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Human exposure pathways to organophosphate flame retardants: Associations between human biomonitoring and external exposure

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ABSTRACT

Organophosphate flame retardants (PFRs) have largely replaced the market of polybrominated diphenyl ethers (PBDEs). Concerns about PFR contamination and its impact on human health have consequently increased. A comprehensive investigation on the human exposure pathways to PFRs is to be endeavoured. This study investigated the occurrence of PFR metabolites in human urine, serum and hair, correlating them with external exposure data that was presented in our previous studies. Participants from Oslo (n = 61) provided a set of samples, including dust, air, handwipes, food, urine, serum and hair. Associations between PFR metabolites analyzed in the biological samples and the PFRs in environmental samples were explored. Different sampling strategies for dosimeters (e.g. floor/surface dust, personal/stationary air) were also compared to understand which is better for predicting human exposure to PFRs. Seven out of the eleven target PFR metabolites, including diphenyl phosphate (DPHP) and bis(1-chloro-2-propyl)-1-hydroxy-2-propyl phosphate (BCIPHIPP), were frequently detected (DF > 30%) in urine. DPHP was the most frequently detected metabolite in both serum and hair. Several PFR metabolites had higher levels in morning urine than in afternoon urine. Floor dust appeared to be a better proxy for estimating PFR internal exposure than surface dust, air, and handwipes. Some PFRs in handwipes and air were also correlated with their metabolites in urine and hair. Age, beverage consumption and food consumption were negatively associated with DPHP levels in urine. Discrepancies observed between the external and internal exposure for some PFRs call for further investigation on PFR bioaccessibility and clearance.

1. Introduction

Flame retardants (FRs) have been widely used as additives in various types of commercial products due to fire safety concerns (Alves et al., 2016; van der Veen and de Boer, 2012; Xu et al., 2016). Polybrominated diphenyl ethers (PBDEs) were the most commonly used organic FRs in the past decades, but their usage is nowadays subjected to international elimination because of their negative impacts on the environment and human health (Dodson et al., 2012; Law et al., 2014; van der Veen and de Boer, 2012). Therefore, alternative chemicals, such as organophosphate flame retardants (PFRs), have been gradually introduced as an option of PBDE replacement (van der Veen and de Boer, 2012).

PFRs have been commonly used in textiles, plastics, paints, foams, electronic and electrical products, LCD displays, lubricants, food packaging, and many other products for fire safety reasons (Fang et al., 2013; Kademoglou et al., 2017; Stapleton et al., 2012; van der Veen and de Boer, 2012; Xu et al., 2016). PFRs have also other applications, e.g. tris(2-butoxylethyl) phosphate (TBOEP) has been applied in floor waxes, while triphenyl phosphate (TPHP) is a commonly used plasticizer and also found in nail polish (Kademoglou et al., 2017; Mendelsohn et al., 2016). Polyvinyl chloride (PVC), rubber and some food packaging materials, may contain 2-ethylhexyl diphenyl phosphate (EHDPHP) as a plasticizer (Ballesteros-Gómez et al., 2015a; van der Veen and de Boer, 2012). PFR global consumption in EU, US and Asia reached 200,000 tons (800 million USD) in 2007, accounting for

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almost 12% of the global FR consumption and amounting for 20% of the European FR consumption (Alves et al., 2016; UK-DEFRA, 2010; van der Veen and de Boer, 2012). According to a PINFA report, global phosphorus (organic and inorganic) market size was 400,000 tons in 2016 and will go up to 560,000 tons by 2021, however, this report did not mentioned specific PFR data (PINFA, 2017).

Similar to PBDEs, PFRs can be slowly released into the ambient environment from the commercial products, resulting in indoor and outdoor contamination and eventually threating the health of wildlife and humans (Alves et al., 2016; Su et al., 2017; van der Veen and de Boer, 2012; Wei et al., 2014). In fact, PFRs have been detected in a wide range of environmental media, including air, water, soil, sediment and indoor dust, as well as in biota and human samples (Brandsma et al., 2015; Brandsma et al., 2013a, b; Eulaers et al., 2014; Kucharska et al., 2015; Su et al., 2015; van der Veen and de Boer, 2012; Wei et al., 2014; Xu et al., 2016, 2017; Zheng et al., 2012). Recent studies have revealed different toxicological effects of PFRs. Some PFRs, such as tris(2-chloroethyl) phosphate (TCEP), tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) and TPHP, have been suspected to show different toxic potentials, including mutagenic, carcinogenic, neurotoxic or endocrine disruption (Ta et al., 2014; van der Veen and de Boer, 2012; Wang et al., 2015; Wei et al., 2014). An in vitro study reported agonistic or antagonistic activity of PFRs to different human nuclear receptors (Kojima et al., 2013). TPHP was suspected to activate peroxisome proliferator-activated receptor γ (PPAR-γ), ultimately inducing adipogenesis (Pillai et al., 2014). TCEP and TDCIPP has shown cytotoxicity, to which the exposure might lead to apoptosis, alteration of morphology and significant changes in the gene and protein levels (Ta et al., 2014). Cytotoxicity and genotoxicity have recently been associated with EHDPHP (Zhang et al., 2019). TDCIPP and TPHP in dust were associated with male hormone level alteration and decreasing sperm quality (Meeker and Stapleton, 2010). Although a recent study finds no link between thyroid cancer and PFR exposure, but high urine PFR levels was associated with increasing body mass index in summer (Deziel et al., 2018).

In the past 10 years, research attention predominately focused on external exposure of PFRs, including dust ingestion, inhalation, dietary intake and dermal absorption (Brandsma et al., 2013a, b; Kademoglou et al., 2017; Pawar et al., 2016; Stapleton et al., 2014; van der Veen and de Boer, 2012; Wei et al., 2014; Xu et al., 2016, 2017), but the exposure might be significantly overestimated due to lack of bioaccessibility information of PFRs. PFRs are not persistent and could be metabolized in human bodies in short time, thus, using PFR metabolites as biomarkers has been considered a good approach for internal exposure assessment of PFRs (Ballesteros-Gómez et al., 2015a, b; Bastiaensen et al., 2018; Kojima et al., 2016; van den Eede et al., 2015a, b, 2016b). Until recently, PFRs and their metabolites have been detected in human biomatrices, including hair, nails, urine and serum (Alves et al., 2017; Hoffman et al., 2015a; Kucharska et al., 2015; van den Eede et al., 2015b; Zhao et al., 2016), which allows the exposure assessment of PFRs from the internal point-of-view. However, the relationships between external and internal exposure to PFRs are not yet clearly understood. Among several bio-matrices, urine has become the most straightforward media for PFRs bio-monitoring. Some evidence linked PFR metabolites in urine with external PFR exposure, for example, diphenyl phosphate (DPHP, a metabolite of TPHP) in urine has been associated to TPHP in dust and handwipes (Hoffman et al., 2015b). Higher levels of bis(1,3-dichloro-2-propyl) phosphate (BDCIPP, a metabolite of TDCIPP) and DPHP were observed in child urine in comparable concentrations as in adult's urine (Carignan et al., 2013; Cequier et al., 2015). So far, two types of metabolites have been used for the biomonitoring of PFRs: 1) diesters, such as BDCIPP and DPHP, and 2) hydroxylated PFR (HO-PFR), such as 2-ethyl-5-hydroxyhexyl diphenyl phosphate (5-HO-EHDPHP), 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHIPP) and 4-hydroxyphenyl diphenyl phosphate (4-HO-TPHP) (Bastiaensen et al., 2018). Early studies mainly reported the presence of diesters in urine (Dodson et al., 2014; Hoffman et al., 2014; van den Eede et al., 2013b), while HO-PFRs started to be reported in recent studies (Bastiaensen et al., 2018; Su et al., 2016; van den Eede et al., 2015b; Zhao et al., 2019).

