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ARTICLE

Temporal trends of phthalate exposures during 2007–2010 in Swedish pregnant women

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Abstract

Background The general population is exposed to phthalates, a group of chemicals with strong evidence for endocrine disrupting properties, commonly used in a large number of consumer products. Based on published research and evidence compiled by environmental agencies, certain phthalate applications and products have become restricted, leading to an increasing number of "new generation compounds" coming onto the market during recent years replacing older phthalates. Some examples of such newer compounds are di-iso-nonyl phthalate (DiNP), di-iso-decyl phthalate (DiDP), and most recently di-isononyl-cyclohexane-1,2-dicarboxylate (DiNCH).

Objectives In order to evaluate temporal trends in phthalate exposure, first trimester urinary biomarkers of phthalates were measured in the Swedish SELMA study over a period of 2.5 years (2007–2010).

Methods We collected first morning void urine samples around week 10 of pregnancy from 1651 pregnant women. Spot samples were analyzed for 13 phthalate metabolites and one phthalate replacement and least square geometric mean (LSGM) levels of the metabolites were compared between the sampling years when adjusted for potential confounders.

Results All 14 metabolites were detectable in more than 99% of the SELMA subjects. The levels were generally comparable to other studies, but the SELMA subjects showed slightly higher exposure to butyl-benzyl phthalate (BBzP) and di-butyl phthalate (DBP). Di-ethyl-hexyl phthalate (DEHP) metabolites levels decreased while DiNP, DiDP/di-2-propylheptyl phthalate (DPHP), and DiNCH metabolites levels increased during the sampling period.

Conclusions Urinary metabolite levels of the older phthalates and more recently introduced phthalate replacement compound changed during the short sampling period in this Swedish pregnancy cohort. Our results indicate that replacement of phthalates can make an impact on human exposure to these chemicals. During this particularly vulnerable stage of life, phthalate exposures are of particular concern as the impacts, though not immediately noticeable, may increase the risk for health effects later in life.

Keywords Phthalates · DiNCH · SELMA-Study · Endocrine disrupting chemicals · Pregnant · Exposure · Temporal

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Introduction

The general population is exposed to chemicals that may interact with the endocrine system, known as endocrine disrupting chemicals. One group of chemicals with strong evidence for endocrine disrupting properties is phthalates [1]. Phthalates have been used for the past 70 years in a variety of industrial applications, e.g., plasticizers in polyvinyl chloride (PVC) products such as toys, flooring materials, food packaging, and medical supplies; as well as in personal care products, such as shampoo, lotion, and perfume [2–5].

Since phthalates create weak chemical bonds when they are added into different products, these compounds are

Table 1 Metabolites from eight parent compounds analyzed in prenatal urine from 1651 pregnant women in the SELMA study

Parent compound	Parent compound CAS NO	Metabolite		Metabolite CAS NO
Di-ethyl phthalate (DEP)	84-66-2	Mono-ethyl phthalate	MEP	2306-33-4
Di-n-butyl phthalate (DBP)	84-74-2	Mono-n-butyl phthalate	MnBP	131-70-4
Butyl-benzyl phthalate (BBzP)	85-68-7	Mono-benzyl phthalate	MBzP	2528-16-7
Di-ethyl-hexyl phthalate (DEHP)	117-81-7	Mono-ethyl-hexyl phthalate	MEHP	4376-20-9
		Mono-ethyl-hydroxy-hexyl phthalate	MEHHP	40321-99-1
		Mono-ethyl-oxo-hexyl phthalate	MEOHP	40321-98-0
		Mono-ethyl-carboxy-pentyl phthalate	MECPP	40809-41-4
		Mono-carboxy-methyl-hexyl phthalate	MCMHP	82975-93-7
Di-iso-nonyl phthalate (DiNP)	68515-48-0	Mono-hydroxy-iso-nonyl phthalate	MHiNP	936021-98-6
		Mono-oxo-iso-nonyl phthalate	MOiNP	936022-00-3
		Mono-carboxy-iso-octyl phthalate	MCiOP	936022-02-5
Di-2-propylheptyl phthalate (DPHP)	53306-54-0	Mono-hydroxy-iso-nonyl phthalate	MHiDP	NA
Di-iso-decyl phthalate (DiDP)	26761-40-0	Mono-carboxy-iso-nonyl phthalate	MCiNP	NA
Di-iso-nonyl-cyclohexane-di-carboxylate (DiNCH)	166412-78-8	Mono-oxo-iso-nonyl cyclohexanecarboxylic acid	MOiNCH	NA

