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Urinary Biomonitoring of Phosphate Flame Retardants: Levels in California Adults and Recommendations for Future Studies

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ABSTRACT: Phosphate flame retardants (PFRs) are abundant and found at the highest concentrations relative to other flame retardant chemicals in house dust; however, little is known about the biological levels of PFRs and their relationship with house dust concentrations. These relationships provide insight into major exposure pathways and potential health risks. We analyzed urine samples from 16 California residents in 2011 for 6 chlorinated and nonchlorinated dialkyl or diaryl phosphates (DAPs), the expected major metabolites of the most prominent PFRs, and qualitatively screened for 18 other metabolites predicted from in vitro studies. We detected all 6 DAPs within the range of previously reported levels, although very few comparisons are available. We found weakly positive nonsignificant correlations between urine and dust concentrations and maxima urine corresponding to maxima dust for the pairs bis(1,3-dichloro-2-propyl) phosphate (BDCIPP)-tris(1,3-dichloroisopropyl) phosphate (TDCIPP) and bis(2-chloroethyl) phosphate (BCEP)-tris(2-chloroethyl) phosphate (TCEP). Metabolite levels of PFRs were correlated for many PFR combinations, suggesting they commonly co-occur. As far as we know, this is the first study to measure these 6 DAP metabolites simultaneously and to detect other PFR metabolites in US urine samples. We recommend biomonitoring studies include these 6 DAPs as well as several additional compounds detected through qualitative screening and previous ADME studies. PFRs represent a class of poorly studied commercial chemicals with widespread exposure and raise concerns for health effects including carcinogenicity and neurotoxicity.

INTRODUCTION

Research on residential exposure to flame retardant (FR) chemicals has focused on California because of its unique furniture flammability standard.1,2 For example, polybrominated diphenyl ether (PBDE) levels have been found at much higher levels in Californians and their house dust relative to other regions.3 In a recent study, we investigated a wider range of FRs in California house dust, providing, for the first time, exposure data for a broad suite of 49 FR chemicals, including PBDEs, other brominated flame retardants (BFRs), and phosphate flame retardants (PFRs).4 Of all FRs, PFRs were found at the highest concentrations in dust. This chemical group includes tris(2-chloroethyl) phosphate (TCEP) and tris(1,3-dichloroisopropyl) phosphate (TDCIPP) (or chlorinated “tris”), which are listed as carcinogens under California’s Proposition 65.5 Little is known about the biological levels of these PFRs and their relationship with levels in house dust.

PFRs are mainly used as chemical additives in FR mixtures but also as plasticizers and in hydraulic fluids, solvents, extraction agents, antifoam agents, adhesives, and coatings for electronic devices.6 PFRs have been in use for many years; however, interest in exposures to and risks from PFRs has increased after their use as replacements for the banned Penta-

BDE mixtures. Commercially available PFRs include both halogenated and nonhalogenated compounds, and chemical properties, such as volatility, dictate their use patterns. Available information about the uses and potential health concerns for many PFRs is summarized in van der Veen et al.7 and Dodson et al.4 Briefly, chlorinated PFRs, such as TCEP and TDCIPP are carcinogens,5 and structural similarities suggest that tris(1-chloro-2-propyl) phosphate (TCIPP) would be also.8 Triphenyl phosphate (TPHP), a component of several FR mixtures including Firemaster 550 and widely used as a plasticizer, has been associated with increased prolactin levels and reduced sperm concentration in men,9 as well as with cardiotoxicity and potential endocrine disruption.10,11 Less is known about the health effects of other nonhalogenated PFRs. Auletta et al.12 reported an association between tri-n-butyl phosphate (TNBP) and increased incidence and severity of bladder tumors in rats, and Kanazawa et al.13 reported an association between TNBP and Sick Building Syndrome in humans. Tris(2-butoxyethyl)
phosphate (TBOEP) has been associated with decreased red cell acetylcholinesterase, ataxia, tremors, and increased liver weights in rats.\textsuperscript{14} Clearly, additional research is needed to understand the potential health effects of these PFR chemicals.