To the best of our knowledge, few studies have reported on PFR metabolites in the serum and hair of the general population. Also, few studies have comprehensively profiled all major external and internal exposure of PFRs together. The present study investigated the presence of PFR metabolites in a range of biological matrices (urine, serum and hair) with an attempt to assess their association with the corresponding parent PFRs exposure via different pathways, including indoor dust, handwipes, air and food. We also discuss different sampling strategies (floor dust or surface dust, stationary air or personal air, food or handwipes) as tools for assessing human internal exposure of PFRs. In combining with our earlier studies (Bui et al., 2017; Xu et al., 2016, 2017), a comprehensive picture of human external and internal exposure to PFRs is drawn.

2. Methods and analysis

2.1. Sample collection

All samples were collected in Oslo (Norway) from December 2013 to May 2014, as a part of a sampling campaign in the EU funded A-TEAM project. Details about the sampling campaign were reported elsewhere (Papadopoulou et al., 2016). Ethical approval of the study was granted by the Regional Committees for Medical and Health Research Ethics in Norway (2013/1269). Also, all participants provided written consents prior to participation (Papadopoulou et al., 2016). Briefly, 61 participants were recruited in the sampling campaign, and each of them signed up for the sample collection over a 24-h-period. General information on the study population can be found in Appendix (Table SI-1). All participants provided three urine samples at the requested time intervals, approximately from 15:30–17:30 of day 1 (T1), 05:30–07:30 of day 2 (T2) and 15:30–17:30 of day 2 (T3). Most participants (n = 55) provided hair samples. During the 24 h sampling, exposure probes, including indoor dust (floor and surface dust), air (stationary air and personal air), handwipes and duplicate diet samples were collected from each participant and their residence (Xu et al., 2016, 2017). Personal air samples were available for 31 out of 61 participants. Blood samples were not collected along with other samples within the 24-hour-sampling-collection. However, it was performed as close as possible to the scheduled sampling, and on average 4 days after the first visit, by trained nurse. All samples were kept frozen at −20 °C until chemical analysis. Questionnaires covering diet, personal information, the household and lifestyle were filled by all participants.

2.2. Chemical analysis

The analysis of urine and serum was conducted at the Toxicological Centre of the University of Antwerp (Belgium). All morning urine samples (T2; n = 61) and serum samples (n = 61) were analyzed to evaluate the human exposure to PFRs. Fifteen participants were randomly selected as a subset, whose T1 (n = 15) and T3 (n = 15) urines were also analyzed to study the diurnal variation of in PFR metabolites in urine. Eleven PFR metabolites (HO-PFRs and diesters) were analyzed in serum and urine samples. Deconjugation with β-glucuronidase was conducted for urine and serum to hydrolyse HO-PFRs before SPE and LC-MS/MS analysis. The chemical structures of the major metabolites detected in our samples, as well as of their parent PFR, are presented in Fig. 1.

The hair analysis for four PFR metabolites (di-esters) was conducted with LC-MS/MS, at the Flemish Institute for Technological Research (VITO, Belgium). The analysis of abiotic matrices was conducted in Toxicological Centre of University of Antwerp (Belgium). Details about

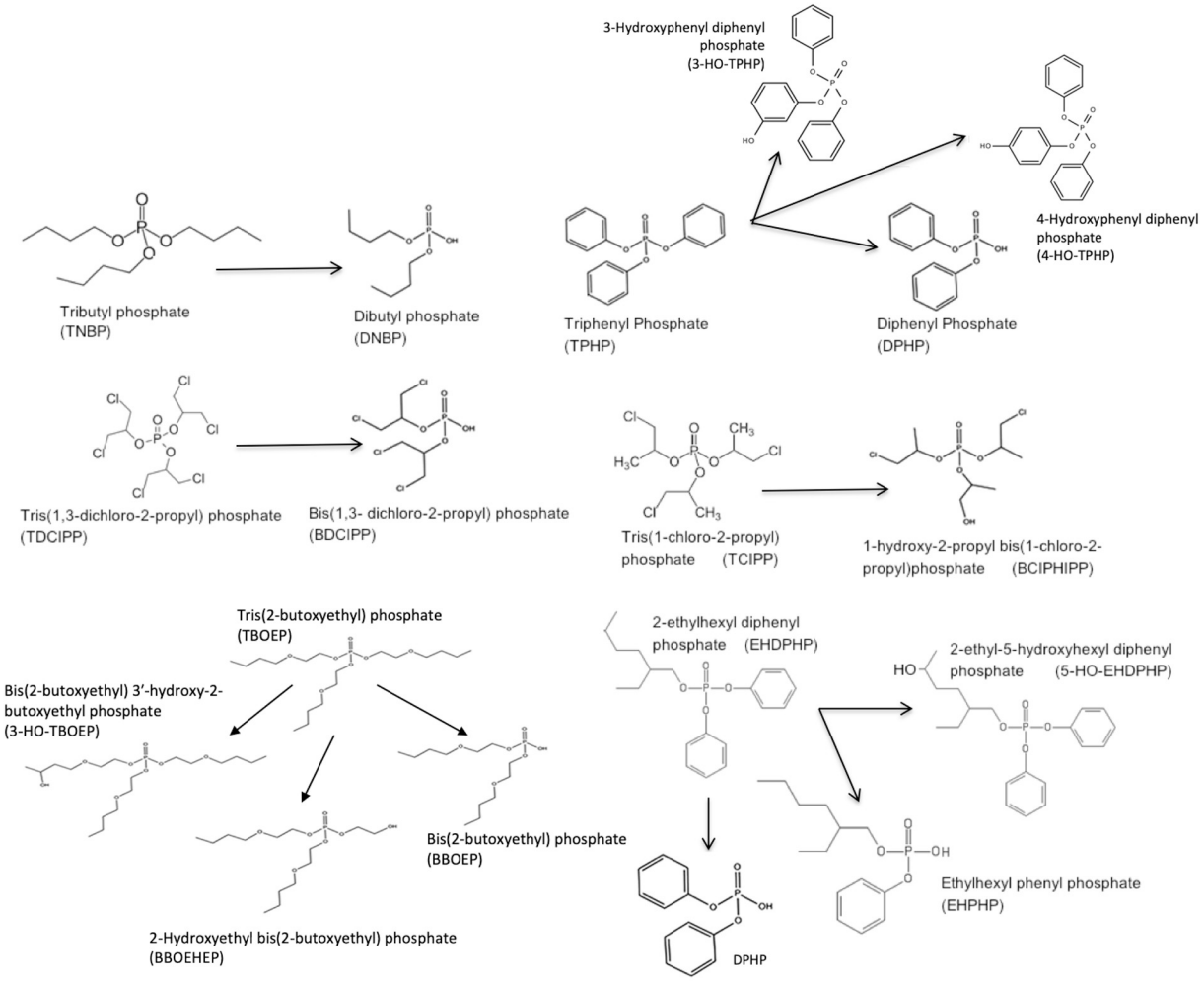

Fig. 1. Chemicals structure of targeted PFRs in this study and their parent compounds.

chemicals, materials and analytical procedure can be found in Appendix and in our early research (Alves et al., 2017; Bastiaensen et al., 2018; van den Eede et al., 2013a, b; Van den Eede et al., 2015b). Data on concentrations of PFRs in dust, air, handwipes and food have been reported in our previous studies, which were analyzed with GC–MS (Xu et al., 2016, 2017). More information about the analytical methods for urine, serum and hair could be found in Appendix.

