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Metabolites of Organophosphate Flame Retardants and 2-Ethylhexyl Tetrabromobenzoate in Urine from Paired Mothers and Toddlers

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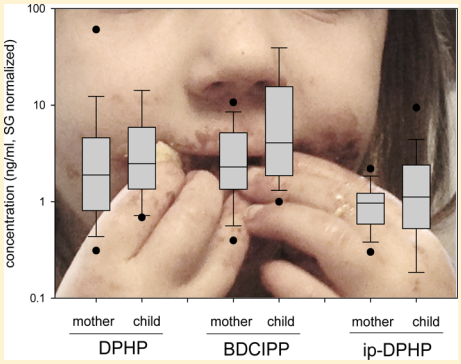
Supporting Information

ABSTRACT: As a result of the polybrominated diphenyl ether (PBDE) ban in the mid-2000s, the chemical flame retardant market has moved toward alterative compounds including chlorinated alkyl and nonchlorinated aryl organophosphate flame retardants (OPFRs) as well as aromatic brominated compounds such as Firemaster 550 (FM550). Recent studies have shown that the OPFRs and Firemaster 550 components are frequently detected in polyurethane foams and in indoor dust. Some OPFRs are considered carcinogenic and/or neurodevelopmental toxicants, and children's exposure to these compounds is a concern. OPFRs are readily metabolized and excreted in the urine as their dialkyl and diaryl compounds which function as biomarkers for OPFR exposure. Limited research has shown that adults are broadly exposed to OPFRs, but nothing is known about children's exposure. Similarly, 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB), a FM550 component, is metabolized to tetrabromobenzoic acid (TBBA). The current study measured levels of bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), bis(1-chloro-2-propyl) phosphate (BCIPP), diphenyl phosphate (DPHP), 2 alkylated DPHPs, and TBBA in urine collected in 2013 from 21 US mother-toddler pairs. BDCIPP, DPHP, and ip-DPHP were detected in 100%, 98%, and 96% of all individuals, whereas BCIPP and *tert*-butyl-DPHP (tb-DPHP) were only detected in 8% and 13%. Further, TBBA was detected in 27% of adults but 70% of children. Overall, children had higher urinary levels of BDCIPP, DPHP, ip-DPHP, and TBBA as compared to their mothers, suggesting higher exposure. For example, on average, BDCIPP levels in children were 4.9 times those of mothers. BDCIPP and DPHP levels in mother's urine were also significantly correlated with levels in children's urine, suggesting similar exposure routes, likely in the home environment. Various potential predictors of OPFR exposure were assessed using a questionnaire. In children some predictors of hand-mouth exposure were associated with elevated BDCIPP and DPHP levels (e.g., less frequent hand washing for BDCIPP). Overall, these trends are consistent with higher flame retardant levels in children as a result of increased hand-mouth behavior and elevated dust exposure.



INTRODUCTION

Chemical flame retardants (FRs) are often added to household products such as furniture foam, textiles, and electronics to meet strict flammability standards. A key regulation was California Technical Bulletin 117 (TB 117), which has recently undergone revision and no longer requires open flame test requirements.[1] The more stringent open flame test led to the high application rates of FRs in polyurethane foam in furniture, but the revised standard − TB117-2013 − focuses on smoldering ignition sources, the main cause of furniture fires, and is expected to reduce the use of FR additives in foam. For many years the dominant FR used in polyurethane foam was the polybrominated diphenyl ether (PBDE) pentaBDE commercial mixture.[2] Concerns regarding their bioaccumulation in human tissues, and potential health effects, resulted in a phase-out of the pentaBDE and octaBDE mixtures in both Europe and the United States during the mid-2000s.

To continue to meet the flammability standards following the pentaBDE phase-out, manufacturers have relied on an increasing variety of alternative FRs. A major class of alternative compounds is the organophosphate flame retardants (OPFRs) which are broadly comprised of the chlorinated alkyl phosphates and nonchlorinated aryl phosphates.[3] Representative OPFR chemicals include tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) and triphenyl phosphate (TPHP). In addition, TPHP has been used as a plasticizer and lubricant.[3] An additional alternative is Firemaster 550 (FM550), which is comprised of TPHP, various isopropylated TPHPs isomers, 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB), and bis(2-ethylhexyl)-2,3,4,5-tetrabromophtalate (BEH-TEBP).[4]

Recent studies from the United States show that OPFRs and FM550 components are the most frequently detected flame retardants in foams from baby products[5] and residential sofas.[2]

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Further, OPFRs are extensively detected in office and house dust[6−8] as well as in children's handwipes.[9] While limited, evidence suggests that OPFR dust levels have increased since the PBDE ban,[6] presumably reflecting the increased use of these chemicals as PBDE replacements.

