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Predictors of urinary flame retardant concentration among pregnant women

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Abstract

Background—Organophosphate compounds are commonly used in residential furniture, electronics, and baby products as flame retardants and are also used in other consumer products as plasticizers. Although the levels of exposure biomarkers are generally higher among children and decrease with age, relatively little is known about the individual characteristics associated with higher levels of exposure. Here, we investigate urinary metabolites of several organophosphate flame retardants (PFRs) in a cohort of pregnant women to evaluate patterns of exposure.

Methods—Pregnant North Carolina women (n=349) provided information on their individual characteristics (e.g. age and body mass index (BMI)) as a part of the Pregnancy Infection and Nutrition Study (2002–2005). Women also provided second trimester urine samples in which six PFR metabolites were measured using mass spectrometry methods.

Results—PFR metabolites were detected in every urine sample, with BDCIPP, DHPH, ip-PPP and BCIPHIPP detected in >80% of samples. Geometric mean concentrations were higher than what has been reported previously for similarly-timed cohorts. Women with higher pre-pregnancy BMI tended to have higher levels of urinary metabolites. For example, those classified as obese at the start of pregnancy had ip-PPP levels that were 1.52 times as high as normal weight range women (95% confidence interval: 1.23, 1.89). Women without previous children also tended to have higher urinary levels of DPHP, but lower levels of ip-PPP. In addition, we saw strong evidence of seasonal trends in metabolite concentrations (e.g. higher DPHP, BDCIPP, and BCIPHIPP in summer, and evidence of increasing ip-PPP between 2002 and 2005).

Conclusions—Our results indicate ubiquitous exposure to PFRs among NC women in the early 2000s. Additionally, our work suggests that individual characteristics are related to exposure and that temporal variation, both seasonal and annual, may exist.

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Keywords

organophosphate flame retardants (PFRs); pregnancy; exposure

1. 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 led to an increased use of alternative flame retardants (Stapleton et al. 2012b; van der Veen and de Boer 2012). Organophosphate flame retardants (PFRs) are now among the most commonly used PBDE alternatives in industries that manufacture residential furniture, electronics (e.g. TVs) and baby products (e.g. nursing pillows). They are commonly added to flame retardant mixtures, such as Firemaster® 550 (FM550), and to other consumer products as plasticizers (Ballesteros-Gomez et al. 2014; Fang et al. 2013; Patisaul et al. 2013; Stapleton et al. 2008; Stapleton et al. 2009; Stapleton et al. 2011).

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 [e.g. (Brandsma et al. 2013; Brommer and Harrad 2015; Cao et al. 2014; Carignan et al. 2013; Cristale et al. 2016; Hoffman et al. 2015b; Stapleton et al. 2008; Stapleton et al. 2009; Stapleton et al. 2011)]. 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. (Butt et al. 2014 and 2016; Cequier et al. 2015; Dodson et al. 2014; Hoffman et al. 2014; Hoffman et al. 2015a; Hoffman et al. 2015b; Kosarac et al. 2016; Meeker et al. 2013a; Van den Eede et al. 2015; Su et al. 2015)). Although data suggest that metabolite levels vary by age, with younger individuals shown to have higher exposures (e.g. Butt et al. 2014 and 2016; Hoffman et al. 2015b; Van den Eede et al. 2015), 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. We focus on widely used PFRs and six metabolites (Figure 1). Identifying factors contributing to higher levels of exposure to these compounds is particularly important because certain PFRs can disrupt normal endocrine function (Liu et al. 2012; Wang et al. 2013; Meeker et al. 2013a) and 2013b), are carcinogenic (Faust and August 2011; Gold et al. 1978), neurotoxic (Dishaw et al. 2011), reproductive toxicants (Meeker et al. 2013a and 2013b; Liu et al. 2013; Farhat et al. 2013), and potentially adipogenic (Patisaul et al. 2013; Pillai et al. 2014). In addition, recent data suggests that PFRs may have similar or greater toxicity than their PBDE predecessors, particularly with respect to neurodevelopmental outcomes (Behl et al. 2015; Behl. et al 2016).

