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Repeated measures analysis of associations between urinary bisphenol-A concentrations and biomarkers of inflammation and oxidative stress in pregnancy

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Abstract

Bisphenol-A (BPA) exposure occurs commonly and may adversely impact pregnancy. Endocrine disruption is posited as the primary mechanism of action, but oxidative stress and inflammation pathways may also be important. We investigated associations between BPA exposure and oxidative stress and inflammation in 482 pregnant women. Participants were recruited early in pregnancy and provided urine and plasma at up to four visits. We measured total BPA and two biomarkers of oxidative stress (8-hydroxydeoxyguanosine and 8-isoprostane) in urine from each visit. Inflammation markers, including C-reactive protein and four cytokines were measured in plasma from the same time points. In adjusted models, an interquartile range increase in BPA was associated with significant increases in both oxidative stress biomarkers (5–9% increase). Additionally, we observed significantly higher IL-6 concentrations in association with an interquartile range increase in BPA (8.95% increase). These systemic changes consequent to BPA exposure may mediate adverse birth outcomes and/or fetal development.

Keywords

BPA; oxidative stress; inflammation; IL-6; pregnancy; biomarkers; longitudinal

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1. Introduction

Bisphenol-A (BPA) is a high production volume chemical that is used in the synthesis of polycarbonate plastics and epoxy resins. These materials are frequently used for food packaging and storage, and leaching of BPA into food and beverages commonly occurs, making the primary route of exposure ingestion.¹ Exposure in the US has declined significantly in recent years, as illustrated by a decrease in geometric mean urinary concentrations from 2.64 ng/mL in 2003–2004 to 1.5 ng/mL in 2009–2010.² However despite lower exposure levels, detection of levels in urine and other biological matrices remains prevalent (~90%) in the US population and elsewhere.^{2,3}

Maternal exposure to BPA during pregnancy has been associated with a number of adverse birth outcomes, including preterm birth,^{4,5} fetal growth restriction,⁶ and also with different facets of child neurodevelopment, behavior, growth, and metabolism.^{7–11} Studies investigating mechanism have highlighted endocrine disruption as a primary pathway of BPA action due to structural similarities between BPA and estradiol and consequent binding affinity for the estrogen receptor.¹² However, a number of *in vitro* and animal studies have also illustrated a capacity for BPA to induce oxidative stress and inflammation. One research group demonstrated that male rats exposed to BPA had increased reactive oxygen species release and lipid peroxidation in both epididymal sperm and liver cells.^{13,14} Turning to inflammatory markers, *in vitro* evidence shows that adipose tissue treated with BPA releases lower levels of adiponectin as well as higher levels of cytokines, including IL-6 and TNF- α , compared to control tissue.^{15,16} These observed effects may be the result of endocrine-related activity, but independent pathways are plausible also. For example, BPA may act as an arylhydrocarbon receptor and/or peroxisome proliferator activated receptor (PPAR) antagonist, preventing normal immune responses and resulting in pro-inflammatory activity.¹⁷

Despite this evidence, few studies have examined associations between BPA exposure and biomarkers of inflammation and/or oxidative stress in human populations. Furthermore, although consequences of exposure during pregnancy is of the utmost interest to both the scientific community and the public, only two small studies to date have examined these relationships in pregnant women.^{18,19} In the present study we examined the relationship between repeated measures of urinary BPA concentrations and a panel of oxidative stress and inflammation biomarkers in a large population of pregnant women in the US. As indicators of oxidative stress we measured urinary concentrations of 8-hydroxydeoxyguanosine (8-OHdG) and 8-isoprostane, which are widely used as indicators of systemic oxidative DNA damage and lipid peroxidation, respectively.^{20–22} To examine inflammation we measured plasma concentrations of C-reactive protein (CRP), an acute phase response protein used widely as a systemic biomarker in the study of cardiovascular disease as well as pregnancy,^{23,24} and have been used as indicators of response to a number of environmental exposures including BPA.^{25,26} Additionally, we measured a panel of plasma cytokines, including IL-1 β , IL-6, IL-10, and TNF- α , all of which have proven useful in previous studies examining associations with environmental contaminant exposures and/or pregnancy outcomes.^{27,28}

2. Materials and methods

2.1. Study population

We obtained IRB approval from the University of Michigan and Brigham and Women's Hospital. This analysis was performed on data collected as part of a nested case-control study designed to assess the relationship between phthalate and BPA exposure during pregnancy in relation to preterm birth. The study design has been described in detail elsewhere.²⁹ The base cohort population included women who delivered live singleton births at Brigham and Women's Hospital (Boston, MA) between 2006 and 2008. At the first study visit (<15 weeks gestation) women provided informed consent. For the nested case-control study, we selected from the study participants who had delivered live singleton newborns 130 women who delivered preterm (prior to 37 weeks completed gestation) and 352 women who delivered after 37 weeks. Urine and plasma samples, collected at four time points during pregnancy (visits 1–4 at median 10, 18, 26, and 35 weeks gestation, respectively), were extracted from –80 degrees Celsius freezer storage for analysis of biomarkers for exposure and outcome assessment purposes.

