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Diverging temporal trends of human exposure to bisphenols and plastizisers, such as phthalates, caused by substitution of legacy EDCs?

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ABSTRACT

Phthalates and phenolic substances were investigated in urine samples from first-time mothers in Uppsala, Sweden, collected between 2009 and 2014. These substances have a comparably fast metabolism and urinary metabolites are predominantly analysed. The main aim was to investigate if measures to decrease production and use of certain phthalates and bisphenol A (BPA) have resulted in decreased human exposure, and to determine if exposures to replacement chemicals have increased. Temporal trends were evaluated for metabolites (n=13) of seven phthalates, a phthalate replacer, four different bisphenols, triclosan, one organophosphate-based flame retardant, and for two pesticides. The results showed downward trends of several phthalates which are in the process of being regulated and phased out. Concomitantly, an increasing trend was seen for a metabolite of the phthalate replacer Di-iso-nonylcyclohexane 1,2-dicarboxylate (DiNCH). Bisphenol A (BPA) showed a downward trend, whereas bisphenol F, identified as one of the substitutes for BPA, showed an increasing trend. The decreasing trend of triclosan is likely due to declining use within the EU. Temporal trend studies of urine samples make it possible to investigate human exposure to rapidly metabolised substances and study how measures taken to regulate and replace problematic chemicals affect human exposure.

1. Introduction

Urine samples can be used for evaluation of temporal trends of non-persistent, rapidly metabolised, contaminants excreted in urine, such as phthalates and bisphenols (Koch and Calafat, 2009; Johns et al., 2015). Many of these widely used industrial chemicals have been identified as potential endocrine disrupters (Gray et al., 2000; Nagao et al., 2001; Borch et al., 2006; Maffini et al., 2006; Lyche et al., 2009; Dann and Hontela, 2011), and there is currently a concern that human exposure to some of these chemicals is high enough to affect human health (Jönsson et al., 2005; Dann and Hontela, 2011; Braun et al., 2013; Chen et al., 2013; Rochester, 2013; Marie et al., 2015).

Phthalates are widely used in industrial and consumer products as plasticizers, solvents and additives, and are commonly found in the human environment (Net et al., 2015). Production and use of some phthalates are currently being phased out, among them di-2-ethylhexyl pthtalate (DEHP), di-n-butyl phthalate (DnBP), and butylbenzyl phthalate (BBzP) (Zota et al., 2014). In this process the old chemicals are substituted with new chemicals with similar function. Di-iso-nonylcyclohexane 1,2-dicarboxylate (DiNCH) was introduced in 2002 to replace DEHP and other phthalates in PVC (Gomez Ramos et al., 2016) as it has been considered to be less toxic (SCENIHR, 2015; ECHA, 2016).

Phenolic substances constitute a broad and heterogeneous group including different bisphenols used as monomers in production of plastic and the anti-bacterial agent triclosan (Soeborg et al., 2014; Rochester and Bolden, 2015). Some chemicals are metabolised to phenolic compounds in the body, for instance pesticides (Nolan et al., 1984; Heudorf and Angerer, 2001).

The aim of the present study was to investigate if measures to decrease production and use of some of these non-persistent chemicals have resulted in decreased human exposure, and to determine if exposure to replacement chemicals has increased. Temporal trends were evaluated for 13 phthalate metabolites of seven different phthalates, di-ethyl phthalate (DEP), DnBP, BBzP, DEHP, di-isononyl phthalate (DiNP), di-iso-decyl phthalate (DiDP) and di(2-propyl heptyl) phthalate (DPHP), one metabolite of DiNCH, four different bisphenols, bisphenol A (BPA), S (BPS) and F (4,4-BPF, 2,2-BPF) and triclosan, one metabolite of the organophosphate-based flame retardant and plasticizer tri-phenylphosphate (TTP), and two metabolites of

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the insecticides chloropyrifos and pyretroids, in urine sampled between 2009 and 2014.