2. Methods

2.1 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 (PIN 2012). 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 2006 (n = 2009; PIN phase 3). This analysis is part of a larger project investigating the impacts of exposure to environmental chemicals on children's growth. This sample is limited to 349 mothers recruited during the final four years of the cohort study, whose children had growth measurements collected at multiple time points (infants born 2002–2005). Self-administered questionnaires, telephone interviews, and home visits were used to collect pregnancy and postpartum health and lifestyle information throughout pregnancy and after the child's birth (PIN 2012). 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.

2.2 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 −80° C until analysis.

Urine samples were extracted using enzyme deconjugation and solid phase extraction (SPE) techniques as previously described (Van den Eede et al. 2013) but adapted for 5 ml of urine (Butt et al. 2016). In brief, samples were thawed, 5 ml of urine were aliquoted into a clean glass test tube, the internal standard mixture was spiked (10 ng of d_{10}-BDCIPP, 8.8 ng of d_{10}-DPHP; 25 ng of d_{12}-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 of 1000 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 37°C 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). Internal standard recovery was quantified by spiking with $^{13}C_2$-DPHP.

Extracts were analyzed using electrospray ionization (ESI) liquid chromatography tandem mass spectrometry (LC-MS/MS) with a Phenomenex Luna C18 column on an Agilent 1100 series LC and an Agilent 6410B tandem mass spectrometer as previously described (Butt et al. 2014 and 2016). 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 d_{10}-BDCIPP, DPHP, ip-PPP and tb-PPP were normalized using d_{10}-DPHP and BCIPHIPP was normalized using d_{12}-TCEP. The mean recovery of the mass-labelled standards in the urine samples (n=349) was 97% (standard error (SE) = 2.1%) for d10-DPHP, 98% (SE=3.0%) for d10-BDCIPP and 34% (SE=1.0%) for d12-TCEP. The low d12-TCEP recovery is partially due to quantification inaccuracies resulting from matrix suppression since the d12-TCEP recovery was 55–73% in the blank

samples (clean water only). Analyte values were blank corrected using the mean laboratory blank levels. Method detection limits (MDLs) were calculated as three times the standard deviation of the laboratory blanks, normalized to the average urine volume (3 ml). Sample were assessed in three batches and MDLs were calculated separately for each batch (MDLs: 136–333 pg/ml for BCIPP, 127–243 pg/ml for DPHP, 60–197 pg/ml for BDCIPP, 37–177 pg/ml for ip-PPP, 213–846 pg/ml for tb-PPP and 3–33 pg/ml for BCIPHIPP.

Specific gravity (SG) was measured in each urine sample prior to analysis using a digital handheld refractometer (Atago). Relative method accuracy was assessed by measuring PFR metabolites in SRM 3673 (n=3). Specific gravity-normalized concentrations were 1.56 ng/ml (SE=0.09) for BDCIPP, 0.44 ng/ml (0.02) for BCIPHIPP, 0.65 ng/ml (0.08) for DPHP and 6.0 ng/ml (0.30) for ip-PPP. These values are similar to those previously reported by our lab for SRM 3673 with the exception of ip-PPP, which was 0.6-times those of the current study (Hammel et al. 2016). BCPP and tb-PPP were not detected in the SRM.

To investigate the impacts of differences in urine dilution on results, we conducted analyses of urinary metabolites using raw PFR metabolite measures as well as using SG-corrected concentrations (Boeniger et al. 1993). Corrected and uncorrected concentrations were very highly correlated [Spearman correlations (r_s) >0.82 for all metabolites] and results were very similar using both methods. Here we present only the results obtained with the specific gravity corrected concentrations to facilitate comparison with previous studies.

2.3 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 $\log_{10}$-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 (see below). Spearman correlations (r_s) were used to assess relationships between urinary PFR metabolites. We used linear regression models with $\log_{10}$-transformed metabolite levels as the outcome to assess maternal predictors of PFR levels. Predictive analyses were conducted only for metabolites detected in at least 80% of the samples. Beta coefficients from these models were exponentiated (10^{β}) 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. In addition to univariate models, we conducted multivariate regression analyses including all variables of interest [age (≤25, 26–30, 31–35 and ≥36), race (white and non-white), education (≤15and ≥16, pre-pregnancy BMI (underweight, normal range, overweight and obese), parity(0 and≥1), gestational duration at the time of sample collection (24–26, 27–28 and 29–30), date of sample collection (continuous measure scaled to years) and season of collection (December–February, March–May, June–August, September–November)] to obtain the independent effect of each variable. Unadjusted and adjusted models produced very similar results. Here, we present adjusted analyses; however, unadjusted results are shown in Supplemental Table 1.

