NATIONAL ENVIRONMENTAL MONITORING COMMISSIONED BY THE SWEDISH EPA

Report to the Swedish EPA (the Health-Related Environmental Monitoring Program)

Concentrations of phthalate metabolites and phenolic substances in urine from first-time mothers in Uppsala, Sweden: temporal trends 2009-2014

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2016-04-29

NATIONELL
MILJÖÖVERVAKNING
PÅ UPPDRAG AV
NATURVÅRDSVERKET

ÄRENDENNUMMER NV-11641-11
AVTALSNUMMER 215 1214
PROGRAMOMRÅDE Hälsorelaterad MÖ
DELPROGRAM Biologiska mätdat
– organiska ämne

Concentrations of phthalates and phenolic substances in urine from first-time mothers in Uppsala, Sweden: temporal trends 2009-2014

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Rapporttitel Concentrations of phthalates and phenolic substances in urine from first-time mothers in Uppsala, Sweden: temporal trends 2009-2014	**Beställare** Naturvårdsverket 106 48 Stockholm **Finansiering** Nationell hälsorelaterad miljöövervakning
Nyckelord för plats Uppsala	
Nyckelord för ämne	
Tidpunkt för insamling av underlagsdata 2009-2014	

Sammanfattning

POPUP studien startade 1996, där blod- och modersmjölksprover regelbundet samlas in från förstföderskor i Uppsala. Sedan 2009 tas också ett urinprov från kvinnorna. I denna studie har ftalater och fenolära ämnen studerats i urinprov insamlade 2009-2014. Dessa ämnen metaboliseras relativt snabbt i kroppen och för flertalet är det en metabolit till själva huvudsubstansen som har analyserats i urinen.

Totalt sett analyserades 13 ftalatmetaboliter till 6 ftalater, en metabolit till en ersättningskemikalie till ftalater, en metabolit till ett fosforbaserat flamskyddsmedel, två pesticidmetaboliter samt 15 fenolära ämnen, t ex triklosan, bisfenol A, S och F, av Lunds universitet. Syftet var att studera tidstrender för de olika ämnena under perioden 2009-2014.

Resultaten visade att flera av de äldre ftalaterna som håller på att fasas ut har en nedåtgående trend. Samtidigt kunde en ökande trend ses för en metabolit till ett ämne som nu används som ersättare till ftalater. Bisfenol A (BPA) som är ett mycket omdiskuterat ämne visade en nedåtgående tidstrend medan bisfenol F som pekats ut som en av ersättarna till BPA istället hade en ökande trend. Även triclosan visade en minskande trend som troligtvis beror på att ämnet har minskat i användning.

Analyser av urin gör det möjligt att studera hur befolkningens exponering för snabbmetaboliserande substanser ser ut. Hur exponeringen förändras med tiden efter att olika åtgärder har satts in för att begränsa problematiska kemikalier samt hur befolkningens exponering för nya ersättningskemikalier har utvecklats.

INTRODUCTION

The Swedish National Food Agency (NFA) has made recurrent sampling of primiparous women in Uppsala since 1996 (POPUP, Persistent Organic Pollutants in Uppsala Primiparas), funded by the Swedish Environmental Protection Agency (EPA) since year 2000. The main aim of the study is to investigate temporal trends of exposure to persistent organic pollutants (POP) among pregnant and nursing women. Since 2009 also urine samples are collected from the women in POPUP at three weeks after delivery, for evaluation of temporal trends of less persistent, rapidly metabolized contaminants excreted in urine, such as phthalates and phenolic compounds as bisphenols. Many of these chemicals have been identified as potential endocrine disrupting chemicals (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 exposures to some of these chemicals are 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).

Phtalates are widely used in industrial and consumer products as plasticizers, solvents and additives, and are commonly found in the human environment. Production and use of some phthalates are currently being phased out, among them di-ethylhexyl pthtalate (DEHP), di-n-butyl phthalate (DNBP), and butylbenzyl phthalate (BBzP). 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 high molecular weight phthalates in PVC (Gomez Ramos et al. 2016).

