Biomonitoring of organophosphorus flame retardants in a Swedish population – Results from four investigations between years 2000 - 2013.

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Abstract

Flame retardants are chemical substances used in furniture, plastics, building materials, several household products and consumer goods to reduce their flammability. Their widespread use has resulted in measurable concentrations of the compounds or their residues in the environment, biota and human biological samples. The previously used brominated flame retardants were found to be persistent and to have bioaccumulative and neurotoxic potential, which raised public awareness. The use of the emerging organophosphorus compounds have increased after phasing out some brominated flame retardants. The chemical properties and health impacts of the organophosphorus flame retardants have not been studied to the same extent. The effects on public health and the environment due to the exposure from their widespread occurrence are therefore unclear.

The aim of this study is to analyse exposure levels of the organophosphorus flame retardants TBP, TPP, TDCIPP and TBOEP in urine samples from a Swedish population of young men. The samples were collected through the enrolment for military service. Urine samples from year 2000, 2004, 2009 and 2013 were analysed for selected biomarkers of exposure with LC-MS/MS. The statistical analysis focused on temporal trends of the measured exposure levels.

The metabolites DBP, DPP and BDCIPP were found in concentrations above LOD (0.03 – 0.1 ng/ml) in the majority of the samples, and DPP in all samples. BBOEP were only found above LOD in some samples and in very low concentrations. There was a statistically significant decreasing trend for the concentrations of DBP over time.

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Abbreviations

PFR = Phosphate flame retardants
TDCIPP = tris(1,3-dichloro-2-propyl) phosphate
TPP = triphenyl phosphate
TBP = tributyl phosphate
TBOEP = tris(2-butoxyethyl) phosphate
BDCIPP = bis(1,3-dichloro-2-propyl) phosphate
DPP = diphenyl phosphate
DBP = di-n-butyl phosphate
BBOEP = bis(2-butoxyethyl) phosphate
pentaBDE = Pentabromodiphenyl ether
LOD = Limit of detection
IS = Internal standard
QC = Quality control
CID = Collision-induced dissociation
SRM = Selected reaction monitoring
ESI = Electrospray ionization

1 Introduction

Flame retardants were introduced in the 1960s in insulating materials and furniture to reduce the flammability in buildings and mitigate the risk of fire (Kemmlein et al., 2003). The compounds are usually mixed into the products and not chemically bound (Marklund et al., 2003). Therefore, leaching may occur during production, use and disposal of the products (Marklund et al., 2003; Cequier et al., 2014b).

Many different chemical compounds are included in the category 'flame retardants'. Brominated compounds, such as pentabromodiphenyl ether (pentaBDE), were previously the most commonly used. When it was discovered that measurable concentrations of these chemicals were found in biota and human samples, concern was raised regarding possible effects of exposure (de Wit, 2002; Kemmlein et al., 2003; Dishaw et al., 2011). The use was restricted or banned in the EU (Directive 2003/11/EC) after discovering that these compounds have possible neurotoxic properties and bioaccumulative potential (Frederiksen et al., 2009; Dishaw et al., 2011; Dodson et al., 2014). They have primarily been replaced with the less persistent phosphorus flame retardants (van der Veen & de Boer, 2012; Dodson et al., 2014; Fang & Stapleton, 2014), such as organophosphate esters. Besides their flame reducing properties, organophosphates are also used as plasticisers and in antifoam agents, coatings for electronics and adhesives in a wide variety of household products (Cequier et al., 2014a; Dodson et al., 2014; Fromme et al., 2014).

The organophosphorus compounds have been reported in widespread presence in indoor environments (Zhou et al., 2016), both in air and dust (Meeker & Stapleton, 2010; Cequier et al., 2014a; Fromme et al., 2014; Tajima et al., 2014; Kojima et al., 2016). Several studies from different countries have also detected measurable levels in human biological samples (Hudec et al., 1981; LeBel & Williams, 1983; LeBel & Williams, 1986; Schindler et al., 2009; Reemtsma et al., 2011; Stapleton et al., 2012; Meeker et al., 2013; Butt et al., 2014; Kim et al., 2014). Previous biomonitoring studies have found significant correlations between the concentrations in indoor dust and metabolite levels in urine (Fromme et al., 2014). Continuous leaching from household products and building materials in the indoor environment are assumed to be the main source of pollution (Carlsson et al., 1997; Marklund et al., 2003; Carignan et al., 2013; Meeker & Stapleton, 2010; van der Veen & de Boer, 2012). A part of the exposure can also be derived from food (Poma et al., 2017), mainly due to the use of PFRs as plasticisers in packaging (WHO, 2000).