All statistical analyses were conducted in SAS (version 9.4; Cary, NC) and statistical significance was set at alpha=0.05.

3. 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 (Table 1). Women included in the present study (Table 1) were more likely to be white, have higher educational attainment, and be older than mothers in the larger PIN cohort (Daniels et al. 2010; PIN 2012). Nearly half of the participants were primiparous (47.6%); and the majority (55.6%) 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.

3.1 PRF Metabolite Levels

BDCIPP, DPHP, ip-PPP and BCIPHIPP were detected frequently in urine samples (92.8%, 83.7%, 99.4% and 98.3%, respectively), and concentrations varied considerably between women. Among these compounds, concentration ranged from non-detectable to approximately 100 ng/mL for all analytes (Table 2). BCIPP and tb-PPP were detected less frequently (48.7% and 2.0% detect, respectively). Correlations between DPHP and other PFR metabolites were generally small, but statistically significant (r_s from 0.11 for ip-PPP to 0.31 for BDCIPP, with $p<0.05$ for both). BDCIPP was additionally correlated with BCIPHIPP ($r_s=0.21$, $p=0.001$) but not ip-PPP, and BCIPHIPP was not correlated with ip-PPP among women in our cohort. The full correlation matrix is shown in Supplemental Table 2. While some overlap in patterns of use or exposure pathways for the parent PFRs is probable, the small magnitude of correlation suggests potential for different sources of exposure or differences in their toxicokinetics (or pharmacokinetics).

Although PRFs 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 indicate that exposures were ubiquitous in the early 2000s, suggesting that PFR use was already common. This finding is consistent with previous work identifying PFRs in National Institute of Standards and Technology Standards Reference Material dust samples collected in the 1990s (e.g. SRM 2585; Van den Eede et al. 2011). Urinary DPHP and BDCIPP levels in the present study were similar to those measured in a 2011–2012 cohort of pregnant women from North Carolina (Hoffman et al. 2014). This was surprising since the present cohort was sampled approximately 6–8 years earlier than the 2011–2012 cohort, and PFR chemical usage is thought to have been increased since the phase-out of Penta BDE(Stapleton et al. 2012b; van der Veen and de Boer 2012). In addition, DPHP and BDCIPP levels in the present cohort are approximately 1 order of magnitude greater than those from a Massachusetts cohort studied during a similar time frame (2002–2007) (Meeker et al. 2013a); however, the Massachusetts cohort was exclusively male which may explain observed differences. Data have previously shown that women have higher levels of DPHP than men (e.g. Hoffman et al. 2015). TPHP was recently detected in nail polish, which has been offered as a possible explanation for this pattern (Mendelsohn et al. 2016); however, we are unaware whether TPHP was used in nail polish in the early 2000s. In

addition BDCIPP levels were also higher in our cohort compared to the men in Meeker et. al. 2013a. Metabolic differences could also explain this pattern. Data from Hays et al. (2015) demonstrate that urine flaw rates differ between males and females (higher in males over 12 years of age), a factor which may impact urinary PFR metabolite concentrations. The ip-PPP levels were approximately 3–6 times higher than recent cohorts from New Jersey and California (Butt et al. 2014 and 2016). Interestingly, the women in the PIN cohort were also found to have higher levels of PBDEs in their breast milk than women in other similarly timed U.S. cohorts (Daniels et al. 2010).