Several of the OPFRs have been shown to be carcinogenic.[3] TDCIPP is listed as a known carcinogen by the State of California[10] and has been classified as a probable carcinogen by the Consumer Product Safety Commission (CPSC).[11] Some OPFRs have also shown neurodevelopmental effects in cell culture systems as well as in fish models.[3,12,13] Further, levels of TDCIPP and TPHP in house dust have been associated with altered hormone levels and diminished semen quality in men.[14,15] Also, perinatal exposure to FM550 resulted in early puberty, glucose sensitivity, and significant weight gain in rats.[16]

OPFRs are readily metabolized in the body to their diester metabolites, such as bis(1,3-dichloro-2-propyl) phosphate (BDCIPP) and diphenyl phosphate (DPHP) as well as phase II conjugate metabolites.[17] Several recent studies have observed nearly ubiquitous detection of OPFR diester metabolites in adult urine,[18−20] indicating that these compounds are convenient biomarkers of OPFR exposure. Further, *in vitro* studies have shown that EH-TBB is rapidly metabolized to 2,3,4,5-tetrabromobenzoic acid (TBBA) in human and rodent subcellular fractions.[21] In addition, a recent study from our group has shown that TBBA urinary levels are a good biomarker of EH-TBB exposure.[22] Previous studies have observed significantly higher levels of PBDEs in children from mother-toddler pairs;[23] however, there is very little information on OPFR and EH-TBB exposure in children. Children's exposure is of concern due to the potential neurodevelopmental effects[12] as well as the elevated hand-mouth behavior which increases exposure to dust-associated contaminants such as the PBDEs.[23,24]

In this study, we measured levels of BDCIPP, bis(1-chloro-2-propyl) phosphate (BCIPP), DPHP, 2 alkylated DPHPs, and TBBA in urine from 21 mother-toddler pairs (chemical structures of parent compounds and target metabolites shown in Figure 1). The study tested the hypothesis that children will have higher levels of OPFR exposure, presumably due to increased hand-mouth behavior. Urine samples were collected from mothers and toddlers in a US pediatric clinic. Samples were extracted using either solid-phase extraction (SPE) or liquid−liquid extraction and analyzed by liquid-chromatography tandem mass spectrometry (LC-MS/MS). In addition, a questionnaire was completed to examine several predictors of OPFR exposure such as hand-mouth activity and the presence of foam-containing items in the house.

EXPERIMENTAL SECTION

Study Group. Urine samples were collected from 21 paired mothers and children. In most pairs, only one child per family was sampled, but two children were sampled for 5 pairs (i.e., total parents = 22, total children = 26). Further, 1 mother did not provide her child's urine. In addition, mothers completed a questionnaire to assess factors that may contribute to OPFR/EH-TBB exposure for both themselves and their children (e.g., hand-mouth activity and the presence of foam-containing furniture and foam-containing children's products). Participants were recruited from Princeton Nassau Pediatrics clinic offices in West Windsor and Princeton, NJ between August 2013 and January 2014. Overall, our cohort was highly educated, mostly Caucasian, and of high socioeconomic status (Table 1).