readily leached into the surrounding environment. As a result, they are routinely found in indoor air [6, 7] and dust [8–11] as well as in food and water [12]. Some phthalates have also been found in food products as a result of contamination from its packaging material [13, 14]. Thus, humans are exposed via multiple uptake routes (per-oral, transdermal, and inhalation), and the uptake pathway varies by compound and exposure source. There are also other routes humans can be exposed to phthalates, e.g., phthalates are used in some medical devices such as flexible blood bags, and intravenous tubing [15], or in pharmaceuticals, especially those with enteric coating [16]. Consequently, metabolites of the parent compounds are routinely found in human biological samples such as blood, urine, and breast milk [1]. It has further been reported that phthalates may pass the placenta and expose the unborn fetus [17].

Phthalates have in epidemiological studies been associated with several human health issues including cancer [18], neurodevelopmental outcomes and behavior [19–21], reproductive health and sexual development [22, 23], and asthma and allergies [24]. Embryonic development and early life stages are critical windows of phthalate exposure, which may have irreversible impacts on development and physiological functions later in life [25].

In the European Union (EU), the usage of defined phthalates has been restricted in children's toys since 1999 with di-ethyl-hexyl phthalate (DEHP), butyl-benzyl phthalate (BBzP), and di-butyl phthalate (DBP) restricted in all toys, and di-iso-nonyl phthalate (DiNP), di-iso-decyl phthalate (DiDP), and di-n-octyl restricted in toys that can be put into mouths [26]. This restriction also states that the amount of these phthalates may not exceed 0.1% mass percent of the plasticized part of the toy [27]. In 2015, the EU commission has published a new Directive (EU 2015/863) to amend Annex II to EU RoHS 2 (Directive 2011/65/EU) by adding another four phthalates (DEHP, BBzP, DBP, and di-isobutyl phthalate) onto the list of restricted substances, which will be restricted from 22 July 2019 for all electrical and electronic equipment, except from Category 8 (medical devices) and Category 9 (monitoring and control equipment), which will have an additional 2 years to comply (by 22 July 2021) [28].

Due to health concerns, regulatory actions and public opinion, the industry—which use phthalates as important ingredients in many products—continuously introduces new phthalate derivatives and alters the chemical formulations. The Swedish Chemical Agency (KEMI) reported results from a survey on the Swedish annual use of phthalates in industrial production in 2015. The data indicated that many Swedish companies have replaced DEHP with newer phthalates such as DiNP, DiDP and di-2-propylheptyl phthalate (DPHP), or more recently, with chemically distinct plasticizers that are not chemically defined as phthalates such as di-iso-nonyl-cyclohexane di-carboxylate (DiNCH) [29–31].

In this study, we characterized 14 urinary metabolites of seven phthalates and one phthalate replacement (DiNCH), and assessed 1st trimester exposures to well-characterized phthalates of concern and their emerging replacements among a sample of 1651 Swedish pregnant women over a period of 2.5 years (2007–2010).

Table 2 Distribution of analyzed urinary levels of phthalate metabolites expressed as crude (ng/mL) and creatinine adjusted (nmol/mmol creatinine) concentrations in 1651 pregnant women in the SELMA study

	Diester name	DEP	DBP	BBzP	DEHP				DiNP			DiDP /DPHP		DINCH
	Variable name	MEP	MBP	MBzP	MEHP	MEHHP	MEOHP	MECPP		MCMHP	MHiNP	MOiNP	MCiOP	MHiDP
MCiNP	MOiNCH													
Crude (ng/mL)	*N* (% above LOD)	1651 (100)	1651 (100)	1651 (100)	1651 (100)	1651 (100)	1651 (100)	1651 (100)	1651 (100)	1651 (100)	1651 (100)	1647 (99.8)	1814 (99.6)	1634 (99.0)
	LOD	0.01	0.1	0.04	0.1	0.02	0.03	0.02	0.068	0.02	0.01	0.02	0.031	0.031	0.023
	Min	1.3	3.0	0.65	0.14	0.86	0.47	0.73	0.21	0.13	0.11	0.59	0.016	0.016	0.012
	Median	62	72	17	3.8	16	11	16	5.9	6.1	2.7	8.9	1.2	0.64	0.25
	95th	530	220	92	16	67	45	63	28	55	19	74	6.6	2.9	4.5
	Max	4400	2700	3500	210	1000	610	760	430	1700	640	1700	140	54	260
	Geometric mean (95% confidence interval)	69 (65–72)	68 (66–71)	16 (15–17)	3.8 (3.6–4.0)	16 (15–17)	11.1 (10.6–11.6)	15.8 (15.2–16.5)	6.3	(6.1–6.6)	6.2 (5.9–6.6)	2.9 (2.7–3.1)	9.8 (9.3–10.3)	1.22 (1.16–1.28)	0.67 (0.64–0.70)
0.30 (0.28–0.32)															
Creatinine adjusted (nmol/mmol creatinine)	Min	1.1	3.2	0.40	0.076	0.62	0.40	0.60	0.39	0.062	0.048	0.21	0.0024	0.0023	0.0013
	Median	33	33	6.7	1.4	5.6	3.8	5.2	1.9	2.0	0.92	2.7	0.38	0.20	0.057
	95th	250	86	30	6.1	23	16	19	8.9	18	6.4	21	2.1	0.98	1
	Max	3500	1300	560	74	330	200	180	100	550	180	410	27	14	31
	Geometric mean (95% confidence interval)	38 (36–39)	33 (32–34)	6.7	(6.4–7.0)	1.45		(1.40–1.51)	5.9 (5.7–6.2)	4.1 (3.9–4.2)	5.5 (5.3–5.7)	2.2 (2.1–2.3)	2.1 (2.0–2.3)	1.00 (0.96–1.06)	3.2 (3.1–3.4)
0.40 (0.39–0.42)	0.21 (0.20–0.22)	0.073													(0.069–0.078)