3.2 Predictors of PFR levels

We found little evidence of association between PFR metabolites and maternal age at the start of pregnancy after adjusting for other factors. Only associations between maternal age and BCIPHPP remained suggestive of an inverse association after adjustment; though confidence intervals were imprecise (Table 3). Although past research has shown that metabolite concentrations decrease with age (Van den Eede et al. 2015 and Hoffman et al. 2015b), the age range of participants in our cohort was relatively narrow, potential limiting our ability to detect real difference occurring with age. Women experiencing their first pregnancy had lower levels of ip-PPP (10^{β}=0.83; 95% CI: 0.71, 0.97; p=0.02), but significantly higher levels of urinary DPHP (10^{β}=1.27; 95% CI: 1.04–1.55; p=0.02). Information on differences in consumer patterns between primiparous women and those with previous children could be helpful in identifying drivers of these associations.

Neither race nor education were strongly associated with urinary levels. However, the cohort was primarily white and well-educated women, reducing our power to fully investigate patterns by these demographics. Still, we did observe higher levels of ip-PPP among women with less education (25% higher for women with less than a 4 year college degree; 10^{β}=1.25; 95% CI: 1.01, 1.56; p=0.04). Educational attainment, a marker of socioeconomic position (SEP), has previously been associated with biomarkers of PBDE exposure in children (e.g. Stapleton et al. 2012a).

Compared to women with a normal pre-pregnancy BMI, those who were overweight or obese prior to pregnancy had higher levels of urinary BDCIPP, DPHP and ip-PPP. For example, obese women had urinary ip-PPP levels 1.52 times those of women with pre-pregnancy BMIs in the normal range (95% CI: 1.23, 1.89; p=0.0002). Our previous research indicates that rats exposed to FM550 in early-life gain weight more readily, suggesting that FM550's components may be obesogenic (Patisaul et al. 2013). 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 which is a critical nuclear receptor in adipocyte differentiation and lipid storage (Belcher et al. 2014; Fang et al. 2015; Pillai et al. 2014). However, it is also possible that PFR metabolism or excretion is intrinsically associated with BMI. For example, previous work from Hays et al. (2015) indicated that failing to account for urine dilution could induce correlation between categories of BMI and urinary BPA metabolites. Although we have conducted analyses both with and without correction for dilution (i.e. specific gravity) and observed similar associations with both methods, other factors linking BMI and

PFR metabolism or excretion could be at play. Alternatively, differences in behavior and activity patterns that are associated with BMI may explain differences in PFR exposure. Based on our study design we are unable to distinguish which factors may be causal in associations between urinary metabolites and BMI; however, this is an important consideration for future research.

The week of gestation during which the urine sample was collected tended to be inversely associated with BDCIPP and DPHP; however, associations were imprecisely estimated and not statistically significant. If real, differences in kidney function and metabolism during pregnancy may explain these patterns. These results are an important consideration 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 may be an important factor driving measured concentrations.

Season of collection was the strongest predictor of urinary PFR metabolite concentrations. Compared to samples collected in the winter, samples collected in the summer had significantly higher concentrations of BDCIPP, DHPH and BCIPHIPP. For example, BDCIPP concentrations in samples collected in the summer were approximately 3 times higher in the summer ($10^{\beta} = 3.97$; 95% CI: 2.96, 5.32; $p<0.0001$). Figure 2 depicts individual concentration measures as well as the average month temperature in our study area. This pattern suggests that behavior or exposure varies with temperature. Past research has shown that indoor dust samples collected in China had lower PFR concentrations in the summer months (Cao et al. 2014). This could mean that PFRs are partition into air more readily in warmer summer months, potentially increasing inhalation exposure which is increasingly recognized as an important exposure route for PFRs (e.g. Xu et al. 2016). However, although we did not directly evaluate indoor temperatures, they are likely to be relatively stable over time in North Carolina (central heating and air conditioning are exceedingly common in the study area) which suggests that exposure in other environments (e.g. outdoors or in vehicles) could be a substantial contributor to total the body burden. For example, PFRs are commonly detected in dust samples collected in cars (e.g. Harrada, 2016). It is possible that exposure inside cars in the summer is higher due to higher temperatures. In contrast, concentrations of ip-PPP were lower in the spring and summer than in the winter. Cumulatively, these results indicate that season of collection could be an important confounder in future epidemiology studies using spot urine samples as a proxy for longer-term PFR exposure. Despite a relatively narrow time frame of sample collection, we also observed statistical evidence of increases in urinary ip-PPP concentration; levels of ip-PPP increased by 18% per year ($10^{\beta} = 1.18$; 95% CI: 1.08, 1.28; $p=0.0003$).

