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## Phenols and parabens in relation to reproductive and thyroid hormones in pregnant women

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## ABSTRACT

**Introduction:** Phenols and parabens are ubiquitous environmental contaminants. Evidence from animal studies and limited human data suggest they may be endocrine disruptors. In the current study, we examined associations of phenols and parabens with reproductive and thyroid hormones in 106 pregnant women recruited for the prospective cohort, "Puerto Rico Testsite for Exploring Contamination Threats (PROTECT)".

**Methods:** Urinary exposure biomarkers (bisphenol A, triclosan, benzophenone-3, 2,4-dichlorophenol, 2,5-dichlorophenol, butyl, methyl and propyl paraben) and serum hormone levels (estradiol, progesterone, sex hormone-binding globulin (SHBG), free triiodothyronine (FT3), free thyroxine (FT4) and thyroid stimulating hormone) were measured at up to two time points during pregnancy (16–20 weeks and 24–28 weeks). We used linear mixed models to assess relationships between exposure biomarkers and hormone levels across pregnancy, controlling for urinary specific gravity, maternal age, BMI and education. In sensitivity analyses, we evaluated cross-sectional relationships between exposure and hormone levels stratified by study visit using linear regression.

**Results:** An IQR increase in methyl paraben was associated with a 7.70% increase (95% CI 1.50, 13.90) in SHBG. Furthermore, an IQR increase in butyl paraben was associated with an 8.46% decrease (95% CI 16.92, 0.00) in estradiol, as well as a 9.34% decrease (95% CI –18.31, –0.38) in estradiol/progesterone. Conversely, an IQR increase in butyl paraben was associated with a 5.64% increase (95% CI 1.26, 10.02) in FT4. Progesterone was consistently negatively associated with phenols, but none reached statistical significance. After stratification, methyl and propyl paraben were suggestively negatively associated with estradiol at the first time point (16–20 weeks), and suggestively positively associated with estradiol at the second time point (24–28 weeks).

**Conclusions:** Within this ongoing birth cohort, certain phenols and parabens were associated with altered reproductive and thyroid hormone levels during pregnancy. These changes may contribute to adverse health effects in mothers or their offspring, but additional research is required.

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**Abbreviations:** 2,4-DCP, 2,4-dichlorophenol; 2,5-DCP, 2,5-dichlorophenol; BP-3, benzophenone-3; BPA, bisphenol A; BPB, butyl paraben; EDCs, endocrine disrupting compounds; E/P, ratio of estradiol to progesterone; ER, estrogen receptor; Ft4, free thyroxine; FT3, free triiodothyronine; GM, geometric mean; GSD, geometric standard deviation; IQR, interquartile range; LOD, limit of detection; MPB, methyl paraben; NHANES, National Health and Nutrition Examination Survey (NHANES); PPB, propyl paraben; PROTECT, Puerto Rico Testsite for Exploring Contamination Threats; SHBG, sex hormone-binding globulin; SG, specific gravity; TCS, triclosan; TSH, thyroid stimulating hormone

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## 1. Introduction

Phenols and parabens are endocrine disrupting compounds (EDCs) widely used in various consumer products, such as personal care products and plastics. Reports from the National Health and Nutrition Examination Survey (NHANES) show that the majority of the U.S. population have detectable levels of a range of phenols and parabens in their bodies (Centers for Disease Control and Prevention, 2015).

Bisphenol A (BPA) is a high volume chemical used in the manufacture of polycarbonate plastics and epoxy resins in many consumer products. BPA has also been shown to have weakly

estrogenic properties (Centers for Disease Control and Prevention, 2015), and has been associated with changes in thyroid and reproductive hormone levels in animals (Peretz et al., 2014), elevated risks of low birth weight, smaller size for gestational age, preterm birth, as well as increases in the levels of leptin and adiponectin among male neonates (Cantonwine et al., 2015; Chou et al., 2011; Huo et al., 2015; Miao et al., 2011).

Benzophenone-3 (BP-3) is a UV-filter used in cosmetics and sunscreens and some plastics (Centers for Disease Control and Prevention, 2015; Krause et al., 2012). Endocrine disruptive properties of BP-3 have been shown in a variety of animals and populations. For example, BP-3 was associated with a decrease in percent fat mass in children after prenatal exposure (Buckley et al., 2016), thyroid receptor agonistic effects in vitro assays (Schmutzler et al., 2007), altered transcription of hormonal receptors in invertebrates (Ozáez et al., 2016), and a decrease in spermatozoa among male fish (Chen et al., 2016). While few studies have assessed the impact of BP-3 exposure specific to reproductive health, it is suggested to be weakly estrogenic and antiandrogenic.

Triclosan (TCS) is an antibacterial and antifungal ingredient added to many consumer products to reduce bacterial contamination. It can be found in personal care products such as soaps, toothpaste, and deodorants, as well as, toys and kitchenware (Dann and Hontela, 2011). Its chemical structure is similar to that of anthropogenic estrogens, and evidence suggests TCS disrupts hormone metabolism, displaces hormones from receptors and disrupts steroidogenic enzyme activity (Wang and Tian, 2015). There is also evidence from animal and in vitro studies of changes in reproductive hormone levels caused by TCS, albeit the evidence from the studies has varied (Wang and Tian, 2015).