Materials and methods

SELMA is a pregnancy cohort study designed to investigate early life exposure to environmental chemicals and health outcomes in offspring children related to growth, developmental and chronic diseases. The SELMA study recruited pregnant women in the county of Värmland, Sweden between September 2007 and March 2010. Women who could read Swedish and were not planning to move out of the county were recruited at their first antenatal care visit; 8394 pregnant women were identified, 6658 were eligible and 2582 (39%) agreed to participate. Detailed recruitment selection criteria and sample collection procedures have been published previously [32].

Collection of samples

First morning void urine samples were obtained from 2325 pregnant women (out of the 2582 participating women) in week 3–27 of pregnancy (median week 10, and 96% of the samples were taken before week 13) at their first visit to the antenatal care center, i.e., at enrollment to the study [32]. Urine samples were collected at home, in supplied glass containers and transferred into polypropylene tubes. Samples were stored at –20 °C before being processed and analyzed at the laboratory at Occupational and Environmental Medicine, Lund University, Sweden [33]. In addition, blood samples were taken at enrollment and the serum aliquots were stored at −80 °C in a biobank for analysis of cotinine.

Self-administered questionnaires distributed at the time of enrollment were collected for background information such as type and location of dwelling, mother's education level, and several other parameters that may impact chemical exposures [32]. Data on the mother's weight at enrollment were imported from the Swedish National Birth Register.

Analysis of phthalate metabolites in urine

Metabolites from seven phthalates and one phthalate replacement (Table 1) were analyzed according to the method presented by Gyllenhammar et al. [34]. Aliquots of 0.2 mL of urine were mixed with 0.1 mL of ammonium acetate (1 M; pH 6.5) and 0.01 mL β-glucoronidase (E-coli) and thereafter incubated at 37 °C for 30 min. Then 0.05 mL of a 50:50 (v:v) water and acetonitrile solution of labeled (^{3}H or ^{13}C) internal standards (IS) of all analyzed compounds were added and the samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Levels of detection limits (LODs) are included in Table 2. The LOD was determined as the concentration corresponding to three times the standard deviation of the ratio of the peak at the same retention time as the analyzed compounds and the corresponding IS determined in the chemical blank samples. An in-house prepared quality control (QC) sample and chemical blank samples were analyzed two times within each sampling batch (96 samples including standards, QCs and lab blanks). The relative standard deviation during all 31 batches were between 4 and 10% for the analyzed metabolites. The samples were analyzed in a randomized order. The creatinine concentrations were analyzed according to an enzymatic method described by Mazzachi et al. [35].

DiDP and DPHP metabolites were reported as a sum (DiDP/DPHP). Since chromatographic separation of several DPHP metabolites from a single DIDP metabolite is difficult, and requires long liquid chromatography separations or analysis using gas chromatography—mass spectrometry [36].

In order to take individual differences in urine dilution into consideration, creatinine corrections were applied in two different ways. To compare the urine metabolite levels with those reported from other studies, traditional creatinine adjustments were applied to individual metabolite concentrations (Table 2). In the regression models, we used creatinine as a co-factor since creatinine may be related to individual characteristics such as age, education, body weight, and smoking. All urinary levels of metabolites were 10 log-transformed to improve the approximation of a normal distribution.