Phenolic substances are a heterogeneous group including different bisphenols used as monomers in production of plastic, the anti-bacterial agent triclosan, degradation products of phenol ethoxylates (surfactants), brominated and chlorinated phenols used as flame retardants and wood preservatives, and the antioxidants and preservatives butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Some chemicals are metabolized to phenolic compounds in the body, for instance pesticides and the contaminants polycyclic aromatic hydrocarbons (PAH).

The following report presents results of analysis of temporal trends of 13 phthalate metabolites from 6 different phthalates, 1 metabolite to a chemical replacing phthalates, 1 metabolite to an organophosphate-based flame retardant, 2 pesticide metabolites and 15 different phenolic substance in urine sampled between 2009 and 2014. The aim is to investigate if measures to decrease production and use of some of the chemicals have resulted in decreased human exposure, and to determine if exposures to replacement chemicals have increased.

MATERIALS AND METHODS

Recruitment and sampling

Mothers were randomly recruited among first-time mothers who were Swedish by birth and delivered at Uppsala University Hospital. In total, 30 women were sampled every year between 2009 and 2014 and the participating rate was 52 %. A midwife visited the participating mothers three weeks after delivery collecting spot urine samples. Data on age, weight, length, lifestyle, medical history, food habits etc. of the mothers were obtained from questionnaires (Table 1).

Table 1. Characteristics of the mothers 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		
	1-3 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.

Analysis

In the present study urine metabolites of di-ethyl phthalate (DEP): monoethyl phthalate (MEP); DnBP: mono-n-butyl phthalate (MnBP); BBzP: monobenzyl 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 analysed. Also three metabolites of the di-iso-nonyl phthalate DiNP: mono-hydroxyisononyl phthalate (MHiNP), monooxoisononyl phthalate (MOiNP), and mono carboxyisooctyl phthalate (MCiOP), two metabolites of a mix of DiDP and DPHP: monocarboxyisononyl phthalate (MCiNP) and 6-hydroxy propylheptyl phthalate (MHiDP), and the DiNCH metabolite: 2-4-methyl-7-oxyooctyl-oxycarbonyl-cyclohexanecarboxylic acid (MOiNCH). One metabolite of the organophosphate-based flame retardant tri-phenylphosphate (TPP): di-phenylphosphate (DPP), and two metabolites to the insecticides chloropyrifos and pyretroids: respective trichloropyridinol (TCP) and 3-phenoxybencoic acid (3-PBA), were analysed. In total 15 phenolic substances were analysed including, seven different alkyl phenols: triclosan (TCS), four different bisphenols (BPA, BPS, 4,4-BPF, and 2,2-BPF), 4-tert-butylphenol (4-t-BP), and 4-tert-octylphenol (tOP), two metabolites 2-OH-phenantrene (2-OH-PH) and 1-hydroxypyren (1-HP) used as biomarkers for PAH exposure, two food additives used as antioxidants 3-tert-butyl-4-hydroxyanisole (BHA) and 2,6-di-tert-butyl-4-methylphenol (BHT), three brominated phenols 4-bromophenol (4-BP), 2,4-dibromophenol (2,4-DBP), 2,4,6-tribromophenol (2,4,6-TBP), and pentachlorophenol (PCP).

These samples were analysed by a modified method for phthalate metabolites (Bornehag et al. 2015). Briefly, urine were added with ammonium acetate (pH 6.5) and glucoronidase (E-coli) and thereafter incubated at 37°C in 30 min. 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, with the exception of BHA, PCP, bromophenol and butylphenol. A C18 column was used prior to the injector to reduce the interferences of contaminants in the mobile phase. The phthalate metabolites in the samples were separated on a C18 column. The mobile phases were water and acetonitrile with 0.08% formic acid or water methanol with 0.1% ammonia. The samples were analysed on a Shimadzu UFLC system (Shimadzu Corporation, Kyoto, Japan) coupled to a QTRAP5500 triple quadrupole linear ion trap mass spectrometer equipped with a TurboIon Spray source (LC-MS/MS; AB Sciex, Foster City, CA, USA). The samples with individual IS, were analysed in duplicate and all samples were analysed in a randomized 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 method is not validated for BHA, BHT, PCP, 4-t-BP, 4-BP, 2,3-DBP, 2,4,6,- DBP, and tOP and therefore the coefficient of variation is not reported in Table 2 for these substances. 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. Urine concentrations adjusted to urine density were calculated according to Carnerup et al. (2006).

