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Exposure to bisphenol A, chlorophenols, benzophenones, and parabens in relation to reproductive hormones in healthy women: A chemical mixture approach

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Abstract

Background: Little is known about the associations of bisphenol A, chlorophenols, benzophenones, and parabens with reproductive hormone levels in women. Our goal was to evaluate the associations between repeated measures of these chemicals and their mixtures with reproductive hormones in women.

Methods: Longitudinal urine samples from healthy, premenopausal women ($n = 143$ with 3–5 urine samples each) were measured for bisphenol A, five chlorophenols (2,4-dichlorophenol (2,4-DCP), 2,5-dichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, triclosan), two ultraviolet (UV) filters (benzophenone-1, benzophenone-3), and eight parabens and their metabolites (benzyl, butyl, ethyl, heptyl, methyl, propyl, 4-hydro-xybenzoic acid (4-HB), 3,4-dihydroxybenzoic acid (3,4-DHB)) over two menstrual cycles. Estradiol, progesterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH) were measured in blood up to 8 times each menstrual cycle. Linear mixed models were used for both single and multi-chemical exposures estimated using principal component analysis. Four factors were identified including: paraben; paraben metabolites and BPA, phenols, and UV filters. Models were adjusted for creatinine, age, race, and body mass index and weighted with inverse probability of exposure weights to account for time varying confounding.

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Competing financial interests

The authors have no competing financial interests to disclose.

Appendix A. Supplementary material

Supplementary material to this article can be found online at <https://doi.org/10.1016/j.envint.2018.07.028>.

Results: In single-chemical models, 3,4-DHB was associated with estradiol (0.06 (95% confidence interval (CI):0.001, 0.12)), 2–4-DCP with increased progesterone 0.14 (0.06, 0.21) and decreased FSH -0.08 (-0.11 , -0.04), and 4-HB was associated with increased FSH 0.07 (0.01, 0.13). In multi-chemical models, all factors were associated with increased progesterone (beta coefficient range: 0.15 for UV filter factor to 0.32 for paraben factor). The paraben factor and the paraben metabolite and BPA factor were associated with increased estradiol[0.21 (0.15, 0.28); 0.12 (0.07, 0.18)]. The phenol and UV filter factors were associated with decreased estradiol, FSH, and LH. The UV filter factor showed the strongest inverse association with estradiol -0.16 (-0.22 , -0.10), FSH -0.12 (-0.17 , -0.07), and LH -0.17 (-0.23 , -0.10).

Conclusion: Mixtures of phenols were associated with changes in reproductive hormones. Such changes could contribute to adverse health in women but additional research is necessary.

1. Introduction

Endocrine disrupting chemicals (EDCs) are exogenous substances which can influence endogenous hormone regulation (Richter et al., 2007; Vandenberg et al., 2007). Humans are widely exposed to EDCs, which include phenolic chemicals. In fact, exposure to parabens, one example of such chemicals, is much higher in women than in men due to the use of these estrogenic chemicals in many cosmetics and personal care products (Wang et al., 2013). Exposure to environmental phenolics is continuous as they are widely detectable despite being rapidly metabolized (CDC, 2009; Wang and Kannan, 2013). It has been suggested that BPA can affect hypothalamic-pituitary-ovarian axis function and estrous cycling in animals (Ziv-Gal and Flaws, 2016). Similar experimental studies show that parabens are estrogen agonists (Darbre and Harvey, 2008; Sun et al., 2016), antiandrogenic (Chen et al., 2007) and inhibited cytochrome P450 substrates (Ozaki et al., 2016). In women, endocrine disrupting effects have been observed among women undergoing IVF, pregnant women, and women with and without occupational exposures, although findings are equivocal (Aker et al., 2016; Hao et al., 2011; Miao et al., 2015; Mínguez-Alarcón et al., 2017; Mok-Lin et al., 2010; Nishihama et al., 2016; Smith et al., 2013).

However previous studies tended to rely on single measures of exposure and evaluate individual chemicals, without consideration of mixtures that characterize real-world exposures. Furthermore, two potentially endocrine-active paraben metabolites (4-HB and 3,4-DHB) have not previously been studied in relation to reproductive hormones in women. Estimating exposure to chemical mixtures is critical to evaluate real-life contexts, which include simultaneous exposure to multiple chemicals. Understanding the relationships of bisphenol A, chlorophenols, benzophenones, and parabens with hormone levels among healthy women is critical to disentangling potential effects on other reproductive and endocrine influenced health outcomes, such as endometriosis, fertility, and hormonally influenced diseases.

Additionally, no studies that we are aware of have evaluated mixtures of multiple longitudinal measures of bisphenol A, chlorophenols, benzophenones, and parabens among non-pregnant or non-fertility treatment seeking women with reproductive hormone levels timed to key periods of variability across the menstrual cycle. Therefore, our goal was to

evaluate the associations between repeated measures of bisphenol A, chlorophenols, benzophenones, and parabens and their mixtures with reproductive hormones, among reproductive-aged women.

