Demographic and Dietary Risk Factors in Relation to Urinary Metabolites of Organophosphate Flame Retardants in Toddlers

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Abstract

Demographic and Dietary Risk Factors in Relation to Urinary Metabolites of Organophosphate Flame Retardants in Toddlers

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Organophosphate flame retardants (OPFRs), including Tris (1,3-dichloro-isopropyl) phosphate) (TDCPP), triphenyl phosphate (TPP), and isopropylated triphenyl phosphate (ITP), are increasingly used in consumer products because of the recent phase out of polybrominated diphenyl ether (PBDE) flame retardants. OPFRs have been widely detected in adults and have been linked to reproductive and endocrine changes in adult males. Carcinogenicity and damage to, immunological, neurologic and developmental systems have been observed in human cell lines.

Young children are especially vulnerable to OPFR exposure, but little is known about exposure levels or exposure risk factors in this population. We examined parent-reported demographic and dietary survey data in relation to OPFR urinary metabolite concentrations in15- to 18-month old toddlers (n=41) to. OPFR metabolites were detected in 100% subjects. The metabolite of TPP, diphenyl phosphate (DPP) was detected most commonly (100%), with TDCPP metabolite, bis(1,3-dichloro-2-propyl) phosphate (BDCPP), detected in 85-95% of samples and ITP metabolite, monoisopropylphenyl phenyl phosphate (ip-DPP), detected in 81% of samples (n=21).

Toddlers of mothers earning <$10,000 annually had average DPP concentrations 70% higher (p=.05) than toddlers of mothers earning <$10,000/year. While no dietary factors were significantly associated with OPFR metabolite concentrations, results suggested meat and fish consumption may be associated with higher DPP and BDCPP levels, while increased dairy and fresh food consumption may be associated with lower DPP, BDCPP, and ip-DPP levels. Research with larger sample sizes and more detailed dietary data is required to confirm these preliminary findings.

Introduction

OPFRs are added to numerous consumer products and building materials, resulting in widespread detection of OPFRs in air, water, soil, indoor environments and human food sources. Yearly manufacture of FRs worldwide is predicted to approach 6 billion pounds by 2019, with phosphorus-based flame retardants projected to account for 16% of global market share.[1] Prior to 2004, three types of flame retardants (FRs) were used in the US; the penta and octa forms of polybrominated diphenyl ethers (PBDEs), and Tris (1,3-dichloro-isopropyl) phosphate) (TDCPP). Accumulating evidence of environmental persistence and toxicity to human and animal health lead to a national phase out of all penta and octa PBDEs by 2012.[2] Since the PBDE phase out, the use of TDCPP and other organophosphate flame retardants (OPFRs) has increased.[3-6] However, concerns have mounted regarding the safety of these replacement FRs.[7, 8]

Most FRs are not tightly bound to product matrices and therefore, migrate easily into the surrounding environment.[9] They may be carried long distances, by air and water, or accumulate in indoor environments.[9] Exposure occurs through inhalation, ingestion and dermal absorption and the primary routes of human exposure are thought to be ingestion and inhalation of household dust.[9] While potentially a large source of exposure, ingestion of contaminated food has received less attention. One study found OPFRs in 100% of 125 food samples in China.[10] However, studies linking human OPFR exposure with dietary patterns are limited. In a study of 50 human placentas, levels of triphenyl phosphate (TPP) varied with maternal meat and vegetable consumption patterns.[11] Another study found no association between 24-hour diet recall and school aged children's urinary OPFR levels.[12]

In animal studies, OPFRs have induced immunologic, metabolic, genetic and endocrine disruptive changes.[13-18] Toxicity data is incomplete but OPFRs are linked to reproductive and endocrine changes in humans.[19] Carcinogenicity and damage to, immunological,

neurologic and developmental systems have been observed in human cell lines.[20, 21] Children have higher exposure concentrations than adults and are more vulnerable because of decreased metabolic efficiency.[12, 22, 23] Additionally, young children are undergoing sensitive periods of development, which may be disrupted by OPFR exposure.[23] Currently, very little data exists regarding early childhood exposure concentrations or the risk factors for exposure.