Calculations and statistics

A total of 178 women were included in the data set. When urine concentrations were below LOD, reported concentrations were used, except for BHA, PCP, TBP, and 4-BP where LOD/√2 were used.

Temporal trends were investigated for the study period 2009-2014. Multiple linear regressions (MINITAB 15® Statistical Software for Windows) were used to analyse associations between logarithmically transformed and density adjusted urine concentrations and sampling year. Covariates in the multiple linear regression analyses 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. Smoking was not significantly associated with urine concentrations of any substance and was therefore excluded. A sensitivity test was performed where observations with standardized residuals ≥3 were excluded from analysis.

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)oxy) carbonyl)cyclohexanecarboxylic acid	MOiNCH	0.08	6
Di-phenylphosphate	DPP	0.03	10
Triclosan	TCS	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
2-OH-phenantrene	2-OH-PH	0.005	4
1-Hydroxypyren	1-HP	0.02	6
Trichloropyridinol	TCP	0.02	2
3-Phenoxybencoic acid	3-PBA	0.03	3
3-tert-Butyl-4-hydroxyanisole	BHA	0.30	*
Pentachlorophenol	PCP	0.30	*
4-*tert*-Butylphenol	4-t-BP	5.0	*
4-Bromophenol	4-BP	0.30	*
2,4-Dibromophenol	2,4-DBP	>20	*
2,4,6-Tribromophenol	2,4,6-TBP	0.5	*
2,6-Di-tert-butyl-4-methylphenol	BHT	>20	*
4-tert-Octylphenol	tOP	1.0	*

*Not validated

RESULTS AND DISCUSSION

Urine concentrations of all analysed substances are presented in ng/ml in Table 3 and detectable substances are also presented as density adjusted concentrations in Table 4.

Urine phthalate concentrations

Almost all samples had phthalate metabolite concentrations above LOD except for MEHP for which 96 out of 178 samples were below a relatively high LOD of 5.0 ng/ml. For MCiNP, MHiDP, and MOiNCH, respectively, 2, 1, and 17 out of 178 samples were below LOD (Table 3). MnBP were found at the highest concentrations, followed by MEP, MCiOP, and MEHHP (Table 3 and 4). The urine concentrations in the POPUP women are in the same range as in previous studies of Swedish women (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 were in the same range as European mothers, whereas MEP and MNBP were lower and MBzP higher (Den Hond et al. 2015).

Urine concentrations of phenolic substances

All urine samples had detectable concentrations of DPP, 2-OH-PH and TCP. For BPA, 4,4-BPF, 1-HP, and 3-BPA almost all samples were over LOD, 170, 175, 164, and 175 out of 178 samples respectively. For TCS, BPS, 2,2-BPF, and BHA, respectively, 146, 121, 119, and 136 were above LOD. Only a few samples (8-15) were above LOD for PCP, 4-t-BP, and 4-BP and none for 2,4-DBP, 2,4,6-TBP, BHT and tOP (Table 3).

DPP is a metabolite to the organophosphate TPP. TPP is used as flame retardants, plasticizers and lubricants in a large variety of products (van der Veen and de Boer 2012). Previous studies from Norwegian mothers and adults from the U.S 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).

Urine concentrations of BPA and TCS have been analysed in several countries (Calafat et al. 2008, Frederiksen et al. 2013, Larsson et al. 2014, Moos et al. 2014, LaKind and Naiman 2015). Median concentrations of BPA and TCS were lower in the present study compared to previous studies of Swedish women (Jönsson et al. 2014, Larsson et al. 2014). Concentrations of BPA in the present study were also lower compared to six other European countries

(Covaci et al. 2015). Studies of urine concentrations of BPS, 2,2-BPF4,4-BPFand tOP are scarce. BPS, 4,4-BPF, and 2,2-BPF have been detected at lower concentrations than the present study in the U.S. and China (Yang et al. 2014, Zhou et al. 2014).

