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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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Keywords

Biomonitoring; BPA; breast milk; lactation; MAMA Study; parabens phenols; serum; urine

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 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 afflictions of 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].

Materials and Methods

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 and inclusion and exclusion criteria and provided 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.

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.

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, prolactin, TNF-α, triglycerides, glucose and estradiol were measured for each MAMA study participant by LabCorp Inc. (Burlington, NC) as defined in the detailed protocols previously reported [28]. Serum was assayed on the same day as or within 24 hours of their co-paired milk samples. Unlike the analytical methods described above, the preservative did not interfere in these assays, therefore the n=31 for V1 milk and n=21 for V2 milk end points. The n for serum samples is identical to those reported above (34 and 30, respectively, for V1 and V2). The assay coefficients of variation and limits of detection have been previously reported [28].

Statistics

We report milk, serum and urine concentrations of benzophenone-3, BPA, methyl paraben and propyl paraben for each woman. The minimum, maximum and 20^{th}-80^{th} percentile concentrations of the endogenous immune-related biomarkers have been previously reported (Table 4 in [28]). Concentration distributions are described for each compound, but comparisons across matrices and across visits were done only for compounds detected in > 50% of samples [36]. Spearman correlations were calculated to evaluate the relationships within and between phenol and paraben concentrations to compare V1 and V2 for the same compound and to examine interrelationships among parabens and phenols. For concentrations below the limit of detection (LOD; reported in Table 1 by analyte and matrix), we assigned a value equal to the LOD divided by the square root of 2 [63]. Comparisons across visits were not performed for milk concentration because only one milk sample was available at V1 and power was limited (total n=10).

Questionnaires were completed at V1 to capture usual behavior and demographic characteristics. We examined questionnaire data to determine associations between 1) demographic, behavioral and dietary characteristics and 2) phenol and paraben concentrations. Spearman correlations and analysis of variance were used to assess relationships between questionnaire variables and the concentrations of the target analytes. Adjustments were not made for multiple comparisons given the exploratory nature of the study and our intent to identify signals that could be useful to suggest future avenues of investigation. In addition, we note that substantial variability was observed and with our limited power, we only comment in the text on questionnaire data that were associated with a compound at both study visits. However, all correlations are reported in supplemental tables (see Supplemental Data Tables S1-S3). We conducted all analyses using SAS Enterprise Guide 4.1 (SAS Inst., Cary, NC). Significance was denoted at $p \leq 0.05$.

Results

Parabens and phenolic compounds detected in MAMA samples

Table 1 lists the detection frequency of the parabens and phenols in specific matrices (milk, serum or urine) by visit. Even though the LODs were comparable across all matrices (Table 1), urine yielded the highest number of detectable concentrations, and for all compounds evaluated, the majority of individual urinary concentrations were detectable. As a chemical class, the parabens were the most frequently detected compounds across all matrices. With the exception of methyl paraben, detected in nearly 100% of all samples in all matrices at all visits, other parabens were more often detectable in urine than in serum. Parabens were detected in the majority of milk samples, but no milk samples contained detectable concentrations of butyl paraben.

For the phenols, BPA was the most frequently detected in milk and urine (80-90%) but was seldom detected in serum. 2,4- and 2,5-dichlorophenol were rarely detected in milk (and were thus not measured in serum), but were detected in the majority of urine samples. Benzophenone-3 was detectable in about half of milk samples, in the majority of urine samples and in less than 30% of serum samples. Triclosan was detected in one third of milk

samples, in nearly 90% of urine samples, and in only one serum sample. Although not shown in Table 1, the chlorinated compound triclocarban was measured but not detected in milk.

Comparison of concentrations of parabens and phenols in various matrices

To better understand the disposition of these chemicals in the individual participants, and how that may vary across the participants, we compared the concentrations of chemicals across matrices. First, we evaluated chemical concentrations in serum and urine of all women with 2 collections (n=30). In Table 2, we show the urine:serum ratio of methyl and propyl paraben, at visit 1 and 2, as they were the only chemicals detected in the serum of at least 50% of the samples. There was wide variation across participants, and for the majority of participants there was a change in concentrations between visits, with both parabens changing in the same direction over time.