2.3. Statistical analysis

Statistical analysis was performed using the statistical package R, version 3.3.2 (R Development Core Team) and JMP Pro 12 (SAS, USA). The null-hypothesis was rejected at $\alpha = 0.05$. The observed and model fitted data were explored for influential outliers and evaluated for their normality and homoscedasticity (Zuur et al., 2010). All chemical data were consequently log-transformed before statistical analysis. Compounds with low detection frequencies (DF < 50%) were excluded from the statistical analysis. The diurnal variation in concentrations of PFR metabolites in urine was investigated using analysis of variance (ANOVA) on linear mixed effect models (package nlme). PFR metabolite concentrations and sampling times were considered as fixed factors and the individual person as a random factor. Post-hoc pairwise contrasts between the different times of sampling were calculated using Least Square Means and evaluated using non-adjusted P values (package lsmeans).

Associations between PFR metabolite levels in urine and concentrations of their parent compounds in different carrier matrices were also investigated using ANOVA. These models allow the prediction of urine PFR metabolite concentrations based on those of their parent PFRs in the different media according to:

$$[PFR\ metabolite\ in\ urine] = \alpha * [FR\ (parent)\ in\ each\ pathway] + \beta$$

where α and β represent the slope and intercept, respectively, of the statistical model for several media investigated in the present study, including floor dust, surface dust, stationary air, personal air and handwipes.

3. Results and discussion

In the present study we determined eleven PFR metabolites in morning urine and serum samples. DNBP, BDCIPP, DPHP, BCIPHIPP, BBOEHEP, and 5-HO-EHDPHP were detected in > 30% of the urine samples, while TBOEP-OH, 3-HO-TPHP, 4-HO-TPHP and BBOEP were detected in < 10 samples ($n = 61$). DNBP, DPHP and 5-HO-EHDPHP were detected in serum, in 20–40% of the samples. Three out of four targeted PFR metabolites were analyzed in hair and only DPHP and BBOEP had DF ≥ 40%. Further information could be found in Appendix.

3.1. PFR metabolites in morning urine samples

Morning urine is typically used to study the extent of chemical exposure in humans (Cequier et al., 2015). Since PFRs are quickly metabolized in humans, T2 (morning urine, $n = 61$) is likely reflecting the

PFR exposure at home, while T1 and T3 (afternoon urine) are likely more indicative of partial outdoor and work indoor exposure. The total concentration of PFR metabolites at T2 ranged from 200 to 62,500 pg/mL, with a median of 1970 pg/mL. BCIPHIPP and DPHP were the two most abundant compounds in urine, accounting for 46% and 39% of the total amount, respectively. BCIPHIPP, DPHP, and 5-HO-EHDPHP were found in almost all morning urine samples (*DF* > 98%), with medians of 720, 610 and 56 pg/mL, respectively, while BBOEHEP was detected in 89% of the samples at a median level of 24 pg/mL (Fig. 2). The maximum levels of DNBP and BDCIPP in T2 were 1300 pg/mL (median: 99 pg/mL, *DF* = 61%) and 560 pg/mL (median: 68 pg/mL, *DF* = 61%), respectively (Fig. 2). Almost 30% of all T2 urine samples contained EHPHP (max: 3100 pg/mL). Other target metabolites were rarely detected (Table SI-3). Fig. 3 shows the relative distribution of PFR metabolites (median) in T2 urine.

In vitro metabolism studies have shown that individual PFRs could have more than one potential metabolite formed and eliminated from our body (Ballesteros-Gómez et al., 2015a; Su et al., 2016; van den Eede et al., 2013a, 2015a, 2016b). However, based on our results it seems that not all metabolites could be used as biomarkers for their corresponding parent PFRs. BBOEHEP, BBOEP and TBOEP-OH are the major metabolites of TBOEP (Van den Eede et al., 2015a). However, as the two other metabolites were rarely detected, BBOEHEP seems to be the most appropriate biomarker for TBOEP. DPHP used to be considered as a good biomarker for TPHP, but studies have showed that other compounds, like EHDPHP and resorcinol bis(diphenyl phosphate), could also metabolize to DPHP (Ballesteros-Gómez et al., 2015b; Zhang et al., 2019). Although 3-HO-TPHP and 4-HO-TPHP were proposed as specific metabolites for TPHP, but only a few studies reported their presence in urine samples (Su et al., 2016; Zhao et al., 2019). Only 13% of morning urine samples were found to have low levels of 3-HO-TPHP (max = 120 pg/mL) and only two samples had detectable 4-HO-TPHP. EHDPHP can be metabolized to EHPHP, DPHP and 5-HO-EHDPHP (Ballesteros-Gómez et al., 2015a; Van den Eede et al., 2016a, b). Since 5-HO-EHDPHP is an exclusive biomarker for EHDPHP and has higher sensitivity than EHPHP in our method, it would be the metabolite of choice for this study.

Table 1 shows the comparison of the morning urine data with other studies for the five most frequently detected urinary metabolites. Cequier et al. (2015) reported similar levels of DPHP and BDCIPP in urine samples of 48 mothers from the Oslo area, but about two times higher levels of these two PFR metabolites (median) were observed in the urine of their children. In the urine of non-smoking Californians, levels and patterns of PFR metabolites were also similar to our T2 samples (Dodson et al., 2014). The highest level of DPHP was reported in Australia, with geometrical means (GM) of 24,400 pg/mL and 63,500 pg/mL, respectively, for two sample groups (Van den Eede et al., 2015b), which is one to two orders of magnitude higher than other studies that we referred in Table 1. For US gymnasts, high level of DPHP (GM = 9000 pg/mL) was found in the urine samples collected after training. The highest level of BDCIPP was reported in the urine of Californian children (GM = 10,900 pg/mL) (Butt et al., 2016), while BDCIPP was 70% lower in the urine of mothers. Among three different studies of populations in North Carolina, BDCIPP found in a similar range, with GMs of 1800 pg/mL, 2300 pg/mL and 2320 pg/mL for pregnant women (Hoffman et al., 2017a), infants (Hoffman et al., 2015a), and the general population (Hammel et al., 2016), respectively. The highest level of BCIPHIPP was reported in one population group from Australia (GM = 18,600 pg/mL), but a ten-fold lower level of BCIPHIPP was reported for another population group in the same study (Van den Eede et al., 2015b). In general, all PFR metabolites in T2 urine had lower concentrations compared to other studies, suggesting that Norwegians may be less exposed to PFRs than people from several other countries. Surprisingly, PFR metabolites in urine from residents in e-waste recycling area in China were not higher than our Norwegian cohort (Lu et al., 2017). The reason for low PFR exposure of Norwegian might be due to their advanced environmental regulation, while not affording of high living standards might attribute to the low PFR exposure of residents in e-waste recycling area.

3.2. Discrepancies between PFR external exposure and urine metabolites levels

Although no study had comprehensively compared external exposure for all pathways with PFR metabolites in urine, the external exposure of PFRs and the excretion of their metabolites were found to have discrepancies. The highest exposure to PFRs does not result in the highest excretion of metabolites. For example, isopropylphenyl phenyl phosphate (ip-PPP) was the most abundant PFR metabolite in urine from US children, but its parent isopropylated triaryl phosphate (ITP) isomers were at less one order of magnitude lower than TDCIPP in both their handwipe and home dust (Phillips et al., 2018). Another study also reported TDCIPP had a higher level among all PFRs in house dust from US, while BDCIPP (geomean) levels were 1/3 of DPHP levels and had similar levels to ip-PPP in urine from residents (Castorina et al., 2017). In this study, TDCIPP in dust showed no correlation with urinary BDCIPP. Possibly, missing exposure pathways, like inhalation or dermal exposure, might also contribute to TDCIPP exposure to significant extent. Therefore, the discrepancies observed between PFR external exposure and excreted metabolites are probably explained by the inability of measuring all possible exposure pathways.