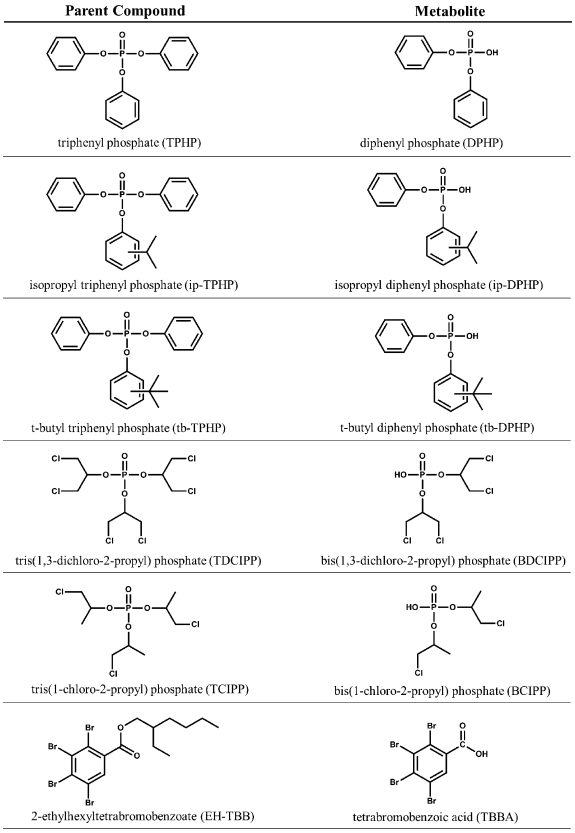

Figure 1. Chemical structures of parent compounds and metabolites.

Table 1. Demographic Characteristics of the Study Cohort

mothers (n = 19)		
race		
	Asian	2
	Hispanic	2
	White	13
	declined	2
education		
	some college	2
	college graduate	8
	graduate degree	7
	declined	2
income (1000s)		
	<50	2
	50−99	1
	100−149	3
	>150	8
	declined	5
children (n = 23)		
age (months)		
	<24	1
	24−35	4
	36−48	14
	>48	3
	declined	1

Mothers were older than 18, and children were between 1 and 5 years of age. Informed consent was obtained from all mothers, and the mothers provided consent for their children. The study design, consent forms, and recruitment materials

were approved by Chesapeake Research Review, Inc. (Chesapeake IRB).

Urine was collected as spot samples in sterile urine specimen collection cups on site at the clinic offices. For the 13 month old child only, urine was initially collected using a urine collection bag and then immediately transferred into a plastic specimen cup. Samples were immediately frozen at −20 °C and shipped to Duke University on dry ice. Samples were kept frozen until extraction and chemical analysis.

Materials. BDCIPP and d_{10}-BDCIPP were purchased from Wellington Laboratories (Guelph, ON). BCIPP and d_{10}-DPHP were synthesized by the Max Planck Institute for Biophysical Chemistry (Goettingen, Germany). Isopropyl DPHP (ip-DPHP), *tert*-butyl DPHP (tb-DPHP), $^{13}C_2$-DPHP, and TBBA were synthesized by the Small Molecule Synthesis Facility at Duke University (Durham, NC). Ammonium acetate, pyrrolidine and 2,3,5-triiodobenzoic acid (TIBA) were purchased from Sigma-Aldrich (St. Louis, MO). StrataX-AW (60 mg, 3 mL) solid phase extraction columns (SPE) and the Luna C18(2) (2.5 μm, 50 × 2 mm) analytical column were purchased from Phenomenex (Torrance, CA, USA). Methanol and acetonitrile were HPLC grade (EMD Millipore Corporation, Bellerica, MA).

Extraction and Instrumental Analysis. Extraction and analysis methods for BCIPP, BDCIPP, DPHP, ip-DPHP, and tb-DPHP were modified from methods previously developed by our lab.[18] Briefly, urine samples were thawed, a 5 ml aliquot was transferred to a clean test tube, spiked with mass-labeled internal standards (d_{10}-BDCIPP = 80 ng, d_{10}-DPHP = 60 ng), acidified to pH <6.5 with formic acid, and diluted 1:1 with water. Urine samples were concentrated and cleaned using StrataX-AW SPE columns as previously described.[18] The eluent from the SPE column was blown to dryness under a gentle nitrogen stream, reconstituted in 500 μL of 1:1 H_2O:MeOH and spiked with the recovery standard ($^{13}C_2$-DPHP = 81.5 ng). Extracts were analyzed by negative electrospray ionization liquid chromatography tandem mass spectrometry (LC-MS/MS). Chromatography was performed using a Luna C18(2) column (50 × 2.0 mm, 2.5 μm particle size, Phenomenex, Torrance, CA) preceded by a SecurityGuard Polar-RP (4 × 2.0 mm) guard cartridge. Methanol and water (modified with 0.8 mM ammonium acetate) were used as the mobile phases, the column oven was 45 °C, the injection volume was 5 μL, and the flow rate was 300 μL/min. Initial conditions were 90:10 water:methanol, held for 3 min, increasing to 5:95 over 4 min, held for 2 min, returning to initial conditions over 2 min, and held for 6 min (representative chromatogram shown in the Supporting Information). Data were acquired under multiple reaction monitoring conditions using optimized parameters. Analyte responses were normalized to internal standard responses. BCIPP and BDCIPP were corrected for recovery using d_{10}-BDCIPP, while DPHP, ip-DPHP, and tb-DPHP were corrected for recovery using d_{10}-DPHP. Specific gravity measurements were taken with a digital refractometer (Atago USA, Inc., Bellevue, WA) prior to analysis.

