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Maternal and fetal exposure to parabens in a multiethnic urban U.S. population

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ABSTRACT

Fetal exposure to five parabens was investigated due to their endocrine-disrupting potential and possible impact on fetal development. Body burdens occurring from real-world exposures were determined typically as total concentrations after conjugate hydrolysis in 181 maternal urine and 38 umbilical cord blood plasma samples from a multiethnic cohort of 185 predominantly-black, pregnant women recruited in Brooklyn, New York between 2007/9. For 33 participants, both sample types (maternal urine and cord blood) were available. Methyl- (MePB), ethyl- (EtPB), propyl- (PrPB), butyl- (BuPB), and benzylparaben (BePB) were detected in 100, 73.5, 100, 66.3 and 0.0% of the urine samples at median concentrations of 279, 1.44, 75.3, 0.39, and <0.02 μg/L, respectively. Median concentrations of MePB and PrPB were, respectively 4.4- and 8.7-fold higher compared to those reported previously for the general U.S. population (NHANES, 2005/6). Listed in the order above, the five parabens were detected in 97.4, 94.7, 47.4, 47.4, and 44.7% of cord blood plasma samples at median total concentrations of 25.0, 0.36, <0.27, <0.09, and <0.10 μg/L, respectively. Free MePB, EtPB, and PrPB were detected in a subset of cord blood plasma samples at, respectively, 3.9, 71.7, and 6.4% of their total concentrations, whereas free BuPB and BePB were not detected. Literature data and those reported here show the urban community studied here to rank highest in the world for MePB and PrPB exposure in pregnant women, whereas it ranks among the lowest for EtPB and BuPB. This study is the first to report the occurrence of parabens in human umbilical cord blood. Maternal exposure to parabens is widespread, and substantial differences were found to exist between communities and countries both in the spectrum and degree of paraben exposures.

1. Introduction

Human exposure to parabens mostly is the result of their use as preservatives in personal care products, pharmaceuticals, and foodstuff, although they also occur naturally in certain fruits and vegetables (Eriksson et al., 2008; Liao and Kannan, 2014; Liao et al., 2013; Guo and Kannan, 2013). Parabens are identified by their aliphatic or aromatic alkyl moieties, with methyl- (MePB), ethyl- (EtPB), propyl- (PrPB), butyl- (BuPB), and benzylparaben (BePB) representing the most commonly used members of this class of alkyl esters of *p*-hydroxybenzoic acid (PHBA) (Bledzka et al., 2014). Parabens have been used for decades and, when used within the recommended doses, are categorized as "generally recognized as safe (GRAS)" by the European Union (SCCS, 2013) and the Food and Drug Administration (Green, 2014; van der Werf et al., 2007). Yet, recent studies have raised awareness for their potential health effects (Bledzka et al., 2014), particularly in children (Boberg et al., 2010) younger than six to twelve months of age whose detoxification systems are still immature (SCCS, 2013).

Parabens can elicit direct and indirect effects on the endocrine system, which may influence fetal development and ultimately adult health (Isling et al., 2013). Specifically, many parabens are ER-α agonists (Routledge et al., 1998), can affect the fate and metabolism of steroid hormones and xenobiotics, and have been implicated in excessive weight gain (Boberg et al., 2010). Their endocrine-disrupting and obesogenic potency typically increases with the size of the alkyl moiety (Fukahori et al., 1996; Pereira-Fernandes et al., 2013; Hu et al., 2013). Additionally, epidemiological surveys have associated individual parabens with a variety of health outcomes, including elevated oxidative stress biomarkers for MePB and EtPB, lower serum thyroid levels for EtPB, aeroallergen sensitization for PrPB and BuPB, and damage of sperm DNA for BuPB (Meeker et al., 2011; Savage et al., 2012; Koeppe et al., 2013; Kang et al., 2013). Taken together, these observations

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warrant monitoring of human exposure to individual parabens and paraben mixtures to inform human risk assessment.