To better understand the potential transfer of the parabens and phenols into breast milk, we also evaluated the ratios of chemicals in all the participants that donated milk in which we could make measurements. In Tables 3–6, we present the concentrations of the parabens or phenols (benzophenone-3, BPA, methyl paraben and propyl paraben) that were detected in greater than 50% of the samples. Corresponding individual urine and serum (total and free) concentrations are also reported in these tables.

For benzophenone-3 (Table 3), the milk:urine concentration ratio was heavily skewed toward urine, with ratio values ranging from 1:57 to 1:738. The milk to urine BPA ratios ranged from 1:1 to 1:80 (Table 4), with 3 of 5 individuals (for which a M:U concentration ratio could be calculated) suggesting a <1:10 relationship. Although methyl paraben (Table 5) was detected in all milk samples, the milk concentrations were 21-764 times lower than the urinary concentrations from the same women. Milk and total serum methyl paraben concentrations were not as varied as the difference in milk and urine concentrations, but concentrations in serum were consistently higher (11-30 fold) than in milk. Comparing across matrices, propyl paraben (Table 6) was detected at the highest concentrations in urine (ranging from 0.5 to 279 μg/L), and for individual participants, the milk and total serum propyl paraben concentrations were comparable, while the milk to urine ratio was heavily skewed toward urine (5 to nearly 700 fold higher).

Correlations of paraben and phenol concentrations across visit

Because of the relatively short biological half-lives of the compounds measured and the likely episodic nature of the exposures, we hypothesized that there would be a large variability between the concentrations of an individual compound between visits (collections). Table 7 shows median, minimum, maximum, and selected percentiles of the nine parabens and phenols measured in urine (total samples: n=33, V1; n=30, V2), separated by visit. The Spearman correlations demonstrate the relationship/ranking of each woman's set of measures, reflecting variation by individual (not with respect to absolute values). These correlations indicate that the relative rank of a woman is significantly related from one visit to the other. Surprisingly, there was a significant correlation between V1 and V2 measurements for 7 of 9 phenols and parabens measured when both collections and all

participants are considered. Benzophenone-3 median concentrations were higher in V2 than in V1 and were not significantly correlated between visits. These findings suggest varied or changing exposures to benzophenone-3 over time, but they may also reflect the variation in timing of sample collection at one or both visits in relation to an individual's potential exposure events. BPA concentrations in urine were also not correlated across time, and our data could not determine if it was due to exposures changing over time or due to the timing of sample collection after a potential exposure.

We made similar comparisons for the concentrations in serum, but were limited in our analysis because from the nine compounds analyzed in serum, only methyl paraben and propyl paraben were detected in greater than 50% of samples. Median, minimum, maximum, and selected percentiles of paraben and phenol concentrations in serum, separated by visit are shown in Table 8. As was the case in urine, there was a significant correlation for both serum methyl paraben and propyl paraben between visits, with the serum range being nearly identical over time for both individual parabens. Butyl paraben was detected in 9% of V1 samples and 17% of V2 samples with a maximum value of 0.7 μg/L. Ethyl paraben was detected in 38% of V1 samples and 35% of V2 samples with a maximum value of 2.4 μg/L. Triclosan was detected in serum samples from two women with a maximum value of 1.5 μg/L, very close to the LOD of 1.1 μg/L.

Only methyl paraben and propyl paraben had detectable concentrations in greater than 50% of both urine and serum samples (as shown in Table 2). Expanding upon this further, methyl paraben urine to serum concentration correlations were significant for V1 (rho=0.40, p=0.02) but not V2 (rho=0.15, p=0.42). Propyl paraben concentrations were not significantly correlated between urine and serum at the same study visit (V1 rho=0.32, p=0.07; V2 rho=0.23, p=0.22). These findings suggest a consistent exposure pattern for the parabens when measured within a matrix over time, but predictions should not be made across matrices based on single collections.