TBBA was analyzed using a novel liquid−liquid extraction technique developed by our group.[22] Briefly, 10 mL of urine was spiked with 5 ng of the internal standard (TIBA), diluted 1:1 with phosphate buffer (pH 7.4, 0.1 M), and acidified with 1 mL of sulfuric acid. The TBBA was extracted by shaking with hexane (×3). The hexane extracts were washed with water (×2), acidified to pH 2−3 with 1 M acetic acid, blown to dryness, and then reconstituted in 1:1 MeOH:water. The extracts were analyzed by ESI(−)-LC-MS/MS using a Synergi Polar-RP column (50 × 2.0 mm, 2.5 μm particle size, Phenomenex). Due to limited sample volume, only 23 children's samples were analyzed for TBBA.

Quality Assurance/Quality Control. Analyte recovery was assessed by spiking 2.5−13 ng of the target compounds into 5 mL of urine (n = 3). Unspiked urine was simultaneously analyzed to correct for the native levels of BDCIPP, DPHP, and ip-DPHP present. Mean recoveries were 125% (standard error = 6%) for BCIPP, 78% (10%) for BDCIPP, 100% (13%) for DPHP, 106% (2%) for ip-DPHP, and 91% (10%) for tb-DPHP.

In the urine samples (n = 56), the mean recovery of the mass-labeled standards was 85% (standard error = 1.9%) for d_{10}-DPHP and 186% (6.5%) for d_{10}-BDCIPP. The apparent high recovery for d_{10}-BDCIPP was because the recovery standard, $^{13}C_2$-DPHP, eluted at an earlier retention time and was subject to increased matrix suppression in the actual urine samples. That is, enhanced suppression of the $^{13}C_2$-DPHP, relative to d_{10}-BDCIPP, resulted in artificially high recovery values for d_{10}-BDCIPP only, but, as shown by the spike and recovery tests, the BDCIPP accuracy was not affected. One replicate and laboratory blank (5 mL Milli-Q water only) sample was extracted with every batch (n = 4). Replicate values were generally within 20%. Very low levels of DPHP (mean = 0.56 ng) and ip-DPHP (0.34 ng) were consistently detected in the laboratory blanks. Urine analyte values were blank corrected using the mean laboratory blank values. Method detection limits (MDLs) were calculated as three times the standard deviation of laboratory blanks normalized to the volume of water extracted (5 mL). MDLs were 117 pg/mL for BCIPP, 22 pg/mL for BDCIPP, 185 pg/mL for DPHP, 90 pg/mL for ip-DPHP, 93 pg/mL for tb-DPHP, and 3.0 pg/mL for TBBA, respectively. Concentrations were normalized to specific gravity as previously described.[18,25]

Statistical Analyses. Descriptive statistics were calculated for OPFR metabolites measured in urine samples from children and adults. These data indicated that the distributions of OPFR metabolite levels were highly skewed. After transformation the values met normality as determined by the Shapiro-Wilk test. Thus, $\log_{10}$-transformed values were used in statistical analyses. A value of MDL/2 was used for all values <MDL. Summary statistics and statistical tests were only performed on analytes with >50% detection frequency. Paired t-tests were used to investigate differences in the geometric mean OPFR metabolite levels in samples collected from children and mothers. Association between maternal and child BDCIPP, DPHP, and ip-DPHP levels were assessed with Pearson ($\log_{10}$-transformed values) and Spearman correlations (nontransformed values). To evaluate whether there were independence concerns resulting from including multiple children from the same family, we also used the average urinary metabolite levels of the siblings in analyses (t-tests and correlations). The results are not reported because they did not differ notably from results including all pairs. Generalized estimating equations (GEEs) were used to examine relationships between continuous measures of OPFRs in urine and children's hand washing and hand-to-mouth behaviors. GEEs are an extension of linear regression models that account for potential residual within-family correlations that may arise from including multiple children from the same family in analyses. For mothers, general linear models were used to examine relationships between continuous measures of OPFRs in urine and hand washing frequency. Beta coefficients from regression models were