The effects of exposure to organophosphate flame retardants have not been studied to the same extent as for the phased-out brominated compounds, and are therefore less well-known (Behl et al., 2016). There are some documented adverse effects from animal studies, especially for TDCIPP that have been reported to have carcinogenic potential (WHO, 1998). Several studies of TDCIPP exposure to zebrafish have shown that environmentally relevant concentrations could have multigenerational effects, reduce fecundity and have developmental neurotoxic and endocrine disruptive potential (Wang et al., 2015a; Wang et al., 2015b; Liu et al., 2016; Yu et al., 2016; Zhu et al., 2016). Some studies have found similar effects for TPP (Liu et al., 2013; Du et al., 2015). In some mammalian studies however, developmental neurotoxic effects have not been supported (Moser et al., 2015). Studies on human health effects have found associations between indoor concentrations of TPP and reduced sperm concentrations and altered hormone levels (Ma et al., 2017). One study also detected endocrine disrupting potential in an *in vitro* study of human cells exposed to TBP, TPP, TDCIPP and TBOEP (Kojima et al., 2013).

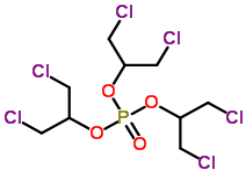
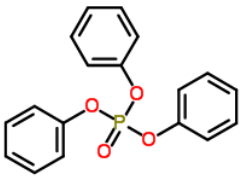
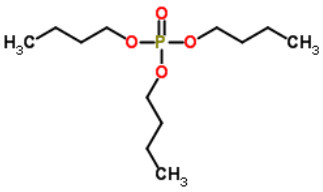
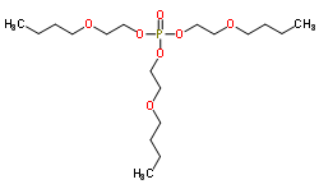
Studies on the metabolism of PFR compounds in animals and *in vitro* studies of human cells have shown that trialkyl and triaryl phosphates mainly are metabolised to and excreted as dialkyl or diaryl phosphates (Lynn et al., 1981; Sasaki et al., 1984; WHO, 1998; Van den Eede et al., 2013; Butt et al., 2014). The biomarkers of exposure in this study are

dialkyl/diaryl metabolites, which have been used in previous biomonitoring programmes (Cooper et al., 2011). The parent compounds and their corresponding biomarkers of exposure measured in this study are listed in Table 1. These compounds and their metabolites seem to have short half-lives based on results from animal studies (WHO, 1998; WHO, 2000). Since organophosphorus flame retardants are used in various products and constantly occur in the surrounding environment there is a continuous exposure. The compounds can therefore be measured in environmental and biological samples, despite their short half-lives.

The targeted organophosphate flame retardants in this study are tris(1,3-dichloro-2-propyl) phosphate (TDICPP), triphenyl phosphate (TPP), tributyl phosphate (TBP) and tris(2-butoxyethyl) phosphate (TBOEP). They are mainly used as plasticizers and in lacquer, paint and glue. All, except TBP, are also extensively used as flame retardants (Marklund et al., 2003). TBP and TPP are components in hydraulic fluids and TBP and TBOEP are used in antifoam agents. None of the compounds are produced in Sweden but have been imported in quantities presented in Appendix I (Swedish Chemicals Agency, 2010). The chemical TDCIPP has not been imported during the years included in this study. The metabolites BCEP, BCPP and BBOEHEP were originally intended to be included in the study, however the analytical method had a too high detection limit for these compounds.

The aim of this study is to biomonitor residues from 4 organophosphorus flame retardants in urine samples from a Swedish population of young men not occupationally exposed.

Table 1. Parent compounds (organophosphates) and corresponding metabolites for measurement.
List of organophosphate flame retardants (right column) and their respective corresponding dialkyl phosphate metabolites used as biomarker of exposure in the analysis (middle column). Chemical formula is shown in the left column.

