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Associations Between Urinary Diphenyl Phosphate and Thyroid Function

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Abstract

Triphenyl phosphate (TPHP) is a commonly used organophosphate flame retardant and plasticizer with widespread human exposure. Data on health effects of TPHP are limited. Recent toxicological studies suggest TPHP may alter thyroid function. We used repeated measures to assess the temporal variability in urinary concentrations of the TPHP metabolite, diphenyl phosphate (DPHP), and to examine relationships between DPHP concentrations and thyroid hormones. We sampled 51 adults at months 1, 6, and 12 from 2010-2011. Urine samples were analyzed for DPHP. Serum samples were analyzed for free and total thyroxine (fT_4, TT_4), total triiodothyronine (TT_3), and thyroid stimulating hormone (TSH). We assessed variability in DPHP using intraclass correlation coefficients (ICCs) and kappa statistics. We used linear mixed-effects models to examine associations between DPHP and thyroid hormones. DPHP was detected in 95% of urine samples. Mean DPHP concentrations were 43% higher in women than men. DPHP showed high within-subject variability (ICC range, 0.13-0.39; Kappa range, 0.16-0.39). High versus low (≥2.65 vs. <2.65 ng/mL) DPHP in all participants was associated with a 0.43 μg/dL (95% confidence interval: 0.15, 0.72) increase in mean TT_4 levels. In sex-stratified analyses, high versus low DPHP was associated with a 0.91 μg/dL (95% CI: 0.47, 1.36) increase in mean TT_4 in women. The association was attenuated in men (βeta= 0.19; 95% CI: -0.15, 0.52). We found no significant associations between DPHP and fT_4, TT_3, or TSH. We found evidence that TPHP exposure may be associated with increased TT_4 levels, especially in women.

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Keywords

Organophosphate flame retardants; triphenyl phosphate; diphenyl phosphate; thyroid hormones

1. Introduction

Organophosphate flame retardants are widely used in commercial and consumer products (van der Veen and de Boer 2012). Their use has increased over the past decade, in part due to the phase out of certain polybrominated diphenyl ethers (PBDEs) such as PentaBDE (EPA 2008). Triphenyl phosphate (TPHP, CAS no. 115-86-6) is an organophosphate ester used both as a flame retardant and plasticizer, and has been applied to polyurethane foam, resins, polyvinylchloride (PVC), hydraulic fluids, lacquers, and nail polish (Mendelsohn et al. 2016; van der Veen and de Boer 2012). Like PBDEs, TPHP is used as a chemical additive, implying that it is not chemically bound to the source material and can escape from products and enter the surrounding environment. TPHP has been detected frequently in the indoor environment (Dodson et al. 2014; Hoffman et al. 2015b; Meeker et al. 2013b). Humans may be exposed to TPHP through inhalation, ingestion of indoor dust, and dermal absorption through contact with dust, source materials, or direct partitioning from vapor (Pillai et al. 2014). The half-life of TPHP in humans is unknown, but is thought to be on the order of hours to days (Hou et al. 2016). However, because of its widespread use and ubiquity in the indoor environment, exposures may be relatively constant over time. Constant exposure could theoretically create “pseudo-persistence” in the human body, meaning levels of urinary metabolites of TPHP would be fairly stable, despite TPHP's short half-life.

Methods have been developed to measure diphenyl phosphate (DPHP), a urinary metabolite of TPHP. As a biomarker of TPHP exposure, urinary DPHP concentrations have been characterized in a growing number of studies, with most U.S. studies reporting ubiquitous detection of DPHP (Butt et al. 2014, 2016; Dodson et al. 2014; Hoffman et al. 2014, 2015b; Meeker et al. 2013b). While the majority o fU.S. studies have been conducted in adults, three recent studies measured levels of urinary DPHP in children (Butt et al. 2014, 2016; Hoffman et al. 2015a). Four studies have assessed intra-individual variability of urinary DPHP via repeated measures (Cequier et al. 2015; Hoffman et al. 2014, 2015b; Meeker et al. 2013b). However, most of these studies had small sample sizes with short sampling periods that may not capture potential long term or seasonal variability.

