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Exposure levels, determinants and risk assessment of organophosphate flame retardants and plasticizers in adolescents (14–15 years) from the Flemish Environment and Health Study

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ABSTRACT

The ubiquitous use of organophosphate flame retardants and plasticizers (PFRs) in a variety of consumer products has led to widespread human exposure. Since certain PFRs are developmental and carcinogenic toxicants, detailed exposure assessments are essential to investigate the risk associated with environmental exposure levels. However, such data are still lacking for European countries. In this study, concentrations of thirteen PFR metabolites were measured in urine samples from 600 adolescents from Flanders, Belgium. 1-Hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHIPP), diphenyl phosphate (DPHP), bis(1,3-dichloro-isopropyl) phosphate (BDCIPP), 2-hydroxyethyl bis(2-butoxyethyl) phosphate (BBOEHEP), 2-ethylhexyl phenyl phosphate (EHPHP) and 2-ethyl-5-hydroxyhexyl diphenyl phosphate (5-HO-EHDPHP) were frequently detected (>83%) in all participants. Comparisons with study populations from outside the EU showed that urinary levels of DPHP, BDCIPP and BCIPHIPP were generally within the same range. Only exposure to 2-ethylhexyl diphenyl phosphate (EHDPHP) was presumably higher in Flemish adolescents. However, determinants analysis through multivariate regression analyses did not reveal significant predictors that may explain this finding. Significantly higher levels of BDCIPP were observed in participants with new decorations at home, while adolescents with highly educated parents had higher levels of BBOEHEP and BDCIPP. Furthermore, multiple PFR metabolite concentrations followed a seasonal pattern. Estimated daily intakes (EDIs) were calculated from the internal dose by including fractions of urinary excretion (F_{UE}) estimated in *in vitro* metabolism studies. EDIs ranged from 6.3 ng/kg bw/day for TBOEP to 567.7 ng/kg bw/day for EHDPHP, which were well below the available oral reference doses for all investigated PFRs. This suggests that the associated risk is low at present. This is the first report on internal exposure to seven commonly used PFRs in a European population.

1. Introduction

Flame retardants are substances added to textiles, furniture, plastics, electronic devices, upholstery and other consumer goods to reduce the risk of fire spreading and to meet flammability standards (van der Veen and de Boer, 2012). Until recently, polybrominated diphenyl ethers (PBDEs) were the most popular flame retardants used worldwide. However, the production and use of PBDE mixtures such as Penta- and

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Deca-BDE is now prohibited due to their listing as persistent organic pollutants (UNEP, 2009, 2017). Some of these mixtures already contained organophosphate flame retardants (PFRs), a class of organic chemicals that has recently overtaken PBDEs as most frequently used flame retardants (van der Veen and de Boer, 2012). PFRs are tri-esters of phosphate with varying side-chains. As a result, they are applied in a wide variety of products such as furniture, textiles, decoration and building materials, paints, floor polish, resins and polyvinyl chloride (PVC) plastics for their fire safety and/or plasticizing properties (Wei et al., 2015). Chlorinated PFRs, such as tris-2-chloroethyl phosphate (TCEP), tris(1-chloro-isopropyl) phosphate (TCIPP) and tris(1,3-dichloro-isopropyl) phosphate (TDCIPP) and non-halogenated alkyl- and aryl-phosphates, such as triphenyl phosphate (TPHP), tri-n-butyl phosphate (TNBP), tris(2-butoxyethyl) phosphate (TBOEP) and 2-ethylhexyl diphenyl phosphate (EHDPHP) have been found in high levels in indoor dust, air and consumer products because PFRs are additive rather than reactive chemicals (Stapleton et al., 2009; Cequier et al., 2014; Kim et al., 2019; Li et al., 2019c). Potential routes of human exposure to PFRs not only include the ingestion of dust, inhalation of air or dermal contact (Xu et al., 2016), but also the intake of contaminated food as they have also been found in several food categories as a result of contamination during industrial processing (e.g., plastic packaging) (Poma et al., 2018; Li et al., 2019a). Levels of PFRs in the indoor environment now exceed those of PBDEs due to a sharp increase in PFR usage since the beginning of this century (Dodson et al., 2012). Exact numbers on production volumes are scarce, but all the PFRs of interest to this work are currently registered as high production volume chemicals under the European REACH regulation (ECHA, 2020). The total consumption of flame retardants in Europe was 465,000 tons in 2006 (of which 20% were PFRs) and is likely several times higher at this moment. Moreover, the worldwide consumption of flame retardants is expected to grow and exceed 4 million tons by 2025 (Grand View Research 2017).

Although PFRs were introduced as suitable alternatives to PBDEs, evidence from *in vitro* and *in vivo* toxicity tests suggest that exposure to these chemicals can lead to adverse health effects for humans and biota due to PFRs' carcinogenic, neurotoxic and endocrine disrupting potential (van der Veen and de Boer, 2012; Wei et al., 2015). Epidemiological evidence is scarce, but exposure to PFRs has been associated with adverse reproductive outcomes (Carignan et al., 2017; Ingle et al., 2018), increased odds of papillary thyroid cancer (Hoffman et al., 2017b), allergic symptoms (Araki et al., 2018) and increased oxidative stress biomarkers (Ait Bamai et al., 2019). As such, reliable exposure and risk assessments of human populations have gained significant scientific interest in recent years. Exposure to environmental contaminants is commonly monitored through the measurement of biomarkers in urine or blood. *In vitro* and *in vivo* studies have shown that PFRs are rapidly metabolized to dialkyl and diaryl phosphates (DAPs) such as diphenyl phosphate (DPHP), bis(butoxyethyl) phosphate (BBOEP), bis(1,3-dichloro-isopropyl)phosphate (BDCIPP), and to different hydroxylation products (HO-PFRs), like 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHIPP) and 2-ethyl-5-hydroxyhexyl diphenyl phosphate (5-HO-EHDPHP), and 2-hydroxyethyl bis(2-butoxyethyl) phosphate (BBOEHEP) (Van den Eede et al., 2013; Ballesteros-Gómez et al., 2015; Völkel et al., 2018). These urinary metabolites have been used as biomarkers of exposure in studies from the US (Hoffman et al., 2018; Ospina et al., 2018), Australia (He et al., 2018a, 2018b), China (Chen et al., 2018; Sun et al., 2018; Ding et al., 2019) and Japan (Bastiaensen et al., 2019; Bastiaensen et al., 2020a). Although exposure levels of PFRs in humans are increasing as shown for populations in the US and Japan (Hoffman et al., 2017a; Bastiaensen et al., 2020a), the number of biomonitoring studies investigating PFR exposure in Europe is still very limited (Fromme et al., 2014; Cequier et al., 2015). Comprehensive analyses of internal exposure are needed to determine the extent of human exposure and the associated risk, especially in industrialized areas such as Europe. In the present study, we report the urinary concentrations of thirteen metabolites of seven frequently used PFRs in adolescents from Flanders, Belgium. We also investigated which factors could significantly impact PFR exposure and if current exposure levels are of concern through the calculation of estimated daily intakes (EDI) and comparison with available reference doses (RfD).