2.2. Exposure biomarker assessment

Total (free plus conjugated) BPA was measured in each urine sample collected (N=1695 samples, N=482 subjects) by NSF International (Ann Arbor, MI, USA) using isotope dilution liquid chromatography with tandem mass spectrometry. Total BPA was selected for measurement for consistency with gold standards established by the Centers for Disease Control and Prevention, and methods were developed based on their protocols as described elsewhere.³⁰ A panel of nine phthalate metabolites were also measured in urine samples by NSF International using similar methods; a detailed description as well as limits of detection, and distributions of levels have been previously published.^{30,31} Measurements of BPA below the limit of detection (LOD) were replaced with the LOD divided by the square root of 2. At the time of BPA measurement urine sample specific gravity was assessed using a digital refractometer (ATAGO Company Ltd., Tokyo, Japan). For the purposes of characterizing BPA distributions individual measurements were corrected for specific gravity using the following formula: $BPA_c = BPA[(1.015-1)/(SG-1)]$, where BPA_c indicates the specific gravity corrected concentration, BPA indicates the raw urinary BPA concentration, 1.015 is the median specific gravity level in all samples measured, and SG is the specific gravity of the individual sample. For modeling purposes, uncorrected BPA concentrations were used and specific gravity was included as a covariate.

2.3. Outcome biomarker assessment

Urine samples with sufficient remaining volume were also utilized for measurement of two biomarker of oxidative stress (N=1678 samples, N=482 subjects) including 8-isoprostane and 8-OHdG. Both were measured using enzyme immunoassay by Cayman Chemical Company (Ann Arbor, MI, USA). Inflammation biomarkers, including CRP as well as the cytokines IL-1 β , IL-6, IL-10, and TNF- α were measured in plasma from blood samples collected from participants at the same time as urine samples (N=1585 samples, N=482 subjects). CRP was measured using enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA) and cytokines were measured using the Milliplex MAP High

Sensitivity Human Cytokine Magnetic Bead Panel (EMD Millipore Corp., St. Charles, MO, USA). Analyses were performed at the Cancer Center Immunology Core at the University of Michigan (Ann Arbor, MI, USA). All outcome biomarker levels reported below the LOD were kept as is and others were imputed as the LOD divided by the square root of 2.

2.4. Statistical analysis

All analyses were performed using R version 3.1.0. Additionally, unless otherwise noted, all analyses were adjusted for the study design by using inverse probability weightings so that the results would not be impacted by the disproportionate number of preterm cases.³² We examined distributions of urinary BPA concentrations by demographic characteristics with geometric means and geometric standard deviations of specific gravity corrected concentrations in all samples measured, and tested for differences across groups using linear mixed models with random intercepts for subject ID number to account for repeated measures. Covariates examined included maternal age; race/ethnicity (White, African-American, or Other); education level (high school, technical school, junior college or some college, or college graduate and above); health insurance provider (public—Medicaid, Supplemental Security Income, or MassHealth, or private—including HMO or self-pay); body mass index (BMI) at visit 1 (<25 kg/m²; 25 to <30 kg/m²; and >30 kg/m²); tobacco use during pregnancy (yes or no); alcohol using during pregnancy (yes or no); parity (nulliparous or parous); and fetal sex (male or female). Also in descriptive statistics we examined Spearman correlations between urinary BPA concentrations and levels of urinary phthalate metabolites, which had previously been examined in association with the inflammation and oxidative stress biomarkers included in the present analysis.^{31,33}

Linear mixed models were built using the nlme package in R to examine associations between urinary BPA concentrations and each individual outcome biomarker. All models included subject-specific random intercepts and slopes. First, models were created with adjustment for urinary specific gravity and gestational age at sample collection only (Model 1). Second, models were created with adjustment for covariates that were associated with both BPA and the outcome biomarker of interest (Model 2). Finally, a third model was created that included one of nine urinary phthalate metabolites measured, selected based on highest correlation with BPA and by strongest association with oxidative stress and inflammation markers in previous analyses. For all models results were presented as percent increase in outcome biomarker in association with an interquartile range (IQR) increase in BPA or, for Model 3, phthalate metabolite.

In sensitivity analyses, we additionally examined associations stratified by fetal sex, as previous studies have observed differences in BPA associations based on this variable. Additionally, we examined associations stratified by study visit in attempt to identify windows of vulnerability. Finally, for significant associations observed in linear analyses, we explored nonlinear associations using generalized additive mixed models.