2,4-dichlorophenol (2,4-DCP) is a metabolite of the widely used herbicide 2,4-dichlorophenoxyacetic acid, and 2,5-dichlorophenol (2,5-DCP) is a metabolite of 1,4-dichlorobenzene, a compound used in mothballs and room deodorizers (Centers for Disease Control and Prevention, 2015). 2,4-DCP and 2,5-DCP exposure in utero has been linked to decreased birthweight in humans (Phillipat et al., 2012). 2,5-DCP was also associated with earlier breast development in young girls (Wolff et al., 2015), and greater percent fat mass in children (Buckley et al., 2016), and was theorized to be a thyroid agonist (Wolff et al., 2015).

Due to their antimicrobial properties, parabens are found in personal care products, pharmaceuticals, and food products (Błędzka et al., 2014). While parabens have low binding affinity to estrogen receptors, they have been documented to have full agonist properties, particularly with longer exposure durations (Darbre and Harvey, 2008). In animal and human studies, methyl paraben (MPB) and butyl paraben (BPB) have been linked to various adverse health effects, including increased birthweight (Phillipat et al., 2014), decreased odds of live births (Dodge et al., 2015), increases in dam uterine weights (indicating estrogenic effects) (Taxvig et al., 2008), and changes in various reproductive hormone levels (Taxvig et al., 2008; Zhang et al., 2014).

To our best knowledge, there have been no reports of repeated measure studies aimed to explore the effects of phenols and parabens on reproductive and thyroid hormones during pregnancy. The Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) Program is an ongoing prospective birth cohort in Northern Puerto Rico that was initiated in order to explore the impact of environmental contaminants, including phenols, parabens, and other EDCs, on reproductive health. We previously observed higher urinary concentrations of TCS, BP-3, 2,4-DCP and 2,5-DCP, and similar concentrations of BPA, 2,4-DCP and parabens in the PROTECT cohort compared to U. S. women of reproductive age (Meeker et al., 2013); therefore, this cohort provides an opportunity to study relationships between phenol and paraben exposure and hormone levels during pregnancy. In the present

study, we assessed associations among a range of phenols and parabens, and reproductive and thyroid hormones within a sample of the PROTECT cohort.

## 2. Methods

### 2.1. Study participants

The present study included participants from an ongoing prospective cohort of pregnant women in Puerto Rico, and was designed to explore associations between environmental contaminants and adverse birth outcomes. Details on the recruitment and inclusion criteria have been described previously (Cantonwine et al., 2014; Meeker et al., 2013). In brief, the study participants included in the present analysis were recruited from 2010 to 2012 at  $14 \pm 2$  weeks gestation from two hospitals and five affiliated prenatal clinics in Northern Puerto Rico, and were aged 18–40 years. Women who lived outside the region, had multiple gestations, used oral contraceptives within three months prior to getting pregnant, got pregnant using in vitro fertilization, or had known medical health conditions (diabetes, hypertension, etc.) were excluded from the study. Demographic information was collected via questionnaires at the initial study visit. Spot urine samples and blood samples were collected at three separate study visits (Visit 1: 16–20; Visit 2: 20–24; Visit 3: 24–28 gestation weeks), and blood samples were collected during the first and third visits. The timing of the visits were aimed to coincide with periods of rapid fetal growth and routine clinical visits.

This preliminary analysis includes the first 106 women recruited into the study for whom both total phenol/paraben concentrations and hormone measurements from at least one study visit were available. This study was approved by the research and ethics committees of the University of Michigan School of Public Health, University of Puerto Rico, Northeastern University, and participating hospitals and clinics. All study participants provided full informed consent prior to participation.

### 2.2. Urinary phenol and paraben measurement

After collection, spot urine samples were divided into aliquots and frozen at  $-80^\circ\text{C}$  until they were shipped overnight to the CDC for phenol and paraben analysis. Urine samples were analyzed for five phenols (BPA, TCS, BP-3, 2,4-DCP, and 2,5-DCP) and three parabens (BPB, MBP and propyl paraben (PPB)) using online solid phase extraction-high-performance liquid chromatography-isotope dilution tandem mass spectrometry (Watkins et al., 2015; Ye et al., 2006, 2005). Concentrations below the limit of detection (LOD) were assigned a value of the LOD divided by  $\sqrt{2}$ . Urinary specific gravity (SG) was used to account for urinary dilution, and was measured using a digital handheld refractometer (AtagoCo., Ltd., Tokyo, Japan). Exposure biomarkers were corrected for SG using the following formula:

$$P_c = M[(SG_m - 1)/(SG_i - 1)] \quad (1)$$

Equation 1: Specific gravity correction of exposure biomarker. where  $P_c$  is the SG-corrected exposure concentration (ng/mL),  $M$  is the measured exposure concentration,  $SG_m$  is the study population median urinary specific gravity (1.0196), and  $SG_i$  is the individual's urinary specific gravity.