2. Methods

2.1. Study population

The study population has been described in detail previously (Wactawski-Wende et al., 2009). Briefly, participants were women aged 18–44, free of known chronic health conditions, and not using hormonal contraception who were recruited at the University at Buffalo research center from 2005 to 2007. Participants attended up to 8 clinic visits for up to two menstrual cycles of study. The present study utilized a subset of stored urine samples. Urine samples were selected at key menstrual cycle phases among 143 original cohort participants. Clinically relevant time points for hormone levels were sampled in this sub-study. For example, FSH levels are clinically relevant in infertility testing early in the menstrual cycle. Ovulatory function is similarly clinically important and may be evaluated either through extremely well-timed mid-cycle visits or more commonly, through measurement of luteal phase progesterone levels. Ovulatory cycles were sampled in the early follicular phase, at ovulation, and mid-luteal phase in cycle 1 and at ovulation in cycle 2 (Fig. 1). One hundred and twenty women had ovulatory cycles, resulting in 437 samples. The 23 women with anovulatory cycles, resulted in 72 anovulatory samples were additionally sampled at ovulation and mid-luteal phase of cycle 2. All such samples were subject to biospecimen availability (Pollack et al., 2016). A total of 509 urine samples were measured for environmental phenols. The University at Buffalo Health Sciences Institutional Review Board (IRB) approved the study and served as the IRB designated by the National Institutes of Health for this study under a reliance agreement. All participants provided written informed consent.

2.2. Environmental phenol analysis

Bisphenol A, chlorophenols, benzophenone, and parabens were measured, including: benzophenone-1 (BP1), benzophenone-3 (BP3), bisphenol A (BPA), 2,4-dichlorophenol (2,4-DCP), 2,5-dichlorophenol (2,5-DCP), 2,4,5-trichlorophenol (2,4,5-TCP), 2,4,6-trichlorophenol (2,4,6-TCP), triclosan, and six parabens (benzyl (BzP), butyl (BuP), ethyl (EtP), heptyl (HeP), methyl (MeP), propyl (PrP)), and two paraben metabolites (4-hydroxybenzoic acid (4-HB), 3,4-dihydroxybenzoic acid (3,4-DHB)). These chemicals were analyzed from spot urine samples stored in cryovials which were frozen at -80°C until analysis. Samples were shipped to the Wadsworth Center, Albany, New York, where they were analyzed using high-performance liquid chromatography coupled with API2000 electrospray triple-quadrupole mass spectrometry (HPLC-MS/MS) to quantify phenols and parabens (Asimakopoulou et al., 2014; Zhang et al., 2011). Samples were spiked with ^{13}C -labelled internal standards of the target analytes and enzymatically deconjugated and extracted by liquid-liquid extraction and analyzed by HPLC-MS/MS. The LOD was determined from the lowest concentration of the calibration standard and a nominal sample volume of 0.5 mL. All instrument derived values were recorded for those measures that were below the LOD and included in analysis. Quality assurance and control processes were

implemented in each batch and included a method blank, a spiked blank, and a pair of matrix-spiked sample duplicates. The laboratory has participated in several proficiency testing programs including that offered by the Centers for Disease Control and Prevention to validate the assay. Creatinine was measured using a Roche Cobas 6000 chemistry analyzer (Roche Diagnostics Inc., Indianapolis, IN) at the University of Minnesota Laboratory, the difference in total was due to sample volume. The creatinine coefficient of variability was 1.5% at 96.6 mg/dL, 4.3% at 18.4 mg/dL and the LOD was 1 mg/dL. Due to low volume, urines were diluted 1:5 (20 μ L urine +80 μ L saline) to obtain a creatinine value. 43 urine specimens had <20 μ L urine and, thus, did not have a creatinine measurement.

2.3. Hormone analysis

Fasting morning blood draws were immediately processed and frozen at -80°C . Mid-cycle visits were scheduled with the aid of a fertility monitor to improve capturing hormone levels around ovulation (Clearblue Easy Fertility Monitor; Inverness Medical, Waltham, Massachusetts) (Howards et al., 2009). Samples were sent to analytical laboratories after a participant's complete cycle (up to eight samples). Samples were analyzed consecutively, within a single run, to limit within-cycle analytical variability. Estradiol, progesterone, LH and FSH were measured at the Kaleida Health Center for Laboratory Medicine (Buffalo, NY, USA) using solid-phase competitive chemiluminescent enzymatic immunoassay by Specialty Laboratories, Inc. (Valencia, CA, USA) on the Diagnostics Product Corporation Immulite 2000 Analyzer (Siemens Medical Solutions Diagnostics, Deerfield, IL, USA). The interassay maximum coefficients of variation reported by the laboratory were 10% for estradiol, 14% for progesterone, 5% for LH and FSH.