2. Materials and methods

2.1. Study population

The Flemish Environment and Health Study (FLEHS), established in 2002, generates information on the presence and distribution of biomarkers of environmental pollutants relevant for public health. The study was implemented, funded and steered by the Environment, Nature and Energy Department of the Flemish government and carried out by a research consortium, the Centre for Expertise on Environment and Health. In previous cycles (2002–2015), reference values for persistent organic pollutants (POPs), metals, pesticides, phthalates, bisphenol A and polycyclic aromatic hydrocarbons (PAHs), among others, have been established for newborns, adolescents and adults (Schoeters et al., 2012). In the fourth cycle (FLEHS IV, 2016–2020), 600 adolescents (14–15 years old) were recruited in two parts as a sample of the general population of Flanders (northern part of Belgium): 200 newborns of the first FLEHS campaign (2002–2006, now adolescents) were asked to participate in FLEHS IV, as well as 400 adolescents newly recruited through schools. One of the objectives of the current program was to investigate the internal exposure to emerging contaminants, such as PFRs, new/alternative plasticizers and perfluoroalkyl substances in relation to (eco-)behavior and potential health effects. Participants were recruited through 20 schools from all five provinces of Flanders between September 2017 and June 2018. Inclusion criteria were: participants had to reside in Flanders for at least 5 years and had to be able to fill in questionnaires in Dutch. Each participant provided urine, hair and blood samples; these were immediately processed during fieldwork at school. Urine samples were collected in clean polyethylene containers, aliquoted into glass vials and stored frozen (-20 °C) until analysis by the responsible laboratory. The body weight (bw) and height of the adolescents were measured by trained nurses during fieldwork. Participants (adolescents and/or parents) completed self-administered questionnaires on personal and lifestyle factors, such as education, income, smoking and eating habits, and on the use of certain materials or products in their home environment (digital or on paper, carried out at home). The characteristics of the study population are shown in Table SI-1. Adolescents and their parents provided a written informed consent. The study protocol was approved by the Ethical Committee of the Antwerp University Hospital (Belgian Registry Number: B300201732753). All data were pseudonymized.

2.2. Measurement of PFR biomarkers in urine

Target analytes in this study were 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHIPP), bis(1-chloro-2-propyl) phosphate (BCIPP), diphenyl phosphate (DPHP), 4-hydroxyphenyl phenyl phosphate (4-HO-DPHP), hydroxyphenyl diphenyl phosphate (HO-TPHP), dibutyl phosphate (DNBP), bis(1,3-dichloro-isopropyl) phosphate (BDCIPP), tris(2-chloroethyl) phosphate (TCEP), 2-hydroxyethyl bis(2-butoxyethyl) phosphate (BBOEHEP), bis(butoxyethyl) phosphate (BBOEP), (3-hydroxy)-2-butoxyethyl bis(2-butoxyethyl) phosphate (3-HO-TBOEP), 2-ethylhexyl phenyl phosphate (EHPHP) and 2-ethyl-5-hydroxyhexyl diphenyl phosphate (5-HO-EHDPHP).

The method used for the extraction and quantification of PFR metabolites in urine has been discussed in detail elsewhere (Bastiaensen et al., 2018). Briefly, urine samples (2 mL) were spiked with mass-labelled internal standards (5 ng), adjusted to pH 6 with phosphate buffer (1 M) and deconjugated with ß-glucuronidase (2 mg/mL). Samples were extracted on Bond-Elut C18 cartridges (3 mL, 200 mg, Agilent, Santa Clara, USA), conditioned with 3 mL of methanol and 2 mL of

water. Target analytes were eluted with methanol (3 mL) and filtered in a micro-centrifuge (0.2 μm nylon, VWR, Leuven, Belgium). Final extracts (water:methanol 1:1) were injected on a Agilent 1290 Infinity liquid chromatography system coupled to a triple quadrupole mass spectrometer (ESI-6460, Agilent). Separation was obtained by a biphenyl column (2.1 mm × 100 mm, 2.6 μm; Phenomenex Kinetex; Torrance, USA) and mobile phases of water with 2% methanol (A) and methanol with 2% water (5 mM ammonium acetate as additive). Target analytes were identified through dynamic multiple reaction monitoring (dMRM) in positive and negative ionization.

Quantification of target analytes was performed with calibration curves in neat solvents ranging from 0.04 to 10 ng/mL, except for BCIPP and 4-HO-DPHP for which the calibration ranged from 0.2 to 50 ng/mL. Procedural blanks were analyzed to check for background contamination. DPHP, DNBP and TCEP were found at trace levels in procedural blanks (0.14, 0.08 and 0.03 ng/mL, respectively). These blank concentrations were subtracted from values in urine samples. Limits of quantification (LOQ) were determined as three times the standard deviation of procedural blank concentrations (for DPHP, DNBP and TCEP) or as the concentration corresponding to a signal-to-noise ratio of 10 in spiked urine samples. Successful participation in inter-laboratory comparison exercises (HBM4EU ICI/EQUAS and OSEQAS, 2018–2020) assured additional external quality control for several target analytes (DPHP, BDCIPP and BCIPP). Metabolites and corresponding parent chemicals targeted in this study are shown in Table SI-2. The performance of the analytical method is summarized in Tables SI-2 and 3. Specific gravity was determined by refractometry at Algemeen Medisch Labo (AML, Antwerp, Belgium).