### 2.3. Reproductive hormone measurement

Estradiol, progesterone, and sex hormone-binding globulin (SHBG) were measured in serum samples using a

chemiluminescence immunoassay (DPC Immulite, Diagnostic Products Corporation). Analyses were performed by the Bioanalytical Core Laboratory at Georgetown University (Washington, DC). There were differences in the number of samples per hormone due to volume limitations in some of the samples. In addition, the ratio of estradiol to progesterone (E/P) may be a better indicator of adverse pregnancy outcomes (such as preterm birth) than the individual hormones alone (Castracane, 2000), so the ratio of the two hormones was calculated for the purposes of this analysis.

#### 2.4. Thyroid hormone measurement

Free triiodothyronine (FT3), free thyroxine (FT4), and thyroid-stimulating hormone (TSH) were measured at the Bioanalytical Core Laboratory of Georgetown University (Washington, DC). Ultrafiltration steps were conducted to separate free hormones, and then isotope dilution liquid chromatography tandem mass spectrometry was used to measure FT3 and FT4 as described previously (Johns et al., 2015; Kahric-Janjic et al., 2007; Soldin et al., 2004; Soldin and Soldin, 2011). A solid-phase immunochemiluminometric assay (DPC Immulite, Diagnostic Products Corporation) was used to measure TSH.

#### 2.5. Statistical analyses

Descriptive analyses were performed on all the variables used in the present analyses. Distributions of all exposure biomarkers were positively skewed and were natural log-transformed. Serum concentrations of progesterone and TSH were also skewed, and therefore natural log-transformed, whereas estradiol, SHBG, E/P, FT3 and FT4 approximated a normal distribution and were left untransformed.

Pearson correlation coefficients were used to assess bivariate relationships between continuous variables. Categorical covariates were examined in linear regression models to assess associations with hormone measures. We also calculated Spearman correlation coefficients between the SG-corrected exposure biomarkers (because they were all skewed to the left), and Pearson correlation coefficients between the hormone levels.

The intraclass correlation coefficients (ICC) of the exposures in this cohort previously published showed relative low agreement at the different time points, with ICC values ranging from 0.24 (BPA) to 0.62 (BP-3) (Meeker et al., 2013). To account for and take advantage of this intra-individual correlation and variation of repeated measures over time, linear mixed effects models with a random intercept for subject ID were used to regress each serum hormone on each exposure biomarker individually. Crude models were first constructed, adjusting for SG only. Final models were adjusted for SG, study visit, maternal age, pre-pregnancy BMI, and maternal education. Only variables that were found to change the main effect estimate by > 10% were retained in the final models. To assess potential windows of vulnerability in pregnancy, we stratified the model to conduct cross-sectional multiple linear regression models by study visit of sample. The first cross-sectional analyses regressed the hormone levels measured at visit 1 on the individual phenol/paraben level at visit 1; and the second cross-sectional analyses regressed the hormone levels measured at visit 3 on the individual phenol/paraben level at visit 3. Cross-sectional analyses were preferred over the addition of interaction terms because the results from cross-sectional models are more readily interpretable. However, interaction terms of the exposure biomarker and the visit number were included in the mixed models to quantitatively compare differences in associations between time points. To improve interpretability, all regression coefficients were transformed into percent changes (and

associated 95% confidence intervals) in hormone concentration associated with an interquartile range (IQR) increase in metabolite concentration relative to the population median hormone level. The alpha level was set at 0.05.

### 3. Results

The 106 women had a mean age of 27 years and were well-educated (79% had a college degree). Only 11.3% of the women had a pre-pregnancy BMI over 30 kg/m<sup>2</sup> (Table 1). Additional demographic details have been described elsewhere (Cantonwine et al., 2015).

All samples had detectable levels of all measured hormones (Supplemental Table 1). Of the serum hormones, only estradiol and progesterone were strongly correlated (Pearson  $r=0.65$ ,  $p<0.0001$ ) (Supplemental Fig. 1). Among the phenols, only urinary 2,4-DCP and 2,5-DCP were strongly correlated with each other (Spearman  $r=0.79$ ,  $p<0.0001$ ) (Supplemental Fig. 2). Parabens also showed some correlation: MPB and PPB were strongly correlated (Spearman  $r=0.77$ ,  $p<0.0001$ ), and PPB and BPB were moderately correlated (Spearman  $r=0.40$ ,  $p$  value  $<0.0001$ ).

All hormones were significantly different in visit 3 as compared to visit 1, except for TSH and FT3 (Supplemental Table 1). As expected, the levels of estradiol and progesterone increased rapidly across the two time points, approximately doubling from visit 1 to visit 3, whereas SHBG had a more modest increase in visit 3. The levels of FT3 and FT4 modestly decreased at visit 3 versus visit 1 ( $p=0.08$  and  $0.001$  respectively), while TSH levels remained the same.