In a recent study, we measured concentrations of 4 halogenated and 9 nonhalogenated PFRs, among other flame retardant chemicals, in house dust collected in 16 California homes in 2006 and again in 2011.\textsuperscript{4} We found TBOEP at the highest concentrations in 2006 and 2011; it had the highest median (almost 4× higher than any other) and highest maximum (1.2× higher than any other) in 2011. TCEP, TCIPP, TDCIPP, and TPHP were also found at higher levels than other FR chemicals, with concentrations into the mg/g range or >0.1%. Best practices for biomonitoring exposure to PFRs are not well developed. For example, major metabolites have not been conclusively identified, and information to relate pharmacokinetic parameters to metabolite levels is limited.\textsuperscript{15}−\textsuperscript{17} In recent studies, investigators have focused on chlorinated and nonchlorinated dialkyl or diaryl phosphate (DAP) metabolites, because these were the major reported metabolites for TNBP and TDCIPP, were reported among the metabolites of TPHP and TCEP\textsuperscript{18}−\textsuperscript{20} and would be expected as hydrolysis products of Phase I metabolism. Cooper et al. measured bis(1,3-dichloro-2-propyl) phosphate (BDCIPP) and diphenyl phosphate (DPHP) in urine of nine nonoccupationally exposed individuals, finding levels in the pg/mL to ng/mL range.\textsuperscript{21} BDCIPP was also found in nearly all urine samples collected from two cohorts in Boston, MA.\textsuperscript{22,23} Carignan et al. reported an association between urinary BDCIPP levels and working in a new office building and noted a positive trend between office dust TDCIPP concentrations and urinary BDCIPP concentrations.\textsuperscript{22} Meeker et al. found a significant relationship between TDCIPP house dust concentrations and urinary BDCIPP concentrations, although not a relationship for another investigated PFR—TPHP and its metabolite DPHP—in a sample of 45 men.\textsuperscript{23} We recently reported concentrations of 6 chlorinated and nonchlorinated DAP metabolites of PFRs in urine samples of 59 obese (mean BMI of 34.5) Belgian adults.\textsuperscript{24}

Several household exposures studies have shown PBDEs and PFRs are abundant in dust, particularly in California due to its unique flammability standards. The objective of this study was to biomonitor the major metabolites of PFRs in people residing in California, where PFRs were found at the highest levels. Specifically, we measured urinary concentrations of bis(2-chloroethyl) phosphate (BCEP), bis(1-chloro-2-propyl) phosphate (BCIPP), BDCIPP, DPHP, dibutyl phosphate (DBP), and bis(2-butoxyethyl) phosphate (BBOEP) in urine samples collected from 16 Californians in 2011. These participants also provided dust samples in 2006 and 2011 and information about home furnishings at both time points. We explored relationships between urinary concentrations of chlorinated and nonchlorinated DAPs and house dust concentrations of the corresponding parent PFRs collected at the same time in 2011. We also screened for 18 additional urinary analytes identified in previous in vitro work.\textsuperscript{24} To our knowledge, this represents the first look at urinary concentrations of several metabolites in a key class of FR chemicals that appears to be increasing in use. We also discuss the best approaches for future flame retardant biomonitoring studies.

### MATERIALS AND METHODS

#### Study Design/Sample Collection

We collected urine samples from 16 nonsmoking adults living in northern California in 2011. Participants were originally enrolled in the California Household Exposure Study conducted in 2006\textsuperscript{4,25,26} and also took part in a follow-up study of flame retardants in California homes.\textsuperscript{4} Among the 16 participants, 12 lived in Richmond, CA, an urban fence-line community, and 4 lived in Bolinas, CA, a rural coastal town. Urine samples were collected in quality certified precleaned 125 mL amber glass jars with Tenlon lids (part # 0125-0050-QC; Environmental Sampling Supply, San Leandro, CA). We stored urine samples at −4 °C before overnight shipment to the Southwest Research Institute where they were thawed, divided into aliquots, and refrozen. We sent 10 mL aliquot to University of Antwerp, Belgium for chemical analysis and a second 1 mL aliquot to LifeLabs (British Columbia, Canada) for creatinine analysis. As reported in Dodson et al.,\textsuperscript{4} we simultaneously collected house dust samples in 2011 using a custom-made crevice tool fitted to a vacuum cleaner, and residents were surveyed about the presence of furniture, carpets, and electronics, particularly if any items were introduced since 2006. The study protocol was approved by Chesapeake Institutional Review Board, and all participants provided informed consent. Participants received their individual study results.