Correlations of parabens and phenols with questionnaire data

Questionnaire data (collected at visit 1) provided information on living, working, and dietary habits, overall health, water source, education, breast feeding practices, how long the participant lived in her locale, and some information on how much time she spent in her car/home/yard, near a computer, and how often she used makeup, nail polish, hair styling products, etc. Those data were analyzed for associations with phenols and parabens concentrations from matrices in which the compounds were detected in more than 50% of the samples (no milk analysis for V1). Detailed questionnaire data results are reported in the supplementary data section of this publication (Supplementary Data Tables S1-S3). Spearman correlation analysis of questionnaire data yielded significant correlations at V1 and V2 for urine methyl paraben and nail polish use (Table S1); urine and serum propyl paraben and nail polish use (Tables S1 and S2); serum methyl paraben and hair styling product use (Table S2); and an inverse correlation between urine concentrations of BPA and education and hair styling product use (Table S3). Other significant outcomes lacked a consistent pattern, with certain correlations appearing at V1 or V2, but not both visits. The

spreadsheets denoting both positive and negative correlations are included in Supplementary Data Tables S1-S3.

Correlation of parabens and phenols concentrations with endogenous immune-related biomarkers

Correlations between the phenol and paraben concentrations and endogenous immune-related biomarkers (cytokines, immunoglobulins, hormones, glucose or triglycerides), which were only measured at visit 1, were explored to try to get a better understanding of potential health related indices associated with these exposures. Data for seven different compounds are reported in Supplementary Data Tables S4-S10. These immune-related biomarkers were measured in all three of the matrices described and significant correlations are denoted based on matrix (see subscripts). Milk IL-6 had significant positive correlations with most of the phenols and parabens measured (all Rho > 0.5): BPA_{U1}, 2,4-dichlorophenol$_{U2}$, 2,5-dichlorophenol$_{U2}$, ethyl paraben$_{S}$1, ethyl paraben$_{U2}$, methyl paraben$_{S2}$, propyl paraben$_{S}$1 and propyl paraben$_{U2}$. Milk sIgA also had significant correlations, some highly correlated (i.e., Rho values of 0.74 and 0.75 with parabens), with many phenols and parabens: BPA_{U1}, 2,4-dichlorophenol$_{U2}$, 2,5-dichlorophenol$_{U2}$, ethyl paraben$_{U2}$, and methyl paraben$_{S}$1. Serum IgA had significant correlation with benzophenone-3_{U1} and ethyl paraben$_{S}$1. Serum and milk IgM had significant correlation with methyl paraben$_{U2}$ and propyl paraben$_{U2}$, respectively. Serum TNF-α had a significant correlation with propyl paraben$_{U1}$. Glucose and triglycerides had negative and positive significant correlations with BPA_{U1} and benzophenone-3_{U1}, respectively. None of the parabens or phenolic compounds had significant correlations with circulating or milk-derived hormone concentrations (estradiol or prolactin).

Discussion

We report concentrations in multiple matrices of parabens and phenols measured at 2 different periods of lactation from individual women. One of our goals was to determine which matrices provide useful data (easily collected, analysis successful in that matrix, and over 50% detects for these chemicals). Urine provided the most useful data, with all parabens and phenols yielding detectable measurements in greater than 2/3 of the individual samples at both visits. Milk concentrations, albeit limited due to the methods development nature of this study, were detectable in >50% of samples for ethyl paraben, methyl paraben, propyl paraben, benzophenone-3 and BPA, suggesting potential usefulness of larger milk biomonitoring efforts on these endocrine active compounds. Measurements in serum were not worthwhile for many of these compounds, as only methyl and propyl paraben were detectable in >50% of samples.