Our study focused on three OPFR compounds: TDCPP, TPP, and isopropylated triphenyl phosphate (ITP) (Table 1).[7, 19, 22, 24-26] TDCPP is the most frequent flame retardant found in US furniture foam, and California's Regulation, Proposition 65, lists TDCPP as a known carcinogen.[21, 26] ITP and TPP, together, make up approximately 50% of the commonly used PBDE replacement Firemaster® 550 (FM 550).[27]

In order to characterize OPFR exposure and exposure risk factors, we measured TDCPP, TPP, and ITP metabolite concentrations in the urine of 41 toddlers in Washington State, USA and investigated associations between dietary practices and demographic characteristics and toddlers' metabolite levels.

Materials and Methods

Study Population.

We recruited a convenience sample of children ages 15-18 months from the Bright Start Study in Seattle, WA. Bright Start was designed to examine the effects of socio-demographic factors on child neurodevelopment. The Bright Start Study recruited English speaking, first-time mothers of healthy infants, ages 18-23. The sample population for the current OPFR study consisted of a randomly selected subset of 41 Bright Start mother/child pairs. Selected subjects were contacted by phone after scheduling their 15-month old Bright Start visit and invited to participate in an environmental exposure study. All study procedures and protocols were approved by the Seattle Children's Research Institute Institutional Review Board.

Questionnaire.

Mothers completed self-administered questionnaires when children were 12-15 months old. Survey information included demographic factors (e.g. race, income, educational status, public assistance), lifestyle factors (e.g. work or school outside home, cohabitation) and dietary factors (e.g. meat, dairy and fresh food consumption over the past month, adequacy of resources to purchase food). Mothers also completed a food journal, detailing everything consumed by their toddlers over the 24 hours before the urine sample.

Urine Collection and Analysis.

Prior to the 15-month Bright Start visit, mothers were mailed a kit to collect urine in a specially padded diaper.[22] If mothers did not bring a urine filled diaper to the visit, study staff collected urine in the diaper during the visit.[22] Urine was squeezed from the diaper into a specimen cup and stored on-site at -20 °C until transferred to either the University

of Washington (UW) Environmental Health Laboratory (n=25) or the Duke University Laboratory (n=21).

We measured levels of urinary metabolites bis(1,3-dichloro-2-propyl) phosphate (BDCPP), diphenyl phosphate (DPP), and monoisopropylphenyl phenyl phosphate (ip-DPP) in urine samples.[22, 28] Analysis of BDCPP, DPP, and ip-DPP at the Duke University Lab was accomplished via negative electrospray ionization liquid chromatography tandem mass spectrometry (LC-MS/MS) and has been detailed elsewhere.[22] Specific gravity measurements were taken prior to analysis. Method detection limits (MDLs) were .06 ng/mL for DPP, .10 ng/mL for BDCPP, and .02 ng/mL for ip-DPP.

UW analyses included DPP and BDCPP. Sample preparation was modeled after methods previously described, with some modification. [29] A smaller bed-size solid-phase extraction cartridge was used (60 mg) because the hand-packed 100 mg StrataX-AW cartridges (Phenomenex, Torrance CA) had irregular flow with lower and more variable recovery. Instrumental analysis used ultra-high-pressure liquid chromatography (LC) tandem mass (MS-MS) spectrometry described by Van den Eede et al.[29] A conventional LC (Agilent 1200) was used with a column, with the same type of packing Synergi Polar-RP (Phenomenex) but with different dimensions (150 x 2.1 mm) and packing size (4 µm). Mobile phases were 10 mM ammonium acetate in water (A) and methanol (B). A gradient method was used: initial conditions 25% B, gradient to 95% B in 5 min, hold for 5 min, hold at 25% B for 5 min; flow rate 0.25 mL/min, and injection volume 5µl; column temperature 35 °C. UW used an Agilent 6460 MS-MS with electrospray-Jet Stream ionization. Conditions were: gas temp. 350 °C, gas flow 7 L/min, nebulizer pressure 40 psi, sheath gas heater 400 °C, sheath gas flow 122 L/min, capillary voltage 4,000V. Stable-isotope dilution quantitation was used. MDLs were .05 ng/mL for BDCPP and .5 ng/mL for DPP.