We calculated a summary metric for DEHP metabolites (ΣDEHP metabolites) equal to the molar sum of mono-ethyl-hexyl phthalate (MEHP), mono-ethyl-hydroxy-hexyl phthalate (MEHHP), mono-ethyl-oxo-hexyl phthalate (MEOHP), mono-ethyl-carboxy-pentyl phthalate (MECPP), and mono-carboxy-methyl-hexyl phthalate (MCMHP) times the molecular weight (MW) of DEHP. The summary metric of DiNP metabolites (ΣDiNP metabolites) was equal to the molar sum of mono-hydroxy-iso-nonyl phthalate, mono-oxo-iso-nonyl phthalate and mono-carboxy-iso-octyl times the MW of DiNP.

Analysis of cotinine in prenatal serum

To assess smoking status, serum levels of the nicotine metabolite cotinine were analyzed using LC-MS/MS. A detailed description of the method is presented in Lindh et al. [37]. Briefly, aliquots of serum were added with labeled IS and proteins were precipitated prior to analysis. The LOD was 0.2 ng/mL. If cotinine levels were below 0.2 ng/mL, subjects were categorized as non-smoker; if cotinine levels were higher than 15 ng/mL, subjects were considered as active smokers; and while in between, subjects were considered as passive smokers [38].

Table 3 Study population characteristics with data from a questionnaire at enrollment for 1651 pregnant women in the SELMA study

Variables	Mean	Std deviation
Mother's Age	31	4.8
Mother's Body weight	69.4	13.5
	N	%
Where is your highest education (mother)?		
With college	1013	61.4
without college	638	38.6
Mother's smoking status		
None	1453	88.0
Passive	86	5.2
Active	112	6.8

Statistical analysis

Pearson correlation, spearman correlation and two-sample t-test were used to examine potential confounders to be included in the regression models. We further applied the method used by Zota et al. [39], i.e., least square geometric mean (LSGM) of phthalates and phthalate replacement metabolites concentrations when comparing the urinary levels between the four different sampling periods (2007, 2008, 2009, 2010). By using LSGM, the geometric means for different time periods (year) could be adjusted for the mother's individual characteristics as well as season for sampling (winter vs. summer). Covariates were selected if they had significant correlation with two or more urinary phthalate metabolite concentrations. From these regression models, LSGM of phthalates and phthalate replacement metabolites concentrations by sampling year was calculated as 10^ (least squares means), with 95% CIs as 10^ (least squares mean ± 1.96 × SE). The least square geometric means is the sampling year specific mean of metabolites concentrations after adjusting for covariates. Percent changes in phthalates and the phthalate replacement metabolite concentrations by sampling year was calculated as $[10^{(\beta)}-1]\times 100\%$ with 95% CIs estimated as $[10^{(\beta \pm 1.96\times SE)}-1]$ where β and SE are the estimated regression coefficient and standard error, respectively.

The statistical analysis was conducted using PROC GLM in SAS version 9.3 of the SAS System for Windows. Copyright © 2012, SAS Institute Inc. Cary, NC, USA.

The Regional Ethical Review Board, Uppsala, Sweden, had approved the SELMA study procedures and all participants signed informed consents prior to the start of data collection.

Results

We have analyzed urinary levels of phthalate metabolites (Table 1) from 2325 SELMA subjects. Out of these 2325 subjects, we had data for all covariates included in the regression modeling from 1651 (71%) pregnant women, which is the study population defined in the current report. The mean age of these SELMA subjects was 31 years with standard deviation of 4.8 years. The mean body weight was 69.4 (±13.5) kg. The majority (88%) of the SELMA subjects were nonsmokers and more than 60% of the subjects had a college degree or a higher education level (Table 3).

Table 4 Distribution of phthalate metabolites concentrations expressed as LSGM (ng/mL) in the urine of 1651 pregnant women in SELMA collected during winter and summer season and percentage changes in relation to winter season, adjusted for urinary creatinine