As a methods development study on novel endpoints, we conducted exploratory analysis without adjustment for multiple comparisons. Because we provided data on only a small number of lactating women, our results should be confirmed in future studies. However, we learned several things that should advance this field: 1) Relatively high detection incidence (56-100%) in breast milk of ethyl, methyl and propyl parabens, BPA and benzophenone-3, so this matrix could be used for exposure analysis in future studies; 2) Relatively low

milk:urine concentration ratio for BPA, so although this is a short-lived compound in the body, it may transfer to milk; 3) Statistically significant between-visit correlations for 7 of 9 of these compounds, suggesting consistent and/or recurrent exposure to these compounds over time; and 4) Consistently positive correlations of some of these compounds with immune end points in the milk of the study participants (see Supplementary data), suggesting further studies into the relationships of phenols and parabens with immune response may be fruitful.

Many of the chemicals measured in these lactating women are hormonally active in laboratory animals. BPA is employed in the manufacture of polycarbonate plastics and epoxy resins used in dental sealants, and as coatings lining food and soda cans [20], among other applications. In this study, individual urinary BPA concentrations were inversely related with the maternal education level and her reported use of hair styling products (Table S3). We did not evaluate the correlation between education and hair styling product use during pregnancy/lactation, but theorize that they are related. In rodent studies, BPA is associated with multiple adverse health outcomes following early life exposures [37]. Our data add to the limited reports of BPA concentrations in individual breast milk samples from US women analyzed mainly for method development studies [7, 30, 38-40]. Other researchers measured BPA in colostrum of Japanese women (n=110) by ELISA [41]. However, ELISA lacks adequate analytical selectivity and specificity, and matrix effects may induce performance anomalies for BPA quantification in human samples [37]. Studies in rats directly exposed to BPA report very low transfer of the compound to milk [42, 43], but the limited number of samples in our study show BPA is present in >50% of milk samples. However, because BPA is a ubiquitous environmental contaminant, we cannot rule out completely the potential for external contamination with BPA during collection, storage, or analysis [33]. We rarely detected total BPA in the serum, but we detected BPA in most urine samples. These data confirm previous reports [44] [45] that serum is not an adequate matrix for biomonitoring of BPA in adults or children, or to estimate dose in most rodent studies.

Benzophenone-3, often used as an ingredient in sunscreen, ultraviolet light stabilizer in plastics, and to inhibit photodegradation [12], has also been shown to be estrogenic [10, 18]. Our report of a mean milk concentration of 3.7 μg/L for the 7/10 samples (from 9 participants) with detectable concentrations adds to the limited data on benzophenone-3 concentrations in milk from US women [30, 39]. Previous studies have suggested that exposure to benzophenone-3 may vary by season [46] and race/ethnicity [19]. The majority of the samples in the current study were collected within the winter and spring season and most women (85%) were white. Benzophenone-3 had been previously reported in milk from Swiss women (n=34) with a median concentration of 19.8 ng/g lipid [6] and detection frequency of ∼18% (LOD = 2 ng/g). The other phenols examined show similar interquartile ranges and medians in urine to data reported in a recent study on pregnant Spanish women and their children [47].

Chlorophenols are found in biocides including pesticides, fungicides, and insecticides [31], and are used in dye synthesis intermediates, moth repellants, room deodorizers, and in treated wood. 2,5-dichlorophenol, the primary metabolite of p-dichlorobenzene, is common

in US populations [1]. Exposure to high doses of 2,4-chlorophenol in laboratory animals causes immunological and liver related effects, in addition to smaller litters and offspring with decreased birth weight [48]. There are a couple of technical manuscripts devoted to analytical method development that report rarely detecting 2,4- and 2,5-dichlorophenol in milk [39, 49]. By contrast, these dichlorophenols are detected in the urine of the majority of the US general population [1], in residents of a California agricultural community [50], and in pooled serum samples of US children [45]. In the present study, we detected 2,4-dichlorophenol in 11% of the milk samples, whereas 2,5-dichlorophenol was undetectable; both compounds were detected in >82% of the urine samples, consistent with previous results.