#### 3.1. Reproductive hormones

In mixed effects models (Table 2), an IQR increase in BPB levels was associated with an 8.46% decrease in estradiol ( $p=0.05$ ) and a 9.34% decrease in E/P ratio ( $p=0.04$ ), while an IQR increase in MPB was associated with a 7.7% increase in SHBG ( $p=0.02$ ). All phenols were associated with decreases in progesterone; however, they did not reach statistical significance.

After stratifying the analyses by visit, differences in the relationships across the two time points appeared. Figs. 1 and 2 show the associations between the exposures and hormones at the two visits (Weeks 16–20 and Weeks 24–28). Parabens MPB and PPB were negatively associated with estradiol and E/P at Weeks 16–20, but became positively associated with the hormones at Weeks 24–28 weeks. The associations of MPB and PPB with E/P was especially pronounced during visit 3 (MPB: 20.6% per IQR

**Table 1**  
Summary demographics of the 106 pregnant women in the sample from PROTECT.

	Mean	Max	Min
<b>Age</b>	27	39	18
		Number of participants	% of participants
<b>Education</b>	< High school	12	11.3
	High school/ equivalent	7	6.6
	College	84	79.2
	Unknown	3	2.8
<b>BMI</b>	≤ 25	59	55.7
	25 – 30	31	29.2
	> 30	12	11.3
	Unknown	4	3.8

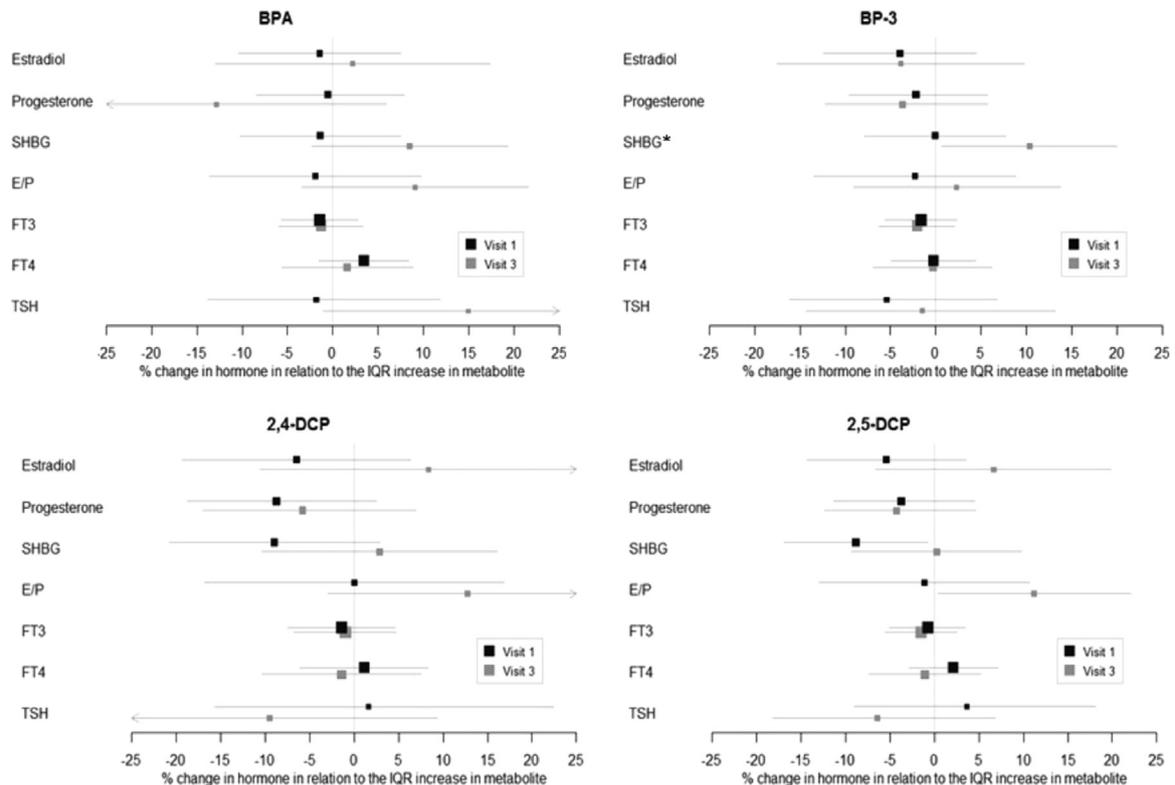
**Table 2**

Linear mixed model results for reproductive hormones adjusted for maternal age, education and BMI: Percent change in hormone level in relation to the interquartile range increase in urinary exposure biomarker.