PAH are formed and emitted into the environment as a result of incomplete combustion of organic materials from natural and human activities. PAH are of public health concern as it is known to be carcinogenic (Bolm-Audorff 1996) and are ubiquitous in outdoor and indoor air and in smoked food. Both PAH metabolites 1-HP and 2-OH-PH were detected in almost all samples. 1-HP concentrations were in the same range as previous studies of young Swedish women (Jönsson et al. 2014) where 2-OH-PH were not analysed. 2-OH-PH were in the same range while 1-HP was possibly a bit lower compared to the general population in the United Kingdom (Aquilina et al. 2010) and both 1-HP and 2-OH-PH were much lower compared to NHANES females in the U.S. (Li et al. 2008).

Concentrations of the metabolites of insecticides chloropyrifos and pyretroids, TCP and 3-PBS were similar as in previous studies of Swedish women (Jönsson et al. 2014). Human exposure to these insecticides is probably from residues in food.

BHA is an antioxidant used as a preservative in for example food, food packaging, and cosmetics. BHA have been detected in low concentrations in the environment (Rosqvist 2004), however to our knowledge no data are available on human tissue concentrations.

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

Substance	Parent compund	Mean	SD	Median	Range	% <LOD
MEP	DEP	58.1	133	24.6	2.30-1374	0
MnBP	DnBP	51.3	46.1	40.0	4.09-371	0
MBzP	BBzP	12.8	18.0	8.13	3.80-190	0
MEHP[a]	DEHP	5.76	5.11	4.57[b] (<LOD)	0.069[b](<LOD)-32.2	55
MEHHP	DEHP	18.0	25.0	10.8	0.73-174	0
MEOHP	DEHP	12.7	18.1	7.45	0.78-148	0
MECPP	DEHP	14.3	19.6	8.66	0.76-164	0
MCMHP	DEHP	4.93	7.19	2.85	0.39-77.4	0
MHiNP	DiNP	20.8	52.1	5.26	0.14-395	0
MOiNP	DiNP	10.1	24.6	3.00	0.08-182	0
MCiOP	DiNP	30.8	56.4	12.0	0.44-382	0
MCiNP	DiDP and DPHP	1.51	3.75	0.58	0.005[b](<LOD)-35.5	1
MHiDP	DiDP and DPHP	7.17	45.9	1.39	0.01[b](<LOD)-606	1
MOiNCH	DiNCH	1.16	3.75	0.33	0.004[b](<LOD)-42.2	10
DPP	TPP	1.69	3.09	0.98	0.064-35.5	0
TCS		12.6	74.2	0.32	0.01[b](<LOD)-732	18
BPA		1.67	2.39	0.87	0.001[b](<LOD)-15.9	4
BPS		0.12	0.20	0.043	0.0003[b](<LOD)-1.38	32
4,4-BPF		1.09	2.51	0.29	0.001[b](<LOD)-24.3	2
2,2-BPF		0.066	0.25	0.015	0.0001[b](<LOD)-2.66	33
2-OH-PH	Phenanthrene	0.20	0.25	0.13	0.011-2.40	0
1-HP	Pyrene	0.094	0.087	0.072	0.0001[b](<LOD)-0.51	8
TCP	Chlorpyrifos	1.90	2.18	1.32	0.086[b](<LOD)-14.2	0
3-PBA	Pyretroids	0.35	0.38	0.22	0.002[b](<LOD)-2.59	2
BHA		2.00	3.76	0.64	0.17[b](<LOD)-26.0	24
PCP					<LOD-2.78	92
4-t-BP					<LOD-44.3	92
4-BP					<LOD-0.89	96
2,4-DBP						100
2,4,6-TBP						100
BHT						100
tOP						100

[a]n = 174
[b]Reported concentration below LOD

Table 4. Density-adjusted concentrations of phthalate metabolites and phenolic substances in urine (ng/ml) from first-time mothers (n=178) in Uppsala sampled 2009-2014.