exponentiated (10^{β}), producing an estimate of the multiplicative change in urinary OPFR levels relative to the reference group. All statistical analyses were performed in SAS (version 9.2; SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Urinary Concentrations in Mothers and Children. Considering all samples from mothers and their children (see Figure 2), BDCIPP, DPHP, and ip-DPHP were detected in

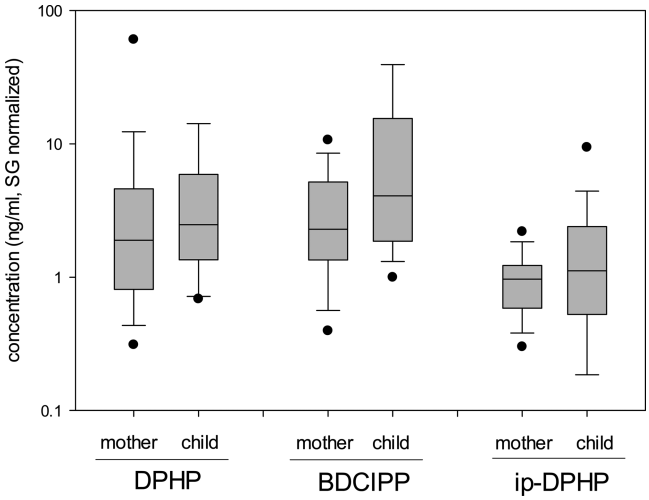

Figure 2. Box and whisker plot comparing OPFR metabolite concentrations (ng/mL, specific gravity normalized) in urine from mothers (n = 22) and their children (n = 26).

100%, 98% (mothers = 95%, children = 100%), and 96% (mothers = 100%, children = 92%) of samples, respectively (Table 2). In contrast, BCIPP and tb-DPHP were only detected in 8% and 13% of samples, respectively. These results, in combination with other small scale exposure studies,[18−20] suggest that exposure to TDCIPP, TPHP, and ip-TPHP is likely ubiquitous in the United States. The low detection frequency of BCIPP was surprising considering that relatively similar levels of TCIPP and TDCIPP are measured in house dust.[6,9] However, urinary BCIPP levels may be limited by the low formation yield of BCIPP from TCIPP, as shown through metabolism studies with human liver microsomes and S9 *in vitro*.[20] TCIPP appears to be metabolized to a dechlorinated carboxylic acid metabolite more than to the dialkyl ester, thus further studies may need to monitor this novel metabolite in urine to fully evaluate exposure to TCIPP.

As we are aware, this is the first report of isopropyl- and *tert*-butyl-TPHP metabolites in humans. Our chromatography method was not capable of separating ortho- and meta-isomers (not shown), and thus the exact structure of the ip-DPHP is not known. Isopropylated-TPHPs are components of the FM550 mixture,[26] and likely other flame retardant and plasticizer mixtures, and they have been detected in furniture foams from the US.[2] Specifically, FM550 contains mono-, di-, tri-, and tetraisopropyl-TPHPs at approximately 32%, 10%, 2.4%, and 0.4%.[26] The present study only measured the monoisopropyl-DPHP, but presumably other isopropylated-DPHPs may be present at lower concentrations. For example, tri-isopropylated- and tri-*tert*-butyl-TPHPs were detected, but at very low frequency, in outdoor air from the Great Lakes.[27]

Adult urinary DPHP levels were within the range reported in recent studies.[18,20] However, BDCIPP levels were 2−6 times greater than in previous studies that measured BDCIPP in urine collected in 2009−2012.[7,18,20] This trend may reflect the increasing use of TDCIPP in household products or higher TDCIPP exposure in our cohort. Children's levels could not be compared to previous reports since the current study is the first to measure OPFR metabolites in children.