Our results represent the first large-scale assessment of individual factors related to the levels of urinary PFR metabolites during pregnancy. These results should be interpreted in the context of several limitations. First, our cohort was relatively homogeneous, with the majority of women reporting white race and having high educational attainment, potentially limiting the generalizability of these results to other populations. However, the cohort's homogeneity may also be an asset for planned future research investigating health impacts of exposure, as it could reduce the potential for confounding. Another limitation is that these results rely on a single spot urine sample. Although we have previously shown that measures

of BDCIPP and DPHP in urine to be relatively stable over the course of pregnancy (Hoffman et al. 2014), and moderately to highly stable over a week (Hoffman et al. 2015b), we were unable to evaluate the potential for differences over time in this cohort. In addition, previous research has shown that urinary metabolite concentrations may vary throughout the day (Cequier et al. 2015; Hoffman et al. 2015b). While we were not able to evaluate diurnal variation because the vast majority of urine samples were collected in the same time window (>95% of samples were collected between 0700 and 1200 hours), the temporal standardization served to control such variation for this comparison. Because samples were mainly collected in the early morning, they are likely to represent exposure over the previous night, which we expect many women would have spent in their homes.

4. Conclusions

Although PFRs are commonly thought to be a replacement for the Penta-BDE mixture, which was phased out of used as flame retardants in the U.S. in the mid-2000s, our results indicate that exposure to PFRs was wide-spread by 2002. We observed strong seasonal trends in metabolite level suggesting season of collection may be an important factor to consider in future epidemiologic investigations. In addition, our results suggest that levels vary by BMI, parity, and education. Additional data are needed to identify the mechanisms explaining observed associations and to determine whether the levels of exposure that we observed are associated with any adverse help impacts among pregnant women of their children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

BCIPHIPP	1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate
BMI	body mass index
BCIPP	bis(1-chloro-2-propyl) phosphate
BDCIPP	bis(1,3-dichloro-2-propyl) phosphate
CI	confidence interval
DPHP	diphenyl phosphate
FM550	Firemaster® 550
GM	geometric mean

ip-PPP	isopropyl-phenyl phenyl phosphate
MDL	method limit of detection
PFRs	organophosphate flame retardants
PBDEs	polybrominated diphenyl ethers
PIN	Pregnancy Infection and Nutrition Study
tb-PPP	tert-butyl phenyl phenyl phosphate

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Highlights

- PFR metabolites were detected in all urine samples provided by pregnant women.
- Geometric mean concentrations were higher than for similarly-timed cohorts.
- Women with higher pre-pregnancy BMI had higher levels of urinary metabolites.
- PFR metabolite concentrations in urine vary seasonally.