2.4. Covariate assessment

Height and weight were measured to obtain body mass index (BMI) at the study enrollment visit by trained study staff using standardized protocols. Age, race, smoking, marital status, medical and reproductive history were obtained using validated questionnaires. All covariates were missing for <5% of participants and was due to non-response.

2.5. Statistical analysis

Descriptive characteristics of the study population and the distribution of phenol and paraben concentrations and hormone concentrations were examined. Correlation between chemicals and intraclass correlation coefficients (ICCs) were determined previously (Pollack et al., 2016). Separately for each outcome hormone including estrogen, progesterone, LH, and FSH; single chemical linear mixed models were run to determine the associations with bisphenol A, chlorophenols, benzophenones, and parabens. These models included natural log transformed hormone and chemical levels, and random intercepts for each woman to account for the dependence of repeated measures. Because concentrations of phenols and parabens were found to vary across multiple time points, we used continuous, time-varying measures of these exposures in our longitudinal models. Both un-adjusted and adjusted models were fitted. Confounding factors were selected based on a priori evidence (Hernán et al., 2002). Models were adjusted for age (continuous), BMI (continuous), race (white/black/other), and urinary creatinine (log transformed continuous). To account for time-dependent confounding from reproductive hormones and other chemicals, marginal structural models

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were used with inverse probability of exposure weights (Robins et al., 2000). Even with high levels of exposure variability, weighted approaches have been recommended (Cole and Hernan, 2008). Chemical-specific weights were generated by menstrual cycle phase, reflecting follicular, ovulatory and luteal phases in the first cycle and the ovulatory phase of the second cycle. By fitting a linear model using the natural log transformation of the hormones and chemicals, we are approximating a nonlinear model. Therefore, the beta coefficients reported in the results can be roughly interpreted as a percent change in outcome in relation to a 1% change in the exposure. Secondary single chemical analyses utilized shifted hormone measures, which were realigned over time to improve the shape of the hormonal profile (Mumford et al., 2011). Based their distribution, chemicals that were below the LOD for >40% of the samples were evaluated as greater than and less than the LOD in relation to reproductive hormones in mixed models.

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To evaluate the association between multiple chemical exposures and hormones, a principal component analysis (PCA) approach was applied. A modified PCA approach was applied as in previous studies of environmental mixtures (Ito et al., 2004; Kioumourtzoglou et al., 2014; Thurston and Spengler, 1985). Briefly, PCA is a dimension reduction approach that reduced the chemicals into a smaller number of un-correlated factors that account for the majority of the variability in the exposures (Jolliffe, 2002). Repeated chemical exposures were coded into phases, with time points 1–3 reflecting follicular, ovulatory and luteal phases of cycle 1 and time 4 reflecting the ovulatory phase of cycle 2. PCA was applied to each time point separately. For each time point, a number of factors were chosen based on the scree plot (Brown) and the number of eigenvalues greater than one (Krall et al., 2017). Then, varimax rotation was applied to obtain more interpretable factors and principal component scores were computed for each participant. Last, to combine and compare principal components across time points, a hierarchical PCA approach (Crainiceanu et al., 2011; Krall et al., 2017) was applied. The resulting four principal components represented multi-chemical exposures and were included in both single factor and multiple factor linear mixed models with random intercepts. Statistical significance was defined as a p-value <0.05. Analyses were performed using the statistical packages SAS version 9.4 (SAS Corp, Cary, NC, USA) and R version 3.4 (R Core Team, Vienna, Austria).

3. Results

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The study participants were largely non-Hispanic white, nulliparous, nonsmokers, with a mean age of 27 years (Table 1). ICCs ranged from 0.04 (95% CI 0.01, 0.25) for BPA to 0.67 (95% CI 0.60, 0.74) for BP-3 (Pollack et al., 2016). These participants are representative of the full study cohort, with similar mean age and BMI, and comparable along race and education as well. All individuals had levels of 2,4,6-TCP, triclosan, methyl paraben and 4-HB above the LOD (Table 2). Benzyl and heptyl paraben and 2,4,5-TCP were below the LOD for >45% of values and were not included in subsequent analyses. Hormone concentrations including range and interquartile range are presented in Table 3.

Single chemical adjusted models for the phenols showed a statistically significant increase in estradiol for 3,4-DHB [β 0.06 (95% confidence interval (CI): 0.001, 0.12)], and an increase in progesterone for 2,4-DCP [β 0.14 (95% CI: 0.06, 0.21)] (Table 4). 2,4-DCP was

associated with a decrease in FSH [β -0.08 (95% CI: -0.11, -0.04)] while 4-HB was associated with increased FSH [β 0.07 (95% CI: 0.01, 0.13)] (Table 5). Bisphenol A, chlorophenols, UV filters, and parabens were not associated with LH in single chemical models (Table 5).

