBDEs. However, with the exception of BDE-100 and the sum PBDE measure, results were not significant ($p>0.05$). The direction of the association of log-transformed TSH across quartiles of PBDE was not monotonic as expected; instead a u-shaped direction was detected across quartiles of all congeners. For both types of models (continuous PBDE or PBDE in quartiles), the adjusted models had substantially stronger positive effects than unadjusted models.

The only covariate that was statistically significantly associated with the outcome across all congeners, whether the exposure was kept continuous or categorical, was time of blood draw. Race/ethnicity was also associated with the outcome for BDE-100, whether the exposure was kept continuous or categorical ($p<0.05$).

Sensitivity Analysis

In our confounding assessment, BMI was not found to be a strong confounder, but in Table 4 we also present results adjusted for BMI due to its association with TSH in the

literature. When limiting analysis to the 72 observations with available BMI z-scores (age 21 months and older) and including BMI as a covariate in the model, the relationship between log-transformed TSH and the log-transformed congeners only slightly increased: BDE-47 (β=0.02), BDE-99 (β=0.16), BDE-100 (β=0.17), BDE-153 (β=0.15), $\sum$PBDE (β=0.19) [Table 4]. Again, none of these, with the exception of the association between TSH and BDE-100 ($p=0.01$), were statistically significant in this pilot study ($p>0.05$). When considering PBDEs as a categorical variable, broken into quartiles, we saw the same general trend of increasing TSH with higher categories of PBDEs, but results were generally not significant ($p>0.05$). However, this was not the case with all congeners, especially BDE-100, the highest quartile of exposure for BDE-99, and the sum measure. The same u-shaped direction across quartiles was less apparent for all congeners as it was when BMI was excluded from the model. Significant monotonic trends were found across quartiles of BDE-99, BDE-100, and $\sum$PBDE ($p<0.1$). As in the primary analysis, for both types of models (continuous PBDE or PBDE in quartiles), adjusted models had substantially stronger positive effects than unadjusted models.

We also categorized age into approximately equal groups, consisting of three levels (1.3-2.5, 4+), and included it as a covariate in a model assessing the association between PBDEs and TSH [Table 5]. When age is categorized, the log-transformed estimates for the various congeners did not meaningfully change from when age is left as a continuous variable. The categorization of age also did not substantially change the association between TSH and PBDEs categorized in quartiles, from when age was left as a continuous covariate.

For our third sensitivity analysis, we categorized the time of blood draw as a categorical variable with one hour and fifty-minute intervals (early morning, late morning, early afternoon, late afternoon) and analyzed the association between PBDEs and TSH [Table 6]. Again, the categorization of blood draw time did not meaningfully impact the association between PBDEs and TSH, whether PBDEs were left as continuous or categorized into quartiles.

Finally, we analyzed only those observations with congener values above the limit of detection (LOD), excluding observations below LOD for each congener [results presented in Table 7, the sum not included]. The relationship between log-transformed TSH and PBDEs only slightly increased in most congeners. Generally, we did not observe meaningful differences in associations between PBDEs and TSH that would change our conclusions. However, this was not the case when considering BDE-100 as a continuous variable or the trend test of categorized BDE-153. After excluding those below LOD for BDE-100, the adjusted estimate was no longer statistically significant in this model ($p>0.05$). For the trend test, we found a linear trend across quartiles of BDE-153 when excluding those below LOD ($p=0.02$).

Discussion

Results of our study investigating serum PBDE levels in relation to TSH levels in young children were generally null. There was some suggestion of increased TSH levels with higher exposure to PBDE. This was significant for BDE-100, but not for any of the other congeners or the sum. However, this was a pilot study with relatively low sample size, so the focus on statistical significance is not as essential. We did not observe

monotonic increases across quartiles of PBDE exposure, except in the case of BDE-100. However, there was some indication that the highest quartile of exposure had higher TSH levels than the lowest quartile of exposure.

Our finding of increased TSH levels with higher levels of PBDE exposure has potential biological significance, especially for developing children. Thyroid hormones are critical to the process of early brain development in children (Dingemans et al., 2011; Horn et al. 2010; Howdeshell et al. 2002). If PBDEs disrupt thyroid function this may pose a serious threat to thyroid development as thyroid production can begin as early as ten weeks of gestation and continue throughout the first few years of life (Dingemans et al., 2011). Even more critical is the particular neurodevelopment window that may be affected by thyroid disruption, which lasts from the last trimester of pregnancy to the first two years of life and is especially susceptible to neurotoxic insults (Dingemans et al., 2011).