Chemical formula	Parent compound	Biomarker of exposure
	**TDCIPP** tris(1,3-dichloro-2-propyl) phosphate	**BDCIPP** bis(1,3-dichloro-2-propyl) phosphate
	**TPP** triphenyl phosphate	**DPP** diphenyl phosphate
	**TBP** tributyl phosphate	**DBP** di-n-butyl phosphate
	**TBOEP** tris(2-butoxyethyl) phosphate	**BBOEP** bis(2-butoxyethyl) phosphate

2 Materials and methods

Collection of samples, sample preparation and analysis and validation of the method is described in detail in this chapter.

2.1 Chemicals and materials

DBP and DPP were purchased from Sigma–Aldrich (St. Louis, MO, U.S.A.). BBOEP, BDCIPP, BBOEP-d4, BDCIPP-d10 and DPP-d10 were synthesized by Vladimir Belov (Max Planck Institute for Biophysical Chemistry, Göttingen, Germany). Methanol, ammonium acetate, and ammonia (25 %) (NH_3) were from Merck (Darmstadt, Germany). β–glucuronidase from E.Coli was purchased from Roche Diagnostics Scandinavia AB (Bromma, Sweden). For sample preparation, 96-position well-plates SQW block with clear glass micro insert vials 1 ml, 45 x 7.6 mm with a welled Blue Molded PTFE/Silicone sealmat block cover was from La-Pha-Pack®GmbH (Langerwehe, Germany).

2.2 Sample collection

The urine samples were collected from young men in the south of Sweden in the enrolment for military service in years 2000 (N = 146), 2004 (N = 197) and 2009 (N = 254). For year 2013 (N = 204), samples were collected from both men and women in upper secondary school. The samples have been stored in a freezer in -20°C since collection and have been used in previous biomonitoring studies for other chemicals (Jönsson et al., 2010; Jönsson et al., 2014).

2.3 Sample preparation

Standard solutions were accurately weighed and dissolved in methanol. The IS and standard stock solutions were diluted further in methanol and stored at - 20°C. The standard solutions were prepared in duplicates. The authentic blank urine samples and the quality control (QC) samples were obtained from healthy volunteers at our laboratory. The authentic blank urine samples were used for the preparation of calibration curves. The QC-samples were also prepared from authentic blank urine and spiked. The QC- samples were divided into aliquots before stored at -20°C. The chemical blank samples were prepared from Millie-Q water instead of urine, and thereafter treated like the other samples. The calibration curve, was prepared in blank urine and spiked giving a urinary concentration between 0.2 and 20 ng/ml. The urine samples and the QC-samples were vortex-mixed after thawing and aliquots of 200 µL were transferred into a 96-well-plate. Then 50 µL of IS solution, 100 µL 1 M ammonium acetate buffer pH 6.5 and 10 µL of the enzyme β–glucuronidase were added. The plate was sealed and mixed thoroughly for about 1 minute before incubation. The enzyme incubation was performed at 37ºC with agitation at 400 rpm for about 30min.

2.4 Instrumentation

Quantitative analysis was conducted using a triple quadrupole linear ion trap mass spectrometer equipped with TurboIonSpray source (QTRAP5500; AB Sciex, Foster City, CA, USA) coupled to a liquid chromatography system with four pumps ($UFLC^{RX}$, Shimadzu Corporation, Kyoto, Japan). The MS/MS analysis was carried out using selected reaction monitoring (SRM) in negative electrospray ionization (ESI) mode. To establish the appropriate SRM conditions, standard solutions were infused into the MS/MS for optimization. Collision-induced dissociation (CID) of each $[M-H]^-$ was performed and the product ions giving the best signal to noise ratio were selected for the SRM analysis. All data acquisition and processing was performed using the Analyst 1.6.3 application software (Applied Biosystems, Foster City, CA, USA).

2.5 Analytical method

The separation of the analytes was carried out, using a Triart-C18 column (2.0 x 50 mm, 3 µm), YMC separation technology (Kyoto, Japan) and an aliquot of 5.0 µL of the sample was injected. The two mobile phases used consisted of 5mM ammonium formate pH 9.2 (mobile phase A) and 10 % mobile phase A in acetonitrile (mobile phase B). The flow rate was 0.6 mL/min. The separation started with isocratic elution with 4 % of mobile phase B for 1.0 min followed by a gradient to 50% B at 3.2 min. The effluent was diverted into the MS between 2.1 and 3.4 min. The LC-MS/MS analysis was performed using SRM transitions and collision energies for BDCPP, DPP, DBP, and BBOEP and the IS as shown in Table 2. The compound DBP *did not* have an internal standard and therefore, only the analyte was quantified in this case.