Using the hierarchical PCA approach on the 13 log transformed phenols and parabens, four factors were identified. Factor 1 was a paraben factor consisting of butyl, ethyl, methyl, and propyl paraben; factor 2 was a phenol factor that consisted of 2,4,6-TCP, 2,4-DCP, 2,5-DCP, and triclosan; factor 3 was a paraben metabolite and BPA factor that consisted of 3,4-DHB, 4-HB, and BPA; factor 4 was a ultraviolet (UV) filter factor that consisted of benzophenone 1 and 3. These factors explained approximately 62–71% of the variability in the chemicals for each exposure time point. The factor loadings are displayed in Supplemental Fig. 1. It is worth noting that because the PCA results are unscaled, the magnitudes of the β coefficients are not directly comparable between the single chemical and multi-chemical approaches. However, the directions of the identified associations can be compared.

The paraben factor and the paraben metabolite and BPA factor were associated with increased estradiol in single factor models, while the phenol factor and the UV filter factor were associated with decreased estradiol in single factor models (Table 6). Including all factors simultaneously, associations were found for increased estradiol and parabens [β 0.21 (95% CI: 0.15, 0.28)] and the paraben metabolite and BPA factor [β 0.12 (95% CI: 0.07, 0.18)]. After controlling for all factors, the phenol and UV filter factors were associated with decreased estradiol [phenol factor β -0.08 (95% CI: (-0.14, -0.02)), UV filter factor β -0.16 (95% CI: (-0.22, -0.10))]. In single factor models, parabens and the paraben metabolite and BPA factor were associated with increased progesterone whereas when modeled together, all factors were associated with increased progesterone (Table 6). The phenol factor and the UV filter factor were associated with decreased FSH and LH in both single factor models and when all factors were modeled together (Table 6). In single factor models, parabens and the paraben metabolite and BPA factors were associated with increased LH.

To further examine whether our findings were influenced by the shape of the hormonal profile, a secondary analysis was undertaken using shifted hormone levels, which have been previously shown to improve the shape of the hormonal profile (Mumford et al., 2011). Propyl paraben was associated with increased estradiol, while BPA was associated with increased progesterone (Supplemental Table 1). 2,4-DCP was consistently associated with increased progesterone and decreased FSH but was also associated with statistically significantly increased LH (Supplemental Table 2). Propyl paraben was associated with statistically significantly increased LH when using the shifted hormone profile levels (Supplemental Table 2). Chemicals with >40% of values below the LOD, which were not included in other models, were not associated with reproductive hormones in models that dichotomized exposure above and below the LOD (Supplemental Table 3).

4. Discussion

To our knowledge, this study is the first to examine mixtures of 13 nonpersistent chemicals including bisphenol A, chlorophenols, benzophenones, and parabens on reproductive hormones in healthy women, using multiple measures of exposure, appropriately addressing time varying confounding and employing a multi-chemical approach. Broadly, the results from the multi-chemical and single chemical approaches generally led to findings in the same direction, although results from single chemical models were not consistently statistically significant. The multi-chemical approach may more closely reflect the biologic pathways that these exposures share. All multi-chemical factors were associated with the ovarian hormones estradiol and progesterone. In the multi-chemical approach, the paraben factor and the paraben metabolites and BPA factor were associated with increased estradiol. The phenol and UV filter factors were associated with decreased estradiol when modeled separately and together. All factors were associated with increased progesterone when modeled together. The phenol and UV filter factors were associated with decreased FSH and LH when modeled together. Given that levels of exposure in the present study are similar to those in nationally-representative samples, these findings await corroboration in other cohorts. Additionally, although the multi-chemical PCA approach is straightforward to apply, the resulting principal component factors can be challenging to interpret because they represent multiple chemical exposures. Therefore, the estimated associations between multi-chemical factors and health should be interpreted with care.

Our findings of increased estradiol in relation to factors consisting of parabens, and paraben metabolites and BPA, in multi-chemical models are consistent with the available evidence. A study found shorter menstrual cycle length in relation to total, ethyl, and butyl paraben exposure (Nishihama et al., 2016), and shorter cycles are related to estradiol levels (Mumford et al., 2012). Experimental studies have similarly found estrogenic effects of paraben exposure (Taxvig et al., 2008). In pregnant women, butyl paraben was associated with decreased estradiol, while methyl and propyl paraben were non-significantly associated with decreased and then increased estradiol later in pregnancy, suggesting changes depending on timing during pregnancy (Aker et al., 2016). A suggested trend of decreased antral follicle count was observed among women seeking fertility treatment in relation to increased levels of propyl paraben (Smith et al., 2013). Among women seeking fertility treatment, BPA was associated with decreased antral follicle count but not with FSH levels (Souter et al., 2013). These findings may not be directly generalizable, given that our study population was not seeking fertility treatment nor pregnant. Our findings underscore that mixtures of phenols and parabens may influence ovarian hormone levels.