To account for differences in urinary dilution, we adjusted raw metabolite concentrations for specific gravity:

$$Mc = (M) \times \frac{(1.024-1)}{(SG-1)}$$

where Mc is the adjusted metabolite concentration, M is the raw metabolite concentration, and SG is urine specific gravity.[22, 28] Specific gravity adjusted metabolite concentrations were used for all descriptive statistics and statistical tests.

Statistical Analysis.

Metabolite measures were found to be right-skewed and were subsequently $\log_{10}$-transformed for statistical analyses. We examined all descriptive statistics and tabulated metabolite concentrations separately for Duke and UW labs to account for inter-lab measurement variation. Urine values below the MDL were calculated as equal to MDL/$\sqrt{2}$.[30] We ran independent two-sample *t*-tests and linear regressions, adjusted for lab, to examine differences in metabolite levels between demographic and dietary risk categories. We examined the following demographic variables in bivariate analysis with metabolite concentrations: sex, race, maternal education, maternal income, mother's marital/cohabitation status, mother's work or school outside of home, public assistance,

adequate resources for at least two meals per day, and food insecurity. We examined the following dietary risk variables: frequency of fresh food, high fat dairy and meat or fish in the last month, and number of fresh food, dairy and meat or fish servings in the last 24 hours. Based on the bivariate analysis results, we chose maternal income and child sex as demographic covariates for the multiple linear regression models. We used multiple linear regression, adjusting for lab, maternal income, and child sex, to evaluate associations between metabolite concentrations and demographic and dietary predictors, such as consumption of meats, high fat dairy, and fresh foods. Statistical analyses were performed using Stata (version 14; StataCorp. College Station, TX).

Results

Study cohort characteristics.

Fifty-four percent of toddlers were male and 56% were of non-Hispanic white ethnicity (Table 2). Mothers were ages of 18-23 years, with 51% having completed high school and 34% having attained a degree beyond high school. Approximately one fourth (24%) of mothers reported an annual income of below $10,000 and 85% of mothers reported working or attending school outside home. When asked how often they had adequate resources for two meals per day, 12% answered “sometimes,” “seldom,” or “not at all.”

Flame retardant metabolite concentrations in urine.

Flame retardant metabolites were detected in 100% of the 41 urine samples (Table 3). DPP was detected all samples and had specific gravity-adjusted geometric mean concentrations of 3.4 and 8.2 ng/mL (Duke, UW). BDCPP was detected in 85 and 95% (Duke, UW) of samples with geometric mean concentrations of 6.8 and 2.7 ng/mL (Duke, UW). Ip-DPP was detected in 81% of samples with a geometric mean concentration of .7 ng/mL.

Predictors of flame retardant metabolite concentrations.

In bivariate analysis, maternal income was significantly associated ($p=.05$) with DPP in a lab-adjusted linear regression model (Table 4). Toddlers of mothers with less than $10,000 annual income had an average DPP concentration (ng/mL) 70% higher (95% CI 0%, 280%) than toddlers whose mothers made more than $10,000 per year.

Overall, multiple regression analyses adjusted for income, child sex, and lab, did not demonstrate significant ($p\leq.05$) associations between OPFR concentrations and self-reported dietary patterns. However, some suggestive trends did emerge (Table 5). Toddlers with higher meat and fish consumption had higher average concentrations of DPP and BDCPP, while toddlers with higher fresh food and dairy consumption had lower DPP, BDCPP and ip-DPP levels. Toddlers eating at least one serving of meat and fish per week had 22% higher DPP (ng/mL, 95% CI -30%, 20%) and 64% higher BDCPP (95% CI -40%, 600%) than toddlers eating meat and fish less than once per week. Conversely, toddlers eating fresh food “usually” or “always” in the month prior to urine sampling, had 13% lower DPP (95% CI -50%, 40%) and 26% lower BDCPP (95% CI -30%, 220%) than toddlers consuming fresh food “sometimes” or “rarely.” Toddlers eating at least 4