#### Analytical Methods

Alkyl phosphate metabolites were analyzed by the Toxicological Center (University of Antwerp, Belgium). DPHP and BBOEP were analyzed via LC-MS/MS based on a method described in Van den Eede et al.\textsuperscript{27,28} Briefly, a 2 mL urine was spiked with 15 ng of IS mixture, adjusted to pH 5, and extracted on Oasis WAX (60 mg, 3 mL). Other potential PFR metabolites were qualitatively screened by mixing 150 mg of urine with 25 ng of internal standard. Five μL of the filtered mixture was analyzed using LC-(ESI)-MS/MS in negative and positive ionization. The same instrument and column were used as for the chlorinated and nonchlorinated DAP analysis though under different conditions (see the Supporting Information). Two specific MRM transitions, which were established using microsomal extracts of the PFRs,\textsuperscript{29} were used for each metabolite. Additional information on materials, LC-MS/MS and GC-MS/MS analysis, and QA/QC protocols are provided in the Supporting Information. Creatinine was measured using a colorimetric Jaffe-based method at LifeLabs.

#### Quality Control

Three pooled urine samples were spiked with metabolites at concentrations of 3 ng/mL and analyzed with the urine samples. Percent recovery for all metabolites was between 78% (DPHP) and 98% (BDCIPP), with relative standard deviations of up to 15% for GC analytes and 2% for LC analytes (see Table SI-2). Method limits of detection (LOD) are also given in Table SI-2. Three blinded field blanks (tap water) were sent to the laboratory to evaluate potential background contamination during transport and handling.
Reported concentrations were below the LOD for all analytes in all three samples.

**Statistical Methods.** We calculated summary statistics without creatinine normalization. All concentrations were blank corrected with mean detected blanks values varying between 0.03 (BCIPP) and 0.37 ng/mL (BBOEP) (see the Supporting Information). BDipp was not detected in the procedural blanks. We calculated summary statistics for urinary concentrations (not creatinine adjusted) using the nonparametric Kaplan–Meier technique recommended by Antweiler and Taylor 2008 and Helsel 2005. Specifically, we used the “censtats” function in the NADA package in R. To investigate relationships between parent PFRs and metabolites and among urinary metabolites and among dust analytes, we calculated Kendall’s tau rank correlation estimates, adjusted for censored data. Kendall’s tau rank correlations, with adjustment for ties, have been shown to provide better estimates compared to Pearson or Spearman correlation coefficients with substitution of arbitrary values (LOD/2 or LOD/√2) for censored data. Kendall’s tau is the most appropriate correlation method for evaluating correlation among our data because our data are likely skewed and are multiply censored. Correlation coefficients were calculated for parents-metabolites and among metabolites and dust analytes when there were at least 50% simultaneous detects and using creatinine-adjusted concentrations. We adjusted urinary concentrations (mass/volume) for dilution by dividing by the creatinine concentration (mass/volume) to obtain a creatinine-adjusted concentration (mass of chemical/mass of creatinine). We set nondetectable concentrations to the method limit of detection (LOD) (see in Table SI-2) for data visualizations and indicate the range of plausible values with lines extending toward zero when graphing. To compare to published TDCIPP-BDipp and TPHP-DHP results, we also calculate Spearman correlation coefficients and develop linear regression models with dust and urine concentrations, setting nondetects to one-half LOD. To evaluate the linear relationship between TDCIPP dust concentrations and urinary BDipp concentrations and to ascertain the predictability of urinary BDipp from dust TDCIPP, we conducted linear regression models with natural log-transformed urinary concentrations as the response variable and natural log-transformed dust concentrations and creatinine as explanatory variables. Creatinine is included as a predictor rather than used to adjust urinary concentrations in linear modeling as recommended by Barr et al. One individual provided a urine sample with insufficient volume for creatinine analysis so values from this participant are not used in analyses with creatinine.

Based on our study findings as well as previous in vitro and in vivo work, we make recommendations for future biomonitoring and research in order to advance exposure assessment of PFRs.