2. Materials and methods

2.1. Recruitment and sampling

Urine samples were collected from primiparous women living in Uppsala County, Sweden, and included in the POPUP study (Persistent Organic Pollutants in Uppsala Primiparas) conducted by the Swedish National Food Agency. Mothers were randomly recruited among first-time mothers who were Swedish by birth and delivered at Uppsala University Hospital (Glynn et al., 2007). All women had to be Swedish by birth as previous studies have shown that levels of POPs varies if the women were born in a Nordic country or not (Glynn et al., 2007). For the rapidly metabolized chemicals in the present study, birth country is probably not of great importance although there may still be life-style differences that influence exposure. Morning spot urine samples were sampled by the mothers themselves in a glass bottle three weeks after delivery and the samples were thereafter biobanked at −20 °C. In total, 30 women were sampled every year between 2009 and 2014 and the participating rate was 52%. Data on age, weight, length, lifestyle, medical history, food habits etc. of the mothers were obtained from questionnaires (Table 1). For details about recruitment and collection of personal characteristics data see Glynn et al. (2007) and Lignell et al. (2009).

2.2. Analysis

In the present study urine metabolites of DEP: mono-ethyl phthalate (MEP); DnBP: mono-n-butyl phthalate (MnBP); BBzP: mono-benzyl phthalate (MBzP), and five metabolites of DEHP: mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxylhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), mono-2-ethyl-5-carboxypentyl phthalate (MECPP), mono-2-carboxymethyl-hexyl phthalate (MCMHP) were analyzed. Also three metabolites of DiNP were analyzed: mono-hydroxy-isononyl phthalate (MHiNP), mono-oxoisononyl phthalate (MOiNP), and mono-carboxy-isooctyl phthalate (MCiOP), as well as two metabolites of a mix of DiDP and DPHP: mono-carboxy-isononyl phthalate (MCiNP) and 6-hydroxy propylheptyl phthalate (MHiDP), and the DiNCH metabolite: 2–4-methyl-7-oxyooctyl-oxycarbonyl-cyclohexane carboxylic acid (MOiNCH). In total five phenols were analyzed: triclosan and four different bisphenols (BPA, BPS, 4,4-BPF, and 2,2-BPF). One metabolite of TPP: di-phenyl phosphate (DPP), and two metabolites of chloropyrifos and pyretroids: trichloropyridinol (TCP) and 3-phenoxybencoic acid (3-PBA) respectively, were analyzed.

Samples were analysed by a modified method for phthalate metabolites using liquid chromatography tandem mass spectromery (Bornehag et al., 2015). Briefly, urine were added with ammonium acetate and glucoronidase (E-coli) and thereafter incubated at 37°. Then a 50:50 (v:v) water and acetonitrile solution of labelled (^{3}H or ^{13}C) internal standards (IS) of all analysed compounds were added. The samples were analysed in duplicate and all samples were analysed in a randomised order. For quality control of the analyses, chemical blanks and in-house prepared quality control samples were analysed in all sample batches. The limit of detection (LOD) was defined as the concentration corresponding to a peak area ratio of three times the standard deviation of the chemical blanks and is shown in Table 2. Furthermore, the imprecisions of the method is shown in Table 2, reported as the coefficient of variation of the quality control sample. The laboratory at Lund University is a reference laboratory for analyses of urinary phthalate metabolites and bisphenol A in a European biomonitoring project (www.eu-hbm.info/cophes). Moreover, the laboratory participates in the Erlangen inter-laboratory comparison program for those compounds where this is possible. Density adjusted urine concentrations were calculated according to Carnerup et al. (2006).

2.3. Calculations and statistics

A total of 178 women were included in the data set. When urine concentrations were below LOD, reported concentrations were used where the blank concentrations were subtracted from the measured concentration of the sample. In the case were the results were zero or negative, the concentration were set to the lowest measured concen-

Table 1
Characteristics of the participating first-time mothers from Uppsala, Sweden sampled in 2009–2014 (n=178).