Despite growing evidence of widespread human exposure, relatively little is known about the potential human health effects of TPHP. Preliminary toxicology studies suggest that TPHP may disrupt thyroid function (Kim et al. 2015; Kojima et al. 2013; Liu C et al. 2013; Liu X et al. 2016). Healthy thyroid function is critical for fetal and child growth and development and for maintaining important bodily systems in adults such as metabolism, mental health and cognition, and reproduction (Taylor et al. 2013). There is increasing evidence that even subclinical changes in adult thyroid function may be associated with adverse effects (Taylor et al. 2013). To date only two studies have examined associations between TPHP exposure and thyroid function in humans (Meeker and Stapleton 2010; Meeker et al. 2013a). These cross-sectional analyses were both conducted in a small cohort

of men from subfertile couples residing in the Boston, Massachusetts (MA) USA area. Additional studies are needed to further explore the potential relationship between TPHP exposure and thyroid function in larger and mixed sex study populations.

TPHP may share exposure sources and routes with PBDEs (Stapleton et al. 2012) and were positively correlated in household dust samples in a recent North Carolina study (Hoffman et al. 2015b). There is a growing literature linking PBDE exposure to thyroid disruption (Czerska et al. 2013; Linares et al. 2015), including previous work in the current study cohort (Makey et al. 2015). Therefore, it is important to consider potential confounding and/or modification by PBDEs when assessing associations between TPHP exposure and thyroid function.

The current study aims to characterize urinary DPHP concentrations in a population of U.S. adults, assess intra-individual variability of repeated DPHP measures, and investigate the association between adult DPHP concentrations and thyroid function. We measured urinary DPHP and serum thyroid hormones in repeated samples from a group of 51 male and female office workers over a one-year period as part of a study investigating exposure patterns and health effects of flame retardant chemicals in the Boston, MA area. We hypothesized that there would be high intra-individual variability in urinary DPHP concentrations over the study period and that DPHP concentrations would be associated with altered serum thyroid hormone levels. We explored whether factors including PBDEs, urinary iodine, age, or sex might modify associations between DPHP and thyroid hormones. Additionally, we characterized urinary DPHP concentrations at a single time point in a subset of the study participants' children.

2. Methods

2.1. Study Participants

Study subjects were part of the Flame Retardant Exposure Study (FlaRE), which has been described previously (Makey et al. 2014). Briefly, a convenience sample of 26 male and 26 female office workers and a subset of their children (n=14), were recruited from the Boston, MA metropolitan area. The fourteen children included some siblings, corresponding to nine FlaRE adults. Eligible adult participants were over the age of 18, nonsmokers, self-described as healthy, and planning to remain in the Boston area for the duration of the study period. Participants were excluded if they had a current or prior diagnosis of thyroid disease, male reproductive disease, or were pregnant. Serum and urine samples were collected from adults during three sampling rounds every six months from January 2010 to May 2011, representing Winter 2010, Summer 2010, and Winter 2011. Questionnaires were administered at each sample round to collect demographic, health, and lifestyle information. During the final sampling round, children provided urine samples, and questionnaires were administered to parents to collect children's demographic and behavioral information. Of the 52 adult participants, 41 completed all three sampling rounds, nine completed two rounds, and two completed one round. Samples were excluded from analysis for the following reasons: pregnancy (1 sample from 1 person), thyroid altering medication use (3 samples from 1 person), and no corresponding blood sample (4 samples from 4 people). PBDE data were excluded for one sample due to suspected field contamination during collection. The

levels of hexaBDEs in the sample were ten times those in the individual's other two samples, while levels of the lower brominated congeners were similar across all three samples, suggesting contamination with residential dust containing the octaBDE commercial mixture. The final study population consisted of 51 adult participants (135 paired urine and serum samples) and 14 children (14 urine samples).

The Boston University Medical Center Institutional Review Board approved the study protocol. All participants provided informed consent and children provided informed assent to participate.

2.2. Urine Samples

A single 90 ml spot urine sample was collected from each participant during each of the three sampling rounds. Samples were measured for specific gravity (SG) using a refractometer and aliquoted before being stored at -20°C prior to analysis. Urinary DPHP was measured at Duke University using previously published methods (Cooper et al. 2011). Briefly, DPHP was extracted from urine using a mixed-mode anion exchange solid-phase extraction cartridge and then analyzed using atmospheric pressure chemical ionization liquid chromatography-tandem mass spectrometry (Cooper et al. 2011). Deuterated diphenyl phosphate (d10-DPHP) was used as the internal standard for quantification. The limit of detection (LOD) was calculated as three times the standard deviation of the laboratory blanks and ranged from 0.13-0.21 ng/mL across rounds. Sample DPHP values were blank corrected and values below the LOD (adults: n=7, 5%; children: n=0, 0%) were replaced with the LOD/√2. All samples were also analyzed for urinary iodine concentrations at the Boston University School of Medicine, Section of Endocrinology, Diabetes, and Nutrition using previously published methods (Valentin-Blasini et al. 2005). The coefficient of variation (CV) for iodine measurements was less than 5%. To account for urinary dilution, we SG corrected DPHP and iodine concentrations in all analyses except for modeling predictors of DPHP, where we included SG as an independent predictor (Boeniger et al. 1993). One sample with an extremely low SG (1.0001) was excluded from SG-corrected analyses due to the uncertainty surrounding the resulting extreme SG-corrected value.