The mechanism of thyroid function that PBDEs are suspected to be disrupting, however, is poorly understood. Although a reduction in T_4 has been well depicted in animal studies, the feedback response of TSH is still not completely clear (Fowles et al., 1994; Hallgren et al., 2001; Zhou et al., 2001, 2002; Ellis-Hutchings et al., 2006; Darnerud et al., 2007; Kuriyama et al., 2007). The biological mechanism in humans is even less clear. However, there have been several epidemiologic studies in humans to address this issue.

Several epidemiologic studies in adults found different results regarding PBDE exposure and thyroid function as measured by TSH. Studies among e-waste workers in

China who are occupationally exposed to PBDEs and adult male sport fisherman in New York found statistically significant inverse associations between PBDE exposure and TSH levels (Wang et al., 2010; Turyk et al., 2008; Yuan et al., 2008), while other studies found no association between PBDEs and TSH (Bloom et al., 2008; Julander et al., 2005). These different results could be due to different sample sizes or study areas. The study area location is crucial in terms of differences in exposure level as the United States has human body burdens that are about ten times higher than the rest of the industrialized world (Sjodin et al., 2008; Birnbaum and Cohen, 2006). This might explain the difference in associations found between prior studies and our study. Another reason for observing a difference in the direction of association between PBDEs and TSH in prior studies of adults and our study could possibly be the result of PBDEs having a different mechanistic effect in children.

The biological mechanism is not clear in humans, but is even more poorly understood in children. Children have rarely been studied, thus, it is not clear if the body is responding differently in children, or if the body is responding at all. There is only one other study of interest that investigates the association between PBDE exposure and thyroid function in children. In a study conducted in Spain, Gascon et al. examined pre- and post-natal exposure to PBDEs and TSH at age 4 in a prospective birth cohort study (n=470 mother-child pairs). The Gascon study results were similar to our findings of increased TSH with higher levels of PBDE exposure (β=0.05, 95% CI: -0.01 to 0.2) (Gascon et al., 2011). This is especially interesting given the Spanish cohort had median levels of exposure for BDE-47 that were around 300 times lower than our median levels of exposure, a maximum BDE-47 exposure that was almost 5 times lower than in our

study (age 4 serum blood BDE-47: median=0.12, max=130.2), and a much smaller sample size of exposed children (n=39). However, similar results to our study that also disagree with those found in the adult literature may support the explanation that PBDEs have a different mechanism of action in children with developing thyroid systems.

There are some limitations to the current study. One limitation was that we did not assess other coexisting environmental contaminants, which may affect thyroid hormone levels. However, since this was a small pilot study, it was beyond the scope of this type of study to investigate other potential contaminants. Also, this study did not assess potential effect modification by other covariates such as sex or age as the study had limited sample size and power to detect interactions. Another limitation was the high number of samples that were below the limit of detection (LOD) for some congeners. In the main analysis, PBDE serum levels were imputed as the LOD/$\sqrt{2}$ and then adjusted for per gram lipid of body fat. Because of this, we may have misclassification of the exposure since the values observed are not the true exposure levels for participants. However, we did run a sensitivity analysis, limiting the analysis to those children who had above detection levels and found that these results did not differ meaningfully from our main analysis.

There are also various strengths to this study. The main strength of the study is that, to our knowledge, it is the largest study to date to assess thyroid stimulating hormone and PBDE exposure in young children. The study targets a population with uniquely high exposure levels due to the hand-to-mouth behavior of young children. A reason for why there have been so few studies that investigated PBDE exposure in children is that enrolling and taking blood samples from this population is difficult. However, an

advantage of our study was the consent rate of 93%, which is attributable to the design of enrolling pediatric patients at CHOA who were already undergoing anesthesia for common childhood surgeries that were thought to be unrelated to both exposure and outcome. This study also has higher exposure levels than other studies in children since residents of the United States are reported to have much higher levels than the rest of the world. Moreover, although this is a cross-sectional study, exposure levels are relatively representative of long-term exposure as PBDEs are considered to be somewhat persistent with an average half-life of around 3 to 6 years, depending on the congener (Geyer et al., 2004). Another strength of the study is the socioeconomically diverse cohort as roughly 60% of participants have Medicaid as their primary insurance and roughly 45% are in the lowest SES category, with 36% in the highest SES category.