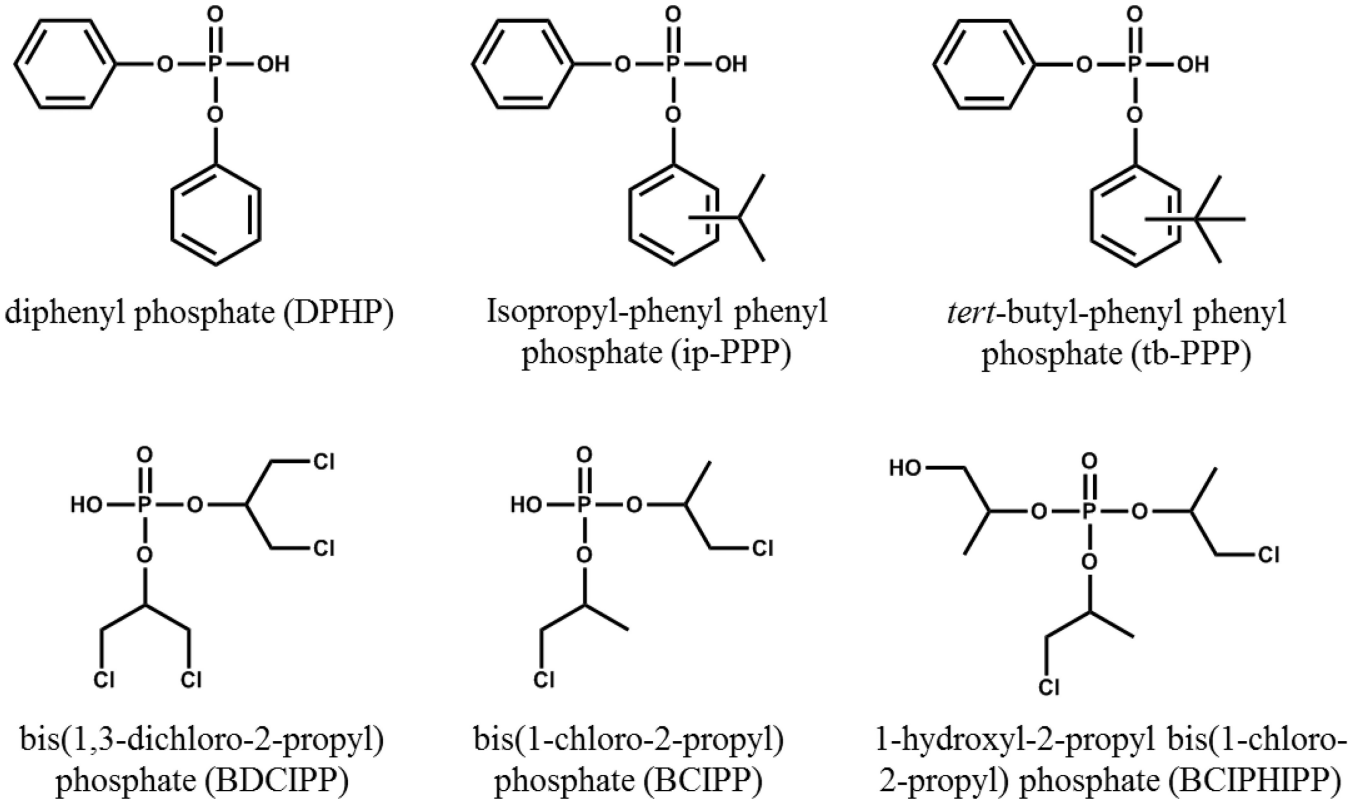

Figure 1.

Chemical structures of urinary PFR metabolites monitored. TPHP metabolite = DPHP; Isopropyl-phenyl diphenyl phosphate metabolite = ip-PPP; Tertbutyl-phenyl diphenyl phosphate metabolite= tb-PPP; TDCIPP metabolite = BDCIPP; and tris(1-chloro-2-isopropyl) phosphate (TCIPP metabolites) = BCIPP and BCIPHIPP.