Our finding that phenols and UV filters were associated with decreased estradiol in multi-chemical models is novel. Human estrogen receptors showed antiestrogenic, estrogenic, and androgenic activity after exposure to UV filters, underscoring that these compounds may act via multiple modes of action (Kunz and Fent, 2006). Estrogen inhibition from triclosan exposure was found in sheep placental tissue, indicating competitive binding by triclosan to the estrogen receptor (James et al., 2010). In human, no differences in hormone levels were observed following sunscreen application (Janjua et al., 2004). Lower birth weight was associated with 2,4-DCP in a prospective cohort study (Philippat et al., 2012), and our

findings of decreased estradiol in relation to a phenol factor support this. Most available human studies of ovarian hormones and phenols are among women seeking fertility treatment (Bloom et al., 2011; Ehrlich et al., 2012; Mínguez-Alarcón et al., 2015b, 2015a; Mok-Lin et al., 2010; Smith et al., 2013). In contrast to our finding of no association in a single chemical model with BPA and estradiol, among girls with precocious puberty, BPA was associated with increased estradiol (Lee et al., 2014). Caution is necessary to avoid over-interpreting these novel multi-chemical findings in relation to single-chemical studies and future studies should consider multi-chemical approaches to confirm these results.

No previous studies that we are aware of have reported associations between multi-chemical models of parabens, benzophenones, and phenols in relation to increased progesterone. Study participants largely had progesterone levels within the clinically normal range, but the implications for the influence of increasing progesterone should be investigated further. Adequate progesterone levels are critical to early pregnancy maintenance and to sufficient luteal phase lengths, perhaps suggesting that these findings may not be harmful. Fertility treatment success was lower among women with higher early follicular phase progesterone levels, which may have occurred due to the cessation of progesterone production from the corpus luteum (Kolibianakis et al., 2004) and a meta-analysis found that elevated progesterone levels in the follicular phase were associated with lower pregnancy rates in women seeking in vitro fertilization treatment (Hamdine et al., 2014).

Phenols were associated with decreased pituitary hormone FSH and LH levels in the present multi-chemical approach, which is novel. Experimental studies show that benzophenones (Kawamura et al., 2003; Suzuki et al., 2005; Weisbrod et al., 2007), dichlorophenols (Ma et al., 2012), and triclosan (Kumar et al., 2009; Stoker et al., 2010) influence the endocrine system (Kumar et al., 2009). Among women undergoing in vitro fertilization, triclosan was associated with a decline in implantation and embryo quality (Hua et al., 2017) and with lower antral follicle count (Mínguez-Alarcón et al., 2017). This is in contrast to our finding of decreased FSH for the phenol factor, which included triclosan. Our findings that in single chemical models, phenols were not associated with estradiol are in agreement with the other available human study (Aker et al., 2016). However, we found that 2,4-DCP was associated with progesterone in single chemical models, in contrast with the findings of Aker and colleagues. This may stem from differences in study population as participants in this study were not pregnant or differences in our use of a multi-chemical approach.

The potential mechanism underlying these associations with reproductive hormones is unclear. Parabens and phenols could influence hormone levels via upregulating estrogen receptor α (Sun et al., 2016; Vo et al., 2011) or by disrupting cholesterol transport, thereby impairing steroid hormone synthesis (Taxvig et al., 2008) and there is also evidence that estrogen receptor β may be preferentially bound by parabens (Watanabe et al., 2013). Taken together, our findings, if confirmed, may have implications at the population level for fertility and other hormonally-mediated chronic diseases.

This study had several strengths. All participants had multiple phenol and paraben measurements taken at key time points of hormonal variability across two menstrual cycles. This enabled the evaluation of associations with chemicals across the menstrual cycle and

improved upon studies that relied on a single or even two measures of exposure of such short-lived chemicals. The study participants were healthy, with no known reproductive pathology, which diminishes uncontrolled confounding playing a role in our findings, although such residual confounding remains a possible explanation for our findings. Smoking levels were extremely low, minimizing the influence of smoking on our findings. The urine samples used for chemical measurement were processed under a standardized protocol and were measured using state-of-the art methods, minimizing important sources of laboratory measurement error. Yet, this study had some limitations. Measurement error may have influenced the chemical or hormone biomarker measurement process (White, 2011). Although conventionally believed to only result in bias toward the null, this is not always the case, and even nondifferential measurement error may lead to bias away from the null, particularly with strongly correlated exposures and correlated error (Pollack et al., 2013). It is important to note that some observed associations may be due to chance, particularly given the number of comparisons. Based upon an alpha cutoff of 0.05, we would expect to find less than five associations by chance, but more than five times that were observed, indicating that such associations were beyond those expected by chance.