3. Results

Distributions of population characteristics by raw numbers are presented in Table 1. The population was primarily White, well-educated, and had primarily private health insurance

providers, indicative of high socio-economic status. Very few of the study participants smoked or used alcohol during pregnancy, and for about half of mothers this was their first pregnancy. With study weightings, the proportion of deliveries that were preterm was consistent with what is approximately expected in the general US population (12% preterm, 88% term).

Of the 1695 samples measured for BPA, 282 (16.6%) were below the LOD. Specific gravity corrected BPA concentrations (geometric means and geometric standard deviations) by population characteristics are also presented in Table 1. BPA concentrations were significantly higher in mothers who identified as African-American or Other race/ethnicity compared to White; mothers with low socioeconomic status, as indicated by education level and health insurance provider; mothers in the highest category of pre-pregnancy BMI; and in mothers who reported smoking during pregnancy. We previously reported that concentrations were similar by study visit (geometric mean 1.32–1.38 ng/mL for specific gravity-corrected concentrations), but had low reproducibility (intraclass correlation coefficient=0.21).⁵

BPA concentrations showed weak to moderate but statistically significant correlations with urinary phthalate metabolites, including: mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), mono-2-ethyl-5-carboxypentyl phthalate (MECPP), mono-benzyl phthalate (MBzP), mono-*n*-butyl phthalate (MBP), mono-iso-butyl phthalate (MiBP), mono-ethyl phthalate (MEP), and mono-carboxypropyl phthalate (MCP) (Table 2). The strongest correlation coefficients were between BPA and MBzP or MBP (Spearman $R=0.25-0.34$, $p<0.01$).

Three different models of the associations between BPA concentrations and each oxidative stress biomarker are presented in Table 3. In Model 1, adjusted for specific gravity as well as gestational age at sample collection, BPA was significantly associated with increases in 8-OHdG as well as 8-isoprostane. The association for 8-OHdG was similar and the association for 8-isoprostane mildly attenuated when models were additionally adjusted for covariates that were associated with both BPA and at least one of the oxidative stress biomarkers, including race/ethnicity, education level, health insurance provider, and pre-pregnancy BMI (Model 2). Because it showed the greatest effect estimates for associations with both oxidative stress biomarkers in our previous analysis,³¹ and showed larger correlation coefficients with BPA compared to other phthalate metabolites, Model 3 additionally included MBzP. Associations in model 3 were reduced for both 8-OHdG (change from 8.95% in model 2 to 5.09% in model 3) and 8-isoprostane (change from 13.8% in model 2 to 8.79% in model 3) but maintained statistical significance.

Models for BPA associations with inflammation biomarkers are presented in Table 4. In Model 1, BPA was positively associated with all inflammation markers, and the association with IL-6 was greatest in magnitude and statistically significant (percent change [%] in IL-6 in association with an IQR increase in BPA=10.9, 95% confidence interval [CI]=3.97, 18.4). This association changed minimally with the addition of covariates in Model 2. Furthermore, the association was robust to the addition of MBzP in Model 3. The effect estimate was only slightly attenuated (% with IQR increase in BPA=8.95, 95% CI=1.81,

16.6), and remained statistically significant ($p=0.01$). The effect estimate for the association between IL-6 and MBzP reported in that model was similar to that previously reported when it was examined in relation to IL-6 alone.³³ Of the phthalate metabolites examined in association with these inflammation biomarkers, the greatest effect estimate for IL-6 was for MCP. Thus, we built an additional model including MCP and BPA as well as covariates in a sensitivity analysis. However, as with the model with MBzP, the association between BPA and IL-6 was similar to that observed in the single pollutant adjusted model (% in IL-6 in association with an IQR increase in BPA=7.81, 95% CI=0.74, 15.4).

In sensitivity analyses, we observed few differences in effects in models stratified by fetal gender. The one exception was that, for the association between BPA and IL-6, associations were stronger in mothers carrying female fetuses compared to males (p for interaction=0.12). When we examined an interaction between BPA and study visit, no significant terms were observed with the exception of the relationship with TNF- α . The interaction term indicated that associations between BPA and TNF- α were greater in magnitude at visits 2–4 compared to visit 1 (β for interaction=0.04, 0.08, and 0.06, respectively; p for interaction=0.05, <0.01, and 0.02, respectively). However, in stratified analyses, associations between BPA and TNF- α were variable and imprecise. Finally, we explored a potential non-linear relationship between BPA exposure and IL-6 using generalized additive mixed models, but this association appeared to be linear based on intersecting confidence intervals (data not shown).