Human exposure to parabens typically occurs through topical contact with or ingestion of paraben-containing products (Eriksson et al., 2008; Liao and Kannan, 2014; Liao et al., 2013; Guo and Kannan, 2013) as well as inhalation of contaminated air and dust (Canosa et al., 2007; Rudel et al., 2010; Wang et al., 2012). Following exposure, human epithelial absorption of parabens and their subsequent metabolism occur rapidly in the liver. Metabolism results in systemic distribution of free parabens and adducts of PHBA, glucuronide, sulfate, and glycine adducts, all of which are excreted principally *via* urine (Boberg et al., 2010; Janjua et al., 2007; Ye et al., 2006). Diverse biomonitoring studies from across the globe have confirmed human exposure to a variety of parabens as being widespread (Kang et al., 2013; Calafat et al., 2010; Frederiksen et al., 2011; Ye et al., 2012; Meeker et al., 2013; Tefre de Renzy-Martin et al., 2014; Casas et al., 2011; Engel et al., 2014; Shirai et al., 2013), and more prevalent and substantial in women compared to men or children (Ye et al., 2012). Select studies investigated human prenatal and neonatal exposures to parabens in the U.S. (Smith et al., 2012; Braun et al., 2013; Mortensen et al., 2014), Puerto Rico (Meeker et al., 2013), Denmark (Tefre de Renzy-Martin et al., 2014), Spain (Casas et al., 2011), China (Engel et al., 2014), Korea (Kang et al., 2013), and Japan (Shirai et al., 2013). In these studies, parabens were confirmed to be distributed systemically, leading to exposure of the human fetus and neonate *via* the placenta (Jimenez-Diaz et al., 2011) and amniotic fluid (Boberg et al., 2010; Kang et al., 2013; Philippat et al., 2013). In children, the estrogenic burden of free parabens and possibly PHBA may become more important than that of endogenous estradiol (SCCS, 2013; Boberg et al., 2010). In adults, frequent use of paraben-containing products may result in steady-state exposure levels to free parabens and PHBA, despite the rapid and efficient metabolism and excretion of parabens upon exposure (Meeker et al., 2013; Engel et al., 2014). In fact, observed changes in the health status of a study population from Puerto Rico were suspected to be due to exposure to endocrine disruptors including, parabens (Meeker et al., 2013). Although children feature an increased susceptibility to endocrine disruption, reports on fetal and neonatal exposures to parabens are still scarce for the populations in the U.S., its territories (Meeker et al., 2013; Smith et al., 2012), and around the world (Kang et al., 2013; Casas et al., 2011; Shirai et al., 2013; Genuis et al., 2013; Philippat et al., 2012). Data often are available only for maternal urine samples, with measurements of paraben levels in placental and amniotic fluid seldom being investigated due to the invasive nature of the sampling of these specimens (Jimenez-Diaz et al., 2011; Philippat et al., 2013).

Here, we report on maternal and fetal body burdens of parabens resulting from real-world exposures. Five parabens were monitored in a section of an urban immigrant population and body burdens in our cohort were compared with those observed in the U.S. general population and pregnant women around the globe. Fetal exposures were assessed using 181 maternal spot urine samples collected once per participant during the second to third trimester and 38 umbilical cord blood plasma collected at birth. Body burdens were determined primarily as total ($\sum$ −) concentrations, accounting for both free product and conjugated species; however, for a random subset of 21 cord blood plasma samples, the concentration of free parabens were also assessed. Human biomonitoring studies usually use cohorts representing the general population to determine the extent and risk of environmental exposures (Calafat et al., 2010). Since pregnant women, infants, and minority populations may differ somewhat in degree and extent of exposure (Meeker et al., 2013; Geer et al., 2012; Hoepner et al., 2013; Just et al., 2010; Teitelbaum et al., 2008), risk (Geer et al., 2012; Perera et al., 2003), or susceptibility, our sampled population requires close scrutiny due to its susceptibility to adverse exposure effects and the paucity of data available today. The present work addresses some of these data gaps by investigating body burdens of pregnant women and neonates in an urban immigrant community using liquid chromatography tandem mass spectrometry (LC–MS/MS) for highly sensitive and selective analysis of the five targeted parabens.