Triclosan is added to some detergents, toothpastes, cosmetics, clothing and plastics to prevent microbial growth. Triclosan has been shown to depress serum testosterone at high doses in male rats without effects on puberty (pre-putial separation) or reproductive organ weight [17]. In female mice, triclosan is an exogenous estrogen enhancer in the weanling uterotrophic assay [11]. The first report of triclosan in human milk was a Swedish study in which five randomly collected samples were analyzed. In 3 of 5 samples triclosan was detected, although the method of sample collection was unknown [51]. Another Swedish study carefully collected samples from 34 women who either used or did not use triclosan containing products [52]. Triclosan was present in the serum of all women tested (even the “controls”) and in the milk of nearly half of the controls and all of the exposed women. When 62 U.S. milk bank samples were tested for triclosan [53], it was present in 51 of them above the LOD of 150 ng/kg and concentrations were highly variable, as was seen in our study. In another study, triclosan was detected (LOD = 1 ng/mL) in two of the four breast milk samples analyzed [30]. In these studies, the milk collection method was unknown. Triclosan has been measured in serum of breast-feeding women [51, 52], in the urine of young American girls [54], and in the general US population [1, 19]. The urinary concentrations of triclosan in these MAMA participants are similar to those reported in NHANES [1] and by Wolff *et al* [54] although these are all vastly different populations (lifestage, sex, age). Also, women from earlier studies had lower milk triclosan concentrations than they did serum concentrations [52]; our data did not allow for these comparisons because we detected triclosan in only one serum sample and in one third of milk samples. In the present study, we assured a fastidious collection procedure with no triclosan-containing product coming in contact with the biological sample or the skin. In our study we detected triclosan in nearly 90% of urine samples, which likely reflects the accurate exposure of the woman to this compound.

Parabens are antimicrobial agents found in personal care products including lotions, cosmetics, medicines, and soaps [55] and certain parabens are approved for food use in the USA [37]. Parabens have weak estrogenic activity [56] and can induce proliferation of breast cancer cells [13]. In male rodents, butyl or propyl paraben exposure decreased sperm production, fetal testosterone, and/or epididymal weight [57, 58], and caused epigenetic changes in sperm [59].

Parabens have been measured in 100% of urine samples from pregnant women and children previously [47], which is identical to the data on lactating women in the present study. There

are currently only two publications that have measured parabens in human milk samples. In the first one, a method development paper, the authors analyzed four samples and detected (LODs = 0.1 ng/mL) methyl paraben in all of them, but propyl paraben in only one [30]. In the second study, methyl, ethyl and propyl parabens were detected in 54 carefully collected breast milk samples [6]. Neither dataset reported detectable concentrations of butyl paraben in milk, identical to our findings. Previous data show methyl paraben and propyl paraben had the highest concentrations in urine with mean values of 43.9 and 9.05 μg/L, respectively [60]. Earlier studies on measures of urine parabens in women and men reported substantial temporal variability [61, 62], and we observed significant correlations within paraben concentration over time, but greater than two collections would give increased clarity to inter-individual variability over time. Interestingly, propyl and methyl paraben had significant correlations with nail product use (Table S1) and methyl paraben concentration was associated with hair product use (Table S2) in our study. Furthermore, the parabens were the class of chemicals with the most correlations with endogenous biomarkers. Consistencies across parabens were seen in correlations with milk IL-6 and milk Ig (sIgA and IgM), and for ethyl, methyl and propyl paraben, these correlations were with urine and serum concentrations of the chemicals. These findings deserve further investigation.