Variable		N	Mean	Median	Range
Age of the mother (year)		178	29.6	29.7	20–41
Pre-pregnancy body mass index (BMI, kg/m^2)		178	23.4	22.7	17–40
Weight gain during pregnancy (% of initial weight)		178	23.8	23.2	−5.9–49
Weight reduction from delivery to sampling (%)[a]		178	9.1	9.0	1.3–25
Variable		**N**	**%**		
Education	max 3–4 yr high school	33	19		
	−3 yr higher education	40	22		
	> 3 yr higher education	105	59		
Smoking[b]	Non-smoker	103	58		
	Former smoker	39	22		
	Smoker	36	20		
Season[c]	Winter	37	21		
	Spring	51	29		
	Summer	24	13		
	Autumn	66	37		

[a] Weight reduction minus birth weight of the child in % of weight just before delivery.
[b] Women who stopped smoking before pregnancy are considered to be former smokers. Women who smoked during pregnancy, even if they stopped smoking during the first or second month of pregnancy, are considered to be smokers.
[c] Time of the year for sampling. Winter=Dec–Feb; Spring=Mar–May; Summer=June–Aug; Autumn=Sept–Nov.

Table 2
Limit of detection (LOD) and the coefficient of variation (CV) for all substances.

Name	Abbreviation	LOD (ng/ml)	CV (%)
Monoethyl phthalate	MEP	0.3	10
Mono-n-butyl phthalate	MnBP	1.6	7
Monobenzyl phthalate	MBzP	0.2	7
Mono-(2-ethylhexyl) phthalate	MEHP	5.0	19
Mono-(2-ethyl-5-hydroxylhexyl) phthalate	MEHHP	0.1	11
Mono-(2-ethyl-5-oxohexyl) phthalate	MEOHP	0.2	7
Mono-(2-ethyl-5-carboxypentyl) phthalate	MECPP	0.07	5
Mono-(2-carboxymethyl-hexyl) phthalate	MCMHP	0.05	5
Monohydroxyisononyl phthalate	MHiNP	0.05	7
Monooxoisononylphthalate	MOiNP	0.05	4
Monocarboxyisooctyl phthalate	MCiOP	0.05	5
Monocarboxyisononyl phthalate	MCiNP	0.05	4
6-Hydroxypropylheptyl phthalate	MHiDP	0.08	9
2-4-Methyl-7-oxyooctyl-oxycarbonyl-cyclohexanecarboxylic acid	MOiNCH	0.08	6
Triclosan		0.10	9
Bisphenol A	BPA	0.22	3
Bisphenol S	BPS	0.03	4
4,4-Bisphenol F	4,4-BPF	0.03	6
2,2-Bisphenol F	2,2-BPF	0.01	3
Di-phenylphosphate	DPP	0.03	10
Trichloropyridinol	TCP	0.02	2
3-Phenoxybencoic acid	3-PBA	0.03	3

tration (< LOD) for each substances. The use of determined values below LOD will result in less statistical bias than if concentration below LOD are replaced with for instance zero, ½ LOD or LOD/√2, since the latter replacements will introduce a systematic error (RSC, 2001; Bergstrand and Karlsson, 2009).

Temporal trends were investigated for the study period 2009–2014. Multiple linear regressions (MINITAB 15® Statistical Software for Windows) were used to analyze associations between logarithmically-transformed and density-adjusted urine concentrations and sampling year. Logarithmically-transformed data were used since the distribution of data closely followed a log-normal distribution. Covariates in the multiple linear regression models were age of the women, pre-pregnancy body-mass-index (BMI), weight gain during pregnancy, weight loss from delivery to time of sampling, education, and season of sampling. These covariates were used as they were significantly associated with urine concentrations of one or more of the analyzed substances in univariate models. Smoking was not significantly associated with urine concentrations of any substance and was therefore not included as a covariate. Women who smoked during pregnancy were considered to be smokers even if they stopped smoking during the first or second month of pregnancy. The urine samples were taken 3 weeks after delivery when probably a very limited number of the women smoked. A sensitivity test was performed where observations with standardized residuals≥3 were excluded from analysis due to their large influence on the regression results. The statistical significance was set to $p \leq 0.05$. The associations between urine concentrations and sampling year are presented as percent change of concentration per year: % change=(1−exp(b)) x100, where b is the regression coefficient for the variable "sampling year".

3. Results

Urine concentrations of all analysed substances are presented as ng/ml and as density-adjusted concentrations in Table 3. MEP, MnBP, and MCiOP were found at highest concentration in urine and BPS, 3-PBA and 2,2-BPF at the lowest (Table 3).