2.3. Blood Samples

A single 30ml non-fasting blood sample was drawn from each study participant during each of the three sampling rounds. Serum samples were stored in amber glass vials at -80°C prior to analysis. Samples from all rounds were analyzed at the Centers for Disease Control and Prevention (CDC) for PBDEs using previously published methods (Sjödin et al. 2004), and for total serum lipids as previously described (Makey et al. 2014). PBDE concentrations were standardized to total serum lipid concentrations (ng PBDE/g lipid). Samples from all rounds were analyzed for thyroid function at the Boston University School of Medicine, Section of Endocrinology, Diabetes, and Nutrition. Thyroid peroxidase antibody (TPOAb) was measured using immunometric enzyme immunoassay (Orgentec Diagnostika). Thyroid stimulating hormone (TSH), free thyroxine (fT_4), total thyroxine (TT_4), and total triiodothyronine (TT_3) were measured using enzyme-linked immunosorbent assays (Immuno-Biological Laboratories, Inc). Assay reference ranges were: TT_4 (women: 4.8-11.6

μg/dL, men: 4.4-10.8 μg/dL), fT_4 (0.8-2.0 ng/dL), TT_3 (0.52-1.85 ng/mL), TSH (0.4-4.2 mIU/L). TPOAb was categorized as normal (≤50 IU/mL) or elevated (>50 IU/mL).

2.4. Statistical Analysis

Concentration distributions of DPHP, TSH, TT_3, and fT_4 were skewed and natural log (ln)-transformed when used as dependent variables. TT_4 was approximately normally distributed and was not transformed for analysis. We calculated Spearman correlation coefficients between DPHP concentrations and covariates to assess the potential for collinearity in our regression models. We calculated descriptive statistics by sampling round for DPHP, thyroid function tests, and other analytes.

We used two methods to assess the temporal variability of the repeated adult urinary DPHP measures over the study period. First, we calculated intraclass correlation coefficients (ICCs) using continuous DPHP concentrations at the three sampling rounds. ICCs represent the ratio of the between-subject variability over the sum of the between-and within-subject variability (total variability). ICCs range from zero to one, with ICCs close to one indicating good reliability and ICCs close to 0 indicating poor reliability (Rosner 2000). ICCs and their 95% confidence intervals (CIs) were calculated with the %ICC9 SAS Macro using mixed effects models in SAS PROC MIXED (Hertzmark and Spiegelman 2010). In addition to ICCs, we assessed the reliability of an individual's exposure category over the study period using kappa statistics. Kappa statistics quantify the amount of agreement between two categorical measurements and range from -1 to 1, with 1 indicating perfect agreement, 0 indicating chance agreement, and values <0 indicating less than chance agreement (Landis and Koch 1977). We calculated kappa statistics between the two winter rounds (rounds 1 and 3) restricted to subjects with urine samples from both rounds (n=35). DPHP concentrations were categorized in two ways: (1) tertiles of all samples (cut points: T1, 1.44 ng/mL; T2, 2.65 ng/mL), and (2) dichotomous categories split at the upper tertile of all samples (“High”, ≥ 2.65 ng/mL; “Low”, <2.65 ng/mL). Weighted kappas were calculated for the tertile categories.

Due to potential correlations between an individual's repeated measures we used linear mixed effects models with random intercepts to assess predictors of repeated DPHP concentrations in adults. Potential predictors included sex, age (years), body mass index (BMI) (kg/m^2), sample collection time, daily handwashing frequency, and nail biting habits. Natural log-transformed uncorrected DPHP levels were modeled as a continuous variable. All models were controlled for sample round and SG.

Due to the small number of children, we only examined univariate associations of each predictor with SG-corrected DPHP concentrations in children using linear regression. Potential predictors included demographic and behavioral characteristics. Due to lack of variability we did not examine sample collection time in children. We did not formally test differences between child and parent mean SG-corrected DPHP levels as we had insufficient power to use a hierarchical model clustered by family to account for potential correlations between the siblings in our sample.