2.3. Statistical analysis

Statistical analyses were carried out for compounds which had exposure values above LOQ in at least 60% of the samples. A truncated lognormal distribution was fitted to the observed values (above the LOQ). This resulted in an estimation of the mean and standard deviation for the distribution of all log-normal transformed values (above and below LOQ). Values below the LOQ were then randomly assigned an imputed value below the log of the LOQ, drawn from the estimated lognormal distribution and retransformed to an imputed value between 0 and the LOQ on the original scale. The description of this imputation protocol and software has been recently communicated in a report for HBM4EU project – Dec 2020 (Statistical analysis plan for the co-funded studies – Deliverable 10.10 – https://www.hbm4eu.eu/deliverables/).

Questionnaire data were used to identify significant determinants of exposure by a stepwise multiple linear regression model per compound using backward selection. Dependent variables were PFR metabolite levels normalized for specific gravity using the following formula: biomarker_SG = $conc_{biomarker}$ * (1.024-1)/(SG-1), and then transformed by the natural logarithm. Independent variables were introduced in the multiple model if the p-value was <0.2 in univariate regression model and if the direction of the association was consistent with mechanistic or epidemiological insights. Correlations among independent variables was also verified by variance inflation factors. Non-significant explanatory variables ($p < 0.05$) were removed one by one until only significant variables were retained. Season and socio-economic variables were only introduced in the final step as they could be proxies for other determinants. The available variables that were tested in univariate regression models are shown in Table SI-4. Specific gravity was also added as an independent variable in each model. The R-square of the model reflects the percentage of variation in PFR metabolite levels that could be explained by the remaining independent variables in the final model.

Estimated daily intakes (EDI in ng/kg bw/day) were calculated based on urinary concentrations of frequently detected metabolites according to the following equation (Fromme et al., 2014; Chen et al., 2018):

$$EDI = \left(\frac{c_{meta} \times V_{urine}}{F_{UE} \times bw}\right) \times \frac{MW_p}{MW_m}$$

where c_{meta} is the specific-gravity corrected metabolite concentration (in ng/mL SG); V_{urine} is the daily excreted volume of urine (estimated at 1200 mL/day for adolescents) (Valentin 2002); F_{UE} is the urinary excretion factor; bw is the body weight of the participant (in kg); and MW_p and MW_m are the molecular weight of the parent compound and its metabolite respectively (in g/mol, Table SI-2). Studies on the toxicokinetics of PFRs in humans are scarce. Therefore, results from *in vitro* human liver microsomes (HLM) and S9 fractions experiments (Van den Eede et al., 2013) were used to estimate the urinary excretion fraction (F_{UE}) as previously suggested by Zhang et al. (2018b). EDIs were calculated based on both scenarios (HLM and S9) to investigate the total daily exposure to PFRs (exact F_{UE} values shown in Table 4). The estimated daily intakes of targeted PFRs were compared to available oral reference doses (RfD). RfD values provide an estimation of the daily exposure for humans that is likely without any adverse effects during a lifetime. As such, RfDs are a valuable tool for risk assessment of human exposure to toxic chemicals (Poma et al., 2019).

3. Results and discussion

3.1. Concentrations of PFR metabolites

The distribution of targeted PFR metabolites in urine of Flemish adolescents is shown in Table 1. Six from the thirteen measured metabolites, originating from seven different parent compounds were detected in the majority of the samples: DPHP as a metabolite of TPHP (detection frequency 99%), BCIPHIPP as a metabolite of TCIPP (96%), BDCIPP as a metabolite of TDCIPP (86%), BBOEHEP as a metabolite of TBOEP (96%), and EHPHP and 5-HO-EHDPHP both metabolites of EHDPHP (99% and 98%, respectively). The high detection frequencies of these metabolites show that participants were widely exposed to all targeted PFRs except TCEP (33%) and TNBP (16%). Detection frequencies of other metabolites derived from the same parent compounds were much lower. EHPHP, DPHP and BCIPHIPP were detected at the highest uncorrected geometric mean concentrations of 3.56 ng/mL, 1.23 ng/mL and 0.79 ng/mL, respectively.

Until now, most studies have focused on diaryl and dialkyl metabolites (DAPs) such as DPHP, BDCIPP, DNBP and BBOEP as targets for human biomonitoring. HO-PFRs have only been measured by a limited number of studies although they are excellent targets for internal exposure assessment as shown by the high detection frequencies of BCIPHIPP, BBOEHEP and 5-HO-EHDPHP in this study. However, not all HO-PFR metabolites are found in detectable levels. For example, exposure to TPHP is commonly monitored by measuring DPHP, but correlations between dust and urine were not statistically significant in most studies (Cequier et al., 2015; Bastiaensen et al., 2019). TPHP is used as a plasticizer and lubricant, but also as a flame retardant in polyurethane foam in combination with chlorinated PFRs (van der Veen and de Boer, 2012). However, other PFRs, such as EHDPHP and resorcinol bis-diphenyl phosphate (RDP) also form DPHP as a metabolite in urine. Thus, DPHP concentrations should be interpreted as the exposure dose resulting from the sum of all PFRs with at least two phenyl substituents (Dodson et al., 2014; Ballesteros-Gómez et al., 2015). Hydroxylated TPHP metabolites (4-HO-DPHP and HO-TPHP) have been proposed as TPHP specific biomarkers, but until now these metabolites have only been reported in low detection frequencies (Bastiaensen et al., 2019; Ding et al., 2019; Li et al., 2019b; Bastiaensen et al., 2020a).

