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Pre- and early post-natal exposure to phthalates and DINCH in a new type of mother-child cohort relying on within-subject pools of repeated urine samples☆

Claire Philippat [a,*], Matthieu Rolland [a], Sarah Lyon-Caen [a], Isabelle Pin [a,b], Amrit K. Sakhi [c], Azemira Sabaredzovic [c], Cathrine Thomsen [c], Rémy Slama [a]

[a] University Grenoble Alpes, Inserm, CNRS, Team of Environmental Epidemiology Applied to Reproduction and Respiratory Health, Institute for Advanced Biosciences (IAB), Grenoble, France
[b] Pediatric Department, Grenoble University Hospital, 38700, La Tronche, France
[c] Norwegian Institute of Public Health, Oslo, Norway

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ABSTRACT

For non-persistent chemicals such as phthalates, a single spot urine sample only reflects exposure in the past few hours. Collecting repeated urine samples for each participant over windows of sensitivity is expected to improve exposure characterization but has rarely been done. We aimed to rely on within-subject pools of repeated urine samples to assess phthalate exposure during pregnancy and infancy.

Women of the French SEPAGES mother-child cohort were asked to collect three urine samples per day over seven consecutive days, twice during their pregnancy (approximatively second (T2) and third (T3) trimesters). For their infants they also collected one sample per day during a week at two (M2) and twelve months (M12). Samples were pooled (within-subject, within-period) prior to phthalate and DINCH metabolite concentrations assessment. Number of pooled samples assayed was 477, 456, 152 and 100 for T2, T3, M2 and M12, respectively. All metabolites were detected in more than 95% of the pooled samples except for the two DINCH metabolites (oh- and oxo-MINCH), MMCHP and oh-MPHP at M2 for which detection frequencies ranged between 64% and 88%. Maternal concentrations of MiBP, MBzP, DEHP metabolites and oxo-MiNP decreased between 2014 and 2017, whereas concentrations of oh-MiNP and the two DINCH metabolites increased (Mann-Kendall p-values $<$ 0.05). While improved compared to studies that relied on spot samples, Intraclass Correlation Coefficients for the pregnancy were below 0.40 for most metabolites. Spearman correlation coefficients between pooled samples collected in infancy were lower than those observed during pregnancy, and were all below 0.30. Exposure to emerging phthalate substitutes such as DINCH and DPHP seems widespread among pregnant women and infants. Collecting repeated urine samples in pregnant women and infants is feasible. The relatively low correlation across trimesters and between maternal and infant samples highlights the need to collect biospecimens in the assumed sensitive time window.

1. Introduction

Phthalates are used as plasticizers in a wide range of consumer products including personal care products, lacquers, varnishes and poly vinyl (PVC) plastics (Hauser and Calafat, 2005). Several phthalates have been classified by the European Commission as having endocrine disrupting or reprotoxic properties (category 1 B). In the European Union, several, but not all, phthalates are now banned from use in cosmetics (benzyl butyl phthalate (BBzP), diethylhexyl phthalate (DEHP), dibutyl phthalate (DBP), bis(2-Methoxyethyl) phthalate (DMEP), EC/1223 /2009). Their use is also regulated in material intended to come into

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* Corresponding author. Institut pour l'Avancée des Biosciences Centre de Recherche UGA / Inserm, U 1209 / CNRS UMR 5309Site Santé - Allée des Alpes, 38700, La Tronche, France.
E-mail addresses: claire.philippat@inserm.fr (C. Philippat), matthieu.rolland@univ-grenoble-alpes.fr (M. Rolland), sarah.lyon-caen@univ-grenoble-alpes.fr (S. Lyon-Caen), ipin@chu-grenoble.fr (I. Pin), AmritKaur.Sakhi@fhi.no (A.K. Sakhi), Azemira.Sabaredzovic@fhi.no (A. Sabaredzovic), Cathrine.Thomsen@fhi.no (C. Thomsen), remy.slama@univ-grenoble-alpes.fr (R. Slama).