In summary, there are strengths of this study that set it apart from others. These strengths include the first report of collection of multiple matrices (urine, serum and breast milk) obtained at two separate visits, from each individual, and analytical measurements by a highly experienced laboratory using validated methods, allowing for an initial assessment of disposition and variability of exposure over time and matrix. There are also limitations. They include the relatively small “n”, with multiple comparisons, and exploratory nature of the exposures and outcomes (e.g., immunological biomarkers). Because this was a pilot study, the milk data are limited but still provide interesting insights for future investigation. Given those limitations, these data suggest that serum is not an appropriate medium for detection of these non-persistent compounds. Urine, and, to a lesser extent milk, represent better matrices for detecting select phenols and parabens. These data provide support to risk assessors working on these non-persistent organic compounds and insight for future studies that may look at the partitioning of phenols and parabens in breast feeding women. Finally, these data suggest that trace levels of phenols and parabens can be present in breast milk, but should not preclude women from breastfeeding. Detection of the total concentrations of these phenols and parabens in breast milk is not proof that a nursing infant will actually absorb the target chemical. The target chemical in breast milk may be inactive, particularly if it is a metabolite. The chemical may be bound to other compounds in milk and have low bioavailability, or it may not be absorbed from the infant GI tract in an active form (e.g., a form capable of binding to target receptors). Breast milk is proven to be an extremely beneficial start to a child's life.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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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

Highlights

- Parabens and phenols were detected in urine of 2/3 of lactating moms in MAMA study
- Ethyl, methyl, or propyl paraben, benzophenone-3 and BPA were detected in breast milk
- BPA and benzophenone-3 exposures could not be predicted by single daily collections
- Correlations between chemical and endogenous immune-related biomarkers are reported

Table 1

Percent of Samples with Detectable Total Concentrations of Phenols and Parabens

Compound	Milk		Urine			Serum		
	LOD (µg/L)	V1+V2 %	LOD (µg/L)	Visit 1 %	Visit 2 %	LOD (µg/L)	Visit 1 %	Visit 2 %
Butyl Paraben	0.1	0	0.2	81	83	0.2	9	17
Ethyl Paraben	0.1	50	1.0	73	53	0.1	38	37
Methyl Paraben	0.1	100	1.0	100	100	0.1	100	97
Propyl Paraben	0.1	100	0.2	97	100	0.2	56	80
2,4-Dichlorophenol	0.16	11	0.17	85	83	NM	NM	NM
2,5-Dichlorophenol	0.42	0	0.12	85	93	NM	NM	NM
Benzophenone-3	0.51	56	0.34	67	80	0.5	17	27
Bisphenol A	0.28	89	0.36	91	80	0.3	3	10
Triclosan	1.5	38	2.27	85	87	1.1	3	3

‘Total’ concentrations refer to the sum of the free plus conjugated species. LOD = limit of detection; NM=not measured (due to very low detects in milk). Milk: n=9 women; 10 samples. Visits denoted by visit 1 (V1) and visit 2 (V2). Urine V1: n=33, V2: n=30. Serum V1: n=34, V2: n=30.

Table 2

Urine:Serum (U:S) total concentration ratios for chemicals present in the serum of 50% of participants

Participant	Methyl Paraben		Propyl Paraben	
	Visit 1 U:S Ratio	Visit 2 U:S Ratio	Visit 3 U:S Ratio	Visit 4 U:S Ratio
1	17	62	22	18
2	22	22	54	20
4	231	43	580	355
5	49	130	<1	<1
6	54	128	100	135
7	429	27	178	35
8	473	22	414	14
9	82	92	31	<1
10	2	4	3	6
11	16	5	23	8
13	28	141	97	25
14	24	68	19	96
15	39	28	11	16
17	38	13	98	3
18	131	21	681	29
19	23	40	7	2
20	265	970	652	242
21	49	13	226	33
22	9	3	15	<1
24	40	2	57	2
25	193	203	418	279
26	104	92	304	246
27	217	135	265	129
28	286	50	470	20
29	312	6081	406	85
30	64	25	18	4
31	39	88	117	347

Participant	Methyl Paraben			Propyl Paraben		
	Visit 1 U:S Ratio	Visit 2 U:S Ratio	Visit 3 U:S Ratio	Visit 4 U:S Ratio		
32	67	67	3	84		
33	24	68	25	116		
34	11	4	1	4		