Our previous studies estimated the external exposure for the same participants via dust, air, handwipes and food, suggesting that EHDPHP, TCPP and TBOEP were three major PFRs to which our participants have been exposed (Xu et al., 2016, 2017). TCPP was a major PFR contaminant in air, dust and handwipes, which coincides with the high level of BCIPHIPP in urine, which accounts for 43% of the total PFR metabolites (Fig. 3). Although TBOEP had the highest level in Norwegian dust and handwipes (but not in air) (Xu et al., 2016), its metabolites, BBOEHEP, BBOEP and 3-HO-TBOEP, were detected with rather low levels or DFs in urine (Fig. 2, Table SI-3). Our previous study estimated that the total exposure (all pathways) of our participants to TCPP and TBOEP were 8.3 ng/kg bw/day and 3.9 ng/kg bw/day, respectively (Xu et al., 2017). By contrast, BCIPHIPP account for 46% of the total metabolites in morning urine, but metabolites of TBOEP were comparably negligible (Fig. 3). Besides, the low detection and levels of TBOEP metabolites were also reported in other studies (Table 1). Possible hypotheses could be: (1) the major metabolite of TBOEP might be not selected. Studies showed that BBOEP, BBOEHEP and 3-HO-TBOEP covered 99% of the depletion of TBOEP in human liver microsome at a high substrate concentration, but about 50% at low concentration (van den Eede et al., 2015a, 2016b); (2) TBOEP metabolites might further undergo rapid metabolism. Although TBOEP has been shown to be quickly depleted in gull and human liver microsomes (Greaves et al., 2016; van den Eede et al., 2016b), information regarding phase II metabolism remains unclear; (3) Considering the low K_{ow} of TBOEP, it may have relatively low bioaccessibility comparing to other PFRs. Further toxicokinetic studies are needed to understand this aspect.

In contrast, our cohort had a lower estimated external exposure of TPHP, but DPHP, its metabolite, had the second highest level in urine, accounting for 39% of the total metabolites in morning urine. As mentioned above, DPHP is not the exclusive metabolite for TPHP, but also other compounds, such as EHDPHP and resorcinol bis(diphenyl phosphate) (Ballesteros-Gómez et al., 2015a, b; Zhang et al., 2019). EHDPHP accounted for 70% of total PFR intake for our cohort (sum of inhalation, diet, dermal absorption and dust ingestion), while total exposure of TPHP was 90-fold lower than EHDPHP (Xu et al., 2017). It could be that majority portion of DPHP in urine samples derived from EHDPHP rather TPHP, resulting much higher DPHP a major PFR metabolites in our urine samples. Most studies have used DPHP as the biomarker for TPHP for exposure assessment, yet this might have to be reconsidered (Castorina et al., 2017; Cequier et al., 2015; Hoffman

Table 1
Comparison of PFR metabolite levels in this study with other studies world-wide (unit for GM and median: pg/mL).

Sample origin	Population		DNBP	DPHP	BDCIPP	BCIPHIPP	BBOEP	Reference
Oslo (Norway)	**Adults**	**Median (pg/mL)**	**99**	**610**	**68**	**720**	**< 35**	**This study (T2)**
	(*n* = 61)	**DF (%)**	**61**	**100**	**61**	**98**	**11**	
Oslo (Norway)	48 mothers (*n* = 244)	Median (pg/mL)	< 120	510	120	NR	< 180	(Cequier et al., 2015)
		DF (%)	8	97	52		32	
	54 children (*n* = 112)	Median (pg/mL)	< 120	1100	230	NR	< 180	
		DF (%)	15	97	61		1	
MA (US)	Office workers (*n* = 29)	GM (pg/mL)	NR	NR	410	NR	NR	(Carignan et al., 2013)[a]
		DF (%)			100			
CA (US)	Non-smoking adults (*n* = 16)	Median (pg/mL)	110	440	90	NR	< 340	(Dodson et al., 2014)
		DF (%)	56	66	94		12	
Queensland (Australia)	Mixed population 1 (*n* = 72)	GM (pg/mL)	< 430	24,400	1000	1740	< 350	(van den Eede et al., 2015b)
		DF (%)	18	97	92	100	6	
	Mixed population 2 (*n* = 23)	GM (pg/mL)	< 430	63,400	660	18,600	< 350	
		DF (%)	4	100	96	100	0	
US	Adults (*n* = 53)	GM (pg/mL)	NR	1020	370	NR	NR	(Hoffman et al., 2015a)
		DF (%)		91	83			
NC (US)	Infants (*n* = 43)	GM (pg/mL)	NR	1000	2300	NR	NR	(Hoffman et al., 2015b)
		DF (%)		93	100			
CA (US)	Mothers (*n* = 28)	GM (pg/mL)	NR	1200	3300	3400	NR	(Butt et al., 2016) [a]
		DF (%)		100%	100	100		
	Children (*n* = 33)	GM (pg/mL)	NR	2900	10,900	2500	NR	
		DF (%)		100	100	100		
Eastern US	11 gymnasts (*n* = 54)	GM (pg/mL)	NR	9000	670	NR	NR	(Carignan et al., 2016)
		DF (%)		100	100			
NC (US)	General population (*n* = 40)	GM (pg/mL)	NR	1137	2320	1103	NR	(Hammel et al., 2016)
		DF (%)		100	100	100		
NC (US)	Pregnant women (*n* = 349)	GM (pg/mL)	NR	1400	1800	500	NR	(Hoffman et al., 2017b)
		DF (%)		84	93	98		
CA (US)	Pregnant women (*n* = 310)	GM (pg/mL)	NR	280	930	NR	NR	(Castorina et al., 2017)
		DF (%)		80	78			
Qingyuan (China)	E-waste area residents (*n* = 211)	Median (pg/mL)	150	550	110	NR	71	(Lu et al., 2017)
		DF (%)	99	100	76		93	

NR: Not reported.
Bold values are significant ($p < 0.05$).
[a] Specific gravity corrected;

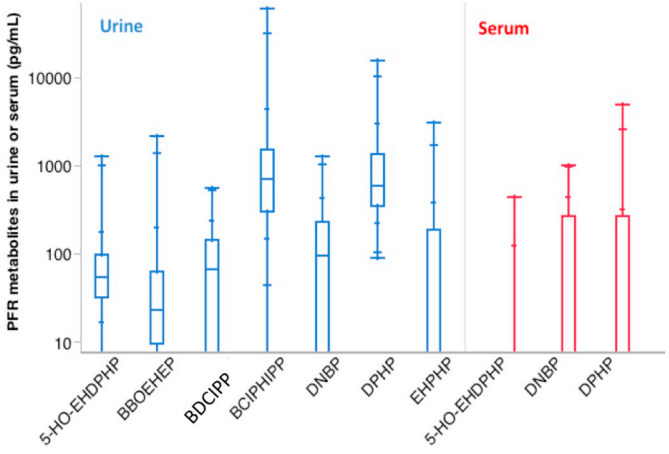

Fig. 2. Levels of the most frequently detected PFR metabolites in morning urine samples ($n = 61$) and serum samples ($n = 61$) are shown in box and whisker plots (Horizontal bars for each compound, from top to bottom, represents 100, 95, 90, 75, 50, 25, 10, 5 and 0 percentile, respectively).