Substance	Parent compound	Mean	SD	Median	Range	% <LOD
MEP	DEP	55.0	110	24.3	3.34-1063	0
MnBP	DnBP	49.6	32.3	42.7	5.95-228	0
MBzP	BBzP	12.7	15.9	8.76	1.09-144	0
MEHP[a]	DEHP	6.70	6.83	4.82[b] (<LOD)	0.038[b](<LOD)-49.4	55
MEHHP	DEHP	16.6	18.2	11.4	1.95-126	0
MEOHP	DEHP	11.9	13.6	7.76	1.42-80.1	0
MECPP	DEHP	13.5	15.1	8.94	1.45-95.6	0
MCMHP	DEHP	4.63	5.31	3.16	0.83-40.9	0
MHiNP	DiNP	18.4	42.4	5.98	0.45-395	0
MOiNP	DiNP	8.80	19.3	3.19	0.22-182	0
MCiOP	DiNP	29.7	50.1	12.0	1.26-382	0
MCiNP	DiDP and DPHP	1.29	2.45	0.63	0.015[b](<LOD)-21.9	1
MHiDP	DiDP and DPHP	5.37	27.5	1.61	0.04[b](<LOD)-359	1
MOiNCH	DiNCH	1.13	3.70	0.37	0.011[b](<LOD)-42.2	10
DPP	TPP	1.64	2.92	1.03	0.22-35.5	0
TCS		12.2	72.3	0.32	0.005[b](<LOD)-650	18
BPA		1.57	1.93	1.02	0.001[b](<LOD)-17.0	4
BPS		0.11	0.19	0.048	0.0007[b](<LOD)-1.46	32
4,4-BPF		1.20	2.63	0.32	0.002[b](<LOD)-18.7	2
2,2-BPF		0.064	0.22	0.017	0.0001[b](<LOD)-2.24	33
2-OH-PH	Phenanthrene	0.18	0.17	0.14	0.030-1.47	0
1-HP	Pyrene	0.089	0.064	0.070	0.0001[b](<LOD)-0.30	8
TCP	Chlorpyrifos	1.98	2.57	1.36	0.16-22.7	0
3-PBA	Pyretroids	0.36	0.45	0.23	0.007[b](<LOD)-3.80	2
BHA		2.23	5.55	0.68	0.14[b](<LOD)-56.8	24

[a]n = 174
[b]Reported concentration below LOD

Temporal trends

Interestingly, negative temporal trends were seen for MEP, MnBP, and MBzP, the metabolites of DEP, DnBP, and BBzP, respectively, and four out of five metabolites of DEHP: MEHHP, MEOHP, MECPP, and MCMHP (Table 5). This shows that efforts to phase out production and use of these phthalates in Europe have resulted in decreased human exposure in Sweden. The usage 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.

For the three metabolites to DiNP: MHiNP, MOiNP, and MCiOP no significant trend was seen (Table 5). 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. MOiNCH, the metabolite to DiNCH, showed a significant increasing temporal trend during the study time (Table 5, Figure 1). 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 increased 47 times between 2011 and 2012 (KEMI 2014), and our results strongly suggests that this has resulted in increased human exposure.

A few other studies have showed decreasing trends of metabolites of DEP, DnBP, BBxP, and DEHP (Wittassek et al. 2007, Göen et al. 2011, Jönsson et al. 2014, Zota et al. 2014). The results for metabolites to DiNP in previous studies are inconsistent with both increasing and decreasing temporal trend (Wittassek et al. 2007, Göen et al. 2011, Zota et al. 2014). In the present study no significant trends were seen for the three metabolites of DiNP. To our knowledge this is the first temporal trend study of metabolites of the phthalates DiDP and DPHP, MCiNP and MHiDP, and the DiNCH metabolite MOiNCH.

Significantly decreasing temporal trends were seen for urine concentrations of TCS, BPA, and 2-OH-PH during the period 2009-2014 (Table 5 and Figure 2). Interestingly 4,4-BPF is showing an increasing temporal trend during the same time period (Table 5, Figure 3). Urine concentrations of the metabolite to the insecticides pyretroids, 3-PBA has also a significant increasing temporal trend. No other statistically significant trends were seen (Table 5).