3.1. Temporal trends

Significantly negative temporal trends were seen for MEP, MnBP, and MBzP, metabolites of DEP, DnBP, and BBzP, respectively, and four out of five metabolites of DEHP: MEHHP, MEOHP, MECPP, and MCMHP (Table 4). For the three metabolites to DiNP: MHiNP, MOiNP, and MCiOP no significant trend was observed (Table 4). Trends of the metabolites of the mix of DiDP and DPHP differed, with MCiNP being significantly negative but with no significant trend for MHiDP. MOiNCH, the metabolite of DiNCH, showed a significant increasing temporal trend during the study time (Table 4, Fig. 1a).

Significantly decreasing temporal trends were observed for urine concentrations of triclosan and BPA, during the study period (Table 4, Fig. 1b). During the same time 4,4-BPF showed an increasing temporal trend (Table 4, Fig. 1c). For 3-PBA a significantly increasing trend was observed when outliers were excluded in the sensitivity test (Table 4). No other statistically significant trends were seen.

4. Discussion

4.1. Concentrations in urine

Human exposure to phthalates and phenols occurs through food and drinking water ingestion, inhalation of air, and dermal contact with products containing these chemicals. Human exposure to chlorpyrifos and pyretroids is probably dominated by residues in food. The substances in the present study are metabolised fairly rapidly in the body and urine concentrations shows recent exposure from hours to days.

Comparisons with results from other studies are hampered by for instance differences in study populations, year of sampling, and the use of different analytical methods. Nevertheless, for phthalate metabolites the urine concentrations in the POPUP women were in the same range as in previous studies of Swedish women sampled during the same time period and analysed with the same method (Jönsson et al., 2014; Larsson et al., 2014). To our knowledge there are no published Swedish data for MCiNP, MHiDP and MOiNCH. In the present study, urine concentrations of DEHP metabolites and MEP were in the same range as from women in several European studies from 2011 to 2012,

Table 3
Concentrations of phthalates and DINCH metabolites, and phenolic substances in urine (ng/ml) from first-time mothers (n=178) in Uppsala sampled 2009–2014.

Substance	Parent compund	< LOD (%)	*Unadjusted*			*Density adjusted*		
			Mean	**Median**	**Range**	**Mean**	**Median**	**Range**
MEP	DEP	0	58.1	24.6	2.30–1374	55.0	24.3	3.34–1063
MnBP	DnBP	0	51.3	40.0	4.09–371	49.6	42.7	5.95–228
MBzP	BBzP	0	12.8	8.13	3.80–190	12.7	8.76	1.09–144
MEHP[a]	DEHP	96 (55)	5.76	< LOD	< LOD–32.2	6.70	< LOD	< LOD–49.4
MEHHP	DEHP	0	18.0	10.8	0.73–174	16.6	11.4	1.95–126
MEOHP	DEHP	0	12.7	7.45	0.78–148	11.9	7.76	1.42–80.1
MECPP	DEHP	0	14.3	8.66	0.76–164	13.5	8.94	1.45–95.6
MCMHP	DEHP	0	4.93	2.85	0.39–77.4	4.63	3.16	0.83–40.9
MHiNP	DiNP	0	20.8	5.26	0.14–395	18.4	5.98	0.45–395
MOiNP	DiNP	0	10.1	3.00	0.08–182	8.80	3.19	0.22–182
MCiOP	DiNP	0	30.8	12.0	0.44–382	29.7	12.0	1.26–382
MCiNP	DiDP and DPHP	2 (1)	1.51	0.58	< LOD–35.5	1.29	0.63	< LOD–21.9
MHiDP	DiDP and DPHP	1 (1)	7.17	1.39	< LOD–606	5.37	1.61	< LOD–359
MOiNCH	DiNCH	17 (10)	1.16	0.33	< LOD–42.2	1.13	0.37	< LOD–42.2
Triclosan		32 (18)	12.6	0.32	< LOD–732	12.2	0.32	< LOD–650
BPA		8 (4)	1.67	0.87	< LOD–15.9	1.57	1.02	< LOD–17.0
BPS		57 (32)	0.12	0.043	< LOD–1.38	0.11	0.048	< LOD–1.46
4,4-BPF		3 (2)	1.09	0.29	< LOD–24.3	1.20	0.32	< LOD–18.7
2,2-BPF		59 (33)	0.066	0.015	< LOD–2.66	0.064	0.017	< LOD–2.24
DPP	TPP	0	1.69	0.98	0.064–35.5	1.64	1.03	0.22–35.5
TCP	Chlorpyrifos	0	1.90	1.32	0.086–14.2	1.98	1.36	0.16–22.7
3-PBA	Pyretroids	2 (2)	0.35	0.22	< LOD–2.59	0.36	0.23	< LOD–3.80