We used linear mixed effects models with random intercepts to assess the potential associations between an interquartile range (IQR) increase in SG-corrected DPHP concentrations and each serum thyroid hormone in adults, using the data from all three sample rounds. All models were controlled for sample round. We further adjusted each model for potential confounding variables including sex, baseline age and BMI (kg/m^2), and time-varying variables at each round including sample collection time, SG-corrected iodine (μg/L), and serum PBDEs (ng/g lipid). Potential confounders were selected *a priori* based on directed acyclic graphs and were included in the final models using both statistical (changes to beta estimates) and theoretical determinations (Hernan et al. 2002). We ran all models adjusting for individual PBDE congeners that had previously been associated with thyroid hormones in this population (BDE-47, -99, -100, -153) (Makey et al. 2015). Results were similar regardless of which congener was used. We included BDE-47 in our final models, as it was most strongly associated with thyroid hormone levels.

We used both continuous and categorical measures of SG-corrected DPHP concentrations in our models of thyroid hormone levels. Because the DPHP distribution was highly skewed, we categorized SG-corrected DPHP concentrations for each individual at each of the three rounds as “High” or “Low” based on the upper tertile (High, ≥ 2.65 ng/mL) and the lower two tertiles, (Low, <2.65 ng/mL) of all urine samples (all three rounds combined); the lower two tertiles had similar concentration levels and were therefore combined. Categorization based on an upper quartile cut point (≥3.28 vs. <3.28 ng/mL) yielded similar results (data not shown). We assessed potential effect measure modification of the associations between DPHP and thyroid hormones by including cross-product terms of DPHP with continuous variables (BDE-47, SG-corrected iodine, age) and by stratifying by categorical variables [sex, age (cut point at median: ≥36 vs. <36 years)]. All data analysis was performed in SAS version 9.3 (SAS Institute Inc., Cary, NC).

It is unlikely that an individual's thyroid function and urinary DPHP concentration would have been related to an individual not providing or missing a sample. We therefore believe our missing data are missing completely at random (MCAR) and should not bias the results of our linear mixed effects models, as they are robust to MCAR data (Fitzmaurice and Laird 1997; Little and Rubin 2002).

3. Results

3.1. Study Population

Demographic characteristics of the study population are summarized in Table 1. The final study population for this analysis consisted of 51 adults and 14 children. Adults were evenly split between men and women, mostly white, highly educated, and ranged in age from 24 to 66 years, with a median of 36 years. Children were evenly split by sex and ranged in age from 3 to 10 years.

Round-specific summary statistics for adult DPHP, iodine, BDE-47 and thyroid hormone measurements are presented in Table 2. Adult geometric mean (geometric standard deviation, GSD) SG-corrected DPHP concentrations at rounds 1, 2, and 3 were: 2.99 (2.98), 1.80 (3.30), and 2.11 (2.48) ng/mL, respectively. Spearman correlation coefficients (p-

values) between SG-corrected DPHP and BDE-47 from rounds 1, 2, and 3 were 0.29 (0.05), 0.23 (0.12), and -0.17 (0.28), respectively. SG-corrected DPHP was not significantly correlated with SG-corrected iodine [round 1: 0.15 (0.30); round 2: 0.10 (0.53); round 3: 0.11 (0.49)]. Geometric mean (arithmetic mean for TT_4) thyroid hormone concentrations were relatively stable across sampling rounds (Table 2), and fell largely within the normal range. Three women had elevated (>50 IU/mL) TPOAb levels (9 samples), but their corresponding thyroid hormone measurements were within the normal range. The FlaRE population was iodine sufficient, with median urinary iodine concentrations >100 μg/L across all three rounds (WHO 2013). Four women (11 samples) reported taking hormonal oral contraceptives, which increases concentrations of thyroxine binding proteins in the blood, and were examined in sensitivity analyses.

In children, uncorrected and SG-corrected GM (GSD) DPHP concentrations were 2.11 (1.84) and 2.40 (2.04) ng/mL, respectively. Parents (n=9) had slightly lower GM (GSD) uncorrected DPHP levels than children (1.74 (2.78) ng/mL) but had comparable GM (GSD) SG-corrected DPHP levels (2.36 (2.30) ng/mL).