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). As reported urine concentrations below LOD were used in the present study some BPA concentrations were very low (Figure 1) which might have influenced the temporal trend. However significant declining trends were seen in the sensitivity analysis (exclusion of outliers) (Table 5) and also when all values below LOD were set to LOD/$\sqrt{2}$ (data not shown). The use of BPA in baby bottles and cosmetics has been banned in the EU and the occurrence of “BPA-free” consumer products have increased during the last years. Other bisphenols like BPS, 4,4-BPF, and 2,2-BPF are now gradually replacing BPA 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 pointing out 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 contrast to our results, increasing temporal trends was seen in U.S. children during 2001-2012 for 1-HP whereas no trends were seen for 2-OH-PH (Jung et al. 2014). 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). To our knowledge no temporal trend studies have been published for 3-PBA. Having this background, it is interesting to note that our data suggest that exposure to pyretroids have increased in Sweden between 2009 and 2015.

Table 5. Regression coefficients (standard error) for the associations between density adjusted urine concentrations (ln-tranformed) and sampling year in first time mothers from Uppsala sampled 2009-2014 (n=178). Multiple regression analysis was used with the covariates: maternal age, pre-pregnancy BMI, years of education, weight gain during pregnancy, weight loss after delivery, and sampling season. Results are shown with all samples, after sensitivity test (exclusion of outliers), and without samples under LOD.

	All samples		*After sensitivity test*				
Substance	**β**	**p**	**n**	**β**	**SE**	**p**	**<LOD[a]**
MEP	-0.10	0.020	176	-0.11	0.041	0.006	0
MnBP	-0.15	<0.001	175	-0.15	0.022	<0.001	0
MBzP	-0.18	<0.001	176	-0.17	0.035	<0.001	0
MEHP[b]	0.0092	0.85	169	-0.017	0.039	0.67	96
MEHHP	-0.18	<0.001	176	-0.17	0.030	<0.001	0
MEOHP	-0.19	<0.001	177	-0.19	0.031	<0.001	0
MECPP	-0.17	<0.001	177	-0.17	0.030	<0.001	0
MCMHP	-0.19	<0.001	176	-0.17	0.027	<0.001	0
MHiNP	-0.038	0.52	177	-0.043	0.057	0.45	0
MOiNP	-0.010	0.85	177	-0.015	0.052	0.77	0
MCiOP	-0.057	0.28	178	-0.057	0.052	0.28	0
MCiNP	-0.083	0.078	174	-0.10	0.043	0.020	2
MHiDP	-0.015	0.76	174	-0.026	0.044	0.56	1
MOiNCH	0.18	0.001	173	0.19	0.048	<0.001	17
DPP	-0.030	0.36	175	-0.0038	0.029	0.90	0
TCS	-0.27	0.001	170	-0.18	0.055	0.001	32
BPA	-0.10	0.029	173	-0.11	0.035	0.003	8
BPS	-0.0080	0.90	177	-0.014	0.059	0.82	57
4,4-BPF	0.18	0.003	176	0.18	0.059	0.002	3
2,2-BPF	0.11	0.095	176	0.097	0.059	0.10	59
2-OH-PH	-0.056	0.054	176	-0.076	0.027	0.006	0
1-HP	-0.017	0.68	175	0.019	0.032	0.56	14
TCP	-0.041	0.27	176	-0.054	0.034	0.12	0
3-PBA	0.030	0.38	173	0.076	0.028	0.008	3
BHA	0.081	0.12	175	0.089	0.047	0.060	42