Multi-chemical approaches better characterize real world exposures and make it possible to evaluate associations with health outcomes thought to come about through a single pathway, rather than considering the influence of such exposures individually. Studies of endocrine disrupting chemicals and human health outcomes that use multi-chemical approaches remain limited. Such approaches have been recommended (Birnbaum et al., 2016; Miller et al., 2017) as they better reflect real-world exposures compared with single chemical models. PCA has several advantages, which include the ability to address multicollinearity because estimated factors are orthogonal. Each PCA factor reflects a weighted combination of the chemicals that is generated without respect to the outcome. This method does have some limitations, such as the inability to evaluate interactions between chemicals and the inability to identify a single chemical that is most related to a particular health outcome. PCA is a straight-forward multi-chemical approach that works well when there is not strong prior knowledge about the nature of multi-chemical exposures. Future research may consider other approaches to handle multiple chemical exposures, such as other dimension reduction approaches (Carrico et al., 2015; Winkquist et al., 2014), variable selection methods (Czarnecka et al., 2015; Lenters et al., 2016), and Bayesian methods (Bobb et al., 2015).

5. Conclusions

In this study population of reproductive-aged women, phenols and parabens were associated with changes in ovarian and pituitary hormone levels. A particular strength of this study was the measurement of exposures across the menstrual cycle, which improved upon research that relied on one or two measures of nonpersistent chemicals. Additionally, this study utilized a multi-chemical approach to reflect real-world environmental exposures. This supports that low-level exposure to mixtures of ubiquitous endocrine disrupting chemicals may play a role in altering reproductive hormone levels, with potential subsequent implications for hormonally-mediated diseases across the life course. However, these findings must be interpreted with caution and await corroboration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Sampling approach for urinary biospecimens by menstrual cycle under study, cycle phase and ovulation status. Light blue bars indicate stored biospecimens, purple dots indicate samples measured for all participants in this sub-study, blue lines indicates additional samples measured for anovulatory cycles.

Table 1Participant characteristics compared to full cohort ($n = 143$).

Characteristic	n (%) or mean $\pm$ SD	Full cohort (%) or mean $\pm$ SD
Age (years)	27 ± 8	27.4 ± 8.2
BMI (kg/m ²)	24 ± 4	24.1 ± 3.9
Race		
White	90 (63)	148 (57)
Black	29 (20)	51 (20)
Other	24 (17)	60 (24)
Married	34 (24)	67 (26)
< High school	19 (13)	32 (13)
Smoker (current)	6 (4)	10 (4)
Nulliparous	107 (77)	187 (74)
Alcohol (avg. drink/day)	0.43 ± 0.6	0.43 ± 0.6

Table 2

Urinary chemical concentrations and percentage of quantifiable samples for bisphenol A, chlorophenols, benzophenones, and parabens ($n = 509$).

Chemical (ng/mL)	LOD ^e	< LOD (%)	Minimum	Q1	Median	Q3	Maximum
Bisphenol A	0.02	0.98	0.007	1.3	2.8	6.8	302.8
Triclosan	0.02	0.00	0.3	7.2	22.2	73.5	1222.2
2,4-DCP ^a	0.50	35.36	0.03	0.03	0.86	2.03	38.6
2,5-DCP ^a	0.50	1.18	0.1	9.9	19.1	34.9	803.5
2,4,5-TCP ^b	0.08	49.12	0.003	0.04	0.08	0.14	2.8
2,4,6-TCP ^b	0.50	0.00	0.03	0.2	0.27	0.5	8.5
Benzophenone-1	0.05	0.79	0.01	1.0	4.1	10.9	117.2
Benzophenone-3	0.02	0.59	0.004	1.2	3.8	14.3	1422.1
Parabens							
Benzyl	0.02	74.66	0.001	0.003	0.004	0.02	1.7
Butyl	0.02	26.33	0.001	0.02	0.5	2.3	132.2
Ethyl	0.05	15.72	0.003	0.1	1.1	4.8	473.8
Heptyl	0.02	98.82	0.002	0.003	0.007	0.008	0.7
Methyl	0.05	0.00	0.6	16.9	59.0	145.1	4802.6
Propyl	0.05	2.95	0.003	3.6	15.1	48.1	2033.3
4-HB ^c	0.20	0.00	15.4	190.8	389.1	889.4	5823.2
3,4-DHB ^d	0.50	0.39	0.2	109.6	344.8	821.1	7739.0

^aDichlorophenol.

^bTrichlorophenol.