4. Discussion

In the present analysis we utilized a large study population with repeated measurements of both exposure and outcome during pregnancy to adequately test this hypothesis. We observed that urinary BPA concentrations were associated with significant increases in two biomarkers of oxidative stress, including 8-isoprostane and 8-OHdG. We also observed a significant association between BPA and the inflammatory cytokine IL-6 which remained after adjusting for correlated phthalate exposure. These results support a role for BPA in increasing maternal systemic oxidative stress and inflammation levels during pregnancy.

Our findings for an association between BPA and IL-6 are consistent with the animal and *in vitro* literature, and additionally correspond to the limited human studies that have investigated this association. One study that examined associations with IL-6 levels specifically was a cross-sectional analysis of adult males ($N=76$), which the authors found that plasma BPA concentrations were positively associated with IL-6 levels.³⁴ Another large cross-sectional study of adults residing in major cities in Korea ($N=485$) observed positive associations between urinary BPA concentrations and CRP, but the association existed only amongst postmenopausal women.³⁵ Finally, a 2008 paper demonstrated significant associations between urinary BPA concentrations and the inflammation marker alkaline phosphatase, but not CRP, in a nationally representative US population.³⁶ As in the latter study, we observed no association between BPA and levels of CRP or other inflammatory cytokines measured in the present study. Multiple factors could explain the associations seen with IL-6 but not other inflammation biomarkers. For example, BPA may be causing changes that specifically stimulate IL-6 production, rather than systemic changes, e.g., the

release of IL-6 from adipocytes, as suggested in a recent review.¹⁶ Alternatively, nuanced changes in the systemic marker CRP in response to BPA may not be detectable when other factors are causing greater fluctuations in the system. Finally, these or other positive or negative findings may be due to chance and replication may be necessary. Further investigation within the context of pregnancy may be worthwhile, as IL-6 shows some of the stronger associations with adverse birth outcomes compared to other markers of inflammation.²⁷

Two of the above studies additionally examined associations between BPA exposure and indices of oxidative stress. The cross-sectional study of adults in Korea identified positive associations between urinary BPA concentrations and malondialdehyde, another biomarker of lipid peroxidation, as well as 8-OHdG, and again these relationships were specific to postmenopausal women.³⁵ Additionally, the nationally representative US study found that BPA was positively associated with gamma glutamyl transferase which is another reputed biomarker of oxidative stress.³⁶ Notably, neither of these studies adjusted for exposure to phthalates which can be correlated to exposure to BPA and have also been associated with malondialdehyde and GGT biomarkers^{37–39}. We too observed significant associations between BPA concentrations and our biomarkers of oxidative stress; and although those associations were attenuated upon addition of phthalate metabolites to the models the effect estimates were still statistically significant. This highlights the fact that BPA, independent of phthalate exposure, may be linked to systemic increases in oxidative stress throughout pregnancy.

Only two other studies to date have explored associations between maternal exposure to BPA in pregnancy and indicators of inflammation and/or oxidative stress. One study measured BPA concentrations in first trimester maternal plasma samples and selected 12 women each in either high or low categories of BPA exposure.¹⁸ They additionally examined plasma concentrations of three markers of tyrosine oxidation, indicative of oxidative protein damage, taken from the same time point and from cord blood at delivery. Elevated 3-nitrotyrosine markers were observed in both first trimester and cord plasma samples from mothers in the high BPA exposure category. The group also observed increases in maternal and umbilical cord plasma 3-nitrotyrosine concentrations from sheep dosed with BPA during gestation. Our findings corroborate these in a larger study population with repeated measures of BPA and different oxidative stress biomarkers.

The second study to examine BPA exposure and these biomarkers was a preliminary analysis published by our group with data from a prospective birth cohort of women in Puerto Rico.¹⁹ BPA levels were measured in urine samples collected from up to three time points during gestation, as were the same biomarkers of inflammation and oxidative stress measured in the present study population. For inflammation, CRP and cytokines were measured in plasma samples taken from up to two time points during pregnancy (N=101 subjects, 181 samples); for oxidative stress, the biomarkers were measured in urine samples taken from up to three time points during pregnancy (N=52 subjects, 140 samples).¹⁹ In that analysis we similarly observed an increase in plasma IL-6 concentrations in association with urinary total BPA in adjusted models (% with IQR increase in BPA=12.5, 95% CI=-2.50, 29.7), along with positive but imprecise associations with the other inflammation markers

measured. Associations were also positive between BPA and both oxidative stress markers in that analysis, and in models adjusted for urinary phthalate metabolites those associations maintained statistical significance.¹⁹