One set of the calibration standards and two sets of the QC-samples were added and analysed with each plate. Concentrations were determined by peak area ratios between the analyte and the IS. All values were corrected for the average concentrations in the chemical blanks, to control for environmental contaminations. The concentrations were determined in the urine used for preparation of standards in each batch and the calibration standard samples were corrected for these concentrations.

The between-run precision was determined from the two QC-samples, in 11 analytical sample batches, prepared and analysed during a period of 3 months. The standard deviation was finally calculated using 22 quantified QC-samples at each concentration level.

Table 2. SRM transitions for selected ions of metabolites and internal standards.
Summary of SRM transitions for all metabolites and marked internal standards used in the LC-MS/MS analysis. The compound DBP did not have an internal standard and the compound was qualified without an IS.

Compound	Transitions (Da)	Collision energy (V)
	Quantifier ions	
BDCPP	319.1 → 35.0	40
[$^2H_{10}$]BDCPP	329.1 → 35.0	45
	Qualifier ions	
BDCPP	317.1 → 35.0	35
[$^2H_{10}$]BDCPP	327.1 → 35.0	50
	Quantifier ions	
DPP	249.1 → 93.0	40
[$^2H_{10}$]DPP	259.1 → 98.0	40
	Qualifier ions	
DPP	249.1 → 155.0	28
	Quantifier ions	
DBP	209.1 → 79.0	60
	Qualifier ions	
DBP	209.1 → 153.1	26
	Quantifier ions	
BBOEP	297.1 → 78.9	60
[$^2H_4$]BBOEP	301.1 → 78.9	60
	Qualifier ions	
BBOEP	297.1 → 196.9	26
[$^2H_4$]BBOEP	301.1 → 199.0	31

2.6 Statistical analysis

Basic descriptive statistics analysis was run in SPSS Statistics (ver 23.0.0.0) for non-adjusted concentrations of each biomarker of exposure from each year of sampling. Percentage above LOD was calculated in Microsoft Excel. In this study, the measured concentrations were used in the statistical analysis for all samples including those below LOD. Negative values were replaced with the lowest measured concentration for the same compound and year divided by $\sqrt{2}$. Linear regression was carried out for density-adjusted values in R and RStudio (ver i386 3.3.2) to look at possible temporal trends of each compound. In the linear regression the median, mean, logarithmic mean and weighted mean of density-adjusted concentrations for each compound were run as dependent variables with the year of sampling as independent variable.

3 Results

Results of the applied analytical method, sample analysis and statistics are presented in this chapter. A comparison with results from similar studies are also included.

3.1 Validation of the analytical method

The LOD in urine was found to be between 0.03 – 0.1 ng/ml, which is sufficiently low for measurements of human exposure. The LOD for each metabolite and the between-run precisions are presented in Table 3.

Table 3. Limit of detection and precision of the method.

Biomarker of exposure	Limit of detection (LOD)	Between run precision	
		QC low	*QC high*
DPP	0.03 ng/ml	7	5
BBOEP	0.03 ng/ml	4	4
BDCIPP	0.05 ng/ml	6	6
DBP	0.1 ng/ml	8	7

3.2 Statistical analysis

Results of measured concentrations in all urine samples divided in years of measurement are presented as descriptive statistics in Table 4. Concentrations that were measured below the limit of detection listed in Table 3 for each biomarker are written as < LOD. The percentage of the samples with concentrations above the detection limit are calculated and presented for each year in the right column of Table 4.

Table 4. Descriptive statistics of concentrations for each biomarker for each year of measurement.
Mean value, standard deviation (SD), median, minimum, maximum and percentiles for the non-adjusted concentrations of each metabolite and year. Percentage of samples with concentrations above limit of detection (% > LOD) is shown in the right column. For year 2000 N = 146, year 2004 N = 197, year 2009 N = 254 and year 2013 N = 204.