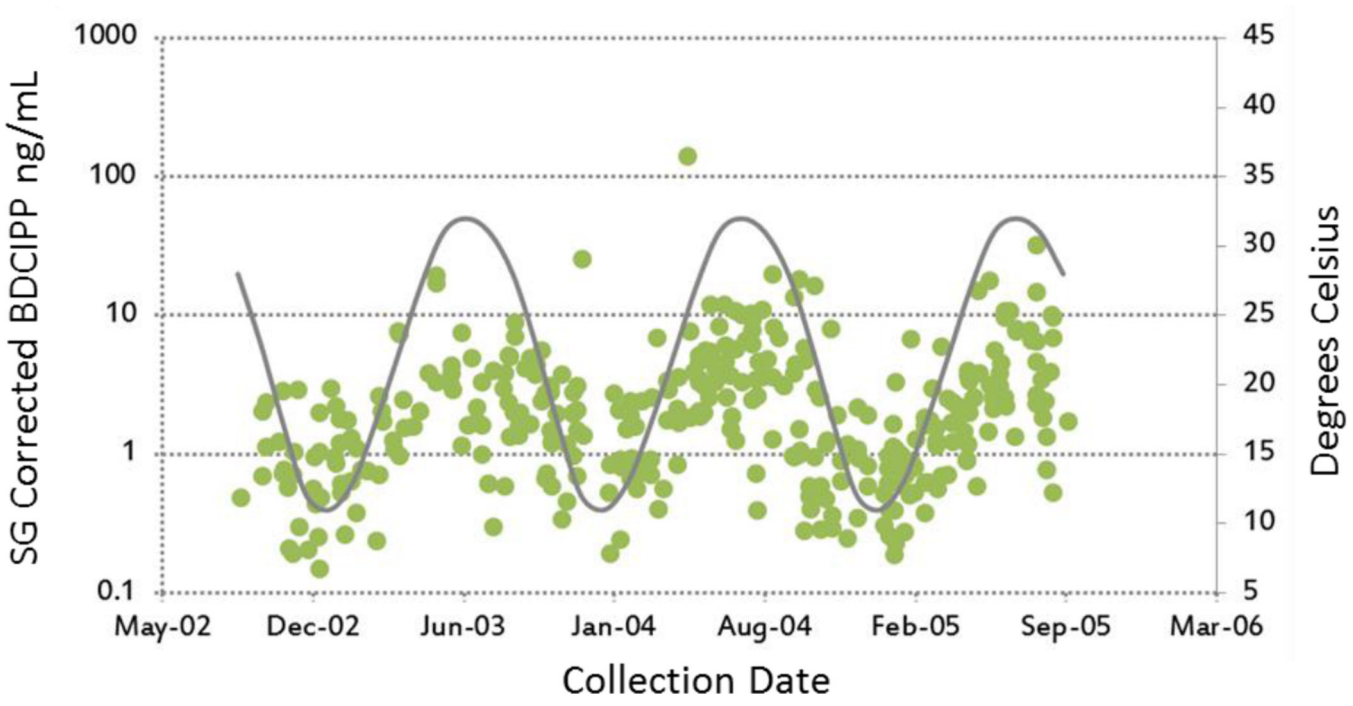

Figure 2.

Individual Urinary BDCIPP (ng/mL) concentrations plotted by date of sample collection (green dots), overlaid with the average month temperature in Chapel Hill, North Carolina (Degrees Celsius; grey line).

Table 1

Selected characteristics of 349 pregnant North Carolina women (2002–2005).

	N	%
Total	349	100.0
Age		
≤25	76	21.8
26–30	126	36.1
31–35	107	30.7
≥36	40	11.5
Race		
white	278	79.7
non-white	71	20.3
Education (years)		
≤15	106	30.4
≥16	243	69.6
Parity		
0	166	47.6
≥1	183	52.4
Pre-pregnancy BMI		
Underweight	46	13.2
Normal range	194	55.6
Overweight	42	12.0
Obese	67	19.2
Gestational weeks at urine sample collection		
24–26 weeks	72	20.6
27–28 weeks	139	39.8
29–30 weeks	138	39.5
Season of urine sample collection		
Winter (Dec – Feb)	81	23.2
Spring (Mar – May)	90	25.8
Summer (Jun – Aug)	94	26.9
Fall (Sep – Nov)	84	24.1

Table 2

Detection frequency, geometric mean and distribution information (ng/mL) for urinary PFR metabolites (N=349). Sample were assessed in three batches and MDLs were calculated separately for each batch (MDL ranges: 136–333 pg/ml for BCIPP, 127–243 pg/ml for DPHP, 60–197 pg/ml for BDCIPP, 37–177 pg/ml for ip-PPP, 213–846 pg/ml for tb-PPP and 3–33 pg/ml for BCIPHIPP.

Metabolite	% Detect	GM[a]	25th %ile	50th %ile	75th %ile	Maximum
BCIPP	48.7	--	--	0.7	1.1	6.1
BDCIPP	92.8	1.8	0.8	1.9	3.6	140
DPHP	83.7	1.4	0.8	1.3	2.7	112
ip-PPP	99.4	6.8	4.2	7.1	10.9	69
tb-PPP	2.0	--	--	--	--	8.6
BCIPHIPP	98.3	0.5	0.2	0.4	0.8	98

[a]GM: geometric mean

Table 3

Multiplicative change (10^{β}) in PFR metabolite concentration by maternal characteristic (simultaneously adjusted for all included factors).