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Acronyms

BBzP	benzyl butyl phthalate
cx-MiNP	Mono-4-methyl-7-carboxyoctyl phthalate
DBP	dibutyl phthalate
DEHP	diethylhexyl phthalate
DiDP	di-isodecyl phthalate
DINCH	1,2-Cyclohexane dicarboxylic acid, diisononyl ester
DiNP	di-isononyl phthalate
DMEP	bis(2-Methoxyethyl) phthalate
DNOP	di-n-octyl phthalate
DPHP	Bis(2-propylheptyl) phthalate
LOD	limit of detection
LOQ	limit of quantification
MBzP	Monobenzyl phthalate
MECPP	Mono-2-ethyl-5-carboxypentyl phthalate
MEHHP	Mono-2-ethyl-5-hydroxyhexyl phthalate
MEHP	Mono-2-ethylhexyl phthalate
MEOHP	Mono-2-ethyl-5oxohexyl phthalate
MEP	Monoethyl phthalate
MiBP	Mono-iso-butyl phthalate
MnBP	Mono-n-butyl phthalate
MMCHP	Mono-2-carboxymethyl hexyl phthalate
oh-MINCH	2-(((Hydroxy-4-methyloctyl) oxy) carbonyl) cyclohexanecarboxylic acid
oh-MiNP	Mono-4-methyl-7-hydroxyoctyl phthalate
oxo-MiNP	Mono-4-methyl-7-oxooctyl phthalate
oxo-MINCH	2-(((4-Methyl-7-oxooctyl) oxy) carbonyl) cyclohexanecarboxylic acid
oh-MPHP	Mono-6-hydroxy-propylheptyl phthalate
SG	specific gravity

contact with food (DEHP, DBP, BBzP, EC/2007/19) as well as in toys and childcare articles (DEHP, DBP, BBP, di-isononyl phthalate (DiNP), di-isodecyl phthalate (DiDP), di-n-octyl phthalate (DNOP), French law 2006–1361). While some of these regulations focus on infancy, a period sensitive to environmental exposures, information on exposure in infants is scarce in the European Union and limited to Germany (Enke et al., 2013; Fromme et al., 2013; Völkel et al., 2014), Czek Republic (Urbancova et al., 2019), Finland (Frederiksen et al., 2014), and Sweden (Carlstedt et al., 2013). In addition, newer phthalates such as bis (2-propylheptyl) phthalate (DPHP) and phthalate's substitutes (1, 2-Cyclohexane dicarboxylic acid diisononyl ester (DINCH)) have recently been marketed. DINCH, mainly used to produce plastics for toys, medical devices and food contact materials was introduced in the European market in 2002 (Kasper-Sonnenberg et al., 2019). Positive temporal trends, suggesting an increase in exposure over time, have been reported for the DINCH metabolites in Sweden (Gyllenhammar et al., 2017) and Germany (Kasper-Sonnenberg et al., 2019), however, to date, no data regarding DINCH exposure in the French general population is available.

Studies describing phthalate exposure in the general population usually assessed their metabolites in spot urine samples. Due to the short half-life of phthalates and the temporal variability in exposure-prone behaviors (e.g., use of personal care products, food intake), a high within-subject variability has been reported in phthalate metabolite urinary concentrations (reviewed by Casas et al., 2018). To improve exposure assessment in biomarker-based studies about such compounds, it has been advocated to collect repeated urine samples per individual (LaKind et al., 2019; Perrier et al., 2016). To limit assay cost, one can pool these repeated samples within subject for each period of interest. Within-subject pooling has not often been used so far, and data on variability of phthalate metabolite concentrations assessed in pools are sparse (Casas et al., 2018; Vernet et al., 2018), especially during infancy.

