ASSOCIATIONS OF MATERNAL URINARY CONCENTRATIONS OF PHENOLS, INDIVIDUALLY AND AS A MIXTURE, WITH SERUM BIOMARKERS OF THYROID FUNCTION AND AUTOIMMUNITY: RESULTS FROM THE EARTH STUDY

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Background and Objective

- **Endocrine disrupting chemicals** (EDCs) such as bisphenol A (BPA), benzophenone-3, parabens, and triclosan may interfere with the endocrine system, leading to detrimental health effects in humans.
- Epidemiologic studies on phenols in relation to thyroid hormones in humans, however, have shown **inconsistent findings** and have not investigated these exposures as a mixture.
- **Subfertile women** have been demonstrated to be at a higher risk for thyroid disease.
- We evaluated **urinary concentrations of BPA, benzophenone-3, parabens, and triclosan**, individually and as a **mixture**, in relation to **thyroid function and autoimmunity biomarkers** among women attending a fertility center.

Study Population

339 women

2009 - 2015

- Observational study of women seeking fertility care at the Massachusetts General Hospital, who enrolled in the Environment and Reproductive Health (EARTH) study between 2009 and 2015.
- Analysis included 339 women with data on phenol concentrations and serum thyroid and autoimmunity biomarker data (Table 1).

Sample Assessment

- We measured single spot urinary concentrations of **six phenol biomarkers**: BPA, benzophenone-3, triclosan, methylparaben, propylparaben, and butylparaben.
- From each participant, **a single non-fasting blood sample** was collected via venipuncture the same day the urine sample was collected to assess **biomarkers of thyroid function and autoimmunity**.
- The six outcomes of interest included serum concentrations of thyroid stimulating hormone (TSH), free and total thyroxine (fT4, TT4), free and total triiodothyronine (fT3, TT3), thyroperoxidase antibody (TPOAb), and thyroglobulin antibodies (TgAb).

Statistical Analysis

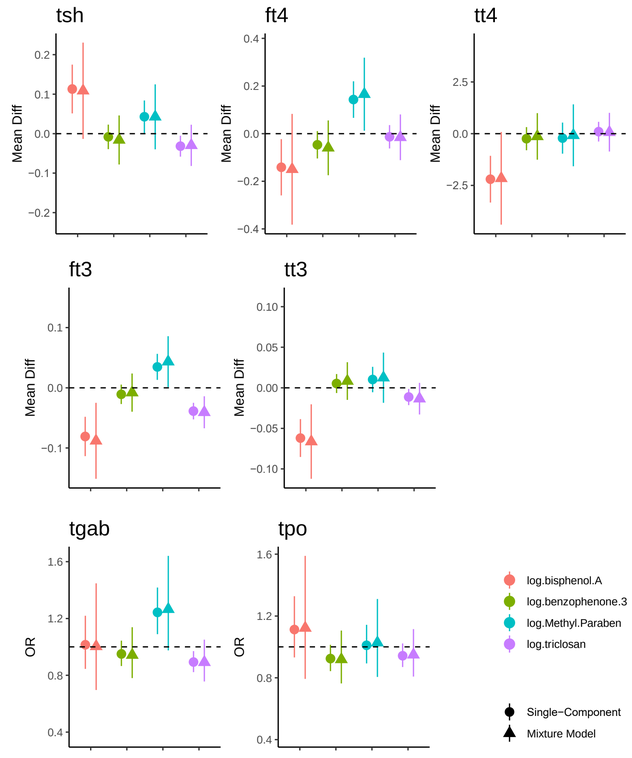
- We first fit **linear models**, regressing each continuous thyroid outcome on the natural log of phenol concentrations.
- We then fit **additive models**, in which the functional relationship between each phenol concentration and the thyroid outcome was allowed to be non-linear and was estimated non-parametrically via penalized splines.
- In the additive models, we also estimated **overall mixture associations**, defined as mean differences in thyroid biomarkers for a simultaneous increase from 25th to 75th percentiles of all mixture exposures simultaneously.
- We further investigated non-additive **interactions** via **Bayesian Kernel Machine Regression** (BKMR) in sensitivity analyses.
- All models - single-exposure and mixture models alike - were **adjusted** for age (years), BMI (kg/m2), and race (white vs. other), and were further adjusted for specific gravity (SG) to account for urine dilution.