[a] n=174.

Table 4
Annual change[a] (% per year and standard error (SE)) in concentrations of phthalates and DINCH metabolites, and phenolic substances in urine from first-time mothers in Uppsala, Sweden, 2009–2014 (n=178).

Substance	< LOD[c] (%)	*All samples*			*After Sensitivity test*[b]			
		Mean (SE)	p	R^2 (%)[d]	n	Mean (SE)	p	R^2 (%)[d]
MEP	0	−9.8 (4.0)	0.020	11	176	−11 (3.7)	0.006	14
MnBP	0	−14 (2.1)	< 0.001	24	175	−14 (1.9)	< 0.001	25
MBzP	0	−16 (3.1)	< 0.001	17	176	−16 (3.0)	< 0.001	18
MEHP[e]	96 (55)	0.93 (4.9)	0.85	10	169	−1.7 (3.9)	0.67	8
MEHHP	0	−16 (2.7)	< 0.001	18	176	−16 (2.6)	< 0.001	19
MEOHP	0	−17 (2.7)	< 0.001	20	177	−17 (2.6)	< 0.001	21
MECPP	0	−16 (2.7)	< 0.001	19	177	−16 (2.6)	< 0.001	20
MCMHP	0	−17 (2.4)	< 0.001	23	176	−16 (2.3)	< 0.001	22
MHiNP	0	−3.7 (5.8)	0.52	5	177	−4.2 (5.6)	0.45	6
MOiNP	0	−1.0 (5.4)	0.85	5	177	−1.5 (5.2)	0.77	6
MCiOP	0	−5.5 (5.0)	0.28	5	178	−5.5 (5.0)	0.28	5
MCiNP	2 (1)	−8.0 (4.4)	0.078	9	174	−9.6 (4.0)	0.020	8
MHiDP	1 (1)	−1.5 (5.1)	0.76	9	174	−2.6 (4.5)	0.56	5
MOiNCH	17 (10)	20 (6.7)	0.001	12	173	21 (5.9)	< 0.001	15
Triclosan	32 (18)	−24 (6.1)	0.001	10	170	−17 (4.7)	0.001	11
BPA	8 (4)	−9.8 (4.3)	0.029	11	173	−10 (3.2)	0.003	10
BPS	57 (32)	−0.80 (6.2)	0.90	6	177	−1.4 (6.0)	0.82	7
4,4-BPF	3 (2)	20 (7.6)	0.003	14	176	20 (7.2)	0.002	13
2,2-BPF	59 (33)	11 (7.2)	0.095	13	176	10 (6.7)	0.10	16
DPP	0	−2.9 (3.2)	0.36	9	175	−0.38 (2.9)	0.90	8
TCP	0	−4.0 (3.6)	0.27	6	176	−5.3 (3.3)	0.12	6
3-PBA	2 (2)	3.1 (3.6)	0.38	9	173	7.9 (3.1)	0.008	17

[a] Multiple regression analysis was used for evaluation of associations between density adjusted urine concentrations (ln-tranformed) and sampling year, with the covariates: maternal age, pre-pregnancy BMI, years of education, weight gain during pregnancy, weight loss after delivery, and sampling season.
[b] Results after sensitivity test with exclusion of outliers.
[c] For values below LOD, reported values were used.
[d] Coefficient of determination for the whole regression model.
[e] n=174.