Specifically, if a chlorinated or nonchlorinated DAP (metabolite) is detected in the urine samples from our study, we suggest additional research and further biomonitoring of these metabolites. We also consider information from previous research identifying metabolites from in vitro liver preparations and the qualitatively screened metabolites in our samples in order to recommend for additional analytical targets. Finally, we note if in vitro or in vivo data in the literature suggest additional potential metabolites.

### RESULTS AND DISCUSSION

**Urinary Concentrations of PFR Metabolites.** This is the first report, to our knowledge, of this extended list of PFR metabolites in US urine samples. We detected BDipp in nearly all urine samples (94%) followed by BCEP (75%), DHP (62%), DBP (56%), BCIPP (31%), and BBOEP (12%) (Table 1). Concentrations were generally in the ng/mL range. DPHP, BDipp, and BCEP were found at the highest concentrations.

The urinary concentrations observed in this study are in the range of previously reported levels; however, limited data are available for comparison (Table SI-3; also Figure SI-1). Maxima for the 6 metabolites in our study were lower than reported for 59 obese Belgian adults. Low detection frequencies preclude comparisons at the mean across all metabolites; however, for the two metabolites with sufficient detection frequencies—BDipp and DHP, means are similar. Four studies provided comparison data for DHP and BDipp. Central tendency estimates were similar (generally within a factor of 4) across studies, whereas maxima were more dispersed. Meeker et al. reported a higher BDipp maximum (25 ng/mL vs 3.9 ng/mL). For BCEP, the only other measurements available are from a German study (n = 30), which had similar detection limits. We observed a higher frequency of detection (75% vs 50%) and median concentration (0.65 vs <0.1 ng/mL); however, the maximum in the German study was 10× higher (2.1 ng/mL versus 27.5 ng/mL), despite the parent chemical, TCEP, apparently no longer being produced in Europe. Overall, central tendency levels for PFR metabolites in our study are similar to levels reported outside of California despite the fact that California participants might have been expected to have higher concentrations based on previously reported findings for PBDEs. Although maximum concentrations in our study did not exceed those reported elsewhere, this may be due to our smaller sample size.

Through qualitative chemical analysis, we detected other PFR metabolites in the urine samples (Table SI-4). We detected hydroxyphenyl phenyl phosphate, a TPHP metabolite, in 53% of the samples and TBOEP metabolites, bis(2-butoxyethyl) 2-ethoxyglucuronide phosphate (7%), 2-butoxyethyl 2-hydroxyethyl phosphate (67%), hydroxybutoxyethyl...
bis(2-butoxyethyl) phosphate (93%), and bis(2-butoxyethyl) 2-hydroxyethyl phosphate (87%) as well (Table SI-4). We also found tris(2-chloroethyl) phosphate (TCEP) in 13% of samples. These compounds were not present in the procedural blanks.

Correlations between Urine and Dust. Correlations between PBDEs in house dust and serum have been previously reported. These and other data support an estimate that approximately 80% of PBDE exposure in the US is from house dust, so we expected similar relationships between environmental (i.e., house dust) and biological (i.e., urine) measurements for these chemicals. However, we found weakly positive nonsignificant correlations and maxima urine corresponding to maxima dust for the metabolite-parent pairs BDCIPP-TDCIPP and BCEP-TCEP. Figure 1 shows scatter plots of the creatinine-adjusted metabolite concentrations and house dust concentrations with Kendall’s tau correlation estimates (95% confidence intervals in parentheses). Dark filled circles indicated detected in urine and dust, lighter filled circles indicate detected in either dust or urine but not both, and open circles indicate not detected in either dust or urine (and values set at detection limit).

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Figure 1. Scatterplots of creatinine-adjusted metabolite concentrations and house dust concentrations with Kendall’s tau correlation estimates (95% confidence intervals in parentheses). Dark filled circles indicated detected in urine and dust, lighter filled circles indicate detected in either dust or urine but not both, and open circles indicate not detected in either dust or urine (and values set at detection limit).
per ng/g in this study versus $6.66 \times 10^{-6}$ ng/mL per ng/g in Meeker et al.). Therefore, for every 10 ng/g increase in TDCIPP concentration in house dust, we found a 0.877 pg/mL increase in its metabolite in urine.