servings of dairy or fresh food in the 24 hours prior to urine sampling, had 23% lower (95% CI -10%, 440%) and 17% lower (95% CI -20%, 370%) BDCPP, respectively, than toddlers consuming no dairy or fresh food in the same period. Urinary concentration of ip-DPP was 21% lower for subjects eating more fresh food (95% CI -30%, 240%) and 50% lower for subjects eating more high fat dairy (95% CI -20%, 140%) in the prior month.

Conclusions

We detected OPFRs in 100% of toddler urine samples, corroborating previous findings indicating widespread human exposure to OPFRs. We found geometric mean concentrations of DPP analyzed by the Duke lab were consistent with those reported by other studies in US toddlers, whereas mean DPP analyzed by the UW lab was higher than previously reported.[22, 24, 25] DPP concentrations were higher than were found in German toddlers (0.8 ng/mL), consistent with previously reported higher FR exposure in US populations compared to Europeans (Table 6).[22, 24, 25, 31, 32] BDCPP concentrations for this study are comparable to levels found in North Carolina and New Jersey cohorts, but lower than were found in a California cohort.[22, 24, 25] This finding is consistent with previously published higher levels of flame retardant concentrations among California subjects, likely due to stringent California furniture flammability regulations.[24, 33] Ip-DPP was lower in this cohort than was found in California and New Jersey studies.[22, 24]

While PBDE exposure has been strongly linked to socioeconomic status, this is the first study to examine OPFRs exposure in regards to very low maternal income.[40] Hoffman et al. found no association between BDCPP and DPP urine levels and income in households earning <$50,000/year. We looked at maternal income <$10,000/year and found low maternal income to be a significant predictor of DPP concentrations in toddlers. Explanations for increased flame retardant exposure among families of lower income have included the presence of poorly made or crumbling foam furniture, poorer ventilation in smaller residences and differences in diet which may either involve ingestion of more highly contaminated foods or an altering of metabolism which may make individuals more vulnerable to flame retardants in indoor air or dust.[40] This is the first study to examine OPFRs in regards to very low maternal income[40] low maternal income to be a significant predictor of DPP concentrations in toddlers. [40]

This is the first study to examine dietary contribution to OPFR metabolite concentrations in toddlers. While dietary contributions to PBDE exposure have been well-documented, we found only three studies reporting on OPFRs in food and only two studies looking at associations between human OPFRs concentrations and diet.[10-12, 33-39] Ding et al. found significantly higher metabolite concentrations of some OPFRs in the placentas of in 50 women in China who consumed more organ meat before and during pregnancy and more vegetables prior to pregnancy. Other meats and fish consumption was not associated with higher metabolite levels.[11] Cequier et al. found no significant associations between urine metabolite levels and 24-hour diet recall records among fifty-four 6-12 year old school children in Norway.[12] In the current study, our data suggested meat and fish consumption

may be associated with higher DPP and BDCPP levels, while increased dairy and fresh food consumption may be associated with lower DPP, BDCPP, and ip-DPP levels. However, no dietary associations were statistically significant. It's possible that diet is not major exposure source for OPFRs, as it is for PBDEs. However, this study, and the two other studies examining diet and OPFR concentrations, had relatively low sample sizes and may have been underpowered to detect significant associations.

