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PREDICTORS OF URINARY FLAME RETARDANT CONCENTRATIONS AMONG PREGNANT WOMEN

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Introduction

Flame retardant chemicals have been added to a variety of household products to meet flammability standards for decades. Until the mid-2000s, polybrominated diphenyl ethers (PBDEs) accounted for a large proportion of flame retardants used in household products including polyurethane foam and electronics; however, regulatory action and concern over the persistence, bioaccumulation, and toxicity of PBDEs has led to an increased use of alternative flame retardants [1, 2]. Organophosphate flame retardants (PFRs) are now among the most commonly used PBDE alternatives in residential furniture, electronics (e.g. TVs) and baby products (e.g. nursing pillows), and are added to flame retardant mixtures, such as Firemaster® 550 (FM550), and to other consumer products as plasticizers [3-6].

PFRs have been detected with high frequency in recent studies of home, office, and automobile dust, demonstrating that they leach from products and suggesting ubiquitous exposure [3-5, 7, 8]. Additionally, an accumulating body of research indicates that the vast majority of U.S. adults (>90%) have detectable levels of PFR metabolites in their urine, and similar detection frequencies have been reported in Canadian, European, Asian and Australian populations (e.g. [8-16]). Although data suggest that metabolite levels vary by age, with younger individuals thought to have higher exposures (e.g [8, 10, 12]), the individual characteristic and behaviors associated with higher levels of exposure are not well understood. In our present work we investigate the levels of exposure in a large pregnancy cohort, and additionally assess factors associated with higher levels of PFR metabolites in urine samples.

Methods

Study Population: The Pregnancy Infection and Nutrition (PIN) Study enrolled a cohort of central North Carolina women in early pregnancy and conducted follow-up through delivery [17]. PIN women were recruited from the University of North Carolina prenatal care clinic, and delivered their infants at University of North Carolina hospitals between 2001 and 2005 (n=2009; PIN phase 3). This analysis is limited to a subset of 349 women that participated in additional follow up components after the birth of their child. Women included in the present study were more likely to be white, have higher educational attainment, and be older than mothers in the larger PIN cohort [17, 18]. Self-administered questionnaires, telephone interviews, and home visits were used to collect pregnancy and postpartum health and lifestyle information throughout the PIN studies[17]. All study protocols were approved by the institutional review board at the University of North Carolina at Chapel Hill and all mothers provided informed consent prior to completing any study activities.

Urine Collection and Analysis: During the late-second or early-third trimester, PIN women collected a spot urine sample in a standard urine collection cup. The time and date of collection was recorded, and urine samples were aliquoted into polyethylene storage tubes and frozen at -80º C until analysis.

Urine samples were extracted using enzyme deconjugation and solid phase extraction (SPE) techniques as previously described [19] but adapted for 5 ml of urine (Butt et al. 2016, submitted). In brief, samples were thawed, 5 ml of urine was aliquoted into a clean glass test tube, the internal standard mixture was spiked (10 ng of d10-BDCIPP, 8.8 ng of d10-DPHP; 25 ng of d12-TCEP) and samples vortexed. After pH adjustment with sodium acetate (1.75 ml of 1 M sodium acetate, pH 5), the enzyme solution was added (250 µl of1000 units/ml µ-glucuronidase, 33 units/ml sulfatase in 0.2 M sodium acetate buffer), and the samples were vortexed and incubated overnight in a 37oC water bath. Samples were extracted and cleaned using SPE with a StrataX-AW (60 mg, 3 ml) column and were reconstituted in 500 ul of 1:1 water:methanol, as previously described (Butt et al. 2016, submitted). Internal standard recovery was quantified by spiking with 13C2-DPHP.

Extracts were analyzed using electrospray ionization (ESI) liquid chromatography tandem mass spectrometry (LC-MS/MS) as previously described [10] (Butt et al., 2016 submitted). Data were acquired under multiple reaction monitoring conditions using optimized parameters. Analyte responses were normalized to internal standard responses. BCIPP and BDCIPP were normalized using d10-BDCIPP, DPHP, ip-PPP and tb-PP were normalized using d10-DPHP and BCIPHIPP was normalized using d12-TCEP. Specific gravity (SG) was measured in each urine sample prior to analysis using a digital handheld refractometer (Atago). To account for differences in urine dilution, we conducted analyses of urinary metabolites using SG-corrected concentrations [20].

Statistical Analysis: Preliminary analyses indicated that urinary PFR metabolite levels were not normally distributed and were positively skewed (i.e. skewed right). Accordingly, we used non-parametric analyses or log10-transformed metabolite concentrations in statistical analyses. We calculated descriptive statistics for each PFR metabolite and conducted additional analyses for those that were detected in >70% of urine samples. For these metabolites, samples with concentrations below the method limit of detection (MDL) were replaced with the MDL/2 prior to adjustment for specific gravity. Spearman correlations were used to assess relationships between urinary PFRs. We used linear regression models with log10-transformed metabolite levels as the outcome to assess maternal predictors of PFR levels. Beta coefficients from these models were exponentiated for interpretation and represent the multiplicative change relative to the reference category for categorical variables, and the multiplicative change for a one unit increase for continuous variables.