Our study had some limitations, including our use of circulating biomarkers of oxidative stress and inflammation, rather than indicators of changes at the maternal-fetal interface which may be more representative of physiological disturbances critical to pregnancy outcomes and aspects of fetal development. We also were unable to adjust for confounding by diet or other environmental exposures (e.g., other phenols such as parabens) which were unmeasured in our study. Additionally, as with all observational studies, we were unable to establish causation. Nevertheless, our study also had a number of strengths, including its ability to address this research question in a large population of pregnant women with repeated measures of urinary BPA levels and biomarkers of both oxidative stress and inflammation. Urinary BPA concentrations are highly variable both in pregnant women and otherwise, so that examining relationships with single spot urine samples can be problematic.⁴⁰ We also measured multiple biomarkers of both oxidative stress and inflammation, each of which may be indicative of different biological pathways. Finally, although this study was performed with a nested case-control study of preterm birth, we were able to apply inverse probability weightings to make the results generalizable to the base Brigham and Women's Hospital population.

In conclusion, maternal exposure to BPA was associated with an increase in circulating plasma concentrations of IL-6 across pregnancy. BPA exposure was also associated with an increase in maternal biomarkers of oxidative stress, including indices of oxidative DNA and lipid damage. Future studies investigating mechanism of BPA effects should attend to inflammatory and oxidative stress processes.

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Abbreviations

BPA	Bisphenol-A
PPAR	peroxisome proliferator activated receptor
8-OHdG	8-hydroxydeoxyguanosine
CRP	c-reactive protein
LOD	limit of detection
BMI	body mass index
IQR	interquartile range
MEHP	mono-2-ethylhexyl phthalate

MEHHP	1 mono-2-ethyl-5-hydroxyhexyl phthalate
MEOHP	mono-2-ethyl-5-oxohexyl phthalate
MECPP	mono-2-ethyl-5-carboxypentyl phthalate
MBzP	mono-benzyl phthalate
MBP	mono- <i>n</i> -butyl phthalate
MiBP	mono-iso-butyl phthalate
MEP	mono-ethyl phthalate
MCCPP	mono-carboxypropyl phthalate

References

1. Kang J-H, Kondo F, Katayama Y. Human exposure to bisphenol A. *Toxicology*. 2006; 226(2):79–89. [PubMed: 16860916]
2. LaKind JS, Naiman DQ. Temporal trends in bisphenol A exposure in the United States from 2003–2012 and factors associated with BPA exposure: Spot samples and urine dilution complicate data interpretation. *Environmental research*. 2015; 142:84–95. [PubMed: 26121292]
3. Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to bisphenol A (BPA). *Reproductive toxicology*. 2007; 24(2):139–177. [PubMed: 17825522]
4. Cantonwine D, Meeker JD, Hu H, Sanchez BN, Lamadrid-Figueroa H, Mercado-Garcia A, et al. Bisphenol a exposure in Mexico City and risk of prematurity: a pilot nested case control study. *Environmental health*. 2010; 9:62. [PubMed: 20955576]
5. Cantonwine DE, Ferguson KK, Mukherjee B, McElrath TF, Meeker JD. Urinary Bisphenol A Levels during Pregnancy and Risk of Preterm Birth. *Environmental health perspectives*. 2015; 123(9):895–901. [PubMed: 25815860]
6. Snijder CA, Heederik D, Pierik FH, et al. Fetal growth and prenatal exposure to bisphenol A: the generation R study. *Environmental health perspectives*. 2013; 121(3):393–398. [PubMed: 23459363]
7. Casas M, Forns J, Martinez D, et al. Exposure to bisphenol A during pregnancy and child neuropsychological development in the INMA-Sabadell cohort. *Environmental research*. 2015; 142:671–679. [PubMed: 26343751]
8. Roen EL, Wang Y, Calafat AM, et al. Bisphenol A exposure and behavioral problems among inner city children at 7–9 years of age. *Environmental research*. 2015; 142:739–745. [PubMed: 25724466]
9. Evans SF, Kobrosly RW, Barrett ES, Thurston SW, Calafat AM, Weiss B, et al. Prenatal bisphenol A exposure and maternally reported behavior in boys and girls. *Neurotoxicology*. 2014; 45:91–99. [PubMed: 25307304]
10. Braun JM, Lanphear BP, Calafat AM, Deria S, Khoury J, Howe CJ, et al. Early-life bisphenol a exposure and child body mass index: a prospective cohort study. *Environmental health perspectives*. 2014; 122(11):1239–1245. [PubMed: 25073184]
11. Valvi D, Casas M, Mendez MA, Ballesteros-Gómez A, Luque N, Rubio S, et al. Prenatal bisphenol a urine concentrations and early rapid growth and overweight risk in the offspring. *Epidemiology*. 2013; 24(6):791–799. [PubMed: 24036610]
12. Takayanagi S, Tokunaga T, Liu X, Okada H, Matsushima A, Shimohigashi Y. Endocrine disruptor bisphenol A strongly binds to human estrogen-related receptor γ (ERR γ) with high constitutive activity. *Toxicology letters*. 2006; 167(2):95–105. [PubMed: 17049190]
13. Bindhumol V, Chitra K, Mathur P. Bisphenol A induces reactive oxygen species generation in the liver of male rats. *Toxicology*. 2003; 188(2):117–124. [PubMed: 12767684]