Biomarker	Year	Mean (SD)	Min	25-perc	Median	75-perc	95-perc	Max	% > LOD
DPP	2000	3.6 (5.7)	0.09	1.1	2.0	3.4	14	43	100
	2004	4.1 (5.3)	0.12	1.3	2.6	5.3	12	48	100
	2009	2.0 (2.6)	0.11	0.84	1.4	2.5	5.4	32	100
	2013	1.6 (1.6)	0.09	0.64	1.2	1.9	4.8	11	100
BBOEP	2000	0.04 (0.06)	< LOD	< LOD	< LOD	0.05	0.17	0.33	35
	2004	0.07 (0.35)	< LOD	< LOD	< LOD	0.04	0.16	4.5	28
	2009	0.12 (1.3)	< LOD	< LOD	< LOD	0.05	0.20	20	35
	2013	< LOD (0.03)	< LOD	< LOD	< LOD	0.02	0.06	0.36	15
BDCIPP	2000	0.39 (0.90)	< LOD	0.07	0.19	0.34	1.3	8.8	82
	2004	0.45 (1.5)	< LOD	0.08	0.21	0.41	1.3	20	81
	2009	0.42 (0.49)	< LOD	0.15	0.27	0.52	1.3	4.4	91
	2013	0.44 (0.89)	< LOD	0.12	0.21	0.43	1.3	8.3	87
DBP	2000	0.48 (0.47)	< LOD	0.19	0.31	0.57	1.6	2.9	93
	2004	0.37 (0.36)	< LOD	0.13	0.28	0.48	1.0	2.5	81
	2009	0.35 (0.43)	< LOD	0.15	0.26	0.40	0.83	4.6	87
	2013	0.26 (0.21)	< LOD	0.12	0.21	0.33	0.62	1.6	82

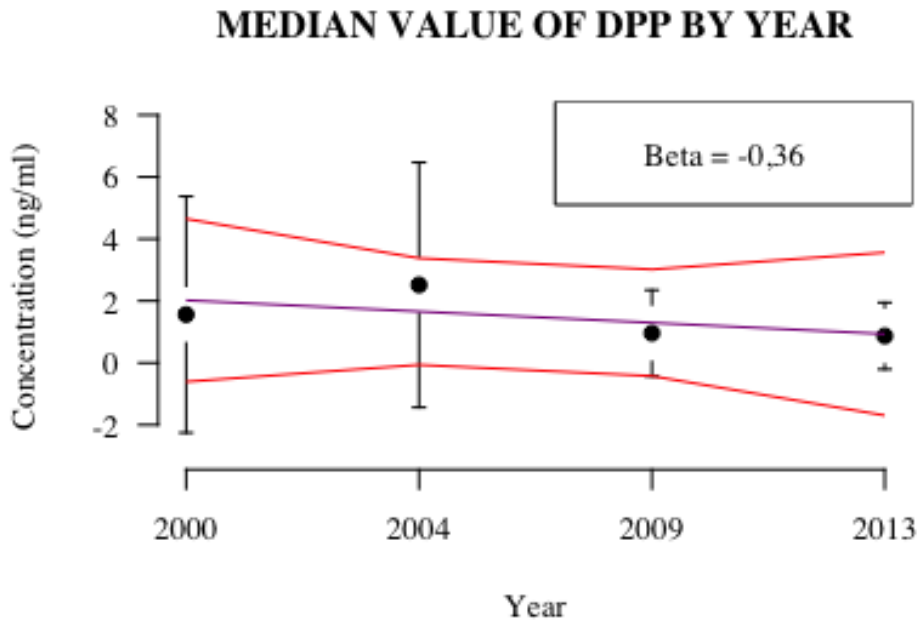


Figure 1. Linear regression of median values of density adjusted concentrations of DPP.
Time trend analysis of median values of density adjusted concentrations of DPP between different years of measurement. Bars represent the standard deviation, purple line shows the slope of change over time and the Beta-value shows the regression coefficient. The red lines show the 95 % confidence interval for the slope. Significance is given as * if $p < 0.05$ and ** if $p < 0.01$.