Results

Table 1. Demographics and reproductive characteristics as well as thyroid biomarkers [median (IQR) or N (%)] among 339 women in the Environment and Reproductive (EARTH) Study.

Age, years	**34 (32, 38)**
White race, N (%)	**281 (83)**
Body Mass Index, kg/m^2	**23.2 (21.2, 26.2)**
Ever smoker, N (%)	**90 (26)**
Education, N (%)	
High school/some college	67 (20)
College graduate	99 (29)
Graduate degree	173 (51)
Initial fertility diagnosis, n (%)	
Male factor	124 (24)
Female factor	146 (28)
Unexplained	250 (48)
TSH (mU/L)	**1.85 (1.40, 2.60)**
Free T4 (pmol/L)	**15.5 (14.1, 16.7)**
Total T4 (nmol/L)	**96.8 (86.5, 110)**
Free T3 (pmol/L)	**4.80 (4.47, 5.21)**
Total T3 (nmol/L)	**1.79 (1.58, 2.06)**
TgAb positivity (>115 IU/mL), N (%)	**37 (11)**
TPOAb positivity (>35 IU/mL), N (%)	**35 (10)**

Figure 1. Associations between exposures and thyroid function in linear models.



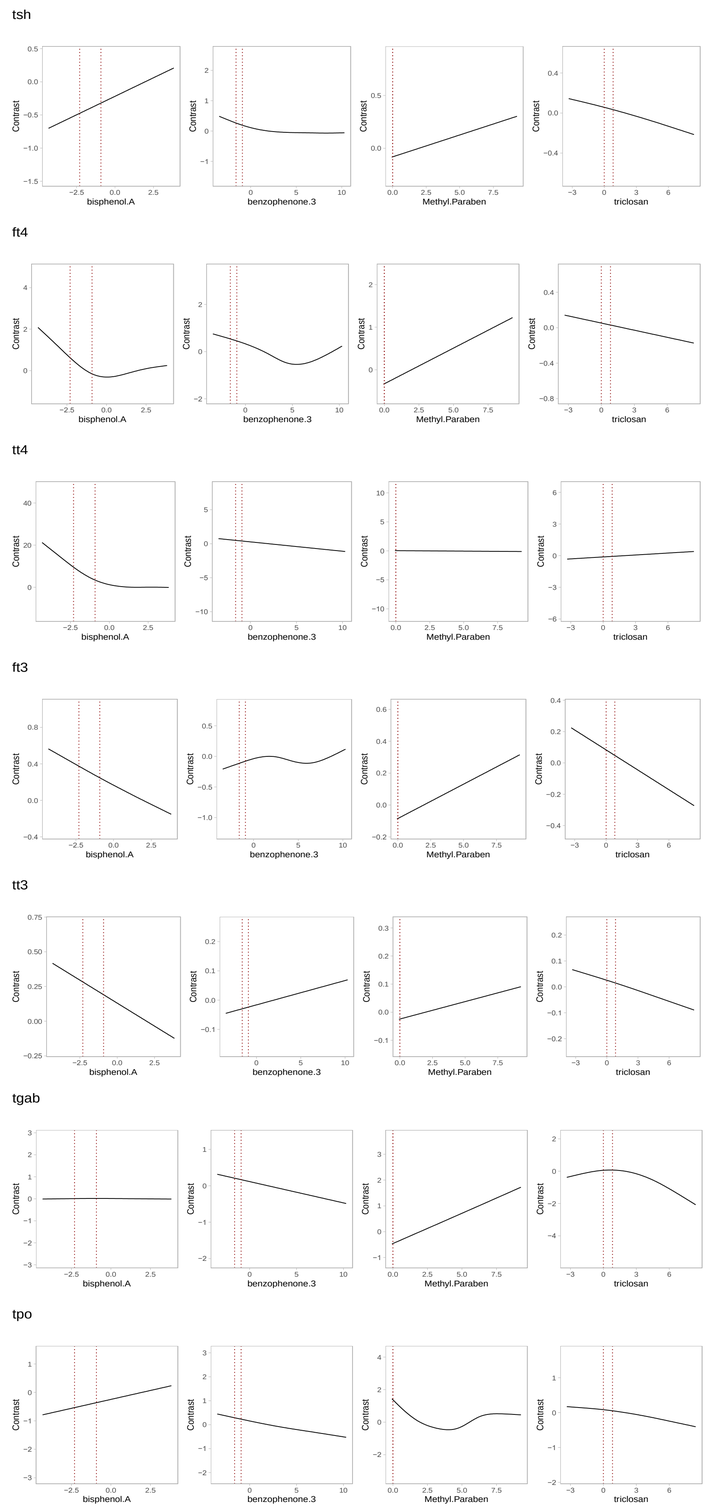
Estimates and corresponding 95% confidence intervals of mean differences (for continuous outcomes) and odds ratios (for binary outcomes) for a 1 log unit increase in concentration. Univariate corresponds to analyses with a single mixture component; multiple corresponds to mixture models with all four components. Models were adjusted for age (years), BMI (kg/m2), race (white vs. other), and specific gravity (SG).

