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Concentrations of environmental phenols and parabens in milk, urine and serum of lactating North Carolina women

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ABSTRACT

Phenols and parabens show some evidence for endocrine disruption in laboratory animals. The goal of the Methods Advancement for Milk Analysis (MAMA) Study was to develop or adapt methods to measure parabens (methyl, ethyl, butyl, propyl) and phenols (bisphenol A (BPA), 2,4- and 2,5-dichlorophenol, benzophenone-3, triclosan) in urine, milk and serum twice during lactation, to compare concentrations across matrices and with endogenous biomarkers among 34 North Carolina women. These non-persistent chemicals were detected in most urine samples (53–100%) and less frequently in milk or serum; concentrations differed by matrix. Although urinary parabens, triclosan and dichlorophenols concentrations correlated significantly at two time points, those of BPA and benzophenone-3 did not, suggesting considerable variability in those exposures. These pilot data suggest that nursing mothers are exposed to phenols and parabens; urine is the best measurement matrix; and correlations between chemical and endogenous immune-related biomarkers merit further investigation.

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1. Introduction

Humans, house pets, and parts of our food chain are exposed to a mixture of man-made chemicals through industrial pollution, pesticide use, consumer and personal care products, house dust, drinking water, and food packaging. The National Health and Nutrition Examination Survey (NHANES), conducted by the Centers for Disease Control and Prevention (CDC), has demonstrated widespread exposure to some of these chemicals, such as phenols (e.g., bisphenol A [BPA], triclosan) and parabens, among the U.S. general population [1]. As these particular chemicals are commonly found in cosmetics, UV filters, anti-microbial soaps, lotions and plastics used in toys and food storage, at-risk populations (i.e., pregnant women, infants, children, and the elderly) may have more potential for exposure due to enhanced use.

Some persistent environmental chemicals, such as brominated flame retardants (BFRs) and perfluoroalkyl substances (PFAS) can be measured at higher serum concentrations in children than adults [2,3]. It is not known whether this is due to different metabolic rates, varied exposure patterns, or smaller blood volumes in children compared to adults. Of note, PFASs and BFRs can also be found in breast milk and can be transferred to the infant [4,5]. However, few studies have examined the extent to which many non-persistent chemicals are found in breast-feeding women and their milk [6–8]. Characterization of chemical exposure in breastfeeding women and the potential for transfer of those chemicals or their metabolites to breast milk would aid in

Abbreviations: BPA, Bisphenol A; CDC, Centers for Disease Control and Prevention; FDA, Food and Drug Administration; GRAS, Generally Recognized as Safe; IgA, Immunoglobulin A; IgG, Immunoglobulin G; IgM, Immunoglobulin M; IL-6, Interleukin 6; LOD, Limit of Detection; MAMA, Methods Advancement in Milk Analysis; NHANES, National Health and Nutrition Examination Survey; PFAS, Perfluoroalkyl substance; s1, Serum from visit 1; s2, Serum from visit 2; sIgA, Secretory Immunoglobulin A; TNF-α, Tumor Necrosis Factor Alpha; u1, Urine from visit 1; u2, Urine from visit 2; US, EPA or EPA United States Environmental Protection Agency; V1, Visit 1; V2, Visit 2.

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exposure assessment in infants/children and is of interest to risk assessors [9].

Certain phenols and parabens have endocrine disrupting effects in cell lines and animal models [10–12]. In laboratory animals, exposures to some phenols and parabens have been linked to pathologies or disorders such as obesity, thyroid dysfunction, and breast cell hyper-proliferation [13–17]. NHANES and other studies have reported the concentrations of certain phenols and parabens in the serum or urine of adults [18–21], but information on the transfer to milk, and the ratios of the chemical concentrations in the various matrices of at-risk populations, such as lactating women [6–8], especially women from the USA, is limited.

Because early life is a critical and influential period for potential health effects of endocrine disrupting factors [22], our goals were to develop or adapt methods to collect biological matrices (i.e., milk, serum, urine) from lactating women and measure the total concentrations of phenols and parabens in these different biological specimens (total and free in serum) at two time points (i.e., visits). These methods are integral for evaluating the effects of environmental exposures in longitudinal health studies, such as the National Children's Study [23,24] or large developmental cohort studies conducted in other countries [25–27]. Those types of studies also have interests in major health outcomes in children, such as puberty timing, obesity, diabetes, allergy and asthma. We had previously validated assays that may serve as health biomarkers and were endogenous components of the matrices we collected [28]. Therefore, we also assessed correlations between phenol and/or paraben concentrations and endogenous components of milk [glucose, triglycerides, secretory immunoglobulin A (sIgA), prolactin, estradiol, interleukin-6, and tumor necrosis factor-alpha (TNF-α)] and serum (including the aforementioned milk biologics with the addition of IgE, IgM, IgG, and IgA instead of sIgA) for the individuals in our study. We did this hypothesizing that there may be significant correlations between these chemical exposures and endogenous components that would mirror correlations reported in animal model studies, especially those indicating estrogen agonist activity (i.e., BPA). We also evaluated correlations between measured concentrations of these chemicals and potential exposure routes, using information gathered from an extensive questionnaire administered at the first of two visits [28].