[a]For values below LOD, reported values were used, except for BHA.
[b]n = 174

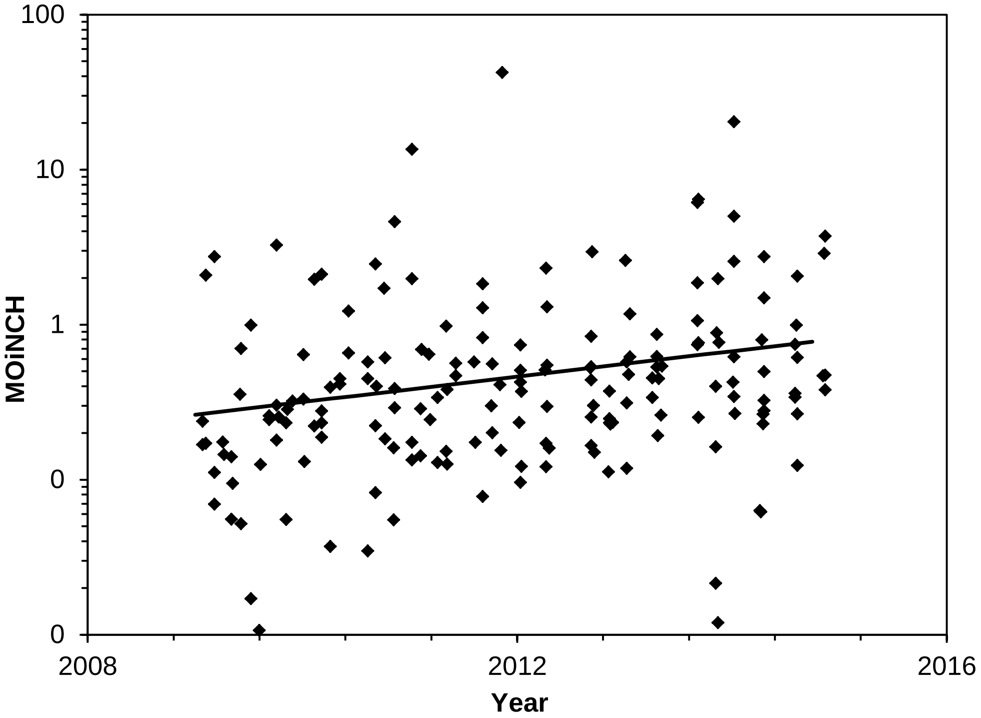

Figure 1. Temporal trend of urine concentrations of MOiNCH (ng/ml), a metabolite to DiNCH, in first-time mothers from Uppsala, Sweden, during the time period 2009-2014.