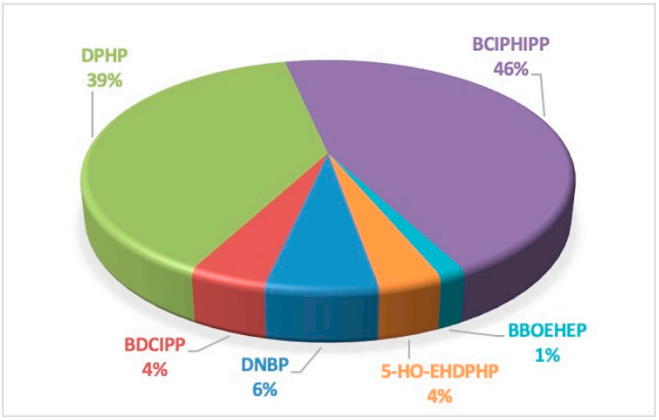

Fig. 3. The relative distribution (based on medians) of individual PFR metabolites in morning urine samples (T2, $n = 61$).

et al., 2015a; Kucharska et al., 2015; van den Eede et al., 2015b). Furthermore, some unclear exposure pathways for TPHP or DPHP, such use of cosmetics and personal care products (Mendelsohn et al., 2016), were not covered by our early assessment, leading to underestimation of total exposure. Therefore, a more specific metabolite should be proposed as TPHP biomarker instead of DPHP.

Quantitatively, total TCPP daily exposure of our participants (8.6 ng/kg bw/day) was nine times lower than total EHDPHP (Xu et al., 2017). Whereas BCIPHIPP (46% of total metabolites) in morning urine was found to be even slightly higher than DPHP (39%) and 5-OH-EHDPHP (4%) combined. Inhalation, dust ingestion and diet were, respectively, the predominate exposure pathway (> 2/3 total exposure) for TCPP, TPHP and EHDPHP (Xu et al., 2017). A hypothesis could be raised that the PFR absorption rate for different pathways might be different.

Apparently, the discrepancies between the internal and external exposure still need to be further investigated. Further information may assist to reduce the uncertainty during exposure assessment, including, but not limited to, bioaccessibility of individual PFR, undefined

Table 2
Pearson correlations (*R*-value) between individual PFR metabolites in morning urine ($n = 61$). Dataset was log-transformed before statistical testing.

log(metabolite)	DNBP	BDCIPP	DPHP	BCIPHIPP	BBOEHEP
BDCIPP	0.06				
DPHP	**0.28***	**0.30***			
BCIPHIPP	0.09	**0.32***	**0.45****		
BBOEHEP	0.18	**0.36****	**0.50****	**0.55****	
5-HO-EHDPHP	−0.04	**0.07****	0.43	0.16	0.08

Bold values are significant ($p < 0.05$).
* $0.01 < P\text{-value} < 0.05$.
** $P\text{-value} < 0.01$.

pathways, bio-clearance rate and PFR metabolism scheme for different pathway. Our previous study tried to bridge this gap through building a toxicokinetic model on the same dataset, but the fitting would be improved if more kinetics parameters were available (Bui et al., 2017). So far, the best option to minimize the discrepancies should be to conduct comprehensive sampling campaigns for exposure assessment.

3.3. Association between PFR metabolites in morning urine and PFR external exposure

Table 2 shows the Pearson correlation matrix for six frequently detected metabolites in morning urine. Significant positive correlations were found between BDCIPP, DPHP, BBOEHEP and BCIPHIPP ($R = 0.30–0.55$, $P < 0.05$), implying their levels may be influenced by similar factors. On the other hand, DNBP and 5-HO-EHDPHP were not well correlated with other metabolites, indicating that their exposure may influence by different factors. Possibly, our participants were exposed to TNBP (inhalation > 90%) and EHDPHP (diet > 95%) via one single pathway; while the exposure profiles for other PFRs were constituted by multiple pathways (Xu et al., 2016, 2017). As a potential metabolite of EHDPHP, DPHP could not be associated with 5-HO-EHDPHP. An explanation could be that DPHP may have other sources, including TPHP, or used as FR itself.

Table 3 elucidates the PFR metabolites in morning urine have been correlated with their parent compounds in different exposure probes (Xu et al., 2016, 2017). In our sampling campaign, for comparison we conducted two sampling strategies for dust (from floor and elevated surface) and air (personal and stationary) collections, along with duplicate diet, handwipe, urine (Papadopoulou et al., 2016).

Dust was collected from floors and elevated surfaces, to elucidate which one is better reflecting the internal exposure. Adults have commonly been considered to primarily be exposed to surface dust due to their above-the-ground activities, while toddlers were considered to have higher exposure to floor dust due to their close-to-floor behaviour (Al-Omran and Harrad, 2016; Bergh et al., 2011). Surface dust had higher levels of PFRs, and due to their smaller particle size, they may be easier attached to hands and thus, more effectively ingested (Xu et al., 2016). Unexpectedly, statistics showed that PFRs in floor dust were significantly associated with their metabolites in morning urine ($R^2 = 0.06–0.21$, $P < 0.02$), except 5-HO-EHDPHP. In contrast, only BCIPHIPP ($R^2 = 0.23$, $P < 0.01$) and DPHP ($R^2 = 0.14$, $P < 0.01$) were positively and significantly correlated with their parent PFRs in surface dust. This suggests that floor dust could be a better indicator than surface dust for the prediction of PFR internal exposure. It also suggests that sampling of floor dust is a more appropriate approach than surface dust for human exposure assessment. PFR profiles in surface dust are more dependent on the nearby emission sources, especially electronic products and upholsters (Brandsma et al., 2013a, b; Rauert and Harrad, 2015). By contrast, floor dust may be more representative for the overall indoor PFR contamination profile. This is probably the reason why PFRs in surface dust were less correlated to urine than floor dust.

Air was sampled as stationary air (from living room with a

Table 3
Associations between concentrations of PFR metabolites in morning urine and the concentrations of the respective parent PFRs in dust (floor and surface), air (personal and stationary), hand wipes, food (concentration in duplicate food samples and estimated dietary exposure), along with association of concentrations of PFR metabolites between morning urine and serum. The dataset was log-transformed before statistical testing.

Metabolites	*log*(metabolite) in urine - *log*(parent PFR or metabolite) in different matrices											
	Floor dust (pg/g)						Surface dust (pg/g)					
	n[a]	F	P	R^2	α	β	n	F	P	R^2	α	β
5-HO-EHDPHP	61	0.54	0.46	0.00	0.10	2.81	61	0.44	0.51	0.00	−0.08	5.18
BBOEHEP	61	16.63	< **0.01**[b]	0.21	0.50	−4.59	61	0.99	0.32	0.00	0.13	1.31
BCIPHPIPP	61	5.05	**0.02**	0.06	0.26	2.86	61	19.25	< **0.01**	0.23	0.35	1.15
BDCIPP	61	6.58	**0.01**	0.09	0.19	1.98	61	1.83	0.18	0.01	0.07	3.55
DPHP	61	8.48	< **0.01**	0.11	0.23	3.45	61	10.65	< **0.01**	0.14	0.32	2.00
	Stationary air (pg/m^3)						Personal air (pg/m^3)					
	n	F	P	R^2	α	β	n	F	P	R^2	α	β
BCIPHPIPP	58	3.32	0.07[c]	0.04	0.24	3.92	29	16.15	< **0.01**	0.35	0.88	−2.53
DPHP	58	0.20	0.65	−0.01	0.07	6.19	29	0.81	0.37	−0.01	0.12	5.72
DNBP	58	1.23	0.27	0.00	0.17	3.29						
	24H duplicated food[d] (pg/g ww)						24H dietary exposure[e] (pg/kg bw/day)					
	n	F	P	R^2	α	β	n	F	P	R^2	α	β
5-HO-EHDPHP	61	0.06	0.80	−0.02	0.03	3.89	49	0.15	0.70	−0.02	0.08	3.19
	Handwipe (pg/wipe)						Serum[f] (pg/mL)					
	n	F	P	R^2	α	β	n	F	P	R^2	α	β
5-HO-EHDPHP	55	3.12	0.08	0.04	−0.23	6.20	12	0.19	0.67	−0.08	0.25	3.01
BBOEHEP	55	6.83	**0.01**	0.10	0.34	−0.14						
BCIPHPIPP	55	13.49	< **0.01**	0.19	0.48	1.75						
DPHP							24	0.77	0.39	−0.01	0.23	5.54
DNBP							21	0.85	0.37	0.00	0.24	5.49