We relied on within-subject pools of many urine samples per subject to describe urinary concentrations of several phthalate and DINCH metabolites among pregnant women and their infants.

2. Methods

2.1. Study population

The SEPAGES cohort is a French couple-child cohort that recruited 484 pregnant women between July 2014 and July 2017 (Lyon-Caen et al., 2019) in the Grenoble area. Inclusion criteria were being pregnant by less than 19 gestational weeks at inclusion, older than 18 years old, reading and speaking French fluently, being affiliated to the French national health security system and having planned to deliver in one of the four maternities of the area. Twin or multiple pregnancies were excluded. Our study population was restricted to the 479 mothers with at least one urine collection during pregnancy.

The SEPAGES study was approved by an ethics committee (CPP: Comité de Protection des Personnes Sud-Est V) and the French data privacy committee (CNIL: Comité National Informatique et Liberté). Both mother and father of the expected child signed an informed consent form prior to inclusion.

2.2. Collection of repeated urine samples during pregnancy and infancy

While doable (Nakiwala et al., 2020; Preau et al., 2010), collecting all the urines produced over several weeks of the pregnancy requires strong implication of the participants, which may lead to high dropout rates and selection bias. For this reason, in SEPAGES, we opted for a lighter urine collection protocol and asked pregnant women to only collect three urine samples per day (morning, midday and evening) during seven consecutive days at two different points in time: during the early second (T2) and third (T3) trimesters of pregnancy. We have shown that, for compounds with similar intra-individual variability than phthalates, exposure proxies obtained with this urine collection protocol correlates well with those obtained with a protocol requesting the collection of all urines produced over a week (Vernet et al., 2019).

After birth, participants were asked to collect their infant's urine (one sample per day over 7 consecutive days) at two (M2) and twelve (M12) months. Infant urine was collected using a cotton gaze inserted in the diaper. If stool was present, collection was performed again. Except diapers, materials used for urine collection were provided to the participants by the study field workers.

Participants were instructed to store each single urine sample in their freezer (−20 °C). For transportation from the participant's home to the certified biobank of the Grenoble University Hospital (bb-0033-00069), the single urine samples were picked up by the study field worker at the end of the collection week and transported in ice boxes.

2.3. Urine samples pooling and assessments of phthalate metabolites

To limit assay cost, spot urine samples were pooled within-subject, within period. This resulted in two pooled urine samples for each woman (second and third trimester) and two pooled samples for each infant (two and twelve months). For the pooling, the single urine samples were thawed overnight at 4 °C and an equal volume of each void was pooled and aliquoted in 1.8 mL cryovials. This equal-volume pooling approach has been previously validated (Philippat and Calafat, 2020). Aliquotes of the pools were subsequently stored at −80 °C. Urine collection tubes and cryotubes were in polypropylene and

high-density polyethylene and certified free of phthalates by the provider.

Aliquotes of the pooled urine samples were sent on dry ice with a temperature sensor to the Norwegian Institute of Public Health in Oslo, Norway where urinary concentrations of 13 phthalate and two DINCH metabolites Table S1 were measured using on-line solid-phase extraction liquid chromatography coupled to tandem mass spectrometer (HPLC-MS/MS), as described elsewhere ((Sabaredzovic et al., 2015), see Supplementary Material, Table S1 for a detailed list of metabolites assessed). The accuracy of the method ranged from 70% to 125% and the precision given as relative standard deviation was below 25% for the phthalate metabolites (Sabaredzovic et al., 2015). In brief, 300 μL urine was pipetted to an Eppendorf vial and labelled internal standard solution and enzyme solution were added to deconjugate glucoronides (betaglucuronidase in ammonium acetate buffer, pH 6.5). The samples were incubated for 1.5 h at 37 °C. After 1.5 h, formic acid (20%) was added, the samples were centrifuged and the supernatant was injected into the HPLC-MS/MS system. Phthalate and DINCH metabolites were measured for all the pregnancy samples (N = 477 and 456 pooled urine samples for the second and third trimesters, respectively). For budgetary reasons, only the 152 first two-month infant pooled urine samples available were assessed, and a random selection of 100 pooled urine samples collected at twelve months from these 152 infants.