Health Implications.
Our study found OPFR's in 100% of urine samples, confirming the ubiquity of OPFR exposure among US toddlers, found by other recent small sample studies.[22, 24, 25] TDCPP is a known human carcinogen and the United States Environmental Protection Agency (EPA) has designated TPP and ITP as moderate carcinogen hazards.[2, 21] TPP is associated with altered hormone levels and decreased sperm count in a sample of US men.[2, 19] Based on animal studies, the EPA has designated ITP a high hazard to human developmental, neurologic, and reproductive systems. Both TPP and ITP induce obesogenic activity in human cells and disrupt cardiac development in non-human vertebrates.[27, 42] Behl et al. compared eleven flame retardant compounds and recommended TPP and ITP for priority action due to evidence of developmental and neurologic toxicity.[41] TDCPP is designated by the EPA as environmentally persistent and a hazard to human reproductive, genetic and developmental functions. Although several US states have banned its use in children's products or residential furniture foam, recent US studies have detected TDCPP in >90% of urine samples from adult men, pregnant women and toddlers.[7, 25, 26] Given the higher exposure levels and unique vulnerability of young children, better understanding of the risk factors for OPFR exposure in young children is needed so as to prevent widespread developmental and health effects among the US population.

Limitations.
Our small sample size reduced the ability to find significant associations and limited the number of covariates we could introduce into our regression models. Given the rapid excretion of OPFR metabolites (Meeker 2013), single spot urine samples may not accurately represent toddler metabolite concentrations over time. However, other studies of OPFR metabolites have been found to be moderately to strongly reliable over months for pregnant women and adult men.[43, 44] Furthermore, our urine samples were analyzed at two different labs, introducing the possibility of inter-lab variability into our results. To account for this, we reported descriptive statistics on metabolites separately by lab and adjusted for lab in all linear regression analyses.

This is the first study to examine OPFRs concentrations in regards to very low maternal income and the first to examine dietary risk factors in toddlers. We detected OPFRs in 100% of toddler urine samples, corroborating previous findings indicating widespread human exposure to OPFRs. Low maternal income was a significant predictor of DPP concentrations in toddlers. While no dietary factors were significantly associated with OPFR metabolite concentrations, our data suggested meat and fish consumption may be associated with higher DPP and BDCPP levels, while increased dairy and fresh food consumption may be associated with lower DPP, BDCPP, and ip-DPP levels. Research

with larger sample sizes and more detailed dietary data is required to confirm these preliminary findings.

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Table 1. Names, abbreviations and parent compounds of studied OPFR metabolites.			
Parent compound	**Abbr.**	**Metabolite**	**Abbr.**
Triphenyl Phosphate	TPP	Diphenyl phospate	DPP
Tris (1,3-dichloro-isopropyl) phosphate	TDCPP	1,3-dichloro-2-propyl	BDCPP
Isopropylated triphenyl phosphate	ITP	monoisopropyl-phenyl phenyl phosphate	ip-DPP
Tris(1-chloro-2-propyl) phosphate	TCPP	bis(1-chloro2-propyl phosphate	BCPP

Table 2. Characteristics of maternal-child pairs (Washington State, USA, 2012-2015, n=41).

		N(%)
Sex	Male	22(54)
	Female	19(46)
Race	White	23(56)
	Asian	1(2)
	Black	6(15)
	Hispanic	8(20)
	Pacific Islander	1(2)
	Other	2(5)
Education	4 yrs college	4(10)
	2 yrs college	10(24)
	High school/GED	21(51)
	< High school	5(12)
	Unknown	1(2)
Annual Income [1]	0-10, 000	10(24)
	10000-25,000	17(41)
	25,000-50,000	10(24)
	50,000-100,000	3(7)
	Unknown	1(2)
Cohabitation	Living with spouse or partner	22(54)
	Not living with spouse or partner	18(44)
	Unknown	1(2)
Work/school outside home	Yes	35(85)
	No	5(12)
	Unknown	1(2)
Public Assistance	Yes	33(80)
	No	7(17)
	Unknown	1(2)
Adequate resources for 2 meals/day [2]	Usually or Always	36(88)
	Not at all, Seldom or Sometimes	5(12)
Food Insecure [3]	Yes	29(71)
	No	10(24)
	Unknown	2(5)
Fresh food	Rarely	1(2)
	Sometimes	13(32)
	Usually	19(46)
	Always	7(17)
	Unknown	1(2)
High fat dairy	Never	4(10)
	Rarely	3(7)
	Once every 2 wks	3(7)
	At least once a wk	15(37)
	Everyday	14(34)
	Unknown	2(5)
Meat and fish	Rarely	2(5)
	Once every 2 wks	5(12)
	At least once a wk	13(32)
	Everyday	20(49)
	Unknown	1(2)
Dairy servings in last 24 hrs	None	8(57)
	High (4)	6(43)
Fresh food servings in last 24 hrs	None	9(50)
	High (4+)	9(50)
[1] US $		

having adequate resources for 2 meals/day not at all, seldom or sometimes.