As mentioned before, comparable results from European studies are scarce, but representative data for adolescents, children, adults and the general population have recently been reported for other countries. Median concentrations of urinary PFR metabolites as reported by various studies are summarized in Table 2. Levels of DPHP in this study

Table 1
Concentrations of PFR metabolites in urine of Flemish adolescents (n = 582, in ng/mL). Geometric means only for compounds with DF > 60%. Imputed values for measurements values < LOQ.

				Percentile						95% CI	
Parent compound	**PFR metabolites**	LOQ (ng/mL)	DF (%)	5th	25th	50th	75th	95th	GM	Lower bound	Upper bound
TPHP	**DPHP**	0.10	99	0.23	0.70	1.22	2.01	4.43	1.23	1.14	1.34
	4-HO-DPHP	0.50	15				n.d.	1.16	n.a.		
	HO-TPHP	0.01	7				n.d.	0.01	n.a.		
TCEP	**TCEP**	0.04	33				0.05	0.15	n.a.		
TCIPP	**BCIPHIPP**	0.04	96	0.07	0.26	0.61	1.42	6.12	0.79	0.69	0.89
	BCIPP	1.00	9				n.d.	2.40	n.a.		
TNBP	**DNBP**	0.15	16				n.d.	0.36	n.a.		
TDCIPP	**BDCIPP**	0.05	83	n.d.	0.12	0.29	0.63	2.20	0.42	0.38	0.46
TBOEP	**BBOEHEP**	0.005	96	0.01	0.02	0.03	0.07	0.24	0.04	0.04	0.04
	BBOEP	0.05	13				n.d.	0.10	n.a.		
	3-HO-TBOEP	0.01	7				n.d.	0.01	n.a.		
EHDPHP	**EHPHP**	0.05	99	0.57	2.18	3.77	6.15	12.90	3.56	3.28	3.85
	5-HO-EHDPHP	0.01	98	0.02	0.04	0.08	0.15	0.34	0.09	0.08	0.09

n.d.: not detected; n.a.: not applicable; LOQ: limit of quantification; DF: detection frequency; GM: geometric mean.

were higher than levels observed in adolescents from China (Ding et al., 2019), but comparable to adolescents from the US (Ospina et al., 2018). BDCIPP and BCIPHIPP levels were lower compared to these study populations, but generally higher in comparison to levels observed in children and adults from Japan (Bastiaensen et al., 2019; Bastiaensen et al., 2020a), Norway (Cequier et al., 2015) and China (Chen et al., 2018; Zhang et al., 2018a, 2018b; Li et al., 2019b). However, higher urinary concentrations have also been reported for BDCIPP and DPHP in children from Australia (He et al., 2018b) and BDCIPP in children from the US (Butt et al., 2016). Differences in internal exposure may be explained by several factors. Firstly, younger children are relatively more frequent exposed to PFRs than adults (Van den Eede et al., 2015). As such, comparisons between different age categories should be performed with caution. Secondly, regulations and usage patterns of chemicals can differ considerably between countries. For example, TCEP use declined in Europe due to stricter legislation. TCEP was classified as a category 2 reproductive toxicant and a category 2 carcinogen (EU 2009)) which led to increased use of TCIPP and TDCIPP. Conversely, no regulations exist for TCEP in various Asian countries which in turn resulted in relatively higher human exposure to TCEP (Li et al., 2019c). Therefore, it is important to consider sampling year and location because PFR consumption patterns change over time.

According to the results of this study, Belgian adolescents were mainly exposed to EHDPHP, DPHP, TCIPP and TDCIPP. Concentrations of EHPHP and 5-HO-EHDPHP were particularly high compared to other studies from China and Japan (Hu et al., 2019; Li et al., 2019b; Bastiaensen et al., 2020a), but similar to results from Belgian adults (Bastiaensen et al., 2018). This indicates that these compounds are present in products, used or present in the life environment of both age groups. EHDPHP is applied as a flame retardant and plasticizer in polyvinyl chloride (PVC), but is also used in paints, rubber, adhesives and food packaging materials (Ballesteros-Gómez et al., 2015). Migration of EHDPHP from packaging to food is likely a major exposure source to this PFR. Recent studies have shown that PFRs, and especially EHDPHP, contaminate food during industrial processing and manipulation (e.g., packaging, drying, canning) (Poma et al., 2018). EHDPHP was the major contributor to the total dietary exposure of PFRs in food basket studies from Belgium (Poma et al., 2018), Sweden (Poma et al., 2017) and Norway (Xu et al., 2017). Dietary intake was responsible for over 95% of the total EHDPHP exposure, compared to limited exposure through dust or air (Xu et al., 2019). We therefore presume that the higher levels of EHPHP and 5-HO-EHDPHP in the urine of Belgian adolescents are associated with dietary exposure. However, we could not identify any significant dietary factors from questionnaires (discussed in Section 3.2). Moreover, future studies are required to confirm these findings since most other studies are not reporting these metabolites (Table 2). EHDPHP is also frequently detected in dust samples, but generally in lower concentrations compared to other PFRs (Li et al., 2019c). However, one study from the UK found that EHDPHP levels in classroom dust significantly exceeded those of all other microenvironments studied (living rooms, offices, cars) (Brommer and Harrad, 2015). It is therefore possible that exposure during school hours also contributed to increased EHDPHP exposure. Food intake is only a minor exposure route for other PFRs, such as TCIPP, TDCIPP, TPHP and TBOEP. Exposure to these PFRs mainly arises from dust ingestion and air inhalation (Xu et al., 2016). TCIPP and TBOEP have been reported in the highest concentrations in dust from houses and other indoor environments in different countries, followed by TDCIPP and TPHP (Li et al., 2019c). TCIPP and TBOEP were also the most abundant PFRs in house dust samples from Belgium in two different studies (Van den Eede et al., 2011; de la Torre et al., 2020). These PFRs are applied in many products present in the indoor environment: chlorinated PFRs, such as TCIPP and TDCIPP are used in polyurethane foam found the upholstery of furniture, TBOEP in floor polish and TPHP as flame retardant in polyurethane foam and as plasticizer in lacquers, hydraulic fluids, resins and PVC (van der Veen and de Boer, 2012). Significant correlations have been reported between TDCIPP, TCIPP and TBOEP in house dust and their metabolites in urine (BDCIPP, BCIPHIPP and BBOEHEP, respectively), which suggest that dust ingestion was an important exposure route to these three PFRs for the participants of this study (Cequier et al., 2015; Bastiaensen et al., 2019). However, levels in dust and urine are not always significantly correlated as shown in children from the US (Phillips et al., 2018).