2. Materials and methods

2.1. Standards and reagents

MePB was purchased from Aldrich (Sigma-Aldrich, St. Louis, MO), and $^{13}C_6$-MePB (99%) was obtained from Cambridge Isotope Laboratories (Andover, MA). EtPB, *n*-PrPB, *n*-BuPB, and BePB were acquired from RT Corp (Laramie, WY) and deuterated standards (EtPB-d_4, *n*-PrPB-d_4, *n*-BuPB-d_4) were purchased from C/D/N Isotopes (Quebec, Canada). LC–MS-grade (99%) methanol, water, and acetic acid were obtained from Fluka, and LC–MS-grade acetone was obtained from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO). Individual stock solutions of the native and stable isotope-labeled compounds were prepared in methanol. All stock solutions were stored at −20 °C in glass vials sealed with polytetrafluoroethylene septa.

2.2. Cohort and sampling procedure

Archived samples of maternal urine and human cord blood plasma originated from an urban immigrant population investigated previously for prenatal exposure to mercury and to the antimicrobials triclosan and triclocarban (Geer et al., 2012; Pycke et al., 2014). These previous studies detail the sampling procedures and cohort descriptors. Table S1 provides a concise overview of the composition of the sample population. The sample population was comprised of individuals with ethnic backgrounds from 19 different countries in the Caribbean, West Indies, Central and South America, and Canada. Briefly, pregnant women (aged 18–45 years) were recruited at the University Hospital of Brooklyn's Prenatal Clinic between October 2007 and December 2009. Random "spot" urine specimens ($n = 181$) were provided once per participant during the 6th to 9th months of pregnancy. A convenience subset of participants were followed to delivery, at which time single umbilical cord blood specimens were collected from the neonates ($n = 38$) for plasma isolation and storage at −80 °C for subsequent lab analysis. Human specimens were collected in polypropylene vials or cups that had not been pre-screened for the presence of target analytes, so a random subset of cord blood plasma samples ($n = 21$) was analyzed for both free and total concentrations. The study protocol was approved by Institutional Review Boards (IRBs) of the State University of New York Downstate Medical Center, and of the New York State Department of Health. Each participant signed an informed consent form prior to participation.

2.3. Extraction procedure

The samples were shipped on dry ice to Arizona State University and archived at −80 °C. To maintain analyte stability, freeze–thaw cycles were minimized by distributing the sample into 2 mL aliquots that were also stored at −80 °C. Human specimens (1 mL of maternal urine or 100 μL of cord blood plasma) were thawed, spiked with a solution containing six isotope-labeled standards (10 μL) as well as a solution containing two hydrolysis standards (50 μL), and diluted with a solution containing hydrolysis enzymes (1 mL). An additional 900 μL MS-grade water was added to the umbilical cord blood samples. The labeled standard solution contained $^{13}C_6$-MePB, EtPB-d_5, *n*-PrPB-d_4, and *n*-BuPB-d_4, and $^{13}C_4$-methylumbelliferone in methanol. The hydrolysis standard contained methylumbelliferone-sulfate and methylumbelliferone-glucuronide in water. Enzyme solution containing 0.5 mg/mL of glucuronidase/sulfatase H1 (1:1) in 1 M ammonium acetate (pH 5.0) was added, where after the mixture was gently mixed and incubated overnight at 37 °C. Target analytes were extracted using 60 mg Oasis HLB (Waters, Milford, MA) solid-phase extraction cartridges as described previously (Pycke et al., 2014). Extracts were dried and