2.4. Statistical analysis

For each phthalate and DINCH metabolite concentrations we presented the percentage of pooled samples above limit of detection (LOD), above limit of quantification (LOQ), along with the 5th, 50th and 95th percentiles. We plotted box-plots and computed Wilcoxon rank sum tests to compare distribution of metabolites between different sampling periods (second and third trimesters of pregnancy, second and twelfth months of infancy). To assess intra-individual variability of biomarker concentrations between the different periods of sampling we relied on Spearman correlation coefficients. To allow comparison across period these correlation coefficients were assessed in the 100 mother-infant pairs with concentration assessment at all four time points (second and third trimesters of pregnancy, second and twelfth months of infancy). For comparison with previous studies (Casas et al., 2018), Intraclass Correlation Coefficients (ICC) were also computed as the ratio of between-subject variance to total variance. They were computed for maternal samples only (N = 454 mothers with a measure at both second and third trimesters) as for infant samples the within-subject variance accounted for nearly all of the variance leading to near null ICCs and unstable confidence intervals. Time trends for maternal concentrations were represented visually with yearly distribution box plots. Trends were tested using the non-parametric Mann-Kendall test. These time trends were computed for pregnancy only, as the collection times of the assayed infant samples spanned a shorter time window (from August 2015 to November 2016 for two-month infants and from February 2016 to October 2017 for twelve-month infants). Finally, within-sample Spearman correlations between metabolites were computed to evaluate co-occurrence of biomarkers.

Statistical analysis was performed using R (version 4.0.2). The R code is available in the following gitlab repository: https://gricad-gitlab.univ-grenoble-alpes.fr/iab-env-epi.

3. Results

3.1. Study population

Most women were highly educated (56% with master's degree or more), they were often primiparous (46%), and only one lived without a partner at enrollment. They were mostly of European ascent (84%) and between 30 and 40 years old (72%, Table 1). Median (IQR) gestational age was 17 weeks (16–18) and 34 weeks (32–35) at the time of maternal urine collection. Median (IQR) infant age at the time of urine collection was 7 weeks (6–8) and 52 weeks (51–54), respectively. Observance to the urine collection protocol was high. Median (IQR) number of samples in each pool was 21 (20–21) for the pregnancy samples and 6 (6–7) for the infant samples.

Table 1
Characteristics of the study population (SEPAGES mother-child cohort, 2014–2017, N = 479 mothers with at least one pooled urine sample during pregnancy).

		N	%
Child sex			
	Boy	254	53%
	Girl	221	46%
	Missing	4	1%
Marital status			
	Has a partner at enrolment	478	100%
	No partner at enrolment	1	0%
Maternal age (years)			
	20 to 29	125	26%
	30 to 39	343	72%
	40 to 46	11	2%
Maternal BMI (kg/m^2)			
	15 to 18.4	29	6%
	18.5 to 24.9	361	75%
	25 to 29.9	65	14%
	30 to 34.9	12	3%
	35 to 45	8	2%
	Missing	4	1%
Maternal education			
	Highschool	28	6%
	Highschool +2 years	54	11%
	Highschool +3–4 years	125	26%
	Highshool +5 or more years	269	56%
	Missing	3	1%
Maternal ethnicity			
	European	401	84%
	Other[a]	15	3%
	Missing	63	13%
Parity			
	First child	219	46%
	Second child	211	44%
	Third child or more	48	10%
	Missing	1	0%

Abbreviation: BMI: Body Mass Index.
[a] includes: Africa (N = 3), America (N = 2), Oriental Mediterranean countries (N = 3), South-East Asia (N = 2), Other (N = 5).