Results and Discussion

Women averaged 29.6 years of age at the time of enrollment and were highly educated, with nearly 70% having a college education. Slightly more than half of the participants had already had at least one child (52.4%), and the majority had a BMI within the normal range at the start of their pregnancy. Urine samples were collected between 24 and 30 weeks gestation, and the average collection time was gestational week 27.

BDCIPP, DPHP, ip-PPP and BCIPHIPP were detected frequently in urine samples and concentrations varied considerably between women (92.8%, 83.7%, 99.4% and 98.3%, respectively). Among these compounds, concentration ranged from non-detectable to approximately 100 ng/mL with geometric means of 1.80, 1.42, 6.80 and 0.51 ng/mL, respectively. Although these compounds are commonly considered replacements for the PentaBDE mixture which was phased out in the U.S. at the approximate time of our sample collection, our results suggest that the PFR metabolites were in common use and that exposures were ubiquitous in the early 2000s. BCIPP and tb-PPP were detected less frequently (48.7% and 2.0% detect, respectively) in urine samples from the women in our cohort and were excluded from additional analyses.

Maternal age at the start of pregnancy tended to be inversely associated with concentrations of urinary metabolites; however, confidence intervals were relatively wide and effect estimates were generally not statistically significant. Using a continuous measure of age, BCIPHIPP concentrations decreased by approximately 3% per year (10^{β}=0.97; 95% confidence interval (CI):0.95, 0.99; p=0.02). This is consistent with the results of past research, which has also shown decreases in metabolite concentrations with age; decreases were similar in magnitude to those reported for pooled samples from the Australian population (e.g. BCIPHIPP decreased approximately 2% per each year increase in the average age of participants in pooled samples)[12] as well as those from our previous work in North Carolina adults (3% and 2% decreases in urinary BDCIPP and DPHP per year, respectively) [8].

In general we observed no associations with maternal race (white v. non-white) among the women in our cohort. This is, perhaps, expected as the cohort is predominantly composed of white women. All other races were combined into a single non-white group for comparison which may be masking important differences among some racial groups. The cohort was also relatively homogeneous with respect to maternal education; however, we did observe higher levels of ip-PPP among women with lower educational attainment (46% higher for women with less than a 4 year college degree; 10^{β}=1.46; 95% CI: 1.23, 1.74) that were statistically significant and also observed higher levels of BDCIPP among these same women (10^{β}=1.23; 95% CI: 0.97-1.51).

The body burden of many persistent organic pollutants has been associated with parity in numerous studies; however, because PFRs are rapidly metabolized and excreted, we did not anticipate that previously giving birth would be associated with higher urinary PFR metabolite levels. Contradictory to this hypothesis, our results suggest that women for whom this was the first birth had lower levels of ip-PPP. However, the opposite trend was observed for DPHP and BCIPHIPP, with women that had not previously given birth having significantly higher levels of urinary biomarkers.

Compared to women with a normal BMI prior to their pregnancy, those that were overweight or obese had higher levels of urinary BDCIPP, DPHP and ip-PPP at the time of the urine sample collection. For example, overweight women had ip-PPP levels 1.49 times those of normal weight range women and obese women had levels 1.73 times those of women with pre-pregnancy BMIs in the normal range (95% CI: 1.16, 1.91 and 1.41, 2.13, respectively). Our previous research indicates that rats exposed to FM550 in early-life gain weight more readily, suggesting that FM550's components may be obesogenic [21]. Additional work with FM550 suggests that the obesogenic potential may be driven by PFRs present in FM550 (e.g. TPHP and ip-TPHP), which are ligands for the peroxisome proliferator-activated receptor gamma, one of the critical nuclear receptors in adipocyte differentiation and lipid storage [22]. However, it is also possible that xenobiotic metabolism is intrinsically associated with BMI.

The week of gestation during which the urine sample was collected was inversely associated with BDCIPP concentrations and, although associations were imprecisely estimated and not statistically significant, DPHP concentrations followed a similar pattern. Differences in kidney function and metabolism during pregnancy may explain these differences. These results are particularly important for epidemiologic studies investigating the consequences of prenatal exposure to PFRs with a single urine sample during pregnancy and suggest that gestational timing of sample collection is an important factor driving measured concentrations.

Although PFRs are thought to be a replacement for the polybrominated diphenyl ethers, which were phased out of used as flame retardants in the U.S. in the mid-2000s, our results indicate that exposure to PFRs was widespread by 2004. In addition, our work suggests that individual characteristic (e.g. BMI, age, educational attainment, and parity) are related to exposure.

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