Predictor	BDCIPP		DPHP		ip-PPP		BCIPHIPP	
	10^{β} (95% CI)	p	10^{β} (95% CI)	p	10^{β} (95% CI)	p	10^{β} (95% CI)	p
Age								
≤25	Reference	--	Reference	--	Reference	--	Reference	--
26–30	1.06 (0.78, 1.45)	0.71	1.12 (0.83, 1.52)	0.44	0.99 (0.79, 1.26)	0.96	0.92 (0.63, 1.34)	0.66
31–35	0.93 (0.66, 1.30)	0.66	0.97 (0.70, 1.35)	0.87	0.84 (0.65, 1.09)	0.20	0.74 (0.49, 1.12)	0.15
≥36	1.04 (0.70, 1.55)	0.84	0.99 (0.68, 1.46)	0.97	1.10 (0.81, 1.49)	0.53	0.67 (0.41, 1.09)	0.11
Race								
white	Reference	--	Reference	--	Reference	--	Reference	--
non-white	1.09 (0.84, 1.42)	0.52	0.91 (0.70, 1.17)	0.45	0.88 (0.72, 1.07)	0.21	0.89 (0.64, 1.22)	0.46
Education (years)								
≤15	1.03 (0.77, 1.38)	0.83	1.07 (0.81, 1.41)	0.65	1.25 (1.01, 1.56)	0.04	0.79 (0.56, 1.13)	0.20
≥16	Reference	--	Reference	--	Reference	--	Reference	--
Parity								
0	0.93 (0.75, 1.14)	0.46	1.27 (1.04, 1.55)	0.02	0.83 (0.71, 0.97)	0.02	1.22 (0.95, 1.57)	0.12
≥1	Reference	--	Reference	--	Reference	--	Reference	--
Pre-pregnancy BMI								
Underweight	1.01 (0.74, 1.37)	0.95	1.12 (0.83, 1.51)	0.45	1.09 (0.86, 1.37)	0.48	0.73 (0.50, 1.07)	0.10
Normal range	Reference	--	Reference	--	Reference	--	Reference	--
Overweight	1.09 (0.79, 1.50)	0.59	1.21 (0.89, 1.65)	0.22	1.36 (1.07, 1.73)	0.01	0.9 (0.61, 1.33)	0.60
Obese	1.17 (0.88, 1.57)	0.28	1.22 (0.92, 1.61)	0.17	1.52 (1.23, 1.89)	0.0002	1.14 (0.80, 1.62)	0.46
Gestational weeks at urine sample collection								
24–26 weeks	Reference	--	Reference	--	Reference	--	Reference	--
27–28 weeks	0.84 (0.64, 1.11)	0.22	0.86 (0.66, 1.13)	0.28	0.89 (0.72, 1.10)	0.30	0.87 (0.62, 1.23)	0.43
29–30 weeks	0.85 (0.65, 1.13)	0.26	0.85 (0.65, 1.12)	0.25	1.07 (0.87, 1.32)	0.52	1.10 (0.78, 1.54)	0.60
Date (years)	1.08 (0.96, 1.21)	0.20	0.96 (0.84, 1.08)	0.54	1.18 (1.08, 1.28)	0.0003	1.08 (0.92, 1.23)	0.33
Season of Sample Collection								
Winter (Dec – Feb)	Reference	--	Reference	--	Reference	--	Reference	--
Spring (Mar – May)	2.73 (2.05, 3.65)	<.0001	1.05 (0.80, 1.39)	0.73	0.67 (0.54, 0.83)	0.0003	1.43 (1.01, 2.03)	0.04

Predictor	BDCIPP		DPHP		ip-PPP		BCIPHIPP	
	10^{β} (95% CI)	p	10^{β} (95% CI)	p	10^{β} (95% CI)	p	10^{β} (95% CI)	p
Summer (Jun – Aug)	3.97 (2.96, 5.32)	<.0001	1.62 (1.22, 2.15)	0.0008	0.75 (0.60, 0.93)	0.01	1.96 (1.37, 2.79)	0.0002
Fall (Sep – Nov)	1.73 (1.29, 2.32)	0.0003	1.15 (0.86, 1.53)	0.34	1.01 (0.81, 1.27)	0.90	1.40 (0.98, 2.01)	0.06