14. Chitra K, Latchoumycandane C, Mathur P. Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. *Toxicology*. 2003; 185(1):119–127. [PubMed: 12505450]
15. Hugo ER, Brandebourg TD, Woo JG, Loftus J, Alexander JW, Ben-Jonathan N. Bisphenol A at environmentally relevant doses inhibits adiponectin release from human adipose tissue explants and adipocytes. *Environmental health perspectives*. 2008; 116(12):1642–1647. [PubMed: 19079714]
16. Ben-Jonathan N, Hugo ER, Brandebourg TD. Effects of bisphenol A on adipokine release from human adipose tissue: Implications for the metabolic syndrome. *Molecular and cellular endocrinology*. 2009; 304(1):49–54. [PubMed: 19433247]
17. Rogers JA, Metz L, Yong VW. Review: Endocrine disrupting chemicals and immune responses: a focus on bisphenol-A and its potential mechanisms. *Molecular immunology*. 2013; 53(4):421–430. [PubMed: 23123408]
18. Veiga-Lopez A, Pennathur S, Kannan K, Patisaul HB, Dolinoy DC, Zeng L, et al. Impact of Gestational Bisphenol A on Oxidative Stress and Free Fatty Acids: Human Association and Interspecies Animal Testing Studies. *Endocrinology*. 2015; 156(3):911–922. [PubMed: 25603046]
19. Watkins DJ, Ferguson KK, Del Toro LVA, Alshawabkeh AN, Cordero JF, Meeker JD. Associations between urinary phenol and paraben concentrations and markers of oxidative stress and inflammation among pregnant women in Puerto Rico. *International journal of hygiene and environmental health*. 2015; 218(2):212–219. [PubMed: 25435060]
20. Montuschi P, Barnes PJ, Roberts LJ. Isoprostanes: markers and mediators of oxidative stress. *The FASEB Journal*. 2004; 18(15):1791–1800. [PubMed: 15576482]
21. Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2'-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis. *Journal of environmental science and health Part C*. 2009; 27(2):120–139.
22. Wu LL, Chiou C-C, Chang P-Y, Wu JT. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetes. *Clinica Chimica Acta*. 2004; 339(1):1–9.
23. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *New England Journal of Medicine*. 2000; 342(12):836–843. [PubMed: 10733371]
24. Tjoa M, Van Vugt J, Go A, Blankenstein M, Oudejans C, Van Wijk I. Elevated C-reactive protein levels during first trimester of pregnancy are indicative of preeclampsia and intrauterine growth restriction. *Journal of reproductive immunology*. 2003; 59(1):29–37. [PubMed: 12892901]
25. Everett CJ, King DE, Player MS, Matheson EM, Post RE, Mainous AG. Association of urinary polycyclic aromatic hydrocarbons and serum C-reactive protein. *Environmental research*. 2010; 110(1):79–82. [PubMed: 19836015]
26. Melzer D, Rice NE, Lewis C, Henley WE, Galloway TS. Association of urinary bisphenol a concentration with heart disease: evidence from NHANES 2003/06. *PloS one*. 2010; 5(1):e8673. [PubMed: 20084273]
27. Wei S-Q, Fraser W, Luo Z-C. Inflammatory cytokines and spontaneous preterm birth in asymptomatic women: a systematic review. *Obstetrics & Gynecology*. 2010; 11(2, Part 1):393–401.
28. van Eeden SF, Tan WC, Suwa T, Mukae H, Terashima T, Fujii T, et al. Cytokines involved in the systemic inflammatory response induced by exposure to particulate matter air pollutants (PM10). *American Journal of Respiratory and Critical Care Medicine*. 2001; 164(5):826–830. [PubMed: 11549540]
29. Ferguson KK, McElrath TF, Ko YA, Mukherjee B, Meeker JD. Variability in urinary phthalate metabolite levels across pregnancy and sensitive windows of exposure for the risk of preterm birth. *Environment international*. 2014; 70:118–124. [PubMed: 24934852]
30. Lewis RC, Meeker JD, Peterson KE, Lee JM, Pace GG, Cantoral A, et al. Predictors of urinary bisphenol A and phthalate metabolite concentrations in Mexican children. *Chemosphere*. 2013; 93(10):2390–2398. [PubMed: 24041567]
31. Ferguson KK, McElrath TF, Chen Y-H, Mukherjee B, Meeker JD. Urinary phthalate metabolites and biomarkers of oxidative stress in pregnant women: a repeated measures analysis. *Environmental health perspectives*. 2014; 123(3):210–216. [PubMed: 25402001]