[a] The number of pairs included in the Pearson correlation analysis.
[b] Significant *P* values are marked in **bold**.
[c] *P* values indicating a trend towards statistical significance are underlined.
[d] The urine metabolite in correlation with PFR concentrations in the pooled 24-h-duplicated food of each participant.
[e] The urine metabolites in correlation with PFR dietary exposure of each participant during sampling period.
[f] Due to low DF (< 50%) of PFR metabolite in serum, data for participants who have detectable metabolites in both urine and serum were selected.

stationary pump) and personal air (the participant carrying a portable pump) (Papadopoulou et al., 2016; Xu et al., 2016). TCPP accounted for over 85% of total PFRs in both stationary air and personal air samples, and inhalation is thought to be its main exposure pathway (Xu et al., 2016). Table 3 showed that BCIPHIPP in morning urine is significantly correlated to TCPP in personal air ($P < 0.01$), while stationary air only showed a potential trend towards a significant correlation ($P = 0.07$). As stationary air was only collected from the living room in the participants residence, personal air, covering all micro environments the participant has been visiting during the sampling period, may better represent the real exposure through inhalation. Other PFRs in air samples were not significantly correlated to their metabolites in urine. From internal exposure point of view, this finding confirms our hypothesis that inhalation might be the major exposure pathway for TCPP, but not for other PFRs.

Handwipes has been considered as a good tool for dermal exposure assessment, as well as an indirect probe for dust ingestion (Hoffman et al., 2015b; Pawar et al., 2016; Phillips et al., 2018; Stapleton et al., 2014). TBOEP and TCPP were the major PFRs found in handwipes of the participants, followed by EHDPHP (Xu et al., 2016). In this study, both BBOEHEP ($R^2 = 0.10$, $P = 0.01$) and BCIPHIPP ($R^2 = 0.19$, $P < 0.01$) in urine were significantly correlated with their corresponding parent PFRs in handwipe. 5-HO-EHDPHP in urine could not be associated with EHDPHP in handwipes, but a trend towards significant was shown ($P = 0.08$). This suggested that the handwipe could be a good predictor for urine excretion of PFRs, especially for TBOEP and TCPP. Due to low DFs in handwipe or urine, the handwipe-urine association could not be assessed for other PFRs. It is thus difficult to compare whether the handwipe is an equally good proxy as floor dust to assess PFR exposure. However, a recent study on toddlers showed contradictive results that PFRs in handwipes were better correlated with PFR metabolites in urine than with floor dust. Possible explanations could be: (1) children have a higher PFR exposure via hand-to-mouth contact than adults; (2) baby products might not significantly influence the PFR profiles in dust, but PFR metabolite levels in children urine might be enhanced due to frequent and close contacts to those products.

Recently, low levels of PFRs in food have been reported in few studies (He et al., 2018; Poma et al., 2017). Considering the large food intake amount, PFR exposure via diet could not be ignored. The total PFR exposure (median) for our cohorts was 110 ng/kg bw/day, of which three quarters were exposed via diet with 73 ng/kg bw/day contributed by EHDPHP (Xu et al., 2017). Although over 95% of the total EHDPHP exposure was estimated to be exposed via diet (Xu et al., 2017), but no correlation could link 5-HO-EHDPHP in urine with EHDPHP their 24 h food duplicate, nor with EHDPHP dietary exposure amount. Neither any other exposure pathway was associated with 5-HO-EDHPHP in urine. Possible explanations for this observation could be: (1) food samples were pooled duplicates of 24-h diet of each participant, but 5-HO-EHDPHP in morning urine might only reflect the exposure from diet of the evening before; (2) the bioaccessibility of EHDPHP via diet remains unknown; (3) the dietary exposure of EHDPHP is a pulsed dosing process, while its body clearance might be a continuous process (unlike other pathways that are constant or frequent processes).

3.4. 24 h variation of PFR metabolite in urine

Four PFR metabolites (DPHP, BCIPHIPP, BBOEHEP and 5-HO-EHDPHP) were frequently observed ($DF > 50\%$) at all three sampling times (Fig. 4 and Table SI-4). DPHP medians were 888, 1464, and 1010 pg/mL for T1, T2 and T3, respectively; while BCIPHIPP medians were 617, 1556, and 757 pg/mL, respectively. The levels of 5-HO-EHDPHP and BBOEHEP in the T1, T2 and T3 samples were one to two orders of magnitude lower than those of BCIPHIPP and DPHP. For DNBP and BDCIPP, T2 samples had a DF above 50%, but neither of them was frequently detected ($30\% \leq DF \leq 40\%$) in T1 and T3.

From the visual inspection of Fig. 4, morning urine (T2) seems to have higher levels and DFs of 5-HO-EHDPHP, BICPHIPP, DPHP and BBOEHEP than afternoon urines (T1 & T3), while their levels seem to be similar between T1 and T3. When applying a linear mix model ANOVA test, no statistical differences between the three time points were found for BBOEHEP ($P = 0.08$), DPHP ($P = 0.06$), or BCIPHIPP ($P = 0.09$), but the *P*-values were close to 0.05 (Table 4). The statistical difference between each pair of samples was assessed using post-hoc pairwise analysis, and significant differences ($P < 0.05$) or trends towards statistical significance ($0.05 < P < 0.1$) were observed between T1 vs T2 or between T2 vs T3 for BICPHIPP, DPHP and BBOEHEP (Table 3). Samples collected at T2 were the first urines after wake-up (morning urine), probably representing the exposure at home the previous night, while T1 and T3 represented the daytime PFR exposure. Since afternoon urine samples (T1 vs T3) were collected at a similar time point (mainly between 15:30–17:30) of two continuous weekdays, participants were likely to have similar activities and thus similar exposure to PFRs during these days. This is also in accordance with the statically tests where no statistical difference in urine concentrations between T1 vs T3 were found ($P > 0.1$).

Comparing the different statistical results between the ANOVA and Post-hoc test, the latter seems to better reveal the 24 h variation in urine for DPHP, BBOEHEP, and BCIPHIPP. Yet, the results of these two tests still left some uncertainty, which could probably be caused by the following: (1) ANOVA compared all three time points at once, finding *P* values close to 0.05 due to similarities between T1 and T3, while in post-hoc tests the difference between individual pairs of time points were evaluated; (2) the small sample size ($n = 15$) selected for this temporal variation study might not be sufficient to show a statistical significance; (3) the urination behaviour of individual participant is different, which might introduce a large variance on metabolite excretion in urine; (4) the urine concentrations were not creatinine-corrected and may potentially introduce additional uncertainty in the dataset. However, not all studies on PFR metabolites in urine are creatinine-normalized, and the necessity for such normalization has not yet been concluded on (Butt et al., 2016; Cequier et al., 2015; Hoffman et al., 2017a; Kosarac et al., 2016; van den Eede et al., 2015b).