3.2. Determinants of exposure

Participants provided detailed information about their home environment and lifestyle habits by answering questionnaires, which could be related to the measured internal exposure. Results of the multiple regression models are summarized in Table 3. Season was a significant determinant for all PFR metabolites. Other studies have reported that concentrations of BDCIPP, BCIPHIPP, DPHP and BBOEHEP were higher in summer (Hoffman et al., 2018; Bastiaensen et al., 2020a). In this study, levels of BDCIPP and DPHP were also higher in warmer months, but the trend was not consistent across all metabolites (e.g. EHPHP, BBOEHEP, 5-HO-EHDPHP). In univariate analysis, we found that BDCIPP, BCIPHIPP and DPHP levels increased with higher average daily outdoor temperature and higher average daily UV radiation (data not shown). Temperature may be an important predictor of exposure to

Table 2
Comparison of median concentrations of PFR metabolites (ng/mL) reported in various studies.

Reference	*n*	Country	Sampling years	Population	TPHP		TDCIPP	TCIPP		TNBP	TBOEP			EHDPHP	
					DPHP	4-HO-DPHP	BDCIPP	BCIPHIPP	BCIPP	DNBP	BBOEHEP	BBOEP	3-HO-TBOEP	EHPHP	5-HO-EHDPHP
This study	*582*	*Belgium*	*2017–2018*	*Adolescents (14–15 y/o)*[a]	*1.37*	*n.d.*	*0.34*	*0.71*	*n.d.*	*n.d.*	*0.04*	*n.d.*	*n.d.*	*4.07*	*0.01*
Ding et al. (2019)	306	China	2015	Adolescents (12–15 y/o)[b]	0.42	n.d.	6.17	1.47	0.18	2.54	n.d.	0.11			
Ospina et al. (2018)	427	USA	2013–2014	Adolescents (12–19 y/o)[b]	1.44		1.43		0.16	0.21					
Ospina et al. (2018)	2666	USA	2013–2014	General population[b]	0.82		0.88		0.16	0.25					
Bastiaensen et al. (2020a)	400	Japan	2012–2017	Children (7 y/o)[b]	0.46	n.d.	0.13	0.38	n.d.	n.d.	0.11	0.11	n.d.	0.31	0.01
Bastiaensen et al. (2019)	128	Japan	2009–2010	Children (7–12 y/[b]o)	0.32	0.22	0.07	0.18	n.d.	n.d.	0.36	0.2	n.d.	n.a.	0.04
Hoffman et al. (2018)	203	USA	2014–2016	Children (3–6 y/o)[a]	2.3		6	1.3	0.46						
Gibson et al. (2018)	90	USA	2015	Children (3-5y/o)[b]	3.2		2.6	0.6	0.9						
Butt et al. (2016)	40	USA	2015	Children (0–3 y/o)[a]	2.5		7.4	2.0	n.d.						
Cequier et al. (2015)	54	Norway	2012	Children (6–12 y/o[a])	1		0.23			n.d.		n.d.			
Chen et al. (2018)	411	China	2015	Children (8–12 y/o)[a]	0.28		0.05		0.15	0.12		0.05			
Fromme et al. (2014)	312	Germany	2011–2012	Children (2–6 y/o)[b]	0.8					0.2		2			
He et al. (2018b)	400	Australia	2014–2015	Children (0-5y/o)[c]	25		2.6	0.43	0.85	0.18	0.075	0.32	0.029		
He et al. (2018a)	61	Australia	2015–2016	Children (0–2 y/o)[b]	1		3.3	0.93	0.68	0.15	0.08	0.1	0.13		
Zhang et al. (2018a)	227	China	2016	Children (0-5y/o)[b]	0.27		0.08		0.69	0.06		0.04			
Sun et al. (2018)	180	China	2016–2017	Adults and children[a]	0.07		n.d.		n.d.	0.00		0.10			
Hammel et al. (2016)	40	USA	2015	Adults[b]	1.16		2.06	1.12	n.d.						
Hu et al. (2019)	15	China	2018	Adults[b]	2.47		0.09	0.49	n.d.	0.16	n.d.	0.12	n.d.	n.d.	n.d.
Bastiaensen et al. (2018)	14	Belgium	2017	Adults[b]	1.09	n.d.	0.19	3.93	n.d.	0.26	0.02	n.d.	n.d.	3.94	0.23
Dodson et al. (2014)	16	USA	2011	Adults[b]	0.44		0.09		n.d.	0.11		n.d.			
Siddique et al. (2020)	120	Canada	2013–2015	Adult women[b]	13.8		1.2	n.d.	0.5		n.d.	n.d.			
Chen et al. (2019)	84	China	2013–2015	Adult women[a]	0.26		0.25		0.14	0.18		0.06			
Li et al. (2019b)	46	China	2017	General population[b]	0.33	n.d.	0.08	0.68	n.d.	n.d.	n.d.	n.d.		0.57	0.004
Zhang et al. (2018b)	323	China	2017	General population[b]	0.30		0.15		0.30	0.29		0.03			

[a] Specific gravity corrected median values.
[b] Uncorrected median values.
[c] Pooled mean values.

Table 3
Multivariate analysis for the identification of determinants of exposure of PFR metabolites (ln-transformed and standardized for specific gravity). Only variables significant at $p < 0.05$ were retained in the model. Estimates are multiplicative factors.