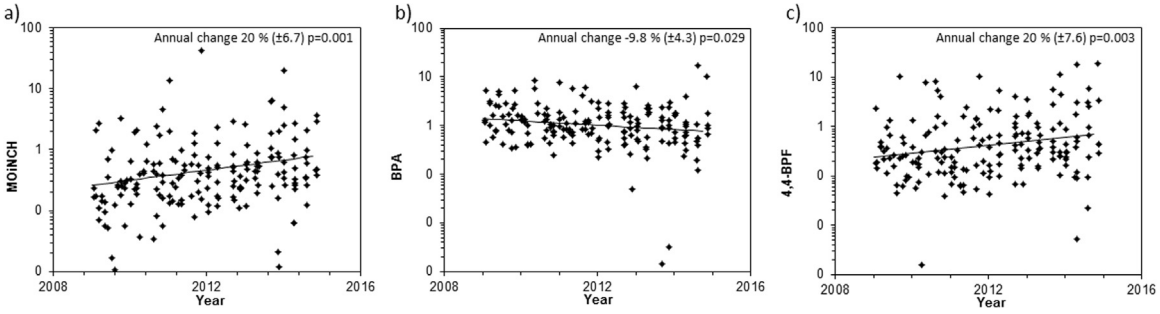

Fig. 1. Temporal trends of urine concentrations of a) MOiNCH, b) BPA, and c) 4,4-BPF (ng/ml) in first-time mothers from Uppsala, Sweden, during the time period 2009–2014. Multiple regression analysis was used for evaluation of associations between density adjusted urine concentrations (ln-tranformed) and sampling year, with the covariates: maternal age, pre-pregnancy BMI, years of education, weight gain during pregnancy, weight loss after delivery, and sampling season.

whereas mean MnBP concentrations were about two-times lower and MBzP about two-times higher (Den Hond et al., 2015), indicating region-specific differences in exposure. Urine concentrations of the metabolites of DiNP were about two to three-fold higher in the present study compared to previous studies in adults from Germany (1988–2008) and the U.S. (2005–2010) (Wittassek et al., 2007; Göen et al., 2011; Zota et al., 2014). Studies of urine concentrations of MCiNP, MHiDP and MOiNCH are scarce. Adults in the U.S. sampled 2005–2010 had higher urine concentrations of MCiNP than the POPUP women (Zota et al., 2014) and both MCiNP and MOiNCH concentrations were higher in Australian adults (2013) than in the present study (Gomez Ramos et al., 2016). Norweigan adults sampled 2013–2014 and German adults sampled in 2012 had urine concentrations of MOiNCH in the same range as the present study (Schutze et al., 2014; Giovanoulis et al., 2016).

Median concentrations of triclosan in the present study were 2.4-times lower compared to young Swedish women sampled in 2013 (Jönsson et al., 2014) but higher compared to a previous study of Swedish mothers sampled 2011–2012 (Larsson et al., 2014). Median concentrations of BPA were in the same range as in young Swedish women and about 1.3-times lower compared to Swedish mothers (Jönsson et al., 2014; Larsson et al., 2014). Concentrations of BPA in the present study were also lower compared to recent studies of 684 women in other European countries sampled 2008 and 2011–2012 (Covaci et al., 2015; Guidry et al., 2015). Studies of urine concentrations of BPS, 2,2-BPF4, and 4-BPF are scarce. BPS, 4,4-BPF, and 2,2-BPF have been detected at lower concentrations than in the present study in the U.S. and China (Yang et al., 2014; Zhou et al., 2014).

DPP is a metabolite of the organophosphate flame retardant and plasticizer TPP. Previous studies from Norwegian mothers (2012) and adults from the U.S. (2013) have shown urine concentrations of DPP in the same range as in the present study (Van den Eede et al., 2013; Cequier et al., 2015).

Concentrations of the metabolites of insecticides chlorpyrifos and pyretroids, TCP and 3-PBA were similar as in a previous studies Swedish women from 2013 (Jönsson et al., 2014). TCP concentrations were lower compared to pregnant Norweigan women (Ye et al., 2009) and higher compared to adults in the U. S. (Barr et al., 2005; Trunnelle

et al., 2014). 3-PBA concentrations were in the same range as in U. S. adults in the NHANES study 2001–2002 (Barr et al., 2010) but lower compared to a more recent study from California (Trunnelle et al., 2014).