Pearson correlations between PFR concentrations at the three different time points were also evaluated (Table 4). Significant correlations between each pair of sampling time for 5-HO-EHDPHP and BBOEHEP were found. This indicates that a participant having high exposure to EHDPHP or TBOEP is likely to have high 5-HO-EHDPHP and BBOEHEP levels in the morning urine if he/she had high concentrations in the afternoon urine. Therefore, PFR exposure may be highly dependent on the personal activity.

So far, only a few studies have reported temporal trends of PFR metabolites in urine, especially for a one-day-period. A study on diurnal variation for mothers and their children also found that morning urine contained higher levels of DPHP and BDCIPP than samples collected at other time points (Cequier et al., 2015). Hoffman et al. (2015a, b) studied the temporal trends of DPHP in urine for five continuous days, but no significant difference was found, possibly due to routine activity of the participants if the sampling hour was constant. Carignan et al. (2016) found a significant increase of DPHP and BDCIPP levels in urine of gymnasts after gym practice. A long-term seasonal variation could be observed for BDCIPP in the urine of pregnant women (Hoffman et al., 2017a).

3.5. PFR metabolites in serum

One study reported parent PFR levels in human blood, in which TPHP (median = 430 pg/mL, DF = 98.4%), TNBP (median = 37,800 pg/mL, DF = 99.6%) and EHDPHP (median = 1220 pg/mL, DF = 100%) were the most abundant and the most frequently detected PFRs (Zhao et al., 2016). In agreement with that study, their respectively metabolites, DPHP

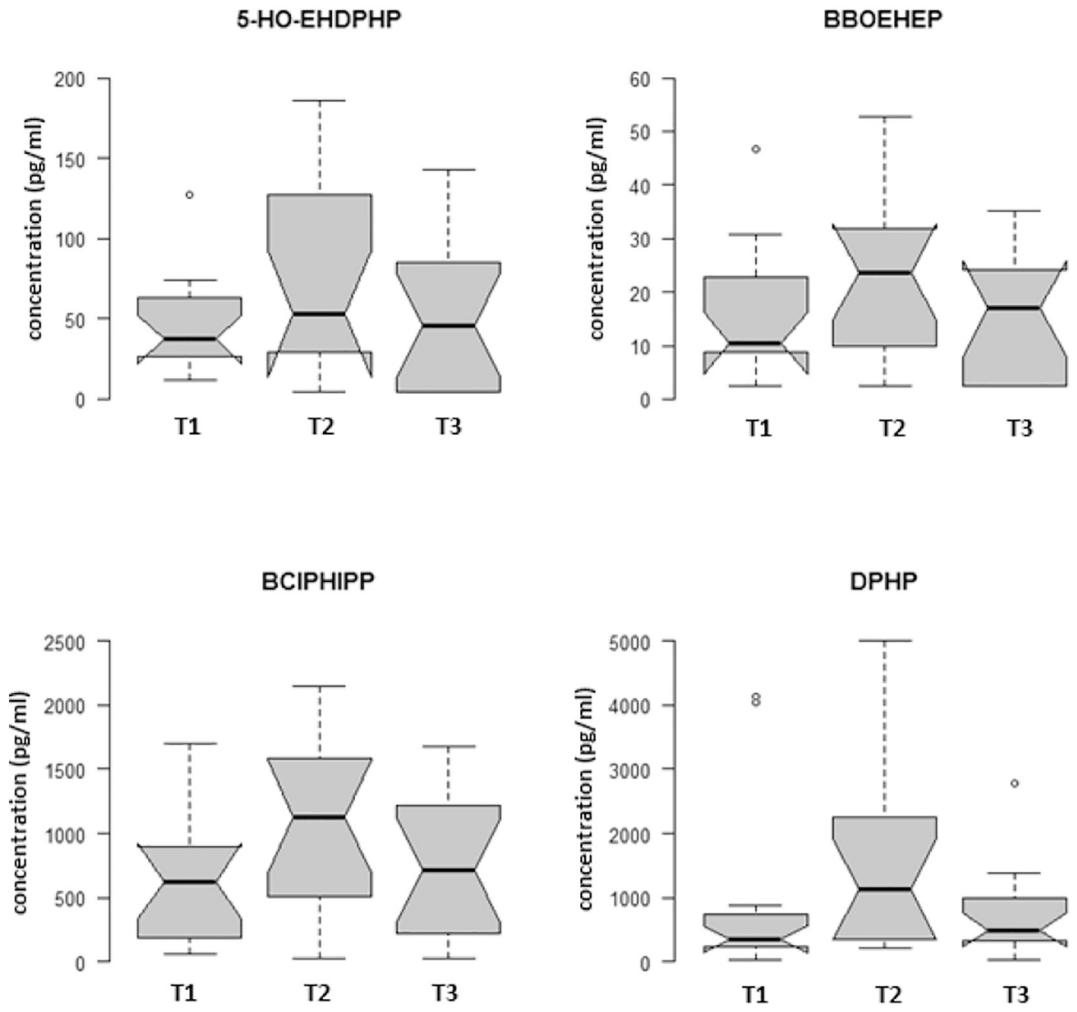

Fig. 4. Concentrations of four PFR metabolites in urine collected at three time points within a 24-hour-period (n = 15) are shown in box and whiskers plots (the top, waist and bottom bars of sandglass shapes represent 75, 50 and 25 percentiles).

Table 4
Comparison of the variation of PFR metabolite concentrations (pg/mL) in urine at different sampling time points (T1, T2, T3) using linear mixed model ANOVA and Post-hoc Pairwise Contrast, as well as Pearson correlation (R-value) between each of the two sampling time points. The dataset was log-transformed before statistical testing.

log(metabolite)	Linear mixed model ANOVA		Post-hoc pairwise contrast						Pearson correlation (R)		
			T1 vs T2		T1 vs T3		T2 vs T3				
	F	P	Δ[a]	P	Δ[a]	P	Δ	P	T1 vs T2	T1 vs T3	T2 vs T3
5-HO-EHDPHP	2	0.16	−0.19	0.64	0.58	0.16	0.77	0.07[b]	**0.69****	**0.52***	**0.68****
BBOEHEP	2.72	0.08	−0.41	0.11	0.14	0.56	0.55	**0.03**	**0.55***	**0.67****	**0.67****
BCIPHIPP	2.6	0.09	−0.6	**0.04**[c]	−0.09	0.74	0.51	0.08	**0.70****	0.19	0.18
DPHP	3.21	0.06	−0.74	**0.02**	−0.12	0.7	0.61	0.06	**0.65****	0.42	**0.57***

[a] **Δ < 0** suggests that the mean of metabolite in urine of earlier time point is lower than later time.
[b] P-value with underline indicates a trend towards statistical significance (0.1 < P-value < 0.05).
[c] Significant P-values (< 0.05) for overall and paired contrast differences are marked in **bold**.
* 0.01 < P-value < 0.05.
** P-value < 0.01.

(max = 5000 pg/mL, DF = 39%), DNBP (max = 1000 pg/mL, DF = 34%) and 5-HO-EHDPHP (max = 450 pg/mL, DF = 20%) were also the most abundant and the most frequently detected compounds in our serum samples. BBOEP, BBOEHEP, EHPHP, and BDCIPP were only found in a few serum samples (Table SI-3).