	IQR <sup>b</sup>	Estradiol (N=175) <sup>a</sup>		Progesterone (N=179)		SHBG (N=147) <sup>a</sup>		Estradiol/Progesterone (N=175) <sup>a</sup>	
		% Δ (95%)	P value	% Δ (95%)	P value	% Δ (95%)	P value	% Δ (95%)	P value
<b>BPA</b>	2.80	1.47 (−5.97, 8.91)	0.70	−4.24 (−9.79, 1.65)	0.15	0.12 (−5.32, 5.56)	0.97	4.19 (−3.64, 12.01)	0.29
<b>BP-3</b>	104	−2.79 (−10.44, 4.86)	0.47	−3.38 (−9.18, 2.80)	0.27	0.67 (−4.75, 6.10)	0.80	1.74 (−6.36, 9.85)	0.67
<b>2,4-DCP</b>	1.85	0.26 (−7.81, 8.33)	0.95	−4.64 (−10.62, 1.74)	0.15	−1.70 (−6.88, 3.47)	0.51	2.85 (−5.67, 11.38)	0.51
<b>2,5-DCP</b>	38.9	−1.15 (−8.94, 6.65)	0.77	−4.31 (−10.12, 1.89)	0.17	−1.92 (−7.01, 3.17)	0.45	2.33 (−5.91, 10.57)	0.58
<b>TCS</b>	110	−3.48 (4.93, −0.03)	0.48	−5.66 (−12.81, 2.08)	0.15	−1.44 (−8.16, 5.27)	0.67	−1.69 (−12.09, 8.71)	0.75
<b>MPB</b>	293	4.13 (−5.14, 13.40)	0.38	−4.35 (−11.23, 3.09)	0.24	<b>7.70 (1.50, 13.90)</b>	<b>0.02</b>	5.83 (−3.97, 15.63)	0.24
<b>BPB</b>	3.30	<b>−8.46 (−16.92, 0.00)</b>	<b>0.05</b>	1.72 (−5.09, 9.01)	0.63	1.26 (−4.99, 7.50)	0.69	<b>−9.34 (−18.31, −0.38)</b>	<b>0.04</b>
<b>PPB</b>	86.4	2.48 (−7.70, 12.66)	0.63	0.09 (−7.81, 8.65)	0.98	3.68 (−3.35, 10.70)	0.30	2.65 (−8.12, 13.43)	0.62

<sup>a</sup> The percent change in hormone level per exposure IQR increase is relative to the median hormone level. Median levels of hormones in ng/mL: estradiol.

<sup>b</sup> Units are in ng/mL.



**Fig. 1.** Results of the stratified linear regressions comparing Visit 1 and Visit 3 associations for BPA, BP-3, 2,4-DCP and 2,5-DCP. Size of the box refers to the precision of the estimate, bars represent the 95% confidence interval of each estimate. Effect estimates are portrayed as percent change in hormone level in relation to the interquartile range increase in urinary exposure biomarker. Asterisk added if the interaction term between exposure\*visit had a p value < 0.05.

change,  $p=0.004$ ; PPB: 21.4% per IQR change,  $p=0.02$ ) (Supplemental Table 1). The interaction terms between MPB & PPB and visit in the mixed effects models were statistically significant for estradiol and E/P ( $p$  values ranged from < 0.0001 to 0.001). On the other hand, some associations became more pronounced at only one of the visits. For example, an IQR increase in BP-3 was not associated with SHBG during the first time point; but was associated with a 10.3% increase in SHBG ( $p=0.04$ ) at the second time point. The interaction term between BP-3 and visit for SHBG was also significant ( $p$  value=0.04).

### 3.2. Thyroid hormones

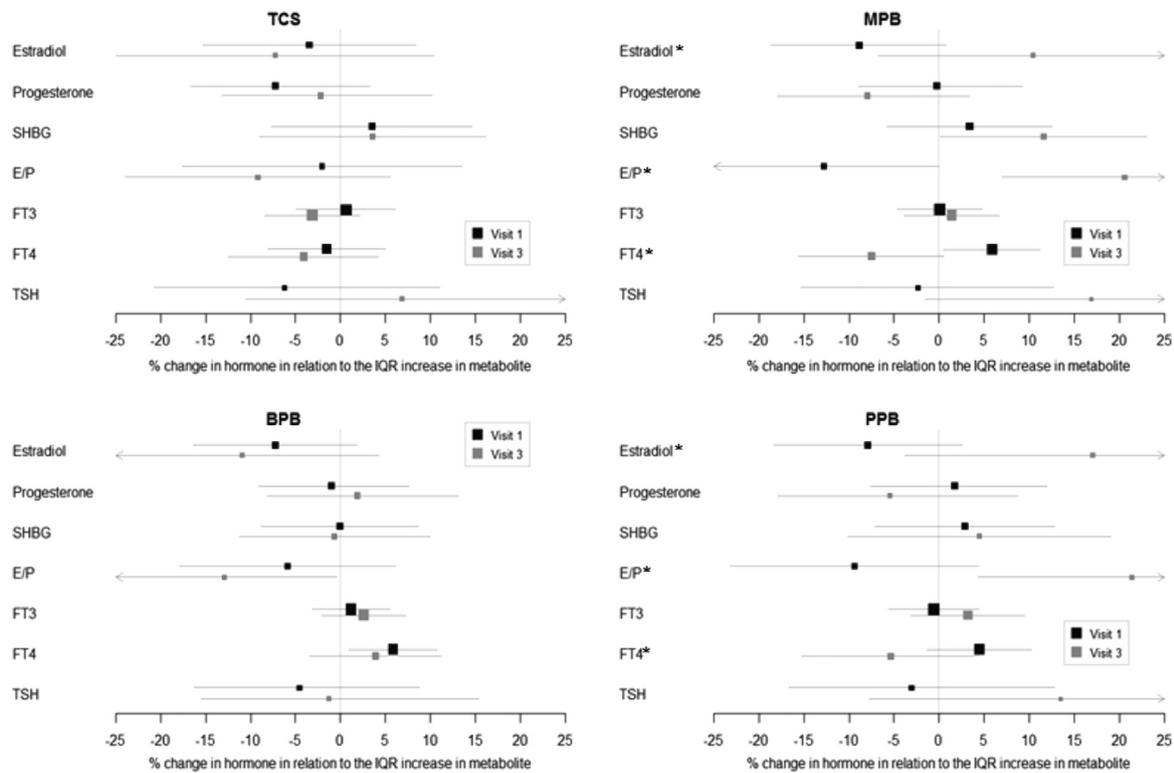
In mixed effects models pertaining to thyroid hormones (Table 3), an IQR increase in BP-3 was associated with a 3.0% decrease in FT3 ( $p=0.04$ ). There was a 4.11% ( $p=0.04$ ) and 5.64%