TBBA was detected in approximately 50% of the combined adult and children urine samples. However, there was a much higher detection frequency in children as compared to adults. Specifically, TBBA was detected in 16 of 23 children samples but in only 6 of 22 adult samples. The geometric mean level in children (7.4 pg/mL, specific gravity normalized) from the present study was comparable to that in adults from a North Carolina cohort (geometric mean = 5.6 pg/mL, specific gravity normalized).[22] Due to the low detection frequency of TBBA in adults from our cohort, the geometric mean was not calculated, and thus we could compare our levels to that from the North Carolina cohort.

A few individuals had high levels of either DPHP, ip-DPHP, BDCIPP, or TBBA but not elevated levels of multiple OPFR metabolites or TBBA. These high levels could not be explained by any of the exposure predictors as assessed through the questionnaire.

Among samples from children, levels of TBBA were not correlated with DPHP (r_p = −0.16, p = 0.45 and r_s = −0.11, p = 0.62) and ip-DPHP (r_p = 0.37, p = 0.08 and r_s = 0.27, p = 0.22); two metabolites potentially originating from the Firemaster 550 mixture were not correlated. The lack of correlation may be

Table 2. OPFR Metabolite Concentrations (ng/mL, Specific Gravity Normalized) and TBBA (pg/mL, Specific Gravity Normalized) in Urine from Mothers and Their Children[a]

		mothers (n = 22)				children (n = 26)[b]				mother-child correlation (n = 23)	
	MDL	detection frequency (%)	geometric mean (95% CI)	min	max	detection frequency (%)	geometric mean (95% CI)	min	max	Spearman coefficient (r_s)	p-value
BCIPP	0.12	14	NA	<0.12	0.64	4	NA	<0.12	0.46	NA	NA
BDCIPP	0.02	100	2.4 (1.5−3.7)	0.37	11.0	100	5.6 (3.2−9.7)	0.89	251	0.51	0.007
DPHP	0.18	95	1.9 (1.1−3.4)	<0.18	68.7	100	3.0 (1.9−4.9)	0.68	140	0.48	0.01
ip-DPHP	0.09	100	0.85 (0.67−1.1)	0.29	2.3	92	1.0 (0.62−1.6)	<0.09	10.1	0.62	0.008
tb-DPHP	0.09	5	NA	<0.09	0.13	19	NA	<0.09	0.48	NA	NA
TBBA	3.0	27	NA	<3.0	62.2	70	7.4	<3.0	84.9	0.52	0.04

[a]Abbreviations: min, minimum; max, maximum; NA, not available (detection frequency was <50%). [b]For TBBA analysis n = 23 due to insufficient volume in three samples.

explained by the multiple DPHP sources, in addition to FM550. Further, considering the combined set of adults and children, BDCIPP, DPHP, and ip-DPHP were not correlated with each other.

Correlations between Mothers and Children. The BDCIPP levels in children's urine were 4.9 times (range: 0.43−45), on average, those of mothers. Using the log-transformed data, a *t*-test showed that these differences were statistically significant (p = 0.01). Similarly, children's DPHP and ip-DPHP levels were 3.0 times (0.09−24) and 1.2 times (0.17−16), on average, than those of their mothers, but the DPHP and ip-DPHP relationships were only suggestive and not statistically significant (p = 0.20 for DPHP and p = 0.28 for ip-DPHP). These trends are consistent with previous studies that show elevated PBDEs levels in children's serum as compared to their mothers[23] as well as nonpaired studies which generally show higher PBDE levels in children relative to adults.[24,28]

Due to the high number of nondetectable TBBA levels in adults, it was not possible to perform statistical tests comparing mother-child levels. Specifically, TBBA was not detected in both adults and children in 6 of the mother-child pairs (n = 21), but children's TBBA levels were higher in all 15 pairs for which there was a detectable level in the child. However, it is worthwhile to note that TBBA was detected in adults from only 6 of these 15 pairs. These results suggest that EH-TBB exposure was higher in children, as compared to their mothers, and are consistent with the elevated DPHP and ip-DPHP levels in children's urine. Taken together, the current study suggests that children have higher exposure to Firemaster 550.