Due to the low DFs, it was difficult to perform statistical analyses for PFR metabolites in serum. We used data from participants who had detectable metabolites in both urine and serum to perform a Pearson correlation analysis (Table 4). However, no significant correlations were found between urine and serum for DNBP (n = 21, P = 0.37), DPHP (n = 24, P = 0.39) and 5-HO-EHDPHP (n = 12, P = 0.67). Possible explanations could be: (1) low DF of metabolites in serum may provide insufficient data for our statistics; (2) this study did not analyse PFRs in serum, which might providing more information for the

Table 5
Associations of DPHP and BBOEP in hair (pg/mL) and their parent PFRs in dust (pg/g), air (pg/m^3) and handwipes (pg). The dataset was log-transformed before statistical testing.

	n[a]	F	P	R^2	α	β
			log(DPHP) in hair - *log*(TPHP) in environmental matrix			
Stationary air	52	0.91	0.35	0.00	0.45	5.33
Personal air	26	0.13	0.72	−0.03	0.19	6.01
Floor dust	55	9.00	< **0.01**[b]	0.13	0.69	−1.20
Surface dust	55	6.69	**0.01**	0.10	0.78	−2.77
			log(BBOEP) in hair - *log*(TBOEP) in environmental matrix			
Floor dust	25	3.04	0.10[c]	0.08	0.27	4.21
Surface dust	25	0.12	0.73	−0.04	−0.04	9.14
Handwipe	25	2.31	0.14	0.05	0.19	6.59

[a] Number of pairs available for Pearson correlation analysis.
[b] Significant P values are marked in **bold**.
[c] P values indicating a trend towards statistical significance are underlined.

relationship between serum and urine; (3) different metabolism rate and elimination rate of each PFR might play a role on failing to find statistical correlation; (4) dietary is the predominate pathway for EHDPHP exposure, and collection of serum samples on a different day from urine collection (for practical reasons) could lead to unmatched data of 5-HO-EHDPHP in urine and serum; (5) serum is often used to assess longer term exposure for (persistent) organic contaminants, like PBDEs and perfluoroalkyl substances (PFASs), while urine is commonly used for shorter term exposure for compounds, like phthalate esters (Alves et al., 2014). As alternative FRs, PFRs do not show as long half-life in human body as PBDEs, but, like phthalate esters, they have relatively faster clearance rates. Thus, for the moment, monitoring PFR metabolites as biomarkers in urine seems to be a more practical approach for PFR exposure assessment than analysing metabolites or parents in serum. Future development of more sensitive methods for the determination of PFR and their metabolites in serum might provide more insights.

One of our previous study used a toxicokinetics model to predict the serum level of 5-HO-EHDPHP via total external exposure of all major pathways from the same cohort, receiving a good fitting with the analytical data reported in the present study (Bui et al., 2017). That model could imply a possible link between PFR external exposure and their serum metabolite levels.

3.6. PFR metabolites in hair

Hair is considered to be a less invasive sampling method for participants and a good indicator for long-term exposure (Alves et al., 2014, 2017; Kucharska et al., 2015). Only four metabolites, e.g. DNBP, DPHP, BDCIPP, and BBOEP, could be analyzed in hair samples due to technical limitations (n = 55; Table SI-5). DPHP was detected in 71% of the samples, with concentrations up to 1,700,000 pg/g (median = 24,000 pg/g); while the DF for BBOEP was 47% (max = 43,000 pg/g). The other two compounds were seldom found in hair. Yet, no significant association between hair and urine was found for DPHP. Possibly, urine reflects short-term exposure, which may largely vary according to the PFR exposure in the past hours, while hair accumulates DPHP over a longer period of time. One study found a correlation between TDCIPP in hair and BDCIPP in urine (Spearman: r = 0.352, P = *0.02*), but no correlation between TPHP in hair with DPHP in urine was observed (Kucharska et al., 2015).

The Pearson correlations between DPHP and BBOEP in hair and their parent PFRs in dust, air and handwipes were evaluated (Table 5). Positive and statistically significant associations between DPHP in hair and TPHP in both dust (floor dust: $P < 0.01$; surface dust: $P = 0.01$) were found, but not between hair and other matrices. Thus, one could speculate if dust and hair could be good indicators for the long term TPHP exposure. Furthermore, no significant correlation could be found between BBOEP in hair and TBOEP in environmental matrices.

3.7. Effect of covariates to internal exposure

Associations between participant condition/behaviour (e.g. gender, age, weight, food consumption amount and beverage consumption amount) and PFR metabolites in morning urine and hair were explored (Table 6). t-Tests showed no significant differences between genders for any PFR metabolites in urine or hair, suggesting that the internal exposure of PFRs might not be gender dependent.

DPHP (P = 0.02) and BBOEHEP (P = 0.02) concentrations in morning urine were negatively correlated with age of the participant

Table 6
Pearson correlations between individual PFR metabolites and physical/dietary conditions. The dataset was log-transformed before statistical testing.

log(metabolite)	Age (year)		Weigh (kg)		*log*(beverage consumption)		*log*(food consumption)	
	R	P	r	P	r	P	r	P
Morning urine (n = 61)								
DNBP	−0.07	0.57	−0.01	0.92	−0.12	0.38	−0.09	0.50
BDCIPP	−0.06	0.62	0.05	0.72	0.03	0.81	−0.10	0.44
DPHP	−0.30	**0.02**[a]	−0.11	0.40	−0.39	< **0.01**	−0.31	**0.02**
BCIPHIPP	−0.15	0.26	−0.09	0.48	−0.16	0.23	−0.11	0.41
BBOEHEP	−0.31	**0.02**	0.00	0.97	−0.13	0.31	−0.22	0.09[b]
5-HO-EHDPHP	−0.13	0.31	−0.05	0.72	−0.20	0.13	−0.23	0.07
Hair (n = 55)								
DPHP	0.01	0.95	0.22	0.11	−0.01	0.93	0.11	0.42

[a] Significant P value are marked in **bold**.
[b] P values indicating a trend towards statistical significance are underlined.

(Table 6). Possibly, personal behaviour/habits due to aging might lead to lower PFR exposure. DPHP in urine was also found to be associated with both food and beverage consumptions during the sampling day, also negatively, possibly because higher urination volume/frequency might dilute the DPHP levels in urine. However, no evidence showed that beverage intake could influence the excretion of other metabolites in morning urine. BBOEHEP ($P = 0.09$) and 5-HO-EHDPHP ($P = 0.07$) were borderline negatively associated with the amount of food consumed. No significant correlations could be found between body weight and any metabolite in urine. Besides, no correlation could link the DPHP level in hair with age, weight, food consumption and beverage consumption.

4. Conclusions

PFR metabolites were detected in urine, serum and hair, raising concerns of indoor and food contamination of PFRs. DPHP was the major metabolite found in urine, serum and hair, which could originate from TPHP, but also from EHDPHP and other PFRs. Morning appears to be a good sample collection time for studying PFR metabolites in urine. The major exposure pathways differed among PFRs, but floor dust seems to be the best proxy of internal exposure to most PFRs. Furthermore, TCPP and TBOEP in personal air and handwipe could be linked to their metabolites in urine. A few PFR metabolites have been found in serum and hair, but further improvement of analytical methods could help understanding the internal exposure of PFR using these matrices. Future research should also focus on the bioaccessibility and the clearance/elimination processes of PFRs, to further explain the discrepancies between the measured external and internal exposure.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.03.053.

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