2. Materials and methods

2.1. MAMA study details

Healthy (no acute illness at the time of sample collection), lactating, English-speaking women between the age of 18 and 38 were recruited for the Methods Advancement in Milk Analysis (MAMA) study by the US Environmental Protection Agency (EPA) contractor, Westat (Chapel Hill, NC). The women visited the US EPA Human Studies Facility clinic in Chapel Hill, NC, between December 2004 and July 2005. Participants ($n = 34$) were asked to fast before sample collection and to avoid the use of breast creams. The method of recruitment and demographic information on the participants has been previously reported [28] with study design including the use of a convenience sampling of women with limited ethnic diversity, e.g., majority Caucasian. The research with human subjects was approved by the Institutional Review Boards (IRBs) of the University of North Carolina-Chapel Hill Medical School under IRB number 03-EPA-207 and the CDC under IRB number 3961. Study volunteers were briefed on the study goals, risks, inclusion and exclusion criteria and participated in informed consent (verbal and written) prior to donation and answering an extensive questionnaire.

Milk, urine and serum were collected at 2–7 weeks and 3–4 months postpartum into polypropylene containers using a previously described protocol [29]. Breasts were cleaned with water and a cloth towel before milk collection. Women provided all of the milk (including hind milk) available at the time of collection (volume was to equal/exceed 3 ounces). A log was kept to record details of the sample collection, including date and time of day. The samples from multiple matrices were collected within an hour of each other. All samples, including freshly collected, mixed milk samples were aliquoted into multiple tubes at collection and stored at or below −20 °C until analysis. Aliquots of each sample were available for endogenous biomarker analyses and analytical chemical analyses. A questionnaire was administered to the women at the first visit and it was aimed at understanding the sources of their potential chemical exposures, including age, race/ethnicity, education, years at current address, personal care product use (i.e., nail polish, hair styling products, hair color, foundation makeup), number of prior children and number breastfed, pregnancy complications (diabetes, preeclampsia, excess weight gain), information on current breastfeeding, source and amount of water consumed daily, and body mass index. Many answers were categorical (i.e., none, seldom, moderate, often) and others were continuous.

2.2. Analytical chemical measurements

Details of the analytical procedures used to measure the total (free plus conjugated) or free concentrations of the environmental chemicals can be found in the Supplementary Data. Specifically, we measured the total (free plus conjugated) concentrations in urine, milk, or serum, but only the free concentrations in serum. Briefly, the target analytes in urine, milk, or serum were pre-concentrated by online solid phase extraction, separated from other matrix components by reverse-phase high performance liquid chromatography, and detected by atmospheric pressure chemical ionization or atmospheric pressure photoionization–isotope dilution–tandem mass spectrometry with peak focusing as described before [30,31]. Because certain compounds measured in this analysis are ubiquitous in the environment, quality control procedures, including the use of blanks, were used at all steps to monitor for BPA contamination from the procedures for sample collection, handling, and analysis [32–34].

The number of samples available for chemical analysis varied due to the method development nature of this study. Two urine, serum and milk ($Milk_A$ = stored at −20 °C; $Milk_B$ = stored at −80 °C to compare stability of these chemicals in milk) aliquots per participant were collected for analyses in this study. One of the two aliquots of serum was previously analyzed for other chemicals (i.e., PFASs) prior to BPA and benzophenone-3 (2-hydroxy-4-methoxybenzophenone) measurements [35]. We measured concentrations of parabens (methyl-, ethyl-, butyl- and propyl), BPA, benzophenone-3, 2,5- and 2,4-dichlorophenol, and triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether) in urine, serum and milk. Measurements of phenols and parabens were made on 1st visit (V1) milk ($n = 1$), 2nd visit (V2) milk ($n = 9$), V1 serum ($n = 34$), V2 serum ($n = 30$), V1 urine ($n = 33$), and V2 urine ($n = 30$) samples. Only 10 milk samples (representative of 9 women) were analyzed for phenols and 8 milk samples for parabens because the initial collection protocol added a preservative (potassium dichromate) to the milk at collection that adversely affected the performance of the method used for analysis of parabens and phenols. This problem was identified after sample collection had begun and the methodology was altered to not include the preservative in the remaining samples.

2.3. Measurements of endogenous immune-related biomarkers

Concentrations of serum IgG, IgM, IgA, IgE, glucose, triglycerides, estradiol, prolactin, IL-6, and TNF-α and milk sIgA, IL-6, leptin,