^^--^^ % Change LSGM (95% CT)^--^^	Winter October–March (*n* = 959)	Summer April–September (*n* = 692)	*p*-value (overall trend)
MEP	Reference	−6.6	0.18
	71(66–75)	66 (61–71)	
MBP	Reference	−1.8	0.57
	69 (66–71)	67(64–71)	
MBzP	Reference	1.6	0.73
	16 (15–17)	16 (15–18)	
MEHP	Reference	11.9	0.006
	3.6(3.4–3.8)	4.0 (3.S-4.3)	
MEHHP	Reference	15.4	0.0002
	15.4(14.7–16.2)	17.8 (16.8–18.9)	
MEOHP	Reference	14.2	0.0006
	10.5 (10.0–11.0)	12.0(11.3–12.7)	
MECPP	Reference	14.8	0.0001
	15 (14–16)	17 (16–18)	
MCMHP	Reference	8.8	0.02
	6.1 (5.8–6.4)	6.6 (6.3–7.0)	
IDEHP	Reference	13.7	0.0004
	68(65–71)	77 (73–81)	
MHiNP	Reference	7.8	0.21
	6.0 (5.6–6.5)	6.5 (5.9–7.1)	
MOiNP	Reference	9.0	0.1
	2.8 (2.6–3.0)	3.0 (2.8–3.3)	
MCiOP	Reference	7.0	0.16
	9.5 (9.0–10.2)	10.2 (9.5–11.0)	
SDiNP	Reference	7.6	0.16
	25 (24–27)	27 (25–29)	
MCiNP	Reference	12.7	0.004
	0.64 (0.60–0.67)	0.72 (0.67–0.76)	
MHiDP	Reference	17.6	0.0005
	1.15 (1.09–1.22)	1.36 (1.26–1.45)	
MOiNCH	Reference	7.5	0.27
	0.29 (0.27–0.31)	0.31 (0.28–0.34)	

Table 5 Levels (LSGM with 95% CIs) of phthalate metabolites in the urine and percentage change of these levels from 1651 pregnant women in the SELMA study during four periods from 2007 to 2010

% Change LSGM (95%CI)	2007 ($n = 271$)	2008 ($n = 683$)	2009 ($n = 593$)	2010 ($n = 104$)	p-value (overall trend)
MEP	Reference	10.2	0.6	−10.6	0.17
	66 (58–75)	73 (67–79)	67 (61–72)	59 (48–72)	
MBP	Reference	9.0*	0.1	16.4*	**0.01**
	65 (60–70)	71 (68–74)	65 (62–68)	76 (67–85)	
MBzP	Reference	11.1	5.4	17.9	0.26
	15 (13–17)	17 (16–18)	16 (15–17)	18 (15–21)	
MEHP	Reference	15.6*	−4.8	−12.2	<0.0001
	3.7 (3.3–4.0)	4.2 (4.0–4.5)	3.5 (3.3–3.7)	3.2 (2.7–3.8)	
MEHHP	Reference	7.5	−11.9*	−15.6	<0.0001
	17 (15–18)	18 (17–19)	15 (14–16)	14 (12–16)	
MEOHP	Reference	8.5	−11.0*	−13.1	<0.0001
	11.3 (10.3–12.4)	12.3 (11.6–13.0)	10.1 (9.5–10.7)	9.8 (8.4–11.4)	
MECPP	Reference	7.1	−11.7*	−14.9*	<0.0001
	16.2 (14.9–17.7)	17.4 (16.5–18.3)	14.3 (13.5–15.2)	13.8 (12.0–15.9)	
MCMHP	Reference	0.5	−19.4***	−25.1***	<0.0001
	6.9 (6.4–7.6)	7.0 (6.6–7.4)	5.6 (5.3–5.9)	5.2 (4.5–6.0)	
∑DEHP	Reference	7.5	−12.1*	−16.2*	<0.0001
	74 (68–80)	79 (75–84)	65 (61–69)	62 (54–71)	
MHiNP	Reference	9.8	23.9*	39.5*	0.02
	5.4 (4.7–6.3)	6.0 (5.4–6.5)	6.7 (6.1–7.4)	7.6 (6.0–9.6)	
MOiNP	Reference	19.3*	29.3***	49.6***	0.001
	2.4 (2.1–2.7)	2.9 (2.6–3.1)	3.1 (2.8–3.4)	3.6 (2.9–4.4)	
MCiOP	Reference	13.8	22.6**	38.6**	0.008
	8.5 (7.5–9.5)	9.7 (9.0–10.4)	10.4 (9.6–11.2)	11.8 (9.7–14.2)	
∑DiNP	Reference	13.5	24.4**	40.6**	0.007
	22 (20–25)	25 (23–27)	28 (26–30)	31 (26–38)	
MCiNP	Reference	10.5	27.2***	36.6***	<0.0001
	0.58 (0.52–0.64)	0.64 (0.60–0.68)	0.73 (0.69–0.78)	0.79 (0.67–0.93)	
MHiDP	Reference	20.2**	29.3***	34.0**	0.002
	1.02 (0.91–1.14)	1.23 (1.15–1.32)	1.32 (1.23–1.42)	1.37 (1.14–1.64)	
MONiCH	Reference	37.5***	108.5***	222.6***	<0.0001
	0.19 (0.16–0.22)	0.26 (0.23–0.28)	0.39 (0.35–0.43)	0.60 (0.47–0.78)	