4.2. Temporal trends

Negative temporal trends were observed for metabolites of DEP, DnBP, and BBzP, and four out of five metabolites of DEHP. For the DEHP metabolite MEHP no temporal trend was detected. For this metabolite 55% of the samples were below LOD and the estimation of the temporal trend is therefore highly uncertain. The mean decreases were estimated to range on average between 11% and 17% per year between 2009 and 2014, which corresponds to half-times of 4–6 years. This shows that efforts to phase out production and use of these phthalates in Europe have resulted in a significantly decreased human exposure in Sweden. There are restrictions on phthalates, in toys and childcare products on the EU market (regardless of manufacturing country) since 2006 and in food contact materials since 2011 (KEMI, 2014). The use of DEHP, DnBP and BBzP is banned in the EU since 2015 unless permission has been sought for specific purposes (KEMI, 2014), however there are no restrictions for imported goods.

MOiNCH, the metabolite of DiNCH, showed a significant increasing temporal trend during the study time. The mean yearly increase was 20%, corresponding to a doubling of exposure every 3.5 years. DiNCH was introduced in 2002 as a replacement to DEHP and other high-molecular weight phthalates in PVC (Gomez Ramos et al., 2016). In Sweden the usage of DiNCH has been estimated to have increased dramatically (47-fold) between 2011 and 2012 (KEMI, 2014), and our results show that this has resulted in significantly increased human exposure in Sweden. Between 2009 and 2014 the adjusted mean urine concentration increased from 0.2 to 0.7 ng/ml.

For the three metabolites of DiNP no significant trend was seen. Trends of the metabolites of the mix of DiDP and DPHP differed, with MCiNP being significantly negative but with no trend for MHiDP. In Europe DiNP, DiDP and DPHP are the most frequently used phthalates whereas in many other countries DEHP is the dominating compound (KEMI, 2014). Our results show that the human exposure to these phthalates in Sweden has not changed dramatically during the study period.

A few other studies have reported decreasing trends of metabolites of DEP, DnBP, BBxP, and DEHP during the last decade in adults from Sweden, Germany, and the U.S. (Wittassek et al., 2007; Göen et al., 2011; Jönsson et al., 2014; Zota et al., 2014), showing that the decreased exposure observed by us is an international phenomenon. The results for metabolites of DiNP in previous studies have shown a small increasing temporal trend in the beginning of the 21th century (Wittassek et al., 2007; Göen et al., 2011; Zota et al., 2014). In the present study temporal trends were studied at a later time period and no significant trends were seen for the three metabolites of DiNP. MCiNP showed a decreasing temporal trend in the present study (2009–2014) compared to an increase in U.S adults between 2005–2006 and 2009–2010 (Zota et al., 2014). German adults sampled between 1999 and 2012 had as in the present study an increasing trend for MOiNCH (DiNCH) (Schutze et al., 2014). To our knowledge this is the first temporal trend study of the metabolite MHiDP (DiDP and DPHP).

Significantly decreasing temporal trends were observed for urine concentrations of BPA during the period 2009–2014, with a half-life of 6.6 years. Interestingly 4,4-BPF showed an increasing temporal trend during the same time period, with a doubling in mean concentrations every 3.8 years. Declining temporal trends for BPA has also been seen in the U.S NHANES study between 2003 and 2012 (LaKind and Naiman, 2015) and also in young Swedish men between 2010 and 2013 (Jönsson et al., 2014). Reported urine concentrations below LOD were used in the statistical trends analysis the present study and these data are more uncertain than data with concentrations≥LOD. Significant declining trends of BPA were seen in the sensitivity analysis (exclusion of outliers) (Table 4) and also when all values below LOD were set to LOD/√2 (data not shown). BPA is a well-debated compound and as a result of consumer concern the industry have begun to remove BPA from their products (Rochester and Bolden, 2015). The use of BPA in baby bottles and cosmetics has been banned in the EU (EC, 2009, 2011). Other bisphenols like BPS, 4,4-BPF, and 2,2-BPF are now gradually replacing BPA (Rochester and Bolden, 2015) and our study show that exposure to at least 4,4-BPF is increasing. Toxicological data on BPS and BPF are scarce but there are studies suggesting that the effects might be similar to those of BPA (Eladak et al., 2015). To our knowledge this is the first study of temporal trends for BPS, 2,2-BPF, and 4,4-BPF.