( $p=0.01$ ) increase in FT4 with an IQR increase in BPA and BPB respectively. TSH was not significantly associated with any of the exposures.

After stratifying the analyses by visit, only the association between MPB and FT4 appeared to differ ( $P$  value for interaction term=0.006). An IQR increase in MPB was associated with a 5.85% increase ( $p=0.03$ ) in FT4 during the first time point, and a 7.53% decrease in FT4 ( $p=0.07$ ) at the second time point. Supplemental Table 3 presents all results of the stratified analyses by visit.

## 4. Discussion

To our knowledge, this is the first study to explore the relationships of phenols and parabens with maternal hormone levels



**Fig. 2.** Results of the stratified linear regressions comparing Visit 1 and Visit 3 associations for TCS, MPB, BPB and PPB. Size of the box refers to the precision of the estimate, bars represent the 95% confidence interval of each estimate. Effect estimates are portrayed as percent change in hormone level in relation to the interquartile range increase in urinary exposure biomarker. Asterisk added if the interaction term between exposure\*visit had a p value < 0.05.

**Table 3**  
Linear mixed model results for thyroid hormones adjusted for maternal age, education and BMI: Percent change in hormone level in relation to the interquartile range increase in urinary exposure biomarker.

	IQR <sup>b</sup>	TSH (n=181)		FT3 (n=181) <sup>a</sup>		FT4 (n=181) <sup>a</sup>	
		% Δ (95%)	P value	% Δ (95%)	P value	% Δ (95%)	P value
BPA	2.80	5.63 (−1.97, 13.83)	0.15	0.81 (−2.01, 3.63)	0.57	<b>4.11 (0.12, 8.10)</b>	<b>0.04</b>
BP-3	104	−3.05 (−10.70, 5.23)	0.45	<b>−3.00 (−5.89, −0.12)</b>	<b>0.04</b>	−0.34 (−4.34, −3.66)	0.87
2,4-DCP	1.85	−0.05 (−8.15, 8.76)	0.99	−1.21 (−4.28, 1.83)	0.43	0.60 (−3.67, 4.87)	0.78
2,5-DCP	38.9	0.78 (−7.19, 9.43)	0.85	−1.19 (−4.16, 1.77)	0.43	1.65 (−2.46, 5.77)	0.43
TCS	110	4.90 (−5.28, 16.17)	0.35	−1.07 (−4.78, 2.64)	0.57	−3.44 (−8.60, 1.71)	0.19
MPB	293	8.46 (−1.74, 19.71)	0.11	0.14 (−3.40, 3.69)	0.94	0.39 (−4.50, 5.28)	0.88
BPB	3.30	−1.59 (−10.24, 7.89)	0.63	−0.11 (−3.35, 3.14)	0.95	<b>5.64 (1.26, 10.02)</b>	<b>0.01</b>
PPB	86.4	0.09 (−19.26, 11.54)	0.98	−0.59 (−4.47, 3.29)	0.77	0.32 (−1.60, 2.25)	0.74

<sup>a</sup> The percent change in hormone level per exposure IQR increase is relative to the median hormone level.

<sup>b</sup> Units are in ng/mL.

at multiple time points during pregnancy. The results of this preliminary study provide evidence of associations between some of the exposure biomarkers and alterations in hormone levels, including changes in estradiol with exposure to parabens, a decrease in progesterone with exposure to phenols, and changes in FT4 and FT3 with exposure to phenols and parabens.