[3] Mother indicated having adequate resources in the last month for 2 meals/day usually or always.

Table 3. Distribution of specific gravity-adjusted flame retardant metabolite concentrations (ng/mL) found in toddler urine (n=41), by lab.

					Selected Percentiles			
Metabolite	**N**	**% >MDL [1]**	**Geometric Mean(SD) [2]**	**Arithmetic Mean (SD) [3]**	**25th**	**50th**	**75th**	**Range**
Lab 1 Duke University								
DPP	21	100	3.37(2.75)	4.63(4.02)	1.65	2.71	6.57	0.81-16.56
BDCPP	21	95	6.81(7.16)	11.83(15.03)	3.52	5.47	9.68	0.86-64.66
IP-DPP	21	81	0.43(0.44)	0.66(0.63)	0.22	0.48	0.84	0.05-2.68
Lab 2; University of WA EHL								
DPP	20	100	8.15(4.68)	9.59(6.17)	6.00	7.72	11.05	2.51-26.93
BDCPP	20	85	2.7(4.93)	17.21(46.55)	1.04	2.01	7.61	0.2-202.4

[1] Values below MDL were estimated using MDL/√2.

[2] $\log_{10}$ -transformed

[3] untransformed

Table 4. Comparison of mean differences in urinary concentrations of DPP, BDCPP, and IP-DPP (ng/mL, specific gravity adjusted) at 15-18 months of age.

		DPP (n=41)[1]			BDCPP (n=41)[2]			IPDPP (n=21)[3]		
		N(%)	N	p-value	N(%)	N	p-value	N(%)	N	p-value
Sex	Male	22(54)	22	0.49	22(54)	22	0.27	15(71)	15	0.95
	Female	19(46)	19		19(46)	19		6(29)	6	
Race	White	23(56)	23	0.96	23(56)	23	0.81	14(67)	14	0.87
	Non-white	18(44)	18		18(44)	18		7(33)	7	
Education	<= High School	26(65)	26	0.30	26(65)	26	0.45	12(57)	12	0.54
	>Highschool	14(35)	14		14(35)	14		9(43)	9	
Annual Income	<10,000	10(25)	10	0.05	10(25)	10	0.47	15(71)	15	0.23
	>10,000	30(75)	30		30(75)	30		6(29)	6	
Living with Spouse or partner	Yes	22(55)	22	0.14	22(55)	22	0.87	10(48)	10	0.91
	No	18(45)	18		18(45)	18		11(52)	11	
Work or school outside home	Yes	35(88)	35	0.34	35(88)	35	0.90	19(90)	19	0.99
	No	5(13)	5		5(13)	5		2(10)	2	
Public Assistance	Yes	33(83)	33	0.59	33(83)	33	0.60	17(81)	17	0.58
	No	7(18)	7		7(18)	7		4(19)	4	
Resources for 2 meals/day[6]	Yes	36(88)	36	0.66	36(88)	36	0.57	17(81)	17	0.42
	No	5(12)	5		5(12)	5		4(19)	4	
Food Insecure[5]	Yes	29(74)	29	0.52	29(74)	29	0.96	14(70)	14	0.51
	No	10(26)	10		10(26)	10		6(30)	6	
Fresh food in last month	Usually|Always	26(65)	26	0.35	26(65)	26	0.51	15(71)	15	0.42
	Sometimes| Rarely	14(35)	14		14(35)	14		6(29)	6	
High Fat dairy in last month	At least 1x/wk	29(74)	29	0.95	29(74)	29	0.66	12(60)	12	0.16
	Less than 1x/wk	10(26)	10		10(26)	10		8(40)	8	
Meat/fish in last month	At least 1x/wk	33(81)	33	0.57	33(81)	33	0.54	17(81)	17	0.13
	Less than 1x/wk	7(17)	7		7(17)	7		4(19)	4	
Dairy servings in last 24 hrs	None	8(57)	8	0.94	8(57)	8	0.61	6(67)	6	0.40
	High (4)	6(43)	6		6(43)	6		3(33)	3	
Fresh food servings last 24 hrs	None	9(50)	9	0.68	9(50)	9	0.56	5(71)	5	0.53
	High (4+)	9(50)	9		9(50)	9		2(29)	2	