3.2. Temporal Variability of Urinary DPHP

ICCs (95% CI) for DPHP concentrations across all three rounds indicated high within-subject variability in DPHP measurement over time [uncorrected: 0.19 (0.06, 0.45), SG-corrected: 0.13 (0.02, 0.52)]. However, ICCs using only data from the two winter rounds (rounds 1 and 3) showed slightly lower variability [uncorrected: 0.39 (0.17, 0.66), SG-corrected: 0.21 (0.03, 0.69)]. Uncorrected DPHP kappas indicated fair to low agreement [tertiles: 0.32 (0.06, 0.58), high/low: 0.20 (-0.13, 0.52)] and SG-corrected kappas indicated poor to fair agreement [tertiles: 0.16 (-0.10, 0.41), high/low: 0.39 (0.08, 0.70)] of individuals' exposure categories between rounds 1 and 3 (Landis and Koch 1977). ICCs for BDE-47, iodine, and thyroid hormone measures were previously reported (Makey et al. 2014; Makey et al. 2015).

3.3. Predictors of Urinary DPHP

Results from the multivariable linear mixed model of adult DPHP concentrations are presented in Table 3. As DPHP concentrations were ln-transformed, we exponentiated the beta estimates to represent the adjusted relative mean difference in DPHP (ng/mL) compared to the reference group for categorical predictors and the relative difference in DPHP (ng/mL) per unit increase in continuous predictors. On average, women had 43% higher DPHP concentrations compared to men (relative mean: 1.43, 95% CI: 0.94, 2.18). DPHP concentrations were higher in samples collected in the afternoon (1-5pm) and evening (5-9pm) compared to the morning (7am-1pm). We did not find meaningful relationships between DPHP concentrations and participant age, BMI, nail biting habits, or daily handwashing frequency.

In children, we found no significant associations between SG-corrected DPHP concentrations and demographic or behavioral predictors (see Table S1).

3.4. DPHP and Thyroid Hormones

Multivariable models of thyroid hormone levels using continuous SG-corrected DPHP concentrations were sensitive to two influential observations with extreme DPHP values. Model results from sensitivity analyses with and without these observations are presented in Table S2. Because these models were strongly influenced by individual observations we present our primary model results using categorized SG-corrected DPHP concentrations as these models were much more robust.

Table 4 presents the adjusted mean difference (TT_4) and relative mean difference (fT_4, TT_3, TSH) in mean hormone levels in the high DPHP group compared to the low DPHP group in the full cohort and stratified by sex. All models were adjusted for sample round, urine collection time (morning, afternoon, evening), SG-corrected iodine, and BDE-47. Models in the full cohort were additionally adjusted for sex. In the full cohort, high urinary DPHP was associated with a 0.43 μg/dL (95% CI 0.15, 0.72) increase in mean TT_4 levels compared to the low DPHP group. The association between DPHP level and TT_4 differed significantly by sex (sex*DPHP interaction p-value=0.03). In women, high urinary DPHP was associated with a 0.91 μg/dL (0.47, 1.36) increase in mean TT_4 levels compared to low DPHP. In men, mean TT_4 levels were only slightly higher in the high compared to low DPHP group (0.19 μg/dL; -0.15, 0.52). We saw small but less precise positive associations between DPHP and TT_3, which did not differ significantly between women and men (sex*DPHP interaction p-value=0.84). We did not find strong associations between DPHP and fT_4 or TSH. Women with high vs. low DPHP had lower mean TSH levels, but this estimate was imprecise. SG-corrected iodine, BDE-47, and age did not modify the associations between DPHP concentrations and thyroid hormone levels (interaction p-value ranges: iodine*DPHP, 0.22 to 0.74; BDE-47*DPHP, 0.34 to 0.89; age*DPHP, 0.13 to 0.94).

We ran sensitivity analyses to assess the influence of extreme DPHP observations (2 samples), women taking oral contraceptives (11 samples), and elevated TPOAb levels (9 samples) on our results. Exclusion of these observations did not meaningfully alter results (data not shown); therefore these observations were included in our final models.

4. Discussion

We detected DPHP in nearly all urine samples from the FlaRE study population. Concentrations were higher in women compared to men, higher in samples collected later in the day, and varied significantly within individuals across sampling rounds. We found a positive association between SG-corrected DPHP concentrations and TT_4 levels among women.

The geometric mean DPHP concentrations in FlaRE adults fell within the range previously reported in studies of U.S. adults (GM ranges: uncorrected, 0.31 to 1.9 ng/mL; SG-corrected, 1.2 to 1.9) (Butt et al. 2016; Hoffman et al. 2014, 2015b; Meeker et al. 2013b). DPHP concentrations in FlaRE adults were considerably higher than in Meeker et al. (0.31 ng/mL), the only other study investigating DPHP and thyroid hormone levels (Meeker et al. 2013a). This difference may be due to the sampling timing; the Meeker et al. population was sampled from 2002 to 2007 and our population was sampled from 2010 to 2011. Dodson et

al. found that levels of TPHP in repeat dust samples were higher in 2011 compared to 2006 in CA homes (Dodson et al. 2012).