To evaluate the linear relationship between TDCIPP dust concentrations and urinary BDCIPP concentrations and to ascertain the predictability of urinary BDCIPP from dust TDCIPP, we created a linear model predicting natural logarithm of BDCIPP (ln[BDCIPP]) urine concentrations with ln[TDCIPP] dust concentrations and creatinine (mmol/L). We found a significant ($p = 0.033$) positive relationship between ln[TDCIPP] and ln[BDCIPP], with approximately 39% of the variance in BDCIPP concentrations explained by its relationship with the natural logarithm dust concentration and measured creatinine (Table SI-5). The discrepancy in conclusions between the rank correlation estimates and linear modeling is likely a result of the latter being a parametric model influenced by the two data points representing the highest concentrations.

The lack of correlation between concentrations of alkyl phosphate metabolites in urine and parent PFRs in dust may be a result of the following: 1. not analyzing the appropriate metabolite; 2. low detection frequencies; 3. shorter exposure period for urine vs dust; 4. contribution of other exposure routes; for example, uses of the chemical other than as a flame retardant, or 5. lack of statistical power. We analyzed for chlorinated and nonchlorinated DAP metabolites expected to be the major metabolites for each of the PFRs in this study; however, there may be other metabolites that, when combined, would provide a better estimate of the total metabolite concentrations. There may be metabolites that are more specific to the parent compound or that have longer half-lives making them more stable measurements to correlate with household dust levels. The appropriateness of the metabolite also affects detection frequency, which is low for some targeted metabolites in this study (e.g., BCIPP and BBOEP), thus decreasing power for seeing correlations with dust levels. House dust represents a long-term reservoir of chemical contami-

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**Figure 2.** Correlation estimates among urinary metabolites (upper left) and among dust PFRs (lower right). Estimates and 95% confidence intervals in parentheses in red indicate significant ($p < 0.05$) Kendall’s tau correlation estimates. Note change in chemical name ordering from previous tables and figures.
nation in the home; whereas the metabolites may reflect short-term exposures due to their relatively short biological half-lives. For example, if a participant spent a considerable amount of time away from the home prior to urine sampling, their urinary levels likely reflect exposures in microenvironments other than the home. Whereas we expect exposure to dust to be the major contributor to total exposure for many FRs, other exposure routes, namely inhalation and dermal exposure to indoor air and direct dermal contact with FR-treated products, may also contribute and dampen the influence of dust on biological levels. Finally, we had data from 16 participants which leads to limited statistical power.

**Source Identification.** Despite the lack of correlation between urine and dust concentrations across the full range of concentrations, the individuals with the highest urine concentrations typically had the highest dust concentration, suggesting the home environment is an important exposure contributor. For example, the individuals with the two highest BDCIPP concentrations also had the highest TDCIPP dust concentrations. Similarly, the individual with the highest BCEP concentration had the highest TCEP dust concentration. These

| Table 2. Parent PFR and Recommended Targets for Future Biomonitoring |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Halogenated PFRs            |                             |                             |                             |
| tris(2-chloroethyl) phosphate (TCEP) | bis(2-chloroethyl) phosphate (BCEP) | bis(2-chloroethyl) phosphate (BCEP) | QN^a |
| CAS: 115-96-8               |                             |                             |                             |
| tris(1-chloro-2-propyl) phosphate (TCIPP) | bis(1-chloro-2-propyl) phosphate (BCIPP) | bis(1-chloro-2-propyl) phosphate (BCIPP) | QN^a |
| CAS: 13674-84-5             |                             |                             |                             |
| tris(1,3-dichloro-2-propyl) phosphate (TCIPP) | bis(1,3-dichloro-2-propyl) phosphate (BDCIPP)^a | bis(1,3-dichloro-2-propyl) phosphate (BDCIPP)^a | QN^a |
| CAS: 13674-87-8             |                             |                             |                             |
| Nonhalogenated PFRs         |                             |                             |                             |
| triphenyl phosphate (TPHP)  | diphenyl phosphate (DPHP)^a,b | diphenyl phosphate (DPHP)^a,b | QN^a |
| CAS: 115-86-6               |                             |                             |                             |
| ethylhexyl diphenyl phosphate (EHPDP) | diphenyl phosphate (DPHP)^b | diphenyl phosphate (DPHP)^b | QN^a |
| CAS: 1241-94-7              |                             |                             |                             |
| tri-n-butyl phosphate (TNBP) | dibutyl phosphate (DBP)^a,c | dibutyl phosphate (DBP)^a,c | QN^a |
| CAS: 126-73-8               |                             |                             |                             |
| tris(2-butoxyethyl) phosphate (TBOEP) | bis(2-butoxyethyl) phosphate (BBOEP) | bis(2-butoxyethyl) phosphate (BBOEP) | QN^a |
| CAS: 78-51-3                |                             |                             |                             |