32. Jiang Y, Scott AJ, Wild CJ. Secondary analysis of case-control data. *Statistics in medicine*. 2006; 25(8):1323–1339. [PubMed: 16220494]
33. Ferguson KK, McElrath TF, Mukherjee B, Loch-Caruso R, Meeker JD. Associations between maternal biomarkers of phthalate exposure and inflammation using repeated measurements across pregnancy. *PloS one*. 2015; 10(8):e0135601. [PubMed: 26317519]
34. Savastano S, Tarantino G, D’Esposito V, Passaretti F, Cabaro S, Liotti A, et al. Bisphenol-A plasma levels are related to inflammatory markers, visceral obesity and insulin-resistance: a cross-sectional study on adult male population. *Journal of Translational Medicine*. 2015; 13(1):1–7. [PubMed: 25591711]
35. Yang YJ, Hong YC, Oh SY, Park MS, Kim H, Leem JH, et al. Bisphenol A exposure is associated with oxidative stress and inflammation in postmenopausal women. *Environmental research*. 2009; 109(6):797–801. [PubMed: 19464675]
36. Lang IA, Galloway TS, Scarlett A, Henley WE, Depledge M, Wallace RB, et al. Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. *Journal of the American medical association*. 2008; 300(11):1303–1310. [PubMed: 18799442]
37. Braun JM, Calafat AM, Berry K, Calafat AM, Berry K, Ehrlich S, et al. Variability of urinary phthalate metabolite and bisphenol A concentrations before and during pregnancy. *Environmental health perspectives*. 2012; 120(5):739–745. [PubMed: 22262702]
38. Ferguson KK, Loch-Caruso R, Meeker JD. Urinary phthalate metabolites in relation to biomarkers of inflammation and oxidative stress: NHANES 1999–2006. *Environmental research*. 2011; 111(5):718–726. [PubMed: 21349512]
39. Ji K, Kho YL, Park Y, Choi K. Influence of a five-day vegetarian diet on urinary levels of antibiotics and phthalate metabolites: a pilot study with “Temple Stay” participants. *Environmental research*. 2010; 110(4):375–382. [PubMed: 20227070]
40. Peretz J, Vrooman L, Ricke WA, Hunt PA, Ehrlich S, Hauser R, et al. Bisphenol A and reproductive health: update of experimental and human evidence, 2007–2013. *Environmental health perspectives*. 2014; 122(8):775–786. [PubMed: 24896072]

Highlights

- BPA is an endocrine disruptor but may also cause oxidative stress and inflammation.
- We used repeated measures models to examine BPA in relation to biomarkers of each.
- BPA was associated with modest increases in biomarkers of oxidative stress.
- BPA was associated with increases in IL-6, an indicator of systemic inflammation.

Table 1

Crude sample sizes and urinary bisphenol-A geometric means and geometric standard deviations

Characteristic		N	BPA (ng/mL)
Race/ethnicity	White (Ref.)	282	1.19 (1.32)
	African-American	77	1.75 (1.33) *
	Other	123	1.49 (1.48) *
Education	High school (Ref.)	68	1.87 (1.43)
	Technical school	77	1.54 (1.35)
	Junior college or some college	139	1.26 (1.35) *
	College graduate	187	1.19 (1.35) *
Health insurance	Missing	11	
	Private insurance/HMO/Self-pay (Ref.)	385	1.23 (1.35)
	Medicaid/SSI/MassHealth	85	1.96 (1.39) *
BMI at visit 1	Missing	12	
	Less than 25 kg/m ² (Ref.)	250	1.22 (1.35)
	25 to less than 30 kg/m ²	123	1.32 (1.43)
	Greater than 30 kg/m ²	102	1.73 (1.32) *
Tobacco use	Missing	4	
	Smoked during pregnancy (Ref.)	31	1.81 (1.40)
	No smoking during pregnancy	445	1.32 (1.37) *
Alcohol use	Missing	6	
	Alcohol use during pregnancy (Ref.)	20	1.20 (1.38)
	No alcohol use during pregnancy	452	1.35 (1.38)
Parity	Missing	10	
	Nulliparous (Ref.)	215	1.30 (1.37)
	Parous	267	1.37 (1.39)
Fetal gender	Male (Ref.)	214	1.30 (1.40)
	Female	268	1.36 (1.36)

* p<0.05 for significant difference in biomarker concentration from reference category, estimated from weighted linear mixed model with random intercepts for subject ID. Ref., reference category.