Significant difference when compared with 2007 indicated with p-value (*<0.05; **<0.01; ***<0.001)

p-value (overall trend) is the significant level for the entire sampling period (2007–2010)

The model adjusted for urinary creatinine, mothers age (years), mother's body weight (kilogram), smoke (non-smoking, passive smoker, or active smoker), mothers education (university vs. other) and season (winter vs. summer)

Phthalate metabolite levels in prenatal urine

The distributions of the urinary concentrations for the metabolites of the 13 phthalates and the phthalate replacement analyzed in all 1651 SELMA subjects are summarized in Table 2. All analyzed metabolites, were detectable in more than 99% of the SELMA mothers. Mono-ethyl phthalate (MEP) (69 ng/mL) and mono-butyl phthalate (MBP) (68 ng/mL) were the phthalate metabolites with the highest geometric mean concentrations. The four

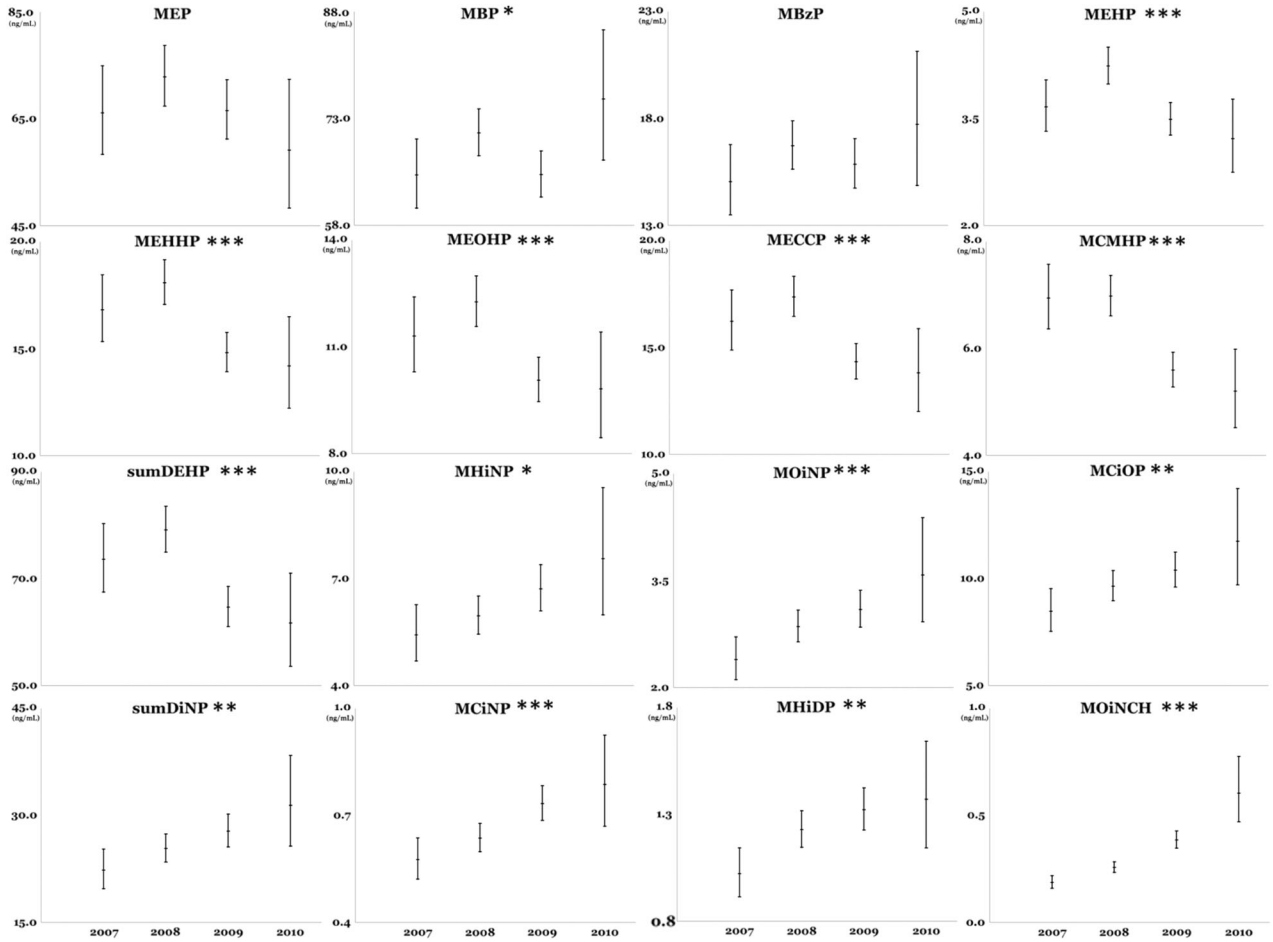

Fig. 1 Urinary levels (LSGM with 95% CIs) of phthalate metabolites during four periods from 2007 to 2010 in 1651 pregnant women in the SELMA study. The model adjusted for urinary creatinine, mothers age (years), mother's body weight (kilogram), smoke (non-smoking, passive smoker, or active smoker), mothers education (university vs. other) and season (winter vs. summer). Adjusted overall significance *p*-value (*<0.05; **<0.01; ***<0.001)

oxidized DEHP metabolites MEHHP (16 ng/mL), MEOHP (11 ng/mL), MECPP (16 ng/mL) and MCMHP (6.3 ng/mL) geometric mean concentrations were numerically higher than the primary monoester MEHP (3.8 ng/mL). The geometric mean concentrations of the three DiNP metabolites ranged from 2.9 to 9.8 ng/mL. The geometric mean concentration of DiNCH, measured as the metabolite mono-oxo-iso-nonyl cyclohexanecarboxylic acid (MOiNCH) was 0.30 ng/mL.

Seasonal variation

When comparing samples collected during the summer (May–September) and winter season (October–April) we found higher urinary levels during the summer for the metabolites from the high molecular weight phthalates DEHP, DiNP, DiDP/DPHP, and DiNCH, though only the DEHP and DiDP/DPHP metabolite levels were significantly different between winter and summer ($p < 0.05$) as shown in Table 4. For the other compounds with lower molecular weights (MEP and MBP), metabolite concentrations during summer season were lower than the winter, but these differences were not significant. No seasonal pattern was suggested for the BBzP metabolite, mono-benzyl phthalate (MBzP).

Time trends