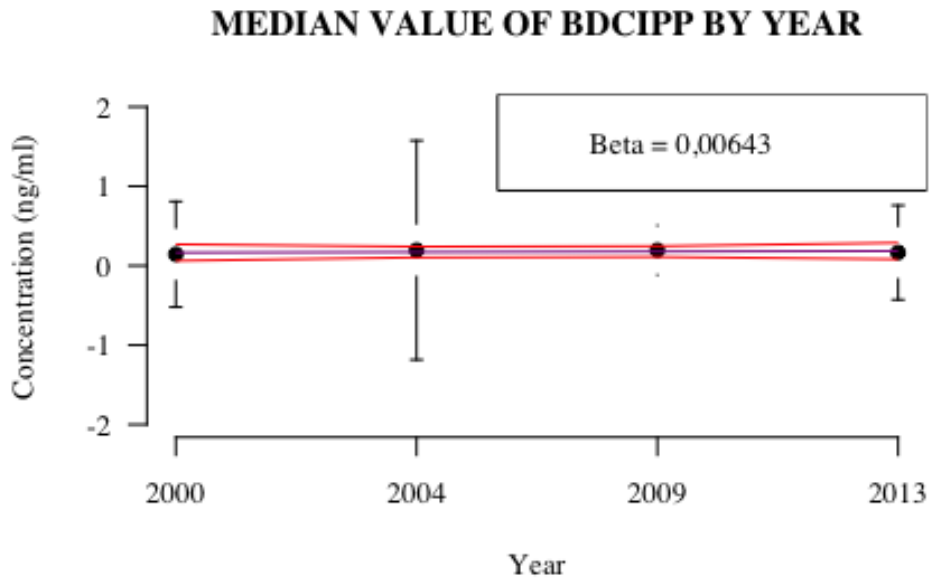


Figure 2. Linear regression of median values of density adjusted concentrations of BDCIPP.
Time trend analysis of median values of density adjusted concentrations of BDCIPP between different years of measurement. Bars represent the standard deviation, the purple line shows the slope of change over time and the Beta-value shows the regression coefficient. The red lines show the 95 % confidence interval for the slope. Significance is given as * if $p < 0.05$ and ** if $p < 0.01$.

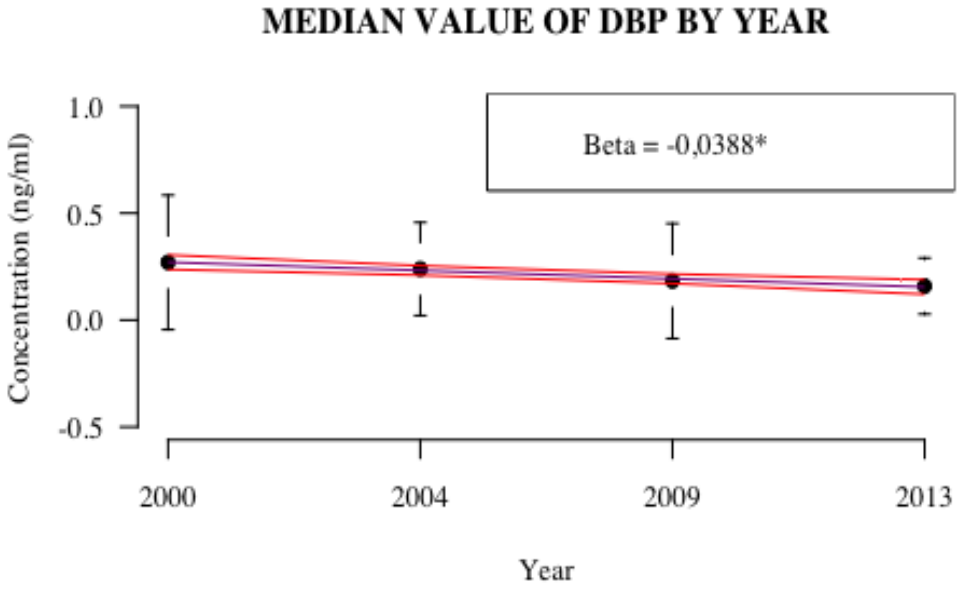


Figure 3. Linear regression of median values of density adjusted concentrations of DBP.
Time trend analysis of median values of density adjusted concentrations of DBP between different years of measurement. Bars represent the standard deviation, purple line is the slope of change over time and the Beta-value shows the regression coefficient. The red lines show the 95 % confidence interval of the slope. Significance is given as * if $p < 0.05$ and ** if $p < 0.01$.

3.3 Comparison of urinary concentrations

A few biomonitoring studies of urinary concentrations of the same biomarkers of exposure have been conducted in Norway, Germany and in the United States. The median values of measured non-adjusted concentrations of each study is summarised in Table 5.

Table 5. Comparison of median urinary PFR metabolite concentrations.
Measured concentrations in ng/ml (non-adjusted) from different studies of the general population. Abbreviations from each study for the detection limit of the method: LOD = limit of detection, DL = detection limit, MDL = minimum detection limit.