In the present study decreasing urine concentrations of triclosan were found during the study period (Table 4) as also seen in previous studies from the U.S. (Mortensen et al., 2014; Han et al., 2016). The declining trend is probably because of declining use, as triclosan have been regulated during the last years. In the EU triclosan has been regulated in cosmetic products since 2014 (EC, 2014) and since 2016 triclosan was not allowed to be used as a biocide in all types of products (product type 1) except cosmetics (EC, 2016). The U.S Food and Drug Administration (FDU) have recently decided that triclosan in antiseptic wash products for consumer will not be allowed on the market from September 2017 (FDU, 2016).

Exposure to the flame retardant and plasticizer TTP did not change markedly between 2009 and 2012, since no significant temporal trend were shown for the metabolite. Similarly we observed no trend for the pesticide metabolite TCP. Urine concentrations of 3-PBA, the metabolite to pyretroids, had a significant increasing temporal trend after exclusion of outliers (Table 4). Thus, our data suggest that exposure to pyretroids have increased in Sweden between 2009 and 2015. To our knowledge no well-designed temporal trend studies have previously been published for 3-PBA, TCP or DPP. In adults in the U.S (NHANES) no differences were seen in 3-PBA concentrations between 1999–2000 and 2001–2002 (Barr et al., 2010).

4.3. Strengths and limitations

In the present study a single spot urine sample was taken from the participating women. The substances included in our study are rapidly metabolised and excreted into urine, and therefore individual concentrations of metabolites show both diurnal and weekly variation. Spot urine samples make it therefore more difficult to discover temporal trends. However, we could anyway observe temporal trends for some of the compounds mainly due to the facts that the first-time mothers from Uppsala is a homogenous population and the temporal trends were adjusted for several life-style/medical factors that could confound the results. Nevertheless, the use of spot samples and the relatively short study period also makes it possible that there are some trends that we could not detect.

The changes in exposure that we observed could be due to changes in life-style, for which we did not have data on, rather than changes in use and production by the industry. However, the present study had a relatively short study period of six years and it is not likely with dramatic life-style changes during such a short period. This study showed results from a population of young women in only one region and there might be regional differences in exposure. For example urine concentrations of MnBP och MBzP was higher in urine from adults living in the northern part of Sweden than among those living in the southern parts (Bjermo et al., 2013). Furthermore, differences in exposure have been observed between populations living on the countryside and in urban areas (Larsson et al., 2014).

5. Conclusion

Urine concentration of metabolites of phthalates, which are in the process of being phased out, such as DEP, DnBP, BBzP, and DEHP, showed declining temporal trends in first time mothers from Uppsala, Sweden, 2009–2014. During the same period the concentrations of the metabolite of the replacement plasticizer DiNCH increased. Also the much debated phenolic substances triclosan and BPA showed a decreasing temporal trend, while 4,4-BPF, a replacement for BPA, increased. Most likely, reduced production and use of certain endocrine-active chemicals have resulted in decreased human exposures. At the same time exposures to some of the replacement chemicals have increased, which may become a problem in the future if the increase continues. Our results show that policy decisions and other external pressure on the industry to phase-out certain problematic chemicals may drive chemical substitution in different directions depending on the availability of alternative chemicals. On one hand, the phase-out of potentially toxic phthalates, such as DEHP, DnBP and BBzP, have resulted in substitution with the apparently less toxic non-phthalate plasticizer DiNCH. On the other hand, replacement of the well-studied BPA with the less studied BPF, as BPA a possible EDC, may not be beneficial from a human health risk point-of-view. A more structured and scientifically sound policy, regulating the process of chemical substitution, is needed for a long-term sustainable chemical production and use world-wide. Moreover, even less problematic chemicals may be harmful to health if exposure is high enough. Therefore it is of vital importance to continue to follow the trends of human exposure to newly introduced chemicals.

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