The LSGM concentrations (adjusted for creatinine and covariates) for each sampling year are shown in Table 5 and Fig. 1. Statistically significant ($p < 0.0001$) downward temporal trends were found in the levels of all DEHP metabolites (MEHP, MEHHP, MEOHP, MECPP, and MCMHP). Compared to 2007, the levels of ΣDEHP metabolites decreased approximately by 16% over the sampling period. During the same sampling period, significant steady upward trends were found for the DiNP and DiDP/DPHP metabolites, where the LSGM concentrations

increased by approximately 34–41%. Finally, the metabolite of the phthalate replacement DiNCH showed the strongest upward temporal trend during the sampling period (2007–2010). In comparison to the 2007 data the LSGM concentrations of MOiNCH increased by 38% ($p < 0.0001$) in 2008, 109% ($p < 0.0001$) in 2009, and by 223% ($p < 0.0001$) in 2010.

For MEP and MBzP metabolite levels, no significant changes could be found in the temporal trend analyses during the sampling period. All regression models were stratified by season, however, the temporal trends persisted (data not shown).

Discussion

To our knowledge, this is the first examination of temporal trends of phthalate and DiNCH metabolites among a sample of pregnant women in Sweden. Despite the short time period from 2007 to 2010 (2.5 years), we observed significant and consistent changes in urinary concentrations of phthalate metabolites. The LSGM levels of DEHP decreased during the sampling period, while the LSGM of the phthalate-based replacements (DiNP, and DiDP/DPHP) and the non-phthalate-based replacement (DiNCH) increased significantly.

Our findings are consistent with biomonitoring assessments in other countries, such as in the US [39] and Germany [40] where decreased trends in DEHP metabolites and increased trends in DiNP and DINCH metabolites have been reported over the last decade.

Our results indicates that the current regulations on phthalate in consumer products, such as toys and products dedicated for small children, as well as changes of plasticizers in other soft PVC materials, such as PVC flooring, may have an impact on the composition of phthalate metabolite levels in pregnant women [26, 27]. The observed trends are most probably related to replacing DEHP by phthalates such as DiNP and DiDP/DPHP and the phthalate substitute DiNCH in products [40, 41]. As indicated by the Swedish Chemical Agency (KEMI), phthalates are the most common plastic softener that is added to PVC, to make hard PVC material into soft PVC used in flooring, car interior material, and coated fabrics [41]. As mentioned above, in Sweden, PVC flooring is widely used, especially in the bedroom. In an investigation of 7694 Swedish homes in 2010 more than 30% of the bedrooms had PVC floorings [42].