Conclusion

Our findings in this pilot cross-sectional study did not show strong evidence of an association between PBDEs and TSH, but there was some suggestion that PBDEs, at the highest levels, are associated with higher TSH levels in children. These preliminary results, although not statistically significant, are consistent with the one other study of PBDEs and TSH in children, and therefore support further investigation in a larger study.

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Appendix

Table 1. BEAT Study demographic and health characteristics for children ages 15 months to 5 years (n=80) undergoing surgery at an ENT practice at Children's Healthcare of Atlanta, January 2011 to December 2011.

Characteristics	Mean (SD) or N (%)	Median (p25, p75)	Min	Max
Demographic Data				
Age (years)	3.4 (1.3)	3.2 (2.4, 4.4)	1.3	6.0
Sex (%)				
Female	34 (42.5%)			
Male	46 (57.5%)			
Socioeconomic Status (%)				
Low	37 (46.3%)			
Middle	14 (17.5%)			
High	29 (36.3%)			
Ethnicity/Race (%)				
African-American	33 (41.3%)			
White	32 (40.0%)			
Other	15 (18.8%)			
Insurance Status (%)				
Private Insurance	30 (37.5%)			
Medicaid	50 (62.5%)			
Income (%)				
Georgia FPL[1] or less	21 (26.3%)			
Greater than Georgia FPL[1]	40 (50.0%)			
Missing	19 (23.8%)			
Health Data				
BMI[2] (kg/m^2)	16.8 (2.4)	16.9 (15.1, 17.9)	13.1	27.9
Breastfed (%)[3]				
None	33 (41.3%)			
Less than 6 months	23 (28.8%)			
6 months or more	24 (30.0%)			
Family history of thyroid disease (%)[4]	25 (31.3%)			
Parent current smoke (%)[5]	14 (17.5%)			
PBDE levels				
∑PBDE[6] level (ng/g lipid)	92.6 (125.8)	63.1 (41.1, 107.8)	5.7	1079.6
BDE-47[7] level (ng/g lipid)	54.6 (76.7)	38.0 (23.0, 60.5)	0.1	642.1
BDE-99 level (ng/g lipid)	15.2 (23.0)	9.9 (6.0, 17.2)	0.3	193.6
BDE-100 level (ng/g lipid)	9.5 (15.1)	6.3 (2.2, 11.5)	0.2	125.8
BDE-153 level (ng/g lipid)	10.5 (12.7)	6.1 (3.4, 12.3)	1.6	78.8
Thyroid Function [8]				
TSH[9] level (µIU/mL)	2.8 (1.8)	2.4 (1.6, 3.6)	0.6	9.6

[1] Federal Poverty Line (FPL) for Georgia: $29, 438
[2] Body mass index (BMI)
[3] Child was breastfed
[4] Family history of hyperthyroidism, hypothyroidism, thyroid disease
[5] Smoking status indicates if any adult living with child currently smokes
[6] Summation of PBDEs across all measured congeners
[7] Bromindated diphenyl ether congener; the same for all congeners below
[8] Geometric mean calculated for mean value
[9] TSH normal range=0.5 to 5.0

Table 2. BEAT Study demographic and health characteristics for children by median PBDE and TSH[9] level (n=80).