#### 4.1. Reproductive hormones

Reproductive hormones have an important role in maintaining pregnancy. Estradiol is essential for the development of oxytocin receptors, for increasing the levels of oxytocin, and in developing gap junctions for myometrial contractions (Castracane, 2000). Progesterone has been implicated in maintaining pregnancies in animals; however, in humans, the role of progesterone is more complex. Evidence suggests that the ratio between progesterone

and estradiol (and/or progesterone and estradiol) is more important in maintaining pregnancy, and deferring labor by promoting uterine relaxation and reducing prostaglandin synthesis, than the level of progesterone on its own (Castracane, 2000; Lachelin et al., 2009).

Parabens were associated with some reproductive hormones in our analyses, in particular estradiol. While BPB maintained a negative association with estradiol at both time points, MPB and PPB had suggestive negative associations with estradiol at 16–20 weeks, and suggestive positive associations with estradiol at 24–28 weeks. MPB was also positively associated with SHBG at both time points (p=0.02). It is unclear why the association between MPB and PPB with estradiol became suggestively positive at the second time point. Estradiol increases throughout pregnancy, with a surge in its level between 17 and 20 weeks, and relative stabilization between 24 and 28 weeks (Tulchinsky et al., 1972). This

potentially signifies different pathways at play between the two time points that the parabens could be targeting differently. However, due to the small sample size, the result could also be a false positive and will be explored further in future studies.

Few studies have examined the effect of paraben exposure on females during pregnancy. In addition, most animal studies studied effects of BPB only, likely due to the fact that BPB has the highest estrogenic potency (El Hussein et al., 2007). It is important to note that while MPB has the lowest estrogenic potency, it also penetrates through the skin at the highest rate as compared to other parabens, and can accumulate in skin layers (El Hussein et al., 2007). We previously observed MPB levels at more than 100 times the level of BPB, and more than three times the level of PPB (Meeker et al., 2013). No other human studies looked at the effects of phenols and parabens on hormone levels in pregnant women, but several animal and in vitro studies have assessed similar endpoints. Two rat studies found no differences in estradiol levels in dam ovaries after in utero exposure to BPB and isobutylparaben (Kawaguchi et al., 2009; Taxvig et al., 2008). However, one of those studies also exposed human adrenocortical carcinoma cells (H295R cells) to parabens to test their interference with steroid hormone biosynthesis, and reported a decrease of estradiol production after exposure to BPB (Taxvig et al., 2008). While this change was not statistically significant, this finding may be consistent with our results. In a study on prepubertal female rats, researchers found decreases in estradiol and progesterone levels after paraben exposure, particularly with ethyl- and isopropyl paraben (Vo et al., 2010a). Conversely, Zhang et al. (2014) identified an increase in estradiol levels among male rat offspring after in-utero exposure to n-BPB (Zhang et al., 2014). The mechanism of action through which parabens impact endocrine function is still unclear. In animal studies, there has been evidence of ER $\beta$  agonistic activity (Watanabe et al., 2013), progesterone mRNA expression via ER $\alpha$  signaling (Vo et al., 2011) (Wróbel and Gregoraszczuk, 2014), and interference with the transport of cholesterol to the mitochondria, leading to an interference with steroidogenesis (Taxvig et al., 2008).

#### 4.2. Thyroid hormones

The balance of thyroid hormones in the mother is essential to the fetus since the fetus depends on maternal thyroid hormones throughout pregnancy. The fetal thyroid only begins to produce hormones at 10–12 weeks gestation; however, thyroid hormones can be detected in the amniotic fluid as early as 8 weeks gestation (Mastorakos et al., 2007; Zoeller et al., 2002). Studies confirm the existence of thyroid hormone responsive genes in the fetal cortex before the onset of the fetal thyroid production (Zoeller et al., 2002). T3, T4 and iodine in the fetus are available from the mother alone for the first trimester, and the mother continues to help maintain thyroid hormone homeostasis in the fetus/newborn for the remainder of the pregnancy and throughout lactation (Hartoft-Nielsen et al., 2011; Krassas et al., 2010). These hormones are essential for fetal neuronal development and brain differentiation, and thus, deficiency in thyroid hormones leads to neurodevelopmental problems (Boas et al., 2012; Hartoft-Nielsen et al., 2011; Krassas et al., 2010; Zoeller et al., 2002). Given this, the mother has to produce more T4 throughout pregnancy to meet new demands (Krassas et al., 2010), making the balance of maternal thyroid hormones essential – even slight alterations may lead to fetal neurodevelopmental concerns (Boas et al., 2012; Hartoft-Nielsen et al., 2011). Maternal hypothyroidism can also lead to maternal morbidity and adverse birth outcomes; however, the evidence with regards to adverse prenatal health effects due to subclinical hypothyroidism remains unclear (Casey, 2006; Krassas et al., 2010; Sheehan et al., 2015). Due to the complexity in the hypothalamic–

pituitary–thyroid axis, thyroid disrupting chemicals could act through several pathways, including hormone synthesis, regulation, transport and metabolism, and/or interference with receptor.

