International Journal of Hygiene and Environmental Health 218 (2015) 212–219

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

Associations between urinary phenol and paraben concentrations and markers of oxidative stress and inflammation among pregnant women in Puerto Rico

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ARTICLE INFO

Article history:
Received 28 July 2014
Received in revised form 15 October 2014
Accepted 11 November 2014

Keywords:
Bisphenol A
Inflammation
Oxidative stress
Parabens
Phenols

ABSTRACT

Phenols and parabens are used in a multitude of consumer products resulting in ubiquitous human exposure. Animal and *in vitro* studies suggest that exposure to these compounds may be related to a number of adverse health outcomes, as well as potential mediators such as oxidative stress and inflammation. We examined urinary phenol (bisphenol A (BPA), triclosan (TCS), benzophenone-3 (BP-3), 2,4-dichlorophenol (24-DCP), 2,5-dichlorophenol (25-DCP)) and paraben (butyl paraben (B-PB), methyl paraben (M-PB), propyl paraben (P-PB)) concentrations measured three times during pregnancy in relation to markers of oxidative stress and inflammation among participants in the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) project. Serum markers of inflammation (c-reactive protein (CRP), IL-1β, IL-6, IL-10, and tumor necrosis factor-α (TNF-α)) were measured twice during pregnancy ($n = 105$ subjects, 187 measurements) and urinary markers of oxidative stress (8-hydroxydeoxyguanosine (OHdG) and isoprostane) were measured three times during pregnancy ($n = 54$ subjects, 146 measurements). We used linear mixed models to assess relationships between natural log-transformed exposure and outcome biomarkers while accounting for within individual correlation across study visits. After adjustment for urinary specific gravity, study visit, maternal pre-pregnancy BMI, and maternal education, an interquartile range (IQR) increase in urinary BPA was associated with 21% higher OHdG ($p = 0.001$) and 29% higher isoprostane ($p = 0.0002$), indicating increased oxidative stress. The adjusted increase in isoprostane per IQR increase in marker of exposure was 17% for BP-3, 27% for B-PB, and 20% for P-PB (all $p < 0.05$). An IQR increase in triclosan (TCS) was associated with 31% higher serum concentrations of IL-6 ($p = 0.007$), a pro-inflammatory cytokine. In contrast, IQR increases in BP-3 and B-PB were significantly associated with 16% and 18% lower CRP, a measure of systemic inflammation. Our findings suggest that exposure to BPA, select parabens, and TCS during pregnancy may be related to oxidative stress and inflammation, potential mechanisms by which exposure to these compounds may influence birth outcomes and other adverse health effects, but additional research is needed.

Abbreviations: 24-DCP, 2,4-dichlorophenol; 25-DCP, 2,5-dichlorophenol; BP-3, benzophenone-3; BPA, bisphenol A; B-PB, butyl paraben; CPR, c-reactive protein; DEHP, di-2-ethylhexyl phthalate; ELISA, enzyme-linked immunosorbent assay; ER, estrogen receptor; GM, geometric mean; GSD, geometric standard deviation; IL, interleukin; IQR, interquartile range; LMM, linear mixed models; LOD, limit of detection; MDA, malondialdehyde; M-PB, methyl paraben; NHANES, National Health and Nutrition Examination Survey (NHANES); OHdG, 8-hydroxydeoxyguanosine; P-PB, propyl paraben; PROTECT, Puerto Rico Testsite for Exploring Contamination Threats; TCS, triclosan; TNF-α, tumor necrosis factor-α.

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http://dx.doi.org/10.1016/j.ijheh.2014.11.001
1438-4639/

Introduction

The rate of preterm birth in Puerto Rico is 17%, one of the highest rates in the U.S. (March of Dimes, 2013) and the world (Blencowe et al., 2012). The island of Puerto Rico also has a high concentration of hazardous waste sites (US EPA, 2014; Padilla et al., 2011), raising the question of whether exposure to environmental chemicals plays a role in the high rates of preterm birth, as well as other prevalent health outcomes among this population. The Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) program is an ongoing prospective birth cohort in Northern Puerto Rico designed to investigate relationships between chemical exposures during pregnancy, preterm birth, and other adverse pregnancy outcomes, as well as potential toxicological mechanisms for these relationships (Meeker et al., 2013). Potential mechanisms of interest include oxidative stress and inflammation, as these have been associated with both environmental contaminants and adverse birth outcomes (Al-Gubory et al., 2010; Bastek et al., 2011; Ferguson et al., 2014c). Previous research within the PROTECT project suggests that pregnant women in Puerto Rico may have higher urinary concentrations of the phenols triclosan (TCS), benzophenone-3 (BP-3), and 2,5-dichlorophenol (25-DCP), and similar urinary concentrations of bisphenol-A (BPA), 2,4-dichlorophenol (24-DCP), and parabens, compared to women of reproductive age from the US general population (Meeker et al., 2013). In the US, recent reports from the National Health and Nutrition Examination Survey (NHANES) suggest that exposure to a number of phenols and parabens is widespread, with detectable urinary concentrations present in the majority of participants (CDC, 2014).