Characteristics	N	Median BDE-47 (ng/g lipid)	Median BDE-99 (ng/g lipid)	Median BDE-100 (ng/g lipid)	Median BDE-153 (ng/g lipid)	Median ∑PBDE	Median TSH (µIU/mL)
Demographic Data							
Age (years)							
1.3-2.4	25	45.6	13.1	6.0	6.9	70.0	2.6
2.5-4	30	33.2	9.0	5.2	5.7	59.8	2.8
4+	25	36.3	8.4	6.9	5.1	62.9	2.1
Sex							
Male	46	29.1	8.0	5.0	4.9	57.6	2.3
Female	34	48.4	12.6	8.0	8.4	82.9	2.7
Socioeconomic Status[1]							
Low	37	33.9	10.3	6.8	8.6	64.3	2.3
Middle	14	42.8	10.2	8.1	5.2	72.5	2.5
High	29	34.0	7.5	5.2	4.6	59.4	2.5
Ethnicity/Race							
Non-Hispanic Black	33	41.5	12.4	9.3	9.7	79.7	2.3
Non-Hispanic White	32	40.1	8.2	5.4	4.8	61.2	2.4
Other	15	32.6	8.8	2.8	4.0	53.2	2.6
Insurance							
Private	30	40.3	8.3	5.4	4.8	62.2	2.5
Medicaid	50	35.1	10.1	6.9	8.2	63.9	2.3
Income							
≤ Georgia FPL[2]	21	29.1	8.8	6.0	7.7	62.5	2.4
> Georgia FPL[2]	40	39.9	9.5	5.8	4.6	60.5	2.4
Health/Study Data							
BMI z-score[3,4]							
≤ -2	3	7.7	3.7	3.4	15.5	58.0	2.9
> -2 to -1	10	26.6	6.9	5.2	4.8	48.8	3.2
> -1 to 0	11	64.9	20.3	14.5	15.1	124.5	2.2
>0 to 1	20	27.0	7.3	4.3	4.6	47.4	2.3
>1 to 2	19	41.5	9.8	7.8	4.6	62.9	1.9
≥ 2	9	36.3	11.2	4.8	4.7	63.4	2.5
Breastfed[5]							
None	33	47.55	11.2	6.8	5.7	70.0	2.2
< 6 months	23	29.09	8.4	5.7	5.7	58.6	3.4
≥ 6 months	24	43.85	9.9	7.4	7.2	78.3	2.2
Family History[6]							
No	55	40.57	11.0	6.5	6.4	65.2	2.4
Yes	25	27.8	7.0	4.8	5.3	57.5	2.5
Smoking Status[7]							
No	66	38.19	10.4	6.3	5.3	63.1	2.5
Yes	14	34.2	7.1	6.8	10.3	69.3	2.1
Time Collected[8]							
Early morning	30	26.99	6.4	5.1	4.4	51.9	3.3
Late morning	17	48.68	12.4	4.8	9.4	79.7	2.1
Early afternoon	22	40.33	10.5	7.9	7.5	65.0	2.1
Late afternoon	11	41.54	9.2	10.3	13.8	70.0	2.4
Surgery Type[8]							
Myringotomy	52	33.24	8.6	5.2	4.8	59.2	2.2
Tonsillectomy	36	52.8	13.3	9.6	9.6	89.4	2.4
Adenoidectomy	44	45.67	10.8	8.1	8.5	79.7	2.4

[1] Socioeconomic status calculated by combining insurance status and reported income for family
[2] Federal Poverty Line (FPL) for Georgia: $29,438
[3] Body mass index (BMI)
[4] Z-score calculated for all children within 3 months of 2 years and above
[4] Child was breastfed
[5] Family history of hyperthyroidism, hypothyroidism, thyroid disease
[6] Smoking status indicates if any adult living with children currently smokes
[7] Time of blood sample collection
[8] Type of surgery conducted for each study participant
[9] TSH normal range=0.5 to 5.0

Table 3. Association between BDEs & ∑PBDE exposure (continuous and categorical) with log-transformed TSH in Emory University's pilot, cross-sectional BEAT Study, 2011 (n=80)[1]

PBDE	BDE-47		BDE-99		BDE-100		BDE-153		∑PBDE	
	Crude	Adj.	Crude	Adj.	Crude	Adj.	Crude	Adj.	Crude	Adj.
Per 1 ln ng/g lipid	-0.03	0.01	0.01	0.08	0.06	**0.14	-0.03	0.12	0.00	0.12
Quartiles										
Q1	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
Q2	0.03	0.14	-0.01	0.16	***0.54	***0.70	0.00	0.15	0.20	**0.39
Q3	-0.12	0.08	-0.17	0.00	0.23	***0.56	-0.03	0.11	0.01	0.24
Q4	0.05	0.25	0.10	0.26	0.29	***0.67	-0.03	0.32	0.16	**0.46
p for trend[2]	0.97	0.26	0.80	0.27	0.35	0.01	0.85	0.16	0.62	0.08

* p-value < 0.1; ** p-value < 0.05; *** p-value < 0.01

[1] Adjusted for gender, age in months, time sample collected, family history, race/ethnicity, breastfeeding time, socioeconomic status, smoking status

[2] P-value for including PBDE quartiles as ordinal value in model

Table 4. Association between BDEs & ∑PBDE exposure (continuous and categorical) with log-transformed TSH with control for Body Mass Index (BMI) (n=72).[1,2]