^cHydroxybenzoic acid.

^dDihydroxybenzoic acid.

^eLimit of detection.

Table 3

Ovarian and pituitary hormone concentrations across the menstrual cycle among premenopausal women ($n = 143$).

Hormone	Minimum	Q1	Median	Q3	Maximum
Progesterone (ng/mL)	0.14	0.50	1.0	4.9	27.7
Estradiol (pg/mL)	6.90	46.0	82.0	151.0	833.8
Follicle stimulating hormone (mIU/mL)	0.40	3.7	5.6	7.70	47.1
Luteinizing hormone (ng/mL)	0.10	3.7	5.7	9.7	101.0

Table 4

Single chemical linear mixed models of natural log-transformed ovarian hormones associated with urinary bisphenol A, chlorophenols, benzophenones, and parabens in premenopausal women ($n = 143$).

Chemical	Estradiol		Progesterone	
	Unadjusted ^a	Adjusted ^b	Unadjusted ^a	Adjusted ^b
Bisphenol A	0.03 (−0.03, 0.10)	0.01 (−0.11, 0.12)	0.06 (−0.03, 0.14)	0.08 (−0.01, 0.18)
Triclosan	−0.005 (−0.06, 0.05)	−0.02 (−0.07, 0.04)	0.010 (−0.06, 0.08)	0.01 (−0.08, 0.06)
2,4-DCP ^c	−0.01 (−0.08, 0.05)	0.01 (−0.05, 0.06)	0.05 (−0.03, 0.14)	0.14 (0.06, 0.21)
2,5-DCP ^c	0.01 (−0.06, 0.08)	−0.03 (−0.10, 0.03)	0.03 (−0.05, 0.12)	−0.06 (−0.14, 0.03)
2,4,6-TCP ^d	0.06 (−0.03, 0.16)	0.092 (−0.01, 0.19)	0.04 (−0.09, 0.16)	−0.011 (−0.14, 0.12)
BP1 ^e	−0.01 (−0.07, 0.04)	−0.01 (−0.06, 0.04)	0.02 (−0.05, 0.08)	0.01 (−0.06, 0.08)
BP3 ^f	−0.002 (−0.05, 0.04)	−0.03 (−0.08, 0.02)	0.03 (−0.03, 0.09)	−0.01 (−0.07, 0.06)
Parabens				
Butyl	0.003 (−0.03, 0.03)	0.005 (−0.02, 0.03)	−0.008 (−0.05, 0.03)	0.01 (−0.03, 0.05)
Ethyl	0.004 (−0.03, 0.04)	−0.009 (−0.04, 0.02)	−0.03 (−0.07, 0.02)	−0.03 (−0.08, 0.01)
Methyl	0.01 (−0.04, 0.07)	−0.014 (−0.07, 0.04)	−0.02 (−0.09, 0.05)	−0.05 (−0.12, 0.023)
Propyl	0.01 (−0.03, 0.06)	0.02 (−0.02, 0.07)	0.002 (−0.06, 0.06)	0.01 (−0.05, 0.07)
4-HB ^g	0.04 (−0.05, 0.12)	0.058 (−0.03, 0.14)	0.11 (0.003, 0.22)	−0.03 (−0.15, 0.08)
3,4-DHB ^h	0.07 (0.01, 0.13)	0.06 (0.001, 0.12)	0.04 (−0.04, 0.12)	0.04 (−0.04, 0.12)

Bold text indicates $p < 0.05$.

^a Adjusted for natural log-transformed creatinine.

^b Adjusted for natural log-transformed creatinine, age, race, and BMI and weighted using inverse probability of exposure weights.

^c Dichlorophenol.

^d Trichlorophenol.

^e Benzophenone-1.

^f Benzophenone-3.

^g Hydroxybenzoic acid.

^h Dihydroxybenzoic acid.

Table 5

Single chemical linear mixed models of natural log-transformed pituitary hormones associated with urinary bisphenol A, chlorophenols, benzophenones, and parabens in premenopausal women ($n = 143$).