Table 3

Individual Benzophenone-3 concentrations (μg/L) by matrices

Individual	Visit	$Milk_A$ 'Total'	$Milk_B$ 'Total'	Serum 'Total'	Serum 'Free'	Urine 'Total'	'Total' M:S:U Ratio
1	1	1.9	1.9	4.6	1.4	108	1:2:57
1	2	<LOD	<LOD	0.8	<LOD	26.1	N/A
2	2	4.9	4.1	6.9	3.2	2710	1:1:661
3	2	<LOD	<LOD	<LOD	<LOD	61.7	N/A
4	2	<LOD	<LOD	<LOD	<LOD	<LOD	N/A
5	2	10.4	10.2	59.2	47.7	1880	1:6:184
6	2	0.7	<LOD	1.4	<LOD	125	N/A
7	2	<LOD	<LOD	<LOD	<LOD	20.8	N/A
8	2	1	1	2.6	1	738	1:3:738
9	2	<LOD	0.5	1.5	<LOD	138	1:3:276

'Total' concentrations refer to the sum of the free plus conjugated species. Raw data only from participants with milk samples available; A and B subscripts denote two aliquots from the same sample ($Milk_A$ and $Milk_B$). A portion of $Milk_A$ was used in previous analyses for other environmental chemicals (vonEhrenstein et al 2010). M:S:U Ratio calculated using $Milk_B$ aliquots (tubes not opened prior to analyses) and Serum 'Total' values.

N/A=not applicable; limit of detection (LOD).

Table 4

Individual Bisphenol A concentrations (μg/L) by matrices

Individual	Visit	$Milk_A$ 'Total'	$Milk_B$ 'Total'	Serum 'Total'	Serum 'Free'	Urine 'Total'	'"Total' M:S:U Ratio
1	1	1.1	<LOD	<LOD	<LOD	0.6	N/A
1	2	0.6	0.6	0.80	0.8	0.7	1:1:1
2	2	0.7	0.5	<LOD	<LOD	4.1	1:<1:8
3	2	<LOD	<LOD	0.3	<LOD	5.0	N/A
4	2	0.7	<LOD	<LOD	<LOD	1.0	N/A
5	2	<LOD	0.3	<LOD	<LOD	1.1	1:<1:4
6	2	0.7	0.5	<LOD	<LOD	7.5	1:<1:15
7	2	0.3	<LOD	0.3	0.3	1.9	N/A
8	2	0.5	0.4	<LOD	<LOD	31.9	1:<1:80
9	2	0.4	<LOD	0.7	<LOD	3.0	N/A

'Total' concentrations refer to the sum of the free plus conjugated species. Raw data only from participants with 603 milk samples available; A and B subscripts denote two aliquots from the same sample ($Milk_A$ and $Milk_B$). A portion of $Milk_A$ was used in previous analyses for other environmental chemicals (vonEhrenstein et al 2010). M:S:U Ratio calculated using $Milk_B$ aliquots (tubes not opened prior to analyses) and Serum 'Total' values.

N/A=not applicable; limit of detection (LOD).

Table 5

Individual methyl paraben concentrations (μg/L) by matrices

Individual	Visit	$Milk_B$ 'Total'	Serum 'Total'	Serum 'Free'	Urine 'Total'	'Total' M:S:U Ratio
1	1	2.3	40.1	7.1	75.3	1:17:33
1	2	0.8	8.8	4.4	17.1	1:11:21
2	2	1.1	13.8	0.3	291	1:13:265
3	2	1.3	24.1	4.9	993	1:19:764
4	2	1.1	24.0	2.4	324	1:22:295
5	2	0.5	5.4	1.8	90.3	1:11:181
6	2	NM	17.2	<LOD	430	N/A
7	2	1.4	42.1	11	968	1:30:691
8	2	NM	11.8	3.5	234	N/A
9	2	0.8	18.5	4	270	1:23:338

'Total' concentrations refer to the sum of the free plus conjugated species. Raw data only from participants with milk samples available; M:S:U Ratio calculated using $Milk_B$ aliquots (tubes not opened prior to analyses) and Serum 'Total' values. N/A=not applicable; limit of detection (LOD); NM=not measured