PBDE	BDE-47		BDE-99		BDE-100		BDE-153		∑PBDE	
	Crude	Adj.	Crude	Adj.	Crude	Adj.	Crude	Adj.	Crude	Adj.
Per 1 ln ng/g lipid	-0.02	0.02	0.06	*0.16	0.08	***0.17	-0.03	0.15	0.04	*0.19
Quartiles										
Q1	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
Q2	-0.03	0.10	0.03	0.26	***0.52	***0.63	0.00	0.15	0.15	0.33
Q3	-0.07	0.17	-0.06	0.16	0.29	***0.65	-0.02	0.17	0.06	0.31
Q4	0.07	0.29	0.19	*0.39	0.30	***0.74	-0.04	0.31	0.18	**0.49
p for trend[3]	0.82	0.15	0.47	0.09	0.23	<0.01	0.81	0.16	0.47	0.03

* p-value < 0.1; ** p-value < 0.05; *** p-value < 0.01

[1] Includes Body Mass Index (BMI) z-score as a covariate, excluding 8 participants

[2] Adjusted for bmi z-score, gender, age in months, time sample collected, family history, race/ethnicity, breastfeeding time, socioeconomic status, smoking status

[3] P-value for including PBDE quartiles as ordinal value in model

Table 5. Association between BDEs & ∑PBDE exposure (continuous and categorical) with log-transformed TSH with control for age as a categorical covariate (n=80)[1,2]

PBDE	BDE-47	BDE-99	BDE-100	BDE-153	∑PBDE
Per 1 ln ng/g lipid	0.01	0.08	***0.14	0.11	0.12
Quartiles					
Q1	ref	ref	ref	ref	ref
Q2	0.16	0.16	***0.74	0.13	**0.41
Q3	0.09	0.01	***0.59	0.10	0.25
Q4	0.27	0.26	***0.69	0.31	**0.47
p for trend[3]	0.28	0.29	0.01	0.17	0.09

* p-value < 0.1; ** p-value < 0.05; *** p-value < 0.01
[1] Includes age as a categorical covariate of 3 groups
[2] Adjusted for gender, age categorized into 3 groups, time sample collected, family history, race/ethnicity, breastfeeding time, socioeconomic status, smoking status
[3] P-value for including PBDE quartiles as ordinal value in model

Table 6. Association between BDEs & ∑PBDE exposure (continuous and categorical) with log-transformed TSH with control for time of blood draw as a categorical covariate (n=80)[1,2]

PBDE	BDE-47	BDE-99	BDE-100	BDE-153	∑PBDE
Per 1 ln ng/g lipid	0.02	0.09	***0.14	0.12	0.12
Quartiles					
Q1	ref	ref	ref	ref	ref
Q2	0.15	0.15	***0.73	0.11	*0.37
Q3	0.04	0.02	***0.55	0.12	0.19
Q4	0.28	0.26	***0.69	0.31	**0.47
p for trend[3]	0.34	0.34	0.01	0.21	0.12

* p-value < 0.1; ** p-value < 0.05; *** p-value < 0.01
[1] Includes time blood samples were collected as a categorical covariate of 4 groups
[2] Adjusted for gender, age in months, time blood sample collected as a categorical variable of 4 groups, family history, race/ethnicity, breastfeeding time, socioeconomic status, smoking status
[3] P-value for including PBDE quartiles as ordinal value in model

Table 7. Association between BDEs & ∑PBDE exposure (continuous and categorical) with log-transformed TSH among participants with PBDE congeners above the limit of detection (LOD)[1,2]

PBDE	BDE-47 (n=78)		BDE-99 (n=79)		BDE-100 (n=66)		BDE-153 (n=53)	
	Crude	**Adj.**	**Crude**	**Adj.**	**Crude**	**Adj.**	**Crude**	**Adj.**
Per 1 ln ng/g lipid	0.02	0.1	0.04	0.1	-0.07	0.08	-0.01	0.22
Quartiles								
Q1	ref	ref	ref	ref	ref	ref	ref	ref
Q2	0.08	0.3	0.01	0.16	0.41	***0.72	0.42	**1.02
Q3	-0.07	0.12	-0.16	0.01	0.1	*0.54	0.36	**1.03
Q4	0.1	0.19	0.12	0.26	0.15	**0.68	0.36	***1.30
p for trend[3]	0.79	0.2	0.72	0.27	0.57	0.32	0.83	0.02

* p-value < 0.1; ** p-value < 0.05; *** p-value < 0.01

[1] Only participants with exposure values above the limit of detection (LOD) are used for analysis of each congener, all other participants are censored

[2] Adjusted for gender, age in months, time blood sample collected, family history, race/ethnicity, breastfeeding time, socioeconomic status, smoking status

[3] P-value for including PBDE quartiles as ordinal value in model