A previous in vitro assay suggested that BPB is a weak thyroid hormone receptor agonist (Taxvig et al., 2008), but we were not able to identify studies that examined the effect of parabens on thyroid hormones during pregnancy. Vo et al. (2010b) found that paraben exposure significantly decreased T4 levels in prepubertal female rats, whereas in our human cohort, parabens were associated with increased FT4, particularly during the first visit at 16–20 weeks. (Taxvig et al., 2008). Using NHANES data on males and females over the age of 12 years, another study found a significant negative relationship between PPB and FT4 and FT3; a significant positive relationship between BPB and FT3; and, a suggestive negative relationship between MPB and FT3 and FT4 (Koeppel et al., 2013). Our results were not consistent with this study, possibly due to the variable hormone levels throughout pregnancy that were not captured by the NHANES study or other differences between the studies. After exposing 26 human males to butyl paraben and phthalates via dermal application, researchers found no effects on reproductive and thyroid hormones, even after detecting the parent compound in serum within an hour (Janjua et al., 2007). A study of men who were partners in couples attending an infertility clinic also found no association between urinary parabens and thyroid hormones (Meeker et al., 2011).

Ambiguity also exists for the relationship between BPA and thyroid hormones. Some studies detected agonistic properties with the thyroid receptors by gene suppression, whereas, others had detected antagonistic interactions (Boas et al., 2012; Hartoft-Nielsen et al., 2011; Rochester, 2013). Two other cross-sectional studies also looked at the association between maternal BPA and thyroid hormones during gestation (Chevrier et al., 2013; Romano et al., 2015), and the only significant association identified was between BPA and a decrease in total T4 (Chevrier et al., 2013). In contrast, we observed a significant positive association between BPA and FT4 in our study. Differences in our results could be due to different study designs and populations. Other cross-sectional studies included non-pregnant women populations. After review, a consistent inverse association between BPA and TSH, and a positive association between BPA and T3 was highlighted (Rochester, 2013). In contrast, our results show a suggestive positive association between BPA and TSH in pregnant women. Differences in our results could be due to the hormonal changes unique to pregnancy or other differences in study design or study populations. No previous studies were identified with regards to the effect of BP-3 on thyroid hormones.

#### 4.3. Strengths and limitations

There were several limitations to this study. As a preliminary study, we had a relatively small sample size, so we may have been unable to detect true associations due to low statistical power. Type I error is also possible in our study due to the large number of comparisons. Thus, some significant associations reported in our exploratory work here may be due to chance. Mixtures of the exposures were also not considered for these preliminary analyses, even though some phenol and paraben mixtures have been associated with adverse reproductive outcomes (Isling et al., 2014; Kim et al., 2012; Popa et al., 2014). We will consider this possibility in our future work with larger sample sizes, as well as explore potential differences by infant sex.

Another limitation was the lack of information on the selenium and iodine status of the women in the cohort, given that deficiencies in either mineral could lead to detrimental effects on thyroid hormone function. However, iodine may act as a mechanistic intermediate between exposure and thyroid disruption

(Koeppel et al., 2013; Rousset, 1981), and controlling for iodine status could lead to further bias.

Despite the limitations, there were several strengths to the study. The PROTECT cohort presents an opportunity to study the effects of exposures on a sub-population with a reported higher rate of some endocrine-related diseases and elevated exposure levels (Centers for Disease Control and Prevention (CDC), 2012; Meeker et al., 2013; Pérez et al., 2008). These analyses took two time points into consideration in order to account for the varying levels of exposures and their short lifespan in the human body across time (Meeker et al., 2013). It also allowed for the examination of the associations with hormone levels at two different times during pregnancy to identify potential windows of susceptibility. The mixed modeling techniques also provided more statistical power by studying repeated measures per individual. Furthermore, the LC/MS analytical techniques used to measure FT3 and FT4 were novel and more precise than traditional immunoassay methods. This is important because levels of thyroxine binding proteins can increase up to 50% more in pregnancy (Soldin and Soldin, 2011). The LC/MS method used is not influenced by serum binding proteins, is more specific, and does not cross-contaminate with other analytes (Johns et al., 2015; Kahric-Janjic et al., 2007; Soldin et al., 2004; Soldin and Soldin, 2011).

## 5. Conclusions

The results of this study provide some suggestive evidence for endocrine disrupting effects of phenols and parabens on thyroid and reproductive hormones during pregnancy. Further studies are required to confirm our findings in larger populations and with more repeated measures across pregnancy. We also hope to better understand the mechanism behind the varying effects at different time points during pregnancy; and how such changes can affect downstream maternal and infant reproductive health outcomes.

## Competing financial interests

The authors declare that they have no competing financial interests.

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## Review by an IRB

This study was approved by the research and ethics committees of the University of Michigan School of Public Health, University of Puerto Rico, Northeastern University, and participating hospitals and clinics. All study participants provided full informed consent prior to participation.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.envres.2016.07.002>.

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