3.2. Urinary concentrations

Frequencies of detection were greater than 95% for all metabolites at all sampling periods except for the following metabolites at two months: MMCHP (85%), oh-MPHP (64%), oxo-MiNP (88%) and the two DINCH metabolites (oxo-MINCH (85%), oh-MINCH (75%)). The three low molecular weight phthalate metabolites (MEP, MiBP and MBP) and one of the DEHP metabolites (MECPP) tended to have the highest median values among all compounds, whatever the period considered, while the lowest medians were observed for oh-MPHP, oxo-MINP and the two DINCH metabolites (Fig. 1, Supplemental Material Table S2).

3.3. Comparison between concentrations at different sampling time points

For most metabolites, median concentrations were similar at the second and third trimesters of pregnancy except for MEHP, oh-MPHP and oh-MINCH, for which higher concentrations were observed at the second trimester, compared to the third (p-values for Wilcoxon rank sum test below 0.05, Fig. 1, Supplemental material Table S2). Marked differences in median urinary concentrations were observed for most metabolites between pregnancy and infancy (Fig. 1). Medians of all low molecular weight phthalate metabolites (MEP, MnBP and MiBP), MEOHP and two of the three DiNP metabolites (oxo-MiNP and cx-MiNP) were lower at one

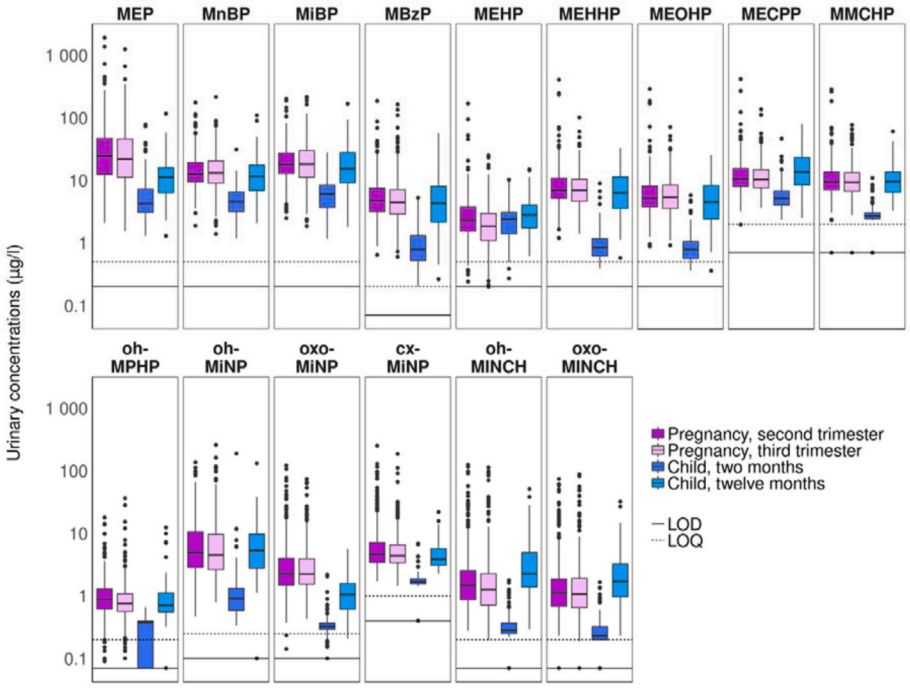

Fig. 1. Distribution of phthalate and DINCH metabolite concentrations (µg/L) during pregnancy and infancy in the SEPAGES mother-child cohort (N_{T2} = 477, N_{T3} = 456, N_{M2} = 152 and N_{M12} = 100 pooled urine samples). Numeric values are provided in Supplemental Table S2.
Abbreviations: LOD: limit of detection, LOQ: limit of quantification, MnBP: Mono-n-butyl phthalate, MiBP: Mono-iso-butyl phthalate, MBzP: Monobenzyl phthalate, MEP: Monoethyl phthalate, oh-MiNP: Mono-4-methyl-7-hydroxyoctyl phthalate, oxo-MiNP: Mono-4-methyl-7-oxooctyl phthalate, cx-MiNP: Mono-4-methyl-7-carboxyoctyl phthalate, oh-MINCH: 2-(((Hydroxy-4-methyloctyl) oxy) carbonyl) cyclohexanecarboxylic acid, oxo-MINCH: 2-(((4-Methyl-7-oxooctyl) oxy) carbonyl) cyclohexanecarboxylic acid, oh-MPHP: Mono-6-hydroxy-propylheptyl phthalate, MEOHP: Mono-2-ethyl-5oxohexyl phthalate, MECPP: Mono-2-ethyl-5-carboxypentyl phthalate, MEHP: Mono-2-ethylhexyl phthalate, MEHHP: Mono-2-ethyl-5-hydroxyhexyl phthalate, MMCHP: Mono-2-carboxymethyl hexyl phthalate.

year compared to pregnancy (p-values < 0.05), while higher concentrations at one year compared to pregnancy were observed for MEHP, MECPP and the two DINCH metabolites (oh-MINCH and oxo-MINCH). All metabolites had lower median urinary concentrations at two months compared to the other periods (p-values < 0.05) except for MEHP, which median at two months was similar to those observed during pregnancy. Comparison of biomarker concentrations assessed in the two-month samples with those assessed at the three other time points however is not straightforward as specific gravity measurements suggested that urine samples collected at two months (mean SG, 1.004) were more diluted than those collected at the other periods (mean SG above 1.012).

We observed differences across periods in the distribution of metabolites of a given phthalate. As an example, the ratio of the monoester (MEHP) to the oxidized DEHP metabolites (MEOHP, MEHHP, MMCHP, MECPP), was higher at two months (median, 0.24) compared to the other sampling periods (median values, 0.07, 0.06 and 0.08 for the second, third trimesters and twelve-month samples, respectively). The ratio of the oxidized metabolites also differed. Higher median value at two months compared to the other periods were indeed observed for the MECPP/MEOHP and MMCHP/MEOHP ratio. Similarly, for DiNP metabolites the cx-MiNP/oxo-MiNP ratio was elevated at two months compared to the other time points (Supplemental Material, Table S3).