OPFR metabolite levels were positively correlated between paired mothers and children for BDCIPP (r_p = 0.50, p = 0.10 and r_s = 0.51, p = 0.007), DPHP (r_p = 0.46, p = 0.02 and r_s = 0.48, p = 0.01), and ip-DPHP (r_p = 0.58, p = 0.002 and r_s = 0.51, p = 0.008) (Figure 3). As well, TBBA levels were positively correlated between mother-child pairs (r_p = 0.62, p = 0.01 and r_s = 0.52, p = 0.04). However, the TBBA correlations should be considered with caution due to the high number of nondetectable levels in adults. Specifically, TBBA was only detected in 6 out of 16 adults among the 16 children had detectable TBBA levels. Overall, these trends indicate that the shared environment between mother-child (i.e., diet, house dust exposure, genes, and behavior) are important determinants of OPFR and EH-TBB exposure. Few studies have investigated FR levels in parent-child pairs, and one study that monitored PBDEs in paired mothers-children did not report correlations.[23] Thus, it is not possible to assess the importance of the shared environment in other studies.

Predictors of OPFR Metabolite Levels in Urine. Various predictors of OPFR exposure (e.g., hand-mouth activity, presence of foam-containing furniture, and children's products) were assessed through a questionnaire. The cohort lacked diversity with respect to education, race, and socioeconomic status, and thus these factors could not be evaluated. Further, most children were similar in age (mean = 3.2 yrs, standard error = 0.18 yrs), and thus associations with children's age could not be evaluated. Overall, the presence and number of foam-containing furniture items in the house were not associated with BDCIPP, DPHP, and ip-DPHP urinary levels in either children or adults.

In children it was shown that some predictors of hand-mouth exposure were associated with urinary BDCIPP and DPHP levels (Table 3). Specifically, parent-reported "frequent" hand-

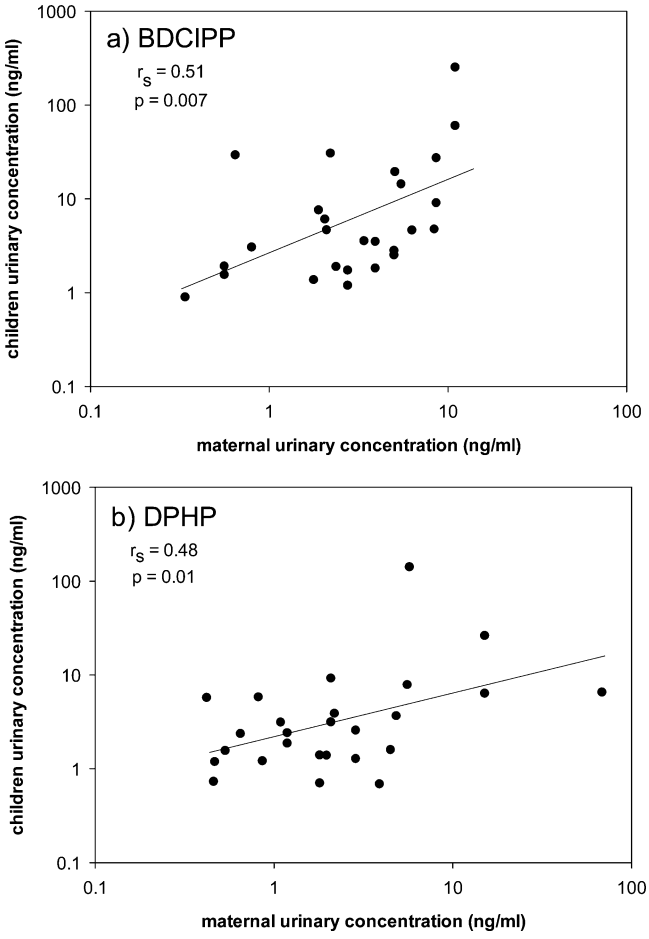

Figure 3. Relationship between a) BDCIPP and b) DPHP urine concentrations (ng/mL, specific gravity normalized) in mothers and their children.

washing in children was suggestive (p = 0.12) of lower BDCIPP levels. The "frequent" hand-washing children had BDCIPP levels that were 0.43 times (95% confidence interval (CI): 0.15, 1.25) those of the "sometimes/hardly ever" children. Also, increased hand-to-mouth contact (>6 times per day) was significantly associated (p = 0.002) with higher DPHP levels, and increased thumb sucking (sometimes/frequently) was suggestive (p = 0.11) of higher DPHP levels. Children with >6 hand-to-mouth contacts per day had DPHP levels that were 1.6 times (95% CI: 1.19, 2.15) those of children that had 0−6 hand-to-mouth contacts per day. Children with "sometimes/frequent" thumb sucking had 3.2 times (0.76−13.5) DPHP levels as compared to those with "hardly ever". The number of hand-to-mouth contacts, number of object-to-mouth contacts, and thumb-sucking frequency were not associated with urinary BDCIPP levels in children, nor were hand washing frequency before eating and object-to-mouth contacts associated with urinary levels of DPHP. No associations were found between ip-DPHP and hand-mouth behavior or hand washing.