Table 2. Estimates of overall mixture association, comparing 75th to 25th percentiles of all mixture components simultaneously.

	Linear		Additive	
	Est	95% CI	Est	95% CI
TSH	0.11	(-0.20, 0.42)	0.13	(-0.70, 0.97)
fT_4	-0.09	(-0.68, 0.49)	-0.13	(-1.36, 1.11)
TT4	-3.57	(-9.25, 2.12)	-3.14	(-6.72, 0.44)
fT_3	-0.19	(-0.35, -0.03)	-0.26	(-0.94, 0.41)
TT_3	-0.09	(-0.21, 0.02)	-0.09	(-0.58, 0.39)
TgAb	0.97	(0.37, 2.54)	0.90	(0.20, 4.07)
TPOAb	0.84	(0.33, 2.17)	1.29	(0.27, 6.25)

Overall associations shown for multiple (generalized) linear models and (generalized) additive models. Est is estimated mean difference (or odds ratio for binary outcomes) comparing 75th to 25th percentiles of exposure biomarker concentration; 95% CI is corresponding confidence interval.

Figure 2. Mixture analysis: Additive model results for continuous and binary outcomes.



Curves represent estimated mean differences compared to median log concentration. Each row corresponds to a different model. Models were adjusted for age (years), BMI (kg/m2), race (white vs. other), and specific gravity (SG).

Discussion

- In models assessing phenols individually (Figure 1), we observed that **BPA was positively associated with TSH and negatively associated with fT4, TT4, fT3 and TT3 concentrations**. Furthermore, **methylparaben was positively associated with serum concentrations of TSH, fT4, fT3 and TgAb, and triclosan was negatively associated with TSH, fT3, TT3 and and TgAb concentrations**. Multi-exposure mixture models yielded similar estimates (Figure 2).
- A strength of this study is the use of several statistical methods to **evaluate biomarker mixtures** including linear models, GAMs, and BKMR.
- Potential limitations include **generalizability** of the results to women in the general population because this study is restricted to women attending a fertility center, and the **cross-sectional** nature of this study. **Non-differential exposure misclassification** - because of the episodic exposure to the phenols examined and their relatively short biological half-lives, especially when only including one urine sample per woman - could bias estimates to the null.

Conclusions

- In a sample of women attending a fertility center, we found that **urinary phenols** - specifically BPA, methylparaben and triclosan - were **associated with several serum markers of thyroid function and autoimmunity** in both single and multi-exposure mixture analyses.
- Further studies should evaluate **long-term consequences** as well as **biological mechanisms** (e.g. omics) explaining the observed findings.

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