HMO, Health Maintenance Organization. SSI, Supplemental Security Income. BMI, body mass index. Bisphenol-A concentrations corrected for urinary specific gravity.

Table 2

Spearman correlations (p values) between specific gravity corrected urinary bisphenol-A (BPA) and urinary phthalate metabolite concentrations by study visit

Phthalate metabolite	Visit 1 (N=479)	Visit 2 (N=422)	Visit 3 (N=412)	Visit 4 (N=380)
MEHP	0.14 (<0.01)	0.15 (<0.01)	0.09 (0.07)	0.22 (<0.01)
MEHHP	0.15 (<0.01)	0.21 (<0.01)	0.14 (<0.01)	0.22 (<0.01)
MEOHP	0.16 (<0.01)	0.20 (<0.01)	0.13 (<0.01)	0.24 (<0.01)
MECPP	0.12 (<0.01)	0.14 (<0.01)	0.12 (0.02)	0.21 (<0.01)
MBzP	0.28 (<0.01)	0.27 (<0.01)	0.25 (<0.01)	0.34 (<0.01)
MBP	0.27 (<0.01)	0.30 (<0.01)	0.25 (<0.01)	0.31 (<0.01)
MiBP	0.20 (<0.01)	0.23 (<0.01)	0.27 (<0.01)	0.27 (<0.01)
MEP	0.15 (<0.01)	0.19 (<0.01)	0.16 (<0.01)	0.10 (0.04)
MCPP	0.27 (<0.01)	0.18 (<0.01)	0.14 (<0.01)	0.22 (<0.01)

Table 3

Percent change in urinary oxidative stress biomarker in association with an interquartile range increase in urinary bisphenol-A (BPA) concentrations. Results from linear mixed effect models with subject-specific random intercepts and slopes

	% change (95% CI) with IQR increase in BPA	p	% change (95% CI) with IQR increase in BPA	p	% change (95% CI) with IQR increase in MBzP	p
8-OHdG	8.62 (3.93, 13.5)	<0.01	8.95 (4.24, 13.9)	<0.01	5.09 (0.54, 9.84)	0.03
8-Isoprostane	16.2 (8.61, 24.4)	<0.01	13.8 (6.03, 22.2)	<0.01	8.79 (1.66, 16.4)	0.02

Model 1 adjusted for specific gravity and gestational age at sample collection (N=1675 observations, 482 subjects). Model 2 additionally adjusted for maternal race/ethnicity, health insurance provider, education level, and pre-pregnancy body mass index (N=1607 observations, 461 subjects). Model 3 additionally adjusted for urinary mono-benzyl phthalate (MBzP) (N=1607 observations, 462 subjects). All models include inverse probability weightings to adjust for case-control study design.

Table 4

Percent change in inflammation biomarker in association with an interquartile range increase in urinary bisphenol-A (BPA) concentrations. Results from linear mixed effect models with subject-specific random intercepts and slopes

	% change (95% CI) with IQR increase in BPA	p	% change (95% CI) with IQR increase in BPA	p	% change (95% CI) with IQR increase in BPA	p	% change (95% CI) with IQR increase in MBzP	p
CRP	3.21 (-3.84, 10.8)	0.39	-0.90 (-7.94, 6.68)	0.81	-1.64 (-8.63, 5.88)	0.66	5.65 (-2.47, 14.5)	0.19
IL-1 β	2.28 (-4.14, 9.14)	0.50	3.36 (-3.41, 10.6)	0.34	3.36 (-3.41, 10.6)	0.34	-0.14 (-7.82, 8.17)	0.97
IL-6	10.9 (3.97, 18.4)	<0.01	9.77 (2.58, 17.5)	<0.01	8.95 (1.81, 16.6)	0.01	5.47 (-2.64, 14.3)	0.18
IL-10	4.14 (-0.95, 9.50)	0.10	3.36 (-1.69, 8.68)	0.19	3.05 (-1.98, 8.35)	0.24	2.51 (-3.37, 8.76)	0.41
TNF- α	1.21 (-2.31, 4.86)	0.50	0.45 (-3.04, 4.07)	0.79	0.30 (-3.18, 3.91)	0.86	1.25 (-2.89, 5.56)	0.58

Model 1 adjusted for specific gravity and gestational age at sample collection (N=1519 observations, 481 subjects). Model 2 additionally adjusted for maternal race/ethnicity, health insurance provider, education level, and pre-pregnancy body mass index (N=1457 observations, 460 subjects). Model 3 additionally adjusted for urinary mono-benzyl phthalate (MBzP) (N=1456 observations, 459 subjects). All models include inverse probability weightings to adjust for case-control study design.