Log adjusted PFR metabolite	Variable (n)	ß (95%CI)	p-Value
BDCIPP	**Highest education level of the mother**		**0.033**
$R^2 = 0.059$	Primary education (42)	Reference	
	Secondary education (84)	1.328 (0.034–1.129)	0.243
	Tertiary education (337)	1.679 (1.065–2.646)	**0.026**
	New decoration in living room (curtains, carpet, wallpapers, etc)		
	No (465)	Reference	
	Yes (98)	1.590 (1.163–2.175)	**0.004**
	Season		**0.001**
	Winter (169)	Reference	
	Spring (191)	1.387 (1.029–1.868)	**0.032**
	Summer (66)	2.291 (1.527–3.438)	**<0.001**
	Autumn (137)	1.319 (0.958–1.817)	0.089
DPHP	**Highest education level of the father**		**0.021**
$R^2 = 0.092$	Primary education (60)	Reference	
	Secondary education (203)	1.198 (0.983–1.459)	0.073
	Tertiary education (231)	1.009 (0.829–1.228)	0.930
	Home with mechanical ventilation		
	No (403)	Reference	
	Yes (91)	0.848 (0.726–0.990)	**0.037**
	Season		**<0.001**
	Winter (145)	Reference	
	Spring (164)	1.384 (1.186–1.613)	**<0.001**
	Summer (61)	1.383 (1.129–1.696)	**0.002**
	Autumn (124)	1.284 (1.090–1.513)	**0.003**
	BMI		**0.032**
	<18.5 kg/m^2 (139)	Reference	
	18.5–25 kg/m^2 (301)	0.884 (0.771–1.013)	0.077
	>25 kg/m^2 (54)	0.757 (0.609–0.939)	**0.012**
BCIPHIPP	**Building type**		
$R^2 = 0.088$	(549) house (549)	Reference	
	(32) apartment (32)	1.679 (1.035–2.721)	**0.036**
	Degree of urbanisation		
	Rural (<600 inhab/km^2) (376)	Reference	
	Urban (>600 inhab/km^2) (205)	0.790 (0.626–0.996)	**0.046**
	Season		**<0.001**
	Winter (173)	Reference	
	Spring (201)	1.319 (0.999–1.742)	0.051
	Summer (66)	1.391 (0.947–2.042)	0.092
	Autumn (141)	0.551 (0.408–0.744)	**<0.001**
5-HO-EHDPHP	**Building type**		
$R^2 = 0.153$	House (509)	Reference	
	Apartment (25)	1.422 (1.023–1.974)	**0.036**
	Insulation/building status		**0.05**
	Others (428)	Reference	
		0.768 (0.612–0.965)	**0.023**
	Newly built after 2006, but no energy-neutral building (56)		
	Energy-neutral building (50)	1.082 (0.856–1.368)	0.511
	Moment of urine collection		**<0.001**
	<10 h (111)	Reference	
	10–12 h (214)	1.080 (0.899–1.298)	0.409
	>12 h (209)	1.493 (1.240–1.800)	**<0.001**
	Season		**<0.001**
	Winter (156)	Reference	
	Spring (185)	0.603 (0.507–0.717)	**<0.001**
	Summer (64)	0.677 (0.536–0.856)	**0.001**
	Autumn (129)	0.957 (0.794–1.153)	0.641
EHPHP	**Season**		**<0.001**
$R^2 = 0.119$	Winter (173)	Reference	
	Spring (202)	0.807 (0.694–0.936)	**0.004**
	Summer (66)	0.839 (0.683–1.033)	0.071
	autumn (141)	1.251 (1.064–1.471)	**0.008**
	BMI		**0.019**
	<18.5 (159)	Reference	
	18.5–25 (359)	0.937 (0.818–1.073)	0.344
	>25 (64)	0.740 (0.600–0.914)	**0.005**
BBOEHEP	**Highest education level of the father**		**0.02**
$R^2 = 0.052$	Primary education (65)	ReferenceReference	
	Secondary education (228)	1.309 (0.983–1.742)	0.065
	Tertiary education (254)	1.484 (1.142–1.968)	**0.006**
	Season		**0.004**
	Winter (162)	Reference	
	Spring (189)	0.785 (0.630–0.977)	**0.031**
	Summer (63)	1.114 (0.824–1.507)	0.481
	Autumn (133)	0.703 (0.554–0.891)	**0.004**

PFRs as seasonality has also been observed in PFR concentrations in indoor dust and outdoor urban air (Cao et al., 2014; Wang et al., 2019a). Temperature and UV radiation can not only change the emission rates of PFRs from applications into the environment, but also the partitioning of PFRs between air and dust (Cao et al., 2014). As such, exposure through inhalation of contaminated air might be higher for more volatile PFRs such as TCIPP and TDCIPP. Indirectly, temperature could also influence the behavior of people with regard to their diet, the clothes they wear, ventilation habits or time spent indoor which, in turn, would result in more frequent exposure to PFRs through different pathways (Hoffman et al., 2018; Bastiaensen et al., 2020a).

BDCIPP and BBOEHEP concentrations were higher in adolescents with mothers or fathers with tertiary education. The same trend has been reported for BBOEP, another TBOEP metabolite, in Japanese children (Bastiaensen et al., 2019) and for BCIPP but not BCIPHIPP in children from the US (Hoffman et al., 2018). Here, DPHP was also associated with education level of the parents, but the trend was different (higher levels for parents with secondary education). These results suggest that educational attainment is a determinant of PFR exposure, but this is likely a proxy for another underlying factor, which could be the use of specific products in these households (such as

Table 4
Estimated daily intakes (EDI in ng/kg bw/day) to organophosphate flame retardants and plasticizers calculated based on urinary metabolite concentrations.