^aIdentified as a major metabolite in vitro by Cooper and Stapleton. ^bPredicted based on in vitro studies. ^cMost likely 4-hydroxyphenyl phenyl phosphate; however, the position of hydroxylation could not be confirmed. ^dChemical structure uncertain. ^QN = quantitatively measured in this study. ^QL = qualitatively detected in this study. ^ND = qualitatively screened for but not detected in this study.
three cases provide an opportunity to investigate influential sources in the home and are intended for hypothesis building since conclusions cannot be drawn from such limited data.

To investigate specific sources in the home that might lead to higher relative dust concentrations, we collected information about furniture, other furnishings, electronics, and additional foam items in the home and recent renovations; collected and tested foam samples of selected items using PIXE; and made observations during home visits (see the Supporting Information). The individual with the highest TDCIPP dust levels reported home renovations in the last 5 years and had acquired several new pieces of upholstered furniture and rugs. In fact, dust levels of TDCIPP in this home increased substantially (3,032 to 44,308 ng/g dust) between 2006 and 2011, and the TDCIPP dust level in 2011 exceeded an estimated risk-based screening level.3 This observation is consistent with our previous finding that levels of Firemaster 550, a replacement FR mixture for PentaBDE, also increased in this and other homes between 2006 and 2011. We hypothesize that this is because FRs in new furniture affect dust levels.4 The individual with the second highest TDCIPP dust levels did not report any renovations or new furniture in the 5 years between dust sampling. In this home, TDCIPP dust concentrations remained relatively stable between 2006 and 2011 (24,200 to 20,600 ng/g dust) and exceeded an estimated risk-based screening level both years. While a foam sample collected from an office chair in this home contained chlorine determined via PIXE analysis, suggesting the possible presence of chlorinated FRs, we did not test foam samples from the rest of the furniture and so the major sources of TDCIPP could not be determined in this home.

For the participant with the highest dust levels of TCEP, which may also be found as an impurity in flame retardant mixture V6,59 the source is not apparent as this resident did not report any relevant recent major renovations or furniture changes. We focused on recent changes in the home, since the levels of TCEP in dust increased substantially between 2006 (6,880 ng/g) and 2011 (110,000 ng/g). House dust concentrations of the Firemaster 550 chemicals as well as TDCIPP also increased in this home during this period. We hypothesize that the Firemaster 550 increase may be a result of the introduction of a new foam mattress top, since foam testing showed the presence of bromine but not chlorine in a sample from this mattress topper. Additional follow-up with this participant did not elucidate any additional potential sources of TCEP.

We explored correlations among urinary PFR metabolites and among PFRs in dust (Figure 2) because correlations suggest a common exposure source and inform consideration of cumulative exposures and health effects. Correlations were stronger among urine metabolites than among PFRs in dust. For example in urine, DPHP was significantly positively correlated with BCEP, BDCIPP, and DBP (τu = 0.49—0.65; p < 0.05). DBP was significantly correlated with DPHP, BCEP, and BDCIPP (τu = 0.59—0.65; p < 0.05). Among parent PFRs in house dust, only TPHP and ethylhexyl diphenyl phosphate (EHDPHP) and TPHP and TDCIPP were significantly correlated (τu = 0.43—0.44; p < 0.05). TPHP and TDCIPP and their metabolites were correlated in dust and urine, respectively, suggesting that these PFRs are commonly used together. The stronger correlations in urine than dust for some PFRs (e.g., TPHP and TCEP) suggest that there are some common exposure sources that do not contribute to house dust, such as workplace or automobile. We speculated that we might see a negative correlation between BCIPP and BCEP, since TCIPP (parent PFR for BCIPP) has been reported as a replacement for TCEP (parent PFR for BCEP),7 but we did not have adequate data to evaluate this.