In adults, we did not find evidence that hand-mouth behavior was associated with BDCIPP or DPHP urinary levels. For example, average hand washing frequency was not associated with BDCIPP or DPHP levels (hand washing frequency: >6 times per day versus 0−6 times per day, p = 0.63 and p = 0.69 for BDCIPP and DPHP, respectively).

Overall, these trends suggest that children's OPFR exposure, but not adult's exposure, are associated with increased hand-

Table 3. Predictors of Specific-Gravity Normalized Urinary BDCIPP, DPHP, and ip-DPHP in Children (n = 23)

		SG-corrected BDCIPP		SG-corrected DPHP		SG-corrected ip-DPHP	
behavior	n	exponentiated beta (95% CI)	p	exponentiated beta (95% CI)	p	exponentiated beta (95% CI)	p
hand washing before eating							
frequently	14	0.43 (0.15, 1.25)	0.12	1.08 (0.32, 3.63)	0.89	0.68 (0.20, 2.33)	0.54
sometimes or hardly ever	9	reference		reference		reference	
hand-to-mouth contacts							
0−6 per day	13	reference		reference		reference	
>6 per day	10	1.48 (0.35, 6.21)	0.59	1.60 (1.19, 2.15)	0.002	0.79 (0.27, 2.26)	0.66
object-to-mouth contacts							
0−3 per day	17	reference		reference		reference	
≥4 per day	6	0.90 (0.38, 2.10)	0.80	1.23 (0.28, 5.47)	0.79	1.71 (0.37, 7.82)	0.49
thumb sucking							
hardly ever	17	reference		reference		reference	
sometimes or frequently	6	1.12 (0.42, 2.98)	0.82	3.21 (0.76, 13.56)	0.11	1.09 (0.28, 4.23)	0.90

mouth behavior. Previous assessments have shown that young children have elevated hand-mouth contacts as compared to older children and adults.[29] Presumably, exposure may originate from contact with contaminated house dust and OPFR-containing products, although these were not analyzed in the current study. This is consistent with previous work from our laboratory which showed toddler's PBDE serum levels were associated with levels in house dust and hand wipes.[24] Regarding OPFR exposure, our previous work also showed that hand-mouth activity was associated BDCIPP urinary levels in adult office workers.[7] Further, house dust was shown to be an important exposure source of TDCIPP, but not TPHP, in adults.[30]

A major strength of the study is that we examined paired mother-child samples, thus reducing confounding effects such as variability in diet and dust exposure. Also, the mother and child samples were collected at the same time. Further, our cohort was relatively homogeneous with respect to socio-economic status which further reduces potential confounding effects but may limit generalizability to other populations. Although we only collected spot measurements, these measurements have been shown to be reliable OPFR indicators over the course of 1 week.[20] Additionally, our small sample size may have limited our statistical power to detect meaningful associations and limited the number of variables we could consider simultaneously in analyses. Lastly, we relied on parent's assessment of child behavior which may underestimate frequency of hand-mouth contacts.

In summary, the current study is the first to measure these FR metabolites in children's urine. Consistent with previous studies in adults, it was shown that children are ubiquitously exposed to OPFRs. Also, it was shown that children's levels were higher than adults, presumably due to increased hand-mouth contact in children. These trends are consistent with PBDEs which show elevated levels in children and may be characteristic for contaminants for which dust is the dominant exposure source. Additional research is needed to investigate how exposure to these fire retardants may affect children's health, particularly since there is evidence that some of them may be carcinogenic and affect hormone signaling and development.

■ ASSOCIATED CONTENT

Supporting Information

LC-MS/MS chromatogram of a calibration standard containing target analytes. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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