Country	Reference	BDCIPP	DPP	DBP	BBOEP
Sweden	*This study, all years (n = 801)*				
	% detect	86	100	85	28
	LOD (ng/ml)	0.05	0.03	0.1	0.03
	median (ng/ml)	0.22	1.6	0.25	< LOD
USA	*Dodson et al., 2014 (n = 16)*				
	% detect	94	62	56	12
	LOD (ng/ml)	0.02	0.23	0.08	0.34
	median (ng/ml)	0.09	0.44	0.11	NA
USA	*Meeker et al., 2013 (n = 45)*				
	% detect	91	96		
	DL (ng/ml)	0.033	0.056		
	median (ng/ml)	0.12	0.27		
USA	*Cooper et al., 2011 (n = 9)*				
	% detect	100	100		
	MDL (pg/ml)	8	204		
	median (ng/ml)	0.083	0.803		
Norway	*Cequier et al., 2015 (n = 54)*[1]				
	% detect	61	97	15	32
	MLD (ng/ml)	0.12	0.03	0.12	0.18
	median (ng/ml)	0.23	1.1	< MLD	< MLD
	Cequier et al., 2015 (n = 48)[2]				
	% detect	52	97	8	< 1
	MLD (ng/ml)	0.12	0.03	0.12	0.18
	median (ng/ml)	0.12	0.51	< MLD	< MLD
Germany	*Fromme et al., 2014 (n = 312)*				
	% detect		91	71	90
	LOD (ng/ml)		0.15	0.1	0,15
	median (ng/ml)		0.8	0.2	2.0

[1] *Urine samples from adults (mothers).*
[2] *Urine samples from children.*

4 Discussion

4.1 Method and Analysis

The four targeted metabolites of organophosphorus flame retardants, DBP, DPP, BBOEP and BDCIPP could be analysed with an estimated LOD between 0.03 and 0.1 ng/ml (Table 3) with excellent between run precisions with CVs in the range 4 – 9 %.

4.2 Comments on the results

The metabolite DPP was detected in concentrations above LOD in all samples from all years (Table 4) and also had the highest median and mean concentrations compared to the other biomarkers of exposure. Triphenyl phosphate has many applications and is used as a plasticiser, flame retardant, in hydraulic fluids and as a compound in several other products. It is imported to Sweden in the highest amounts of the four compounds mentioned (Appendix I).

The higher import levels and widespread use of triphenyl phosphate might be one reason for why it is detected in all samples in this study.

The biomarkers BDCIPP and DBP were also found in concentrations above LOD in the majority of samples with median and mean values between 0.2 and 0.5 ng/ml, e.g. above LOD but representing very low levels of exposure. BBOEP was only detected in 14 - 34 % of the samples and the median were values below LOD for all years (Table 4).

The median levels of DPP, DBP and BDCIPP seem to decline from year 2000 to year 2013 (Figure 1 – 3). For the biomarker DBP, there was a significant decreasing trend of the median (Figure 3), although only a decline of 0.038 ng/ml between the years of measurements. These four compounds are still being used, although the import of tributyl phosphate to Sweden has decreased during the time period of this study (Appendix I). The decreasing exposure levels could reflect the lower import volumes of pure DBP to Sweden.

Worth to mention is that the biomarker BDCIPP is detected in concentrations above LOD in 80-90 % of the samples, even though the organophosphate tris(1,3-dichloro-2-propyl) phosphate has not been imported as a substance to Sweden during the years of measurement according to the Swedish Chemicals Agency's product register. The measurable exposure levels are probably due to imported products and consumer goods with materials that already contain these compounds. The amounts in these products are not included in the product register of the Swedish Chemicals Agency.

Regarding exposure pathways, most studies conclude that the indoor environment is the main source of contamination (Cequier et al., 2014c). However, some studies have found that intake of food such as red meat, poultry and sometimes fish also may contribute to the exposure (Fraser et al., 2009). This might vary for different compounds, and in different countries, depending on how frequently they are used in packaging plastics (WHO, 2000). A recent study of residues of PFRs in food in Sweden has found measurable concentrations of TDCIPP and TPP in several different food categories (Poma et al., 2017). Even though the measured concentrations in house dust usually are much higher than in food, the intake volume of food is much higher than the inhalation of dust.

Fang & Stapleton (2014) states that PFRs seems to have a higher bioaccessibility than BFRs, which is increasing with decreasing particle size of the compounds. Therefore, exposure through house dust could result in higher measurable exposure levels for PFRs than BFRs even if they occur in the same levels in indoor dust. This also contributes to the importance of measuring the exposure levels in further studies.