As reported by KEMI [41], the usage of DEHP and DiNP respectively in PVC has, to a great extent, switched between 2002 and 2005. The production of DEHP was reduced from 12,272 tons in 1999 to 2732 tons in 2002, while DiNP increased from 452 tons to 8246 tons during the same period. This substitution process has continued throughout the past several years, although to a lower extent [41]. It should be noted, PVC flooring is known for its long-lasting durability, and with sustained usage for up to 30–40 years. Therefore, even though the replacement of DEHP by DiNP in new products was introduced in industrial processing around the turn of the century, the older material will be in use for a long time. Another DEHP replacement, which was reported in Van Vliet et al. [30], showed that BASF expanded DiNCH production from 25,000 ton in 2002 to 100,000 ton in 2007.

PVC flooring is one source of human BBzP phthalate exposure in Sweden [2]. Furthermore, several studies have shown that diet is a source for DEHP uptake. Rudel et al. [3] conducted a study where 20 participants had 3 days of "fresh food" diet after their normal diet, and found that DEHP exposure was substantially reduced when participants' diet were restricted to food with limited packaging. Zota et al. [43] indicated fast food might be a source of exposure to DEHP and DiNP.

Zota et al. [39] reported declining trend for di-ethyl phthalate (DEP) metabolites in NHANES. However, we observed non-significant decreasing MEP concentrations in pregnant women in the SELMA cohort over the sampling period. The relatively short 2.5 years SELMA sampling period could be one of the reason for the non-significant decreasing trend.

For a few phthalates, we found that season was a significant exposure predictor, e.g., concentrations of the phthalate metabolites originating from DEHP and DiDP/DPHP were higher during the summer (May-September) season. These results are similar to the P4 study in Ottawa, Canada where urinary DEHP metabolites (MEHP and MCMHP) were higher in the summer [44]. This seasonal trend for DEHP metabolites could be due to diet differences between summer and winter, however, we have no relevant additional information that could explain these results.

Phthalate metabolite levels in urine from this analysis are relatively comparable to results from several other studies in Europe [45–48], USA [49–51] and Canada [52]. However, the levels of MBzP and MBP measured in SELMA were higher, while levels of MEP and MECPP were lower compared with other studies from around the same time [46, 53]. One reason for the higher BBzP metabolite levels in Swedish women could be due to the frequent use of PVC flooring material in homes (mainly in bedrooms, bathrooms, and kitchens). As mentioned above, our previously published data showed that around 30% of 7694 families reported having PVC flooring in the parents' bedrooms in 2010, which is a higher frequency than in most other countries [42]. During the sampling period, LSGM concentrations of BBzP and DBP metabolites did not change significantly as Zota et al. [39] reported. This could be due

to that both BBzP and DBP already had decreased from expected higher levels closer to the turn of the century, and no significant changes could thus be seen during the short sampling period in the current study [51].

One limitation with the present study is that we only have one urine sample from each participating pregnant woman. Calafat et al. [54] indicated that phthalate diesters are non-persistent in the body and metabolize quickly. As a result, the levels in serum can be several orders of magnitude lower than in urine. Such low levels increase the risk for background contaminations, and only the phthalate oxidative metabolites are valid exposure biomarkers in serum [54]. For these reasons, measuring the levels of metabolites in urine has been the choice for many different studies. As mentioned above, the relatively rapid turnover of phthalates in the human body makes variation in exposures. However, we used the first morning void samples in order to decrease the variability in urinary phthalate metabolite levels related to the timing of individual activities such as use of cosmetics, dietary variations, or other very recent potential exposure [55].

Conclusions

Our analysis demonstrates that common phthalates—measured as metabolites in urine—were detectable in almost all of the 1651 pregnant women in the Swedish SELMA-study. Furthermore, we identified a temporal trend indicative of a shift in exposure to individual phthalates during the time period from 2007 to 2010. DEHP levels decreased while DiNP and DiDP/DPHP metabolite levels increased and the DiNCH metabolite level increased dramatically. This is consistent with the substitution of plasticizers in plastics and other products where DiNP and DiDP/DPHP and more recently DiNCH have replaced DEHP in many products. Our results indicate that the replacement of phthalates in consumer products is one way to change the exposure for phthalates. Phthalate exposures are of particular concern as the impacts of exposure though not immediately noticeable at birth, may increase the risk of health effects later in life.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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