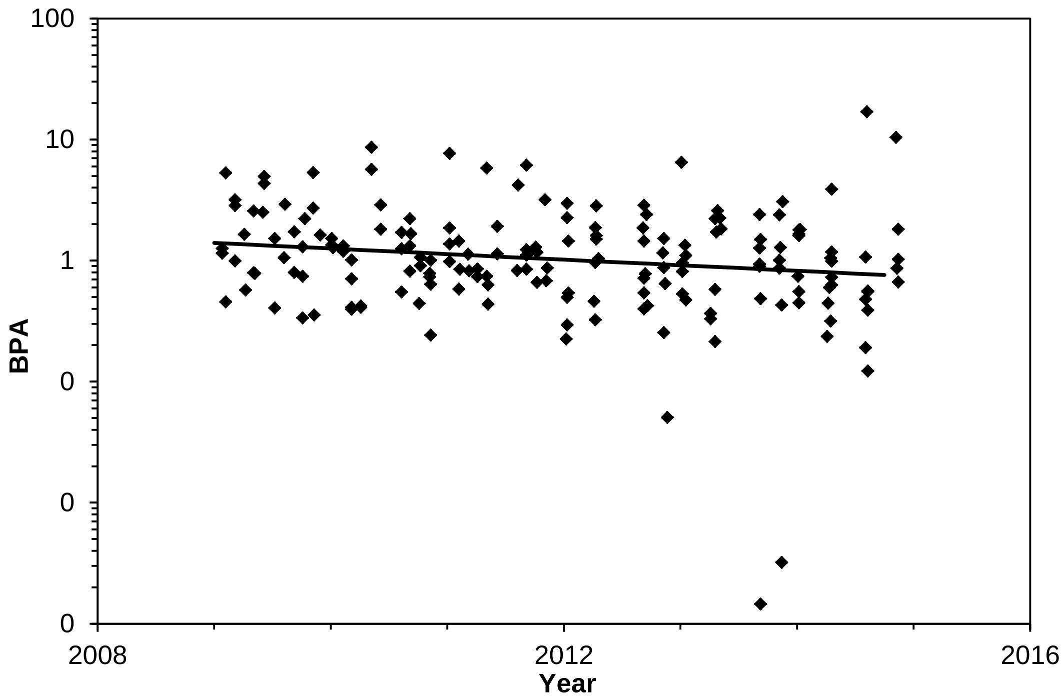

Figure 2. Temporal trend of urine concentrations of BPA (ng/ml) in first-time mothers from Uppsala, Sweden, during the time period 2009-2014.

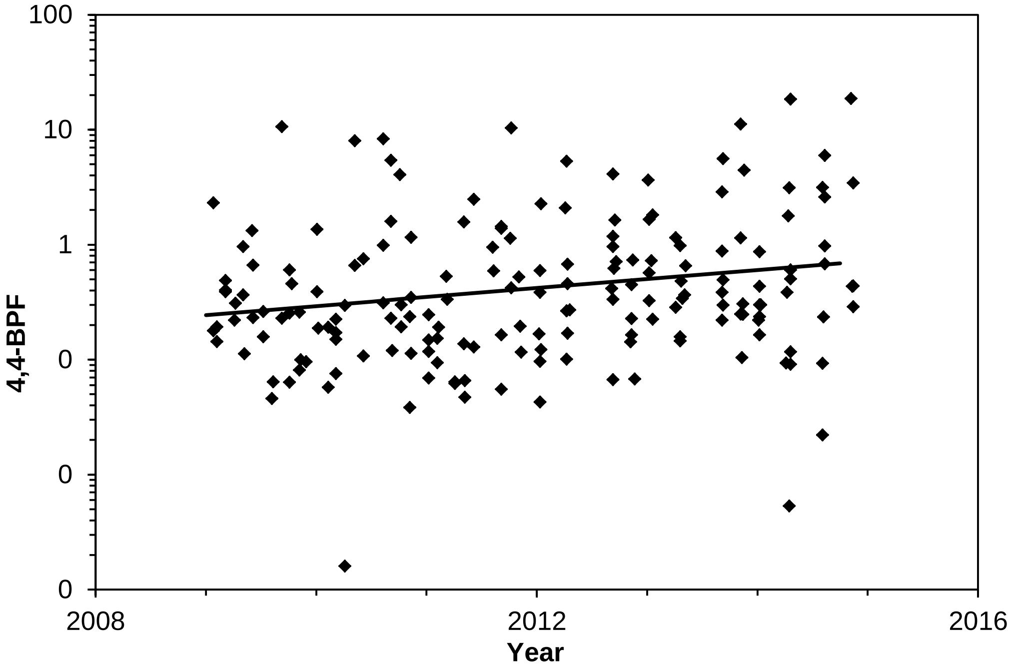

Figure 3. Temporal trend of urine concentrations of the bisphenol 4,4-BPF (ng/ml) in first-time mothers from Uppsala, Sweden, during the time period 2009-2014.

CONCLUSION

Urine concentration of metabolites of phthalates in the process of being phased out, such as DEP, DnBP, BBzP, and DEHP, showed declining temporal trends in first time mothers from Uppsala, 2009-2012. During the same period the trend for the metabolite to the replacement DiNCH was increasing. Also the debated phenol substances TCS and BPA had an decreasing temporal trend while 4,4-BPF, a replacement for BPA, was increasing. We can conclude that reduced use of these substances that could possibly affect human health have resulted in decreased exposure to Swedish humans. At the same time exposures to some replacement chemicals seem to have increased, which may become a problem in the future if the increase continues.

ACKNOWLEDGEMENT

We thank the Swedish EPA (Environmental Protection Agency) for financial support. Appreciation is expressed to the participating women and to Marianne Leimar who assisted in recruitment, interviewing, and sample collection. The laboratory technicians Ingalill Gadhasson and Åsa Amilon are appreciated for technical assistance.

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