**Recommendations for Biomonitoring of PFRs.** Exposure biomonitoring studies for rapidly cleared chemicals require identification of stable major metabolites for parent chemicals of interest. Based on these findings from a small study, as well as limited work identifying metabolites in in vitro liver preparations exposed to these chemicals and limited in vivo work, we recommend further investigation of the six metabolites we detected, as well as the other metabolites we reported qualitatively (Table 2). Specific suggestions include the following:

- We detected BCEP in 75% of urine samples; we also recommend the parent compound (TCEP) be added as a target because in vitro liver metabolism studies suggest a low clearance rate of TCEP,59 and we found it in 13% of our urine samples. Obtaining levels of both BCEP and TCEP in the same urine samples gives a more complete idea of exposure to TCEP.
- In addition to the metabolite BCIPP, detected in 31% of our participants, in vitro studies indicate that an oxidative dehalogenation product, namely bis(1-chloro-2-proplyl)hydroxy-2-propyl phosphate, is a TCIPP metabolite,59 however, we did not detect this latter metabolite in our samples. Therefore, we recommend focusing on BCIPP as a target metabolite for biomonitoring and suggest further work is needed to identify more accurate TCIPP metabolites.
- BDCIPP is the major metabolite of TDCIPP, as suggested by Cooper and Stapleton15 as well as our previous in vitro work.59 In vivo studies of TDCIPP also suggest BDCIPP is a major metabolite.18,40 BDCIPP is also the most appropriate metabolite because it is unique to TDCIPP since no other PFR is transformed or hydrolyzed to BDCIPP.15 BDCIPP was detected in nearly all (94%) of our samples and in 91% of samples in Meeker et al.53
- DPHP is a potential metabolite of several phosphates containing at least two phenyl substituents, including TPHP15,59 and EHDHPH.38 In addition, we detected hydroxyphenyl phenyl phosphate — hydroxylation position unconfirmed — in our participants despite not being identified as a major metabolite in vitro.29 We did not detect diphenyl hydroxyphenyl phosphate and diphenyl sulfophenyl phosphate, which were predicted as major metabolites in vitro. We therefore suggest to monitor both DPHP and hydroxyphenyl phenyl phosphate (TPHP-M1).
- We detected DBP — a metabolite of TNBP — in just over half (56%) of our samples. Limited rodent in vivo and in vitro work suggests that major metabolites of TNBP are DBP, butyl dihydrogen phosphate (also known as mono-n-butyl phosphate), and butyl bis(3-hydroxybutyl) phosphate.38 As we only tested for DBP, we cannot make recommendations for these other metabolites, although at least mono-n-butyl phosphate has been reported elsewhere.41
- Although TBOEP was present at the highest concentration in dust, we detected BBOEP in only 12% of our samples. Liver incubations indicate that other potential metabolites for monitoring include 2-butoxyethyl 2-hydroxyethyl phosphate (TBOEP-M1), bis(2-butoxyethyl) 2-hydroxyethyl phosphate (TBOEP-M9), and bis(2-butoxyethyl) hydroxy-2-butoxyethyl phosphate (TBOEP-M10), all of which we frequently detected.
in our participants using qualitative methods without reference standards.

As far as we know, this is the first study to measure these 6 chlorinated and nonchlorinated DAP metabolites simultaneously and to attempt to qualitatively report other PFR metabolites in US urine samples. Limited biomonitoring data are available for PFRs, a class of metabolites in US urine samples. Limited biomonitoring data previously and to attempt to qualitatively report other PFR extended set of PFRs in California residents, which are in the range of previously reported values, and shows relationships with household dust levels for some participants, indicating that the home can be an important source of exposure. The limited sample size (n = 16) precludes extensive mixtures analysis and limits generalizability. As we did not have reference standards for the qualitative screening of alternative metabolites, we do not know how high or low the method detection limit was for each of the metabolites. As a result, positive hits are likely good candidates for biomonitoring, but nondetects should not be disregarded completely from any future biomonitoring study. As analytical capabilities improve and expand to other PFRs and emerging FRs, a clearer picture of the biological burden of FR in Californians will emerge.

ASSOCIATED CONTENT

Supporting Information
Specific details for analytical methods; methods for quantifying bromine and chlorine content in foam samples; quality control test results; PFR metabolite concentration comparisons to other studies; semiquantitative screening results; household survey. 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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