EDIs in ng/kg bw/day	TPHP		TDCIPP		TCIPP		TBOEP		EHDPHP	
	Based on DPHP		Based on BDCIPP		Based on BCIPHIPP		Based on BBOEHEP		Based on EHPHP + 5-HO-EHDPHP [a]	
	S9[b]	HLM[b]	S9[b]	HLM[b]	S9[b]	HLM[b]	S9[b]	HLM[b]	High F_{ue}[c]	Low F_{ue}[c]
F_{UE}	0.19	0.41	0.68	0.46	0.28	0.33	0.16	0.81	0.2	0.8
min	8.3	3.8	0.1	0.2	0.3	0.2	0.1	0.02	12.0	3.0
5th per	64.3	29.8	0.8	1.2	8.1	6.9	1.2	0.3	194.3	48.6
25th per	124.1	57.5	6.4	9.4	26.2	22.2	3.6	0.7	356.7	89.2
50th per	197.7	91.6	14.5	21.4	57.4	48.7	6.3	1.2	567.7	141.9
75th per	310.9	144.1	29.5	43.6	126.2	107.1	11.9	2.4	905.6	226.4
95th per	640.0	296.6	119.1	176.1	517.5	439.1	44.2	8.7	2004.2	501.1
max	8080.9	3744.8	1958.9	2895.7	11282.4	9573.0	246.3	48.6	3308.2	827.1
Medians										
Male	216.3	100.3	14.3	21.1	61.3	52.1	6.0	1.2	562.1	140.5
Female	181.4	84.1	14.7	21.8	51.3	43.5	6.4	1.3	570.8	142.7
BMI < 18.5	249.5	115.6	18.8	27.7	70.0	59.4	7.6	1.5	694.4	173.6
BMI 18.5–25	188.6	87.4	13.8	20.4	50.7	43.0	5.6	1.1	562.1	140.5
BMI > 25	121.6	56.4	11.0	16.3	48.0	40.7	5.4	1.1	323.3	80.8
RfD (ng/kg bw/day)[d]	2.0×10^4		$2.0x10^4$		1.0×10^4		1.5×10^4		1.5×10^4	
% >RfD	0%		0%		0.17% (1/582)		0%		0%	
Ratio RfD/95th per	31	67	168	114	19	23	340	1720	7	30

HLM: human liver microsome fraction; S9: S9 fraction; bw: bodyweight; per: percentile.
[b] F_{ue} estimations taken from Van den Eede et al. (2013).
[c] No F_{ue} available in literature, therefore low and high F_{ue} scenario.
[d] Oral reference doses (RfDs) taken from Poma et al. (2019).

furniture, floor covering or electronic devices). However, the reasons remain unknown as shown in the results of this determinant analysis.

Other variables included in the final models were more heterogeneous between compounds and are discussed individually below. Adolescents with higher BMI (>25 kg/m^2) had significantly lower levels of DPHP and EHPHP. Wang et al. (2019b) reported that levels of DPHP, BDCIPP and BCIPP were higher in obese individuals, whereas BBOEP levels were lower in obese individuals than those with normal BMI. No differences in PFR metabolite levels were found between boys and girls. BDCIPP concentrations were significantly higher in adolescents living in homes with new decoration such as curtains, carpets and wall papers. TDCIPP is mainly applied in upholstered furniture, but it is possible that it is also used as a flame retardant in decorative materials (Wei et al., 2015). We also found that DPHP levels were lower for adolescents that live in homes with mechanical ventilation. This would suggest that ventilation decreases the concentration of TPHP in indoor dust or air. However, in a previous study by our research group, an opposite trend was observed consistent across multiple PFR metabolites (higher BCIPHIPP, DPHP, BDCIPP, BBOEHEP and BBOEP levels with more frequent ventilation) (Bastiaensen et al., 2020a). In this study, ventilation was only significant for DPHP. All other metabolites were not associated with any of the ventilation variables, neither in univariate analysis (Table SI-4). Concentrations of 5-HO-EHDPHP were lower in adolescents living in a recently constructed home, which could suggest that newer insulation or building materials would not or contain less EHDPHP. Although EHDPHP is possibly applied in building materials as part of PVC polymers, no public data exist on the type of products in which this PFR is used (de la Torre et al., 2020). Finally, there were two significant determinants of TCIPP exposure for which no reasonable explanation could be found: building type (higher levels for adolescents living in apartments) and degree of urbanization (lower levels for adolescents living in urban areas).

Despite the extensive list of available questions and large sample size, the overall proportion of variance predicted by significant variables in multiple models was low (max $R^2 = 0.153$). Because various PFRs are often applied in similar applications (some even within the same product), we hypothesized that some associations would be similar for multiple metabolites. However, there were no consistent associations for characteristics of the indoor environment such as the use of building materials, electronic devices or ventilation. Furthermore, none of the investigated food variables were significant determinants of exposure. Other studies have reported similar difficulties to relate food intake with urinary metabolite concentrations (Thomas et al., 2017a, 2017b; Bastiaensen et al., 2020a). One possible explanation for the lack of association with food is that the questions of this study focused on fresh foods while dietary exposure mainly originates from industrially processed food (such as grains, oils and dairy products) (Poma et al., 2018). Finally, the low predictive value of the constructed models may also be attributable to the short-term variability of PFR metabolite concentrations in urine. In this study, random spot samples were collected at school (9–16 h). Sampling time was a significant predictor for 5-HO-EHDPHP but not for other PFR biomarkers (Table 3). Spot urine sample analysis may not accurately represent exposure due to within-individual variation as a result of the short-biological half-life of PFRs (Wei et al., 2015). However, intraclass correlation coefficient analysis of PFR metabolites in spot urine samples has shown fair-to-good reproducibility of most PFR metabolites with ICC ranging from 0.35 for BBOEP to 0.68 for BDCIPP over 5 weeks (Wang et al., 2019b) and from 0.40 for 5-HO-EHDPHP to 0.60 for BDCIPP over 5 consecutive days, except for DPHP and EHPHP (ICC of 0.30 and 0.23, respectively) (Bastiaensen et al., 2020b).

3.3. Estimated daily exposure

A major advantage of human biomonitoring is that it provides an overall view of the internal dose and, thus, that it encompasses all exposure pathways. However, data on the bioavailability and toxicokinetics of PFRs are still very limited which limits the performance of a thorough risk assessment.