As previously reported, we found higher concentrations of urinary DPHP in women compared to men (Hoffman et al. 2015b; Van den Eede et al. 2015). We did not collect data on TPHP exposure sources and therefore could not determine if higher DPHP in women was due to greater exposure or physiological differences compared to men. As previously reported DPHP concentrations were higher in samples collected in the afternoon and evening compared to the morning (Hoffman et al. 2015b; Meeker et al. 2013a). Because TPHP is thought to have a relatively short half-life, higher DPHP concentrations later in the day may indicate that greater TPHP exposure occurs throughout the day, potentially outside of the home. More exposure source and activity data are needed to investigate these patterns.

To date only three previous studies have reported DPHP concentrations in children in the United States (Butt et al. 2014, 2016; Hoffman et al. 2015a). SG-corrected geometric means were higher compared to the FlaRE children (2.9-3.2 vs. 2.4 ng/mL). However, these studies sampled younger age groups (<1 to 5 years) and DPHP concentrations may be inversely associated with age (Hoffman et al. 2015b; Van den Eede et al. 2015). Butt et al. 2014 and 2016 reported higher concentrations of DPHP in children compared to their mothers (Butt et al. 2014, 2016). In FlaRE, concentrations in children were comparable to those in adults.

Although the half-life of TPHP in the human body is unknown, it is thought to be metabolized and excreted rapidly from the body, which may cause high intra-individual variability in spot urine measures (Aylward et al. 2014; Hou et al. 2016; Muir et al. 1983). We found markedly lower ICCs over our one-year study period compared to previous studies that reported moderate to strong reliability over much shorter time periods (ICC range: 0.35 to 0.7; see Table S4) (Cequier et al. 2015; Hoffman et al. 2014, 2015b; Meeker et al. 2013b). This indicates that DPHP concentrations may be relatively stable in individuals over shorter, but not longer, time periods. Our kappa statistics based on categories of SG-corrected DPHP concentrations showed fair agreement between rounds 1 and 3 and ICCs based on data from just those rounds showed moderate reliability, indicating that there could seasonal variability in exposure sources or patterns. Cao et al. saw lower concentrations of organophosphate flame retardants in office dust in the summer compared to the fall and winter (Cao et al. 2014). We only had one summer sampling round and could not formally test the effect of season on DPHP levels.

Subtle changes in circulating thyroid hormones can occur over a relatively short period of time due to the short half-lives of T_4 (~7 days) and T_3 (18 hours) (Anderson et al. 2003; Russell et al. 2008). Therefore the moderate to strong reliability previously reported for DPHP concentrations over shorter sampling periods may indicate that despite the low ICCs for our one-year study period, our spot DPHP measurements may be adequate for assessing associations with thyroid hormone levels. Spot samples may not be appropriate exposure measures for health outcomes that develop over longer time scales.

We found a significant positive association between high DPHP concentrations and TT_4 levels in women and a suggestive positive association with TT_3, but did not find associations

with TSH or fT_4. The only other study of urinary DPHP and thyroid hormone levels in humans did not report results for TT_4, but found a positive association between DPHP and TT_3 and suggestive positive associations with fT_4 and TSH (Meeker et al. 2013a). Meeker et al. were unable to explore effect modification by sex because the study population was restricted to men (Meeker et al. 2013a). However, we cannot rule out that the lack of association between DPHP and TT_4 seen in men in our study could be due to limited statistical power rather than lack of a true association.

In vitro toxicology studies have shown that exposure to TPHP can alter expression of genes involved in regulation of the hypothalamic pituitary axis (HPT) (Kim et al. 2015; Liu C et al. 2013). In vivo toxicology studies in zebrafish reported increases in circulating T_4 and T_3 following TPHP exposure (Kim et al. 2015; Liu et al. 2016). Similarly to our results, Liu et al. reported a significant increase in circulating T_4 and T_3 following chronic TPHP exposure in female zebrafish, but did not in males (Liu et al. 2016). Because we only saw significant associations between DPHP concentrations and TT_4, an alternative explanation is that the increase in TT_4 is due to an increase in protein binding of T_4 in serum. TPHP increases levels of 17β-estradiol in zebrafish (Liu X et al. 2013; 2016) and large increases in estrogen can increase serum thyroxine binding globulin (TBG) levels (Ain et al. 1987). We did not measure TBG or estrogen levels and were unable to investigate this potential mechanism.