[1,2] Linear regression, adjusted for lab, of speicfic gravity adjusted metabolites. Corrected geometric means of logadjusted specific gravity adjusted metab back calculated.

[3] Linear regression. Geometric mean of untransformed, specific gravity adjusted, metabolite concentrations.

[4] In 12-month old survey, mother indicated child ate high fat dairy or meat at least 1x/wk or engaged in nighttime breastfeeding

[5] Mother indicated either receiving food stamps, or WIC or having adequate resources for 2 meals/day, seldom or sometimes.

[6] In 12-month old survey, mother indicated having adequate resources for 2 meals/day usually or always.

Table 5. Predictors of specific gravity-adjusted urinary DPP, BDCPP, and ip-DPP (ng/mL), adjusted for lab only compared to adjusted for lab, maternal inco sex.

		DPP (n=41)			BDCPP(n=41)			ip-DPP (n=21)		
		N	Unadjusted Beta[1] (95% CI)	Adjusted Beta[2] (95% CI)	N	Unadjusted Beta[1] (95% CI)	Adjusted Beta[2] (95% CI)	N	Unadjusted Beta[1] (95% CI)	Adjusted Beta[2] (95% CI)
Fresh food in last month	Usually or Always	26	0.8(0.5-1.3)	0.87(0.5-1.4)	26	0.71(0.3-2)	0.74(0.3-2.2)	15	0.66(0.23-1.87)	0.79(0.3-2.4)
	Sometimes or Rarely[3]	14			14			6		
High fat dairy in last month	>=1x/wk	29	1.02(0.6-1.8)	0.991(0.6-1.7)	29	0.78(0.2-2.5)	0.81(0.2-2.7)	12	0.51(0.19-0.12)	0.5(0.2-1.4)
	< 1x/wk[3]	10			10			8		
Meat & fish in last month	>=1x/wk	33	1.19(0.6-2.2)	1.22(0.7-2.2)	33	1.48(0.4-5.3)	1.64(0.4-6)	17	insufficient n	
	< 1x/wk[3]	7			7			4		
Dairy servings in last 24 hrs	High (4)	6	0.97(0.4-2.2)	0.98(0.5-2.1)	6	0.62(0.1-4.5)	0.77(0.1-4.4)	3	insufficient n	
	None[3]	8			8			6		
Fresh food servings in last 24 hrs	High (4+)	9	0.89(0.5-1.6)	0.96(0.6-1.6)	9	0.65(0.1-3)	0.83(0.2-3.7)	5	insufficient n	
	None[3]	9			9			2		

[1] Adjusted for lab only (Duke University vs. University of Washington).

[2] Adjusted for lab (Duke University vs. University of Washington, maternal income (>/< US$10,000/yr), and child sex.

[3] Reference goup

Table 6. Summary of published findings of specific gravity adjusted OPFR metabolites in toddler urine. Geometric mean in ng/mL, unless otherwise specified.

Location	Age	n	DPP	BDCPP	ip-DPP	References
Germany	22-80 mos	312	.8 (median)	-	-	Fromme et al. (2014)
New Jersey, US	1-5yrs	26	3	5.6	10	Butt et al. (2014)
North Carolina, US	2-18 mos	43	3.2	7.3	NR	Hoffman et al. (2015)
California, US	2-70 mos	33	2.9	10.9	1.8	Butt et al. (2016)
This study	15-18 mos	21	3.4	6.8	0.43	Duke lab
		20	8.2	2.7	-	UW lab