The daily intakes calculated here should therefore be considered as an estimation and the interpretation should be done cautiously. We opted to discuss only the EDI results based on the F_{UE}-S9-fraction as it contains both phase I and phase II metabolites (contrary to HLM that only contain phase I enzymes) (Richardson et al., 2016). This is of importance since some PFR metabolites are excreted as glucuronide conjugates (Van den Eede et al., 2013).

The highest median EDI was observed for $EHDPHP_{highFue}$ (567.7 ng/kg bw/day). Median EDIs of other PFRs were a factor 10 (TPHP, TDCIPP,

TCIPP) or 100 lower (TBOEP). For TPHP, TDCIPP and TCIPP, the maximum EDIs calculated were several times higher than the 95th percentile, which indicates that some participants had extreme levels of these metabolites in their urine. As reported above, the total daily intake of TPHP and EHDPHP was related to BMI but not to sex (see descriptive results in Table 4). The calculated median EDI for TDCIPP was similar to those reported for adults and children from China and the US based on urinary metabolite levels (Chen et al., 2018; Zhang et al., 2018b; Li et al., 2019b; Wang et al., 2019b). EDIs for TPHP, TCIPP and TBOEP were more heterogeneous between the available studies, but generally within the same range. For EHDPHP however, the median EDI was higher than the one reported for Chinese adults (Li et al., 2019b).

EDIs of this study were much higher compared to results from external exposure assessments for Belgian adults (not available for adolescents). The mean daily intake for Belgian adults via food intake was estimated at 46.6 ng/kg bw/day for TPHP, 9.6 for TDCIPP, 18.5 for TCIPP and 14.9 for EHDPHP (Poma et al., 2018). The median intakes of PFRs by dust ingestion for Belgian adults were between a factor 10 and 100 lower compared to the estimate for food intake (0.1 ng/kg bw/day for TPHP, 0.3 for TDCIPP, 0.5 for TCIPP, 0.7 for TBOEP and 0.1 for EHDPHP) (Van den Eede et al., 2011; de la Torre et al., 2020). However, even when intake from dust and food are combined, the total intake accounted for a fraction of the median intake calculated based on urinary metabolite concentrations (24% for $TPHP_{S9}$, 69% for $TDCIPP_{S9}$, 33% for $TCIPP_{S9}$ and 3% for $EHDPHP_{highFue}$). These results suggested that other exposure pathways such as air inhalation and dermal absorption could contribute considerably to PFR exposure and that some sources of exposure may still be unknown such as direct exposure to diesters (Tan et al., 2019).

Oral reference doses (RfD) for human exposure were used according to Poma et al. (2019) to assess the potential risk associated with the estimated intakes. EDIs of all participants, except one for $TCIPP_{S9}$, were well below the RfDs of the investigated PFRs (Table 4). More precisely, the 95th percentile EDI (high exposure scenario) was 7 ($EHDPHP_{highFue}$) to 1720 ($TBOEP_{HLM}$) times lower than the RfD. Overall, these results suggest that the PFR exposure of Belgian adolescents is well below the risk threshold. However, it is important to note that these RfD values were determined based on toxicological data and should be interpreted within the context of their limitations. When RfDs are revised according to new toxicological evidence they may become stricter. Furthermore, the assessment presented here does also not account for the potential additive effect on health of combined exposure to multiple PFRs and/or other toxic chemicals. Finally, due to the lack of *in vivo* toxicokinetic data of PFRs in humans, the calculated EDI may be over- or underestimated. The toxic characteristics of most PFRs are relatively well documented in *in vitro* and animal studies but reports on the health effects of background levels on humans are limited. Urinary concentrations of PFR metabolites within the same range as the levels observed in this study have been associated with allergic symptoms and oxidative stress biomarkers in Japanese children (Araki et al., 2018; Ait Bamai et al., 2019). EHDPHP concentrations were also significantly correlated with sphingolipid levels in human blood (Zhao et al., 2016). Although these association studies may not be representative to adolescents or other age groups, they do suggest a relationship between exposure and effect. Additional epidemiological research is needed to understand sources of exposure as well as the potential health impact of PFR exposure.

4. Conclusions

This is the first study to report reference values for 13 PFR urinary metabolites originating from 7 parent compounds, determined in European adolescents. Metabolites of EHDPHP, TCIPP, TDCIPP, TPHP and TBOEP were detectable in practically every participant suggesting widespread exposure to PFRs in Flanders, Belgium. Concentrations of DPHP, BDCIPP and BCIPHIPP were generally within the same range as in other study populations from non-European countries, only EHDPHP exposure was presumably higher in this study. However, we could not identify any explanatory factors related to the ingestion of EHDPHP or other PFRs through food. Determinant analysis did show higher levels of BDCIPP with the introduction of new decoration, lower levels of 5-HO-EHDPHP in adolescents living in newly built homes and higher levels of BBOEHEP and BDCIPP in adolescents with highly educated parents. Season was the only determinant significant for several PFR metabolites. The estimated daily intakes for Flemish adolescents were all well below the available reference doses indicating that the risk associated to the measured internal doses is low.

CRediT authorship contribution statement

Michiel Bastiaensen: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Visualization. **Celine Gys:** Conceptualization, Investigation, Writing - review & editing, Visualization. **Ann Colles:** Conceptualization, Investigation, Writing - review & editing. **Veerle Verheyen:** Conceptualization, Writing - review & editing. **Gudrun Koppen:** Conceptualization, Writing - review & editing. **Eva Govarts:** Conceptualization, Investigation, Writing - review & editing. **Liesbeth Bruckers:** Conceptualization, Investigation, Writing - review & editing. **Bert Morrens:** Conceptualization, Writing - review & editing. **Ilse Loots:** Conceptualization, Writing - review & editing. **Annelies De Decker:** Conceptualization, Writing - review & editing. **Vera Nelen:** Conceptualization, Writing - review & editing. **Tim Nawrot:** Conceptualization, Writing - review & editing. **Stefaan De Henauw:** Conceptualization, Writing - review & editing. **Nik Van Larebeke:** Conceptualization, Writing - review & editing. **Greet Schoeters:** Conceptualization, Methodology, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Adrian Covaci:** Conceptualization, Methodology, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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