BPA is a weakly estrogenic, high-volume chemical used to make polycarbonate plastics and epoxy resins that are present in a multitude of consumer products (CDC, 2014). In animal studies, BPA has been shown to affect a number of reproductive endpoints, but human studies have been limited (Cantonwine et al., 2013). BPA has also been associated with a number of markers of oxidative stress in both humans (Yang et al., 2009), and animals (Aboul Ezz et al., 2013; Hassan et al., 2012; Song et al., 2014).

TCS is a broad-spectrum antimicrobial used in a large number of personal care products and consumer goods, including soaps, toothpaste, mouthwash, deodorants, textiles, toys, and kitchenware (Dann and Hontela, 2011). Associations between TCS exposure and reproductive outcomes in humans have not been studied, although TCS is thought to have anti-inflammatory properties (Barros et al., 2010; Elwood et al., 2007; Modeer et al., 1996; Mustafa et al., 2000; Wallet et al., 2013). Given that inflammation is a potential factor in preterm birth (Bastek et al., 2011), we might expect a possible protective relationship between urinary TCS concentrations and this outcome.

BP-3 is a UV-filter commonly used in sunscreens, cosmetics, and plastics (CDC, 2014). Animal studies suggest that BP-3 is weakly estrogenic and antiandrogenic (Krause et al., 2012), but health effects related to BP-3 exposure in humans have not been studied. In *in vitro* studies, BP-3 has been associated with markers of oxidative stress (Gao et al., 2013; Kato et al., 2006), but findings from an animal study suggested that BP-3 may have anti-inflammatory properties (Couteau et al., 2012)

24-DCP is a minor metabolite of 2,4-dichlorophenoxyacetic acid (2,4-D), a herbicide widely used in the US and elsewhere, while 25-DCP is a metabolite of 1,4-dichlorobenzene, a compound used in mothballs and room and toilet deodorizers (CDC, 2014). In human studies, maternal urinary 24-DCP and 25-DCP concentrations during pregnancy have been associated with decreased birth weight in boys (Philippat et al., 2012; Wolff et al., 2008) but not in girls, and one study observed no association between maternal urinary concentrations and gestational age (Wolff et al., 2008). However, 24-DCP has been associated with markers of oxidative stress (Bukowska, 2003).

Parabens are a class of chemicals widely used as antimicrobial preservatives in cosmetics and other personal care products, food, and pharmaceuticals (Calafat et al., 2010). Animal and *in vitro* studies have demonstrated that parabens are estrogenic (Boberg et al., 2010; Karpuzoglu et al., 2013), but studies on reproductive or pregnancy outcomes are limited. In humans, higher urinary concentrations of various parabens have been associated with increases in markers of oxidative stress (Kang et al., 2013).

Few human studies have explored measures of phenol and paraben exposure in relation to oxidative stress and inflammation despite the potential for these processes to be important mediators of numerous health effects, including atherosclerosis, cardiovascular disease, cancer, and pregnancy outcomes such as intrauterine growth restriction and preterm birth. The objective of the current study was to examine these associations by utilizing repeated measures of phenols, parabens, and markers of oxidative stress in urine samples collected up to three times per participant throughout pregnancy, and markers of inflammation in plasma samples collected up to two times per participant throughout pregnancy among women participating in the PROTECT project.

Methods

Study participants

Participants in the present study are pregnant women who have enrolled in the PROTECT project, an ongoing prospective birth cohort in Northern Puerto Rico. Recruitment practices and inclusion criteria have been previously described (Meeker et al., 2013). Briefly, pregnant women 18–40 years of age were recruited at less than 20 weeks gestation from 7 prenatal clinics and hospitals from 2010 to 2012. We excluded women who used oral contraceptives within three months prior to getting pregnant, used *in vitro* fertilization to get pregnant, or had known obstetric or medical health conditions (*e.g.*, heart conditions or diabetes). Women provided spot urine samples at three separate study visits (16–20, 20–24, and 24–28 weeks gestation). We collected blood samples from participants at visits 1 and 3, and administered questionnaires at all three visits. We followed participants until delivery and recorded detailed information on birth outcomes. This analysis comprises the first 141 women recruited into the study for which urinary phenol and paraben measurements and urinary specific gravity (SG) were available from at least one study visit, as participation was ongoing for some women. Markers of either oxidative stress or inflammation were available on 106 of these women. The ethics and research committees of the University of Michigan School of Public Health, University of Puerto Rico, Northeastern University, and participating hospitals and clinics approved all study protocols, and all subjects provided informed consent prior to participation.

Urinary phenols and parabens

Spot urine samples were collected in polypropylene containers, divided into aliquots, and frozen at −80 °C until aliquots were shipped overnight on dry ice to the CDC for phenol and paraben analysis or to the University of Michigan for measurement of oxidative stress biomarkers. At the CDC, urine samples ($n = 375$) were analyzed for five phenols (BPA, TCS, BP-3, 24-DCP, and 25-DCP) and three parabens (butyl paraben (B-PB), methyl paraben (M-PB), and propyl paraben (P-PB)) by online solid phase extraction-high-performance liquid chromatography-isotope dilution tandem mass spectrometry (Ye et al., 2005, 2006). The inter-assay coefficients of variation for urinary phenols and parabens range from 5 to 10%.