Chemical	Follicle stimulating hormone		Luteinizing hormone	
	Unadjusted ^a	Adjusted ^b	Unadjusted ^a	Adjusted ^b
Bisphenol A	−0.03 (−0.08, 0.02)	0.03 (−0.04, 0.10)	−0.01 (−0.08, 0.05)	0.08 (−0.05, 0.21)
Triclosan	0.007 (−0.03, 0.04)	0.003 (−0.03, 0.04)	0.007 (−0.04, 0.05)	−0.01 (−0.05, 0.04)
2,4-DCP ^c	−0.01 (−0.05, 0.04)	−0.08 (−0.11, −0.04)	−0.01 (−0.07, 0.05)	0.002 (−0.05, 0.06)
2,5-DCP ^c	0.02 (−0.02, 0.07)	0.001 (−0.04, 0.05)	−0.03 (−0.09, 0.03)	−0.05 (−0.11, 0.01)
2,4,6-TCP ^d	0.02 (−0.05, 0.09)	0.024 (−0.04, 0.09)	0.02 (−0.07, 0.12)	0.04 (−0.06, 0.15)
BP1 ^e	−0.02 (−0.06, 0.01)	−0.002 (−0.04, 0.03)	−0.01 (−0.06, 0.03)	−0.04 (−0.08, 0.01)
BP3 ^f	−0.02 (−0.05, 0.01)	−0.02 (−0.05, 0.01)	−0.02 (−0.05, 0.02)	−0.04 (−0.08, 0.01)
Parabens				
Butyl	0.007 (−0.01, 0.03)	−0.01 (−0.02, 0.02)	0.002 (−0.02, 0.03)	0.01 (−0.01, 0.04)
Ethyl	0.02 (−0.002, 0.04)	0.01 (−0.02, 0.03)	0.01 (−0.02, 0.04)	−0.003 (−0.03, 0.03)
Methyl	0.04 (0.006, 0.08)	0.01 (−0.03, 0.04)	0.006 (−0.04, 0.06)	−0.002 (−0.05, 0.05)
Propyl	0.04 (0.006, 0.07)	0.01 (−0.02, 0.04)	0.005 (0.005, 0.05)	0.02 (−0.02, 0.06)
4-HB ^g	−0.005 (−0.07, 0.05)	0.07 (0.01, 0.13)	−0.004 (−0.08, 0.07)	0.02 (−0.07, 0.11)
3,4-DHB ^h	0.01 (−0.03, 0.05)	0.01 (−0.04, 0.05)	0.03 (−0.02, 0.09)	0.04 (−0.01, 0.10)

Bold text indicates $p < 0.05$.

^a Adjusted for natural log-transformed creatinine.

^b Adjusted for natural log-transformed creatinine, age, race, and BMI and weighted using inverse probability of exposure weights.

^c Dichlorophenol.

^d Trichlorophenol.

^e Benzophenone.

^f Benzophenone.

^g Hydroxybenzoic acid.

^h Dihydroxybenzoic acid.

Table 6

Association of ovarian and pituitary hormones with bisphenol A, chlorophenol, benzophenone, and paraben factors from principal component analysis in single- and multiple-factor linear mixed models ($n = 143$).

Chemical	Single-factor model β (95% CI)	Multi-factor model β (95% CI)	Single-factor model β (95% CI)	Multi-factor model β (95% CI)
	Estradiol		Progesterone	
Paraben ^a	0.25 (0.20, 0.31)	0.21 (0.15, 0.28)	0.16 (0.08, 0.24)	0.32 (0.23, 0.41)
Phenol ^b	-0.20 (-0.25, -0.15)	-0.08 (-0.14, -0.02)	0.04 (-0.03, 0.11)	0.26 (0.18, 0.35)
Paraben metabolite & BPA ^c	-0.30 (-0.36, -0.24)	0.12 (0.07, 0.18)	0.09 (0.03, 0.15)	0.27 (0.19, 0.35)
UV filter ^d	0.15(0.11, 0.20)	-0.16 (-0.22, -0.10)	-0.01 (-0.09, 0.08)	0.15 (0.06, 0.24)
Chemical	Single-factor model β (95% CI)	Multi-factor model β (95% CI)	Single-factor model β (95% CI)	Multi-factor model β (95% CI)
	Follicle stimulating hormone		Luteinizing hormone	
Paraben ^a	0.02 (-0.02, 0.06)	-0.04 (-0.09, 0.01)	0.10 (0.04, 0.15)	0.05 (-0.01, 0.12)
Phenol ^b	-0.07 (-0.11, -0.03)	-0.11 (-0.16, -0.06)	-0.16 (-0.22, -0.11)	-0.05 (-0.22, -0.09)
Paraben metabolite & BPA ^c	0.02 (-0.01, 0.06)	-0.03 (-0.08, 0.01)	0.11 (0.07, 0.16)	0.05 (-0.01, 0.11)
UV filter ^d	-0.09 (-0.13, -0.04)	-0.12 (-0.17, -0.07)	-0.15 (-0.21, -0.09)	-0.17 (-0.23, -0.10)

All models adjusted for age, BMI, race, and creatinine.

Bold text indicates $p < 0.05$.

^aParaben factor: butyl, ethyl, methyl, and propyl paraben.

^bPhenol factor: 2,4,6-TCP, 2,4-DCP, 2,5-DCP, and triclosan.

^cParaben metabolite and BPA factor: 3,4-DHB, 4-HB, and BPA.

^dUltraviolet (UV) filter factor: benzophenone 1 and 3.