Table 6

Individual propyl paraben concentrations (μg/L) by matrices

Individual	Visit	$Milk_B$ 'Total'	Serum 'Total'	Serum 'Free'	Urine 'Total'	'Total' M:S:U Ratio
1	1	0.2	<LOD	<LOD	<LOD	N/A
1	2	0.1	<LOD	<LOD	0.5	1:<1:5
2	2	0.3	0.4	<LOD	34.3	1:1:114
3	2	0.6	5.4	1.0	279	1:9:465
4	2	0.3	3.5	0.6	77.5	1:12:258
5	2	0.2	1.5	0.7	13.8	1:8:69
6	2	NM	4.8	2.2	187	N/A
7	2	0.3	2.9	0.6	208	1:10:693
8	2	NM	0.9	0.5	41.8	N/A
9	2	0.4	5.2	1.3	151	1:13:377

'Total' concentrations refer to the sum of the free plus conjugated species. Raw data only from participants with milk samples available; M:S:U Ratio calculated using $Milk_B$ aliquots (tubes not opened prior to analyses) and Serum 'Total' values. N/A=not applicable; limit of detection (LOD); NM=not measured.

Table 7

Urine paraben and phenol total concentration (μg/L) quintiles and between visit correlations

Compound	Visit (n)	Min	5th	25th	Median	75th	95th	Max	Significance
Butyl paraben	1 (34)	<LOD	<LOD	0.4	1.4	4.6	16.7	65.5	*
	2 (33)	<LOD	<LOD	0.5	2.9	6.8	69.6	110	
Ethyl paraben	1(34)	<LOD	<LOD	<LOD	5.3	19.4	84	135	***
	2(33)	<LOD	<LOD	<LOD	1.0	29	63.6	497	
Methyl paraben	1(34)	<LOD	11.6	75.3	143	266	1610	1710	**
	2(33)	6.7	17.1	45.8	125	248	968	993	
Propyl paraben	1(34)	<LOD	1	13.8	28.6	69	207	243	**
	2(33)	0.4	0.5	7.5	41.3	93.9	208	279	
Triclosan	1(34)	<LOD	<LOD	8.2	18.4	54.7	405	567	**
	2(33)	<LOD	<LOD	6.3	17.9	71.5	289	295	
Benzophenone-3	1(34)	<LOD	<LOD	<LOD	4.7	22.3	1440	3200	No
	2(33)	<LOD	<LOD	0.7	36.7	158	1880	2710	
Bisphenol A	1(34)	<LOD	0.6	0.6	1.1	4.1	16.9	20	No
	2(33)	<LOD	<LOD	0.5	1.0	3	28.3	31.9	
2,4-Dichlorophenol	1(34)	<LOD	<LOD	0.3	0.5	0.9	3.1	3.2	***
	2(33)	<LOD	<LOD	0.2	0.7	1.1	3.8	19.5	
2,5-Dichlorophenol	1(34)	<LOD	<LOD	0.6	1.9	6.2	53.5	65.3	***
	2(33)	<LOD	<LOD	0.6	3.1	6.9	84.1	778	

'Total' concentrations refer to the sum of the free plus conjugated species. Urine paraben/phenol significant between visit correlation, *p*-value

* <0.05,

** <0.01,

*** <0.001.

Concentrations below the limit of detection (LOD) were assigned a value equal to LOD divided by the square root of 2, for all calculations.

Table 8

Serum paraben total concentration (μg/L) quintiles and between visit correlations

Compound	Visit(n)	Min	5th	25th	Median	75th	95th	Max	%>LOD	Significance
Methyl paraben	1(34)	0.2	0.3	0.8	2.1	5.1	20.9	34	100	**
	2(33)	0.1	0.2	1.7	3.35	4.9	22.2	34.7	97	
Propyl paraben	1(34)	<LOD	<LOD	<LOD	0.3	0.8	8.9	74	56	*
	2(33)	<LOD	<LOD	0.4	0.9	4.9	33.5	80.1	80	

Paraben correlation between visits 1 and 2, *p*-value

* <0.05,

** <0.01.

LOD=limit of detection.