A problem with previous studies of exposure and health effects is that they have not measured the exposure levels in human tissue or samples, only compared concentrations in indoor environments with endpoints such as sperm count and hormone levels (Meeker & Stapleton, 2010). The outcome can therefore not be directly linked or related to the exposure of these compounds (US EPA, 2015). Further research is needed to establish a connection between exposure of PFRs and mechanisms of health effects.

4.3 Comparison with other studies

The concentrations found in this study seem to be consistent with findings in other biomonitoring studies. The metabolites DPP and BDCIPP are detected most frequently in urine samples in the studies conducted in other countries as well (Table 5). The median concentration of DPP is higher in the measurements in this study compared to other studies (Table 5). All studies in the comparison found that the metabolite BBOEP occurred in the lowest concentrations and mainly below LOD.

Remarkable is that median levels of BDCIPP is higher in the studies from Sweden and Norway compared to the studies conducted in the U.S., even though this compound was not imported as a chemical substance in Sweden during the years of sample

collection (Appendix I). Products that contain this compound is still being imported but not included in the product register (Swedish Chemicals Agency, 2010).

In several other studies that have measured the concentrations of different PFRs in indoor environment, TBOEP is frequently detected (Fromme et al., 2014; Langer et al., 2016). It is also one of the PFRs found in the highest concentration in influent and effluent waters from wastewater treatment plants in Sweden (Marklund et al., 2005). The exposure levels of the TBOEP metabolite BBOEP are very low in urine samples of the study populations in all studies included in the comparison here (Table 5). According to WHO (2000) the main exposure for TBOEP in the general population is from food and drinking water and not from indoor or/and surrounding environments. Dodson et al. (2014) measured indoor house dust levels and urinary biomarkers of PFRs and found the same result with high levels of TBOEP in the indoor environment but very low or non-detectable concentrations in the urine samples. Dodson et al. (2014) suggested that the indoor concentrations reflects long-time exposure while the urinary concentrations are in a short-term perspective since the metabolites seem to have short half-lives. Since the urinary concentrations in general only represent the exposure during the last day it could reflect exposure from other microenvironments than their homes. It might also be possible that the uptake of TBOEP from surrounding environments is very low, but this theory needs to be further studied.

In general it's difficult to say where the main exposure comes from since all the suggested exposure sources are very diffuse. The population seems to be constantly exposed since the metabolites of the compounds TDCIPP, TPP and TBP are found in the majority of urine samples from the individuals in this study and the other studies in the comparison (Table 5). It is also known that these compounds are found in food and household products used on a daily basis. Further research is needed for the exposure pathways, uptake, mechanisms and possible health effects of organophosphorus flame retardants.

5 Conclusions

- The metabolites DPP, DBP and BDCIPP were found in concentrations above LOD in the majority of the urine samples from the population in this study.

- The metabolite DPP was detected in concentrations above LOD in all samples in this study.

- The metabolite BBOEP occurred in the lowest concentrations and was only detected above LOD in 14 - 34 % of the urine samples. Similar results have been found in other studies. In comparison to environmental measurements, TBOEP is one of the most abundant PFRs detected, even in sewage treatment plants in Sweden.

- The linear regression showed a significant declining trend for the exposure levels of DBP during the years of measurement. Compared to the import of TBP to Sweden the amounts are decreasing during the same years and might reflect the lower exposure.

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APPENDIX I

Table A. Import volumes of the four organophosphate flame retardants to Sweden year 2000 – 2014.
The imported volumes in tonnes per year for tris(1,3-dichloro-2-propyl) phosphate, triphenyl phosphate, tri(2-butyxoethyl) phosphate and tributyl phosphate. Statistics retrieved from Swedish Chemicals Agency's product register through KemI-stat at www.kemi.se.

CAS-nr	Kemiskt ämne	Benämning	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
13674-87-8	**2-Propanol, 1,3-dichloro-, phosphate**	**TDCIPP**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
115-86-6	**Phosphoric acid, triphenyl ester**	**TPP**	83,8	49	59,8	51,6	58,1	46,8	78,8	86,5	73,9	70	100	64,3	109	147,5	96,4
78-51-3	**Tri(2-butoxyetyl)fosfat**	**TBOEP**	65,8	62,3	71,5	85,4	86,8	95,4	102	81,1	78	46,1	33,8	33,4	32,6	23,4	18,8
126-73-8	**Tributylfosfat**	**TBP**	34,6	31,7	24	28,7	33,2	47,7	47,5	38,4	33,2	32,7	22,2	16,3	13,4	11,2	7,2