Serum PBDE concentrations, which were previously shown to alter thyroid hormone levels in our population (Makey et al. 2015), were positively correlated with urinary DPHP at two of the study rounds. Controlling for BDE-47 strengthened the associations between urinary DPHP concentrations and TT_4 levels in all participants and women, but did not meaningfully change the interpretation of our results. Our study is the first to assess PBDE coexposures; because both chemicals share similar exposure sources and routes and their relationship is still unclear, we believe co-exposures to PBDEs should be considered in future studies.

The present study has several limitations. The child sample size was small, which limited our ability to assess predictors of DPHP concentrations. Because of the highly skewed DPHP distribution and influential points, we categorized DPHP in adults, lowering our statistical power. We saw high intra-individual variability in DPHP concentrations over the study period, but as previously discussed, spot samples may still be sufficient to assess associations with thyroid hormone levels. Recent studies indicate that DPHP could also be a metabolite of other aryl-phosphate flame retardants (Ballesteros-Gomez et al. 2015; Nishimaki-Mogami et al. 1988). Therefore, it is unclear whether TPHP, other DPHP parent compounds, DPHP, or other TPHP metabolites are responsible for the observed associations between urinary DPHP and thyroid hormone levels. Finally, while we did control for exposure to PBDEs, we cannot exclude the possibility of residual confounding due to demographic predictors or co-exposures to additional endocrine disrupting compounds.

The FlaRE study population was relatively homogenous, limiting the generalizability of the study findings to the general population, but potentially reducing residual confounding. However, the concentrations of DPHP in the study were similar to those reported in most other U.S. study populations. This is only the second study assessing associations between

urinary DPHP concentrations and thyroid hormone levels and the first in a mixed sex cohort. We assessed these associations using repeated measures of both exposure and outcome, and controlled for other factors associated with thyroid function including iodine status and co-exposure to PBDEs.

Conclusions

We found evidence that urinary DPHP concentrations are higher in women and may be associated with increased TT_4 levels, especially among women. DPHP was not strongly associated with TSH or FT_4. As TPHP is almost ubiquitously detected in urine and this is only the second study to assess these relationships, further studies in larger, mixed sex populations are needed to elucidate the relationship between urinary DPHP concentrations and thyroid function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Urinary DPHP concentrations showed high within-subject variability over time
- Geometric mean DPHP concentrations were higher in women compared to men
- Higher DPHP levels were associated with lower total thyroxine levels in women

Table 1
Baseline FlaRE population characteristics

Characteristic	n (%) or mean ± SD
Adults (n=51)	
Age (years)	40.0 ± 12.7
Sex	
Male	26 (51)
Female	25 (49)
Race/ethnicity	
White	45 (88)
Other	6 (12)
Education	
≥ College graduate	50 (98)
< College graduate	1 (2)
BMI (kg/m^2)	
< 25	32 (63)
25 – 29.9	17 (33)
≥ 30	2 (4)
Children (n=14)	
Age (years)	6.6 ± 2.2
Sex	
Male	7 (50)
Female	7 (50)
Race/ethnicity	
White	10 (71)
Other	4 (29)

SD, standard deviation

Table 2

Summary statistics of analytes by sample round for adults (51 subjects, 135 paired urine and serum samples)

	Round 1 (n=47)		Round 2 (n=46)		Round 3 (n=42)	
Analytes	**GM (GSD)**	**Range**	**GM (GSD)**	**Range**	**GM (GSD)**	**Range**
Urinary						
DPHP (ng/ml)						
Uncorrected[a]	1.74 (3.29)	0.12 – 162	0.99 (3.77)	0.14 – 62	1.42 (3.00)	0.15 – 25
SG-corrected[b]	2.99 (2.98)	0.39 – 142	1.80 (3.30)	0.17 – 99	2.11 (2.48)[b]	0.46 – 25
Iodide (μg/L)						
Uncorrected[a]	130 (2.19)	27 – 890	102 (2.33)	11 – 382	148 (1.92)	32 – 660
SG-corrected[b, d]	223 (1.62)	78.4 – 815	186 (1.70)	30.3 – 519	240 (1.61)	98.5 – 671
Specific Gravity[c]	1.016 (0.007)	1.004 – 1.028	1.016 (0.009)	1.003 – 1.035	1.016 (0.007)	1.0001 – 1.027
Serum						
Hormones						
TT_4 (μg/dL)[c]	7.30 (1.32)	3.18 – 9.63	7.30 (1.42)	3.9 – 10.2	7.13 (1.24)	4.74 – 9.71
fT_4 (ng/dL)	1.23 (1.16)	0.93 – 2.09	1.20 (1.19)	0.95 – 2.2	1.19 (1.15)	0.96 – 1.61
TT_3 (ng/mL)	1.08 (1.17)	0.78 – 1.54	1.06 (1.18)	0.8 – 1.44	1.06 (1.18)	0.84 – 1.54
TSH (μIU/mL)	0.71 (1.78)	0.17 – 2.65	0.77 (1.81)	0.16 – 2.27	0.88 (1.98)	0.17 – 4.77
BDE-47 (ng/g lipid)[e]	9.52 (2.87)	0.60 – 151	10.26 (2.72)	1.30 – 149	7.93 (2.62)	0.90 – 99

GM, geometric mean; GSD, geometric standard deviation;

[a] Uncorrected for urinary dilution

[b] SG-corrected for urinary dilution

[c] Arithmetic mean and standard deviation

[d] 1 sample excluded during round 3 due to extreme urinary dilution (SG = 1.0001)

[e] 1 sample excluded during round 3 due to suspected contamination

Table 3

Adjusted[a] associations of change in adult urinary DPHP (ln(ng/mL)) concentrations by participant characteristics (51 subjects, 135 samples)

Predictor	$e^{\beta eta}$ (95% CI)[b]
Sex	
Male	1.00 (Ref)
Female	1.43 (0.94, 2.18)
Sample collection time *	
7:00 am–12:59 pm	1.00 (Ref)
1:00–4:59 pm	1.23 (0.75, 2.00)
5:00–9:00 pm	2.37 (1.57, 3.57)
Age (years)	1.00 (0.99, 1.02)
BMI (kg/m^2)	1.02 (0.96, 1.08)
Handwashing frequency (times/day)	
≤ 6	1.00 (Ref)
> 6	1.26 (0.87, 1.84)
Fingernail biter	
No	1.00 (Ref)
Yes	0.77 (0.49, 1.21)

ln, natural logarithm; CI, confidence interval; Ref, reference

[a] Adjusted for SG, sample round, and covariates in table.

[b] $e^{\beta eta}$ represents the relative mean difference in DPHP compared to the reference group for categorical predictors and the relative difference in DPHP per unit increase in continuous predictors.

* Global p-value <0.001

Table 4

Adjusted[a] associations of change in thyroid hormones levels in high versus low SG-corrected DPHP in all subjects and stratified by sex (51 subjects, 133 samples[b])

			Total T_4 (μg/dL)*	Free T_4 ln(ng/dL)	Total T_3 ln(ng/mL)	TSH ln(mIU/mL)
Model	**DPHP[d]**	**n**	**βeta (95% CI)[e]**	**$e^{βeta}$ (95% CI)[f]**	**$e^{βeta}$ (95% CI)[f]**	**$e^{βeta}$ (95% CI)[f]**
All[c]	Low	89	0.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	High	44	0.43 (0.15, 0.72)	1.01 (0.97, 1.05)	1.03 (0.98, 1.08)	1.05 (0.90, 1.23)
Women						
	Low	39	0.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	High	22	0.91 (0.47, 1.36)	1.01 (0.95, 1.07)	1.05 (0.98, 1.13)	0.91 (0.71, 1.68)
Men						
	Low	50	0.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	High	22	0.19 (-0.15, 0.52)	1.02 (0.96, 1.08)	1.04 (0.97, 1.11)	1.06 (0.88, 1.27)

ln, natural logarithm; CI, confidence interval

[a] All models adjusted for sampling round, time of sample collection (morning, afternoon, evening), SG-corrected iodine (μg/L), and BDE-47 (ng/g lipid).

[b] 1 sample excluded due to extreme urinary dilution (SG = 1.0001), 1 sample missing BDE-47 due to potential contamination.

[c] Models additionally adjusted for sex.

[d] "Low" <2.65 ng/mL, "High" ≥ 2.65 ng/mL

[e] βeta represents the absolute mean difference in Total T_4 in the high compared to low DPHP group

[f] $e^{βeta}$ represents the relative mean difference hormone level in the high compared to low DPHP group

* Sex*DPHP interaction p-value = 0.03