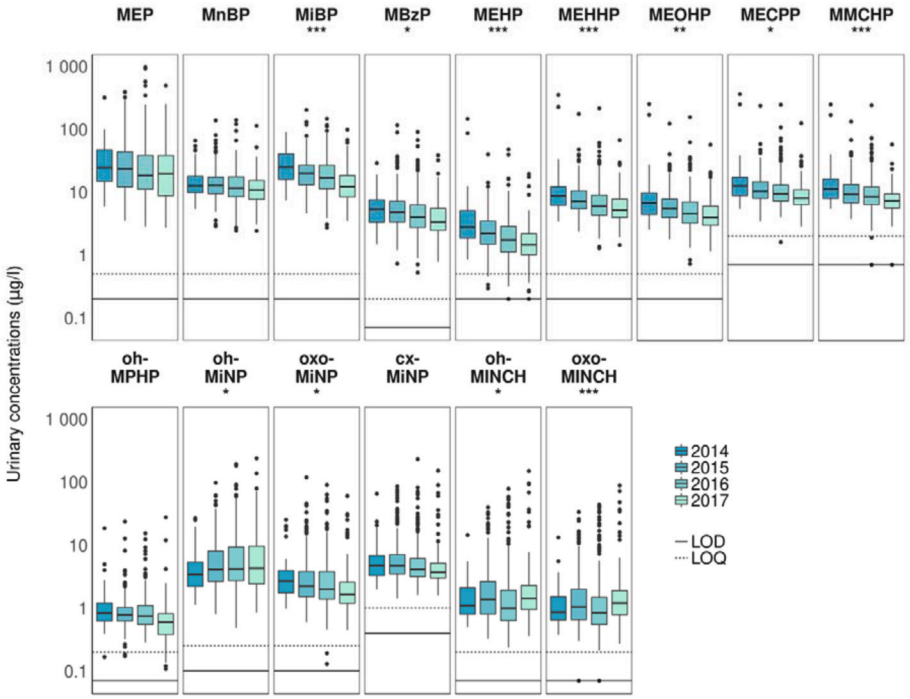

Fig. 2. Yearly maternal urinary concentrations of phthalate and DINCH metabolites (SEPAGES cohort, 2014–2017) (Number of pooled urine samples was respectively 49, 292, 413 and 179 for year 2014, 2015, 2016 and 2017). Stars correspond to the p-value of the Mann Kendall trend test; *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.
Abbreviations: LOD: limit of detection, LOQ: limit of quantification, MnBP: Mono-n-butyl phthalate, MiBP: Mono-iso-butyl phthalate, MBzP: Monobenzyl phthalate, MEP: Monoethyl phthalate, oh-MiNP: Mono-4-methyl-7-hydroxyoctyl phthalate, oxo-MiNP: Mono-4-methyl-7-oxooctyl phthalate, cx-MiNP: Mono-4-methyl-7-carboxyoctyl phthalate, oh-MINCH: 2-(((Hydroxy-4-methyloctyl) oxy) carbonyl) cyclohexanecarboxylic acid, oxo-MINCH: 2-(((4-Methyl-7-oxooctyl) oxy) carbonyl) cyclohexanecarboxylic acid, oh-MPHP: Mono-6-hydroxy-propylheptyl phthalate, MEOHP: Mono-2-ethyl-5oxohexyl phthalate, MECPP: Mono-2-ethyl-5-carboxypentyl phthalate, MEHP: Mono-2-ethylhexyl phthalate, MEHHP: Mono-2-ethyl-5-hydroxyhexyl phthalate, MMCHP: Mono-2-carboxymethyl hexyl phthalate.

Table 2
Spearman correlation coefficients and intraclass correlation coefficients (ICC) across time points.

	Spearman Correlations N = 100[a]				ICC (95%CI) N = 454[b]
	T2-T3	M2-M12	Mother - M2	Mother - M12	T2-T3
MEP	0.58	0.24	0.27	0.19	0.61 (0.55; 0.67)
MnBP	0.51	0.14	0.26	0.39	0.58 (0.52; 0.64)
MiBP	0.50	0.24	0.39	0.38	0.60 (0.54; 0.65)
MBzP	0.63	0.22	0.43	0.29	0.63 (0.57; 0.68)
MEHP	0.53	0.13	−0.06	−0.07	0.42 (0.34; 0.50)
MEHHP	0.38	0.14	0.28	0.09	0.35 (0.27; 0.43)
MEOHP	0.37	0.15	0.20	0.10	0.37 (0.29; 0.45)
MECPP	0.39	0.25	0.23	0.04	0.39 (0.31; 0.47)
MMCHP	0.36	0.22	0.28	0.21	0.31 (0.22; 0.39)
∑DEHP[c]	0.34	0.19	0.29	0.11	0.37 (0.28; 0.45)
oh-MPHP	0.41	0.20	0.27	0.16	0.20 (0.10; 0.28)
oh-MiNP	0.23	0.22	0.23	0.20	0.20 (0.12; 0.30)
oxo-MiNP	0.28	0.15	0.28	0.17	0.21 (0.12; 0.30)
cx-MiNP	0.34	0.22	0.00	0.10	0.20 (0.11; 0.29)
∑DiNP[c]	0.25	0.20	0.19	0.19	0.18 (0.09; 0.27)
oh-MINCH	0.29	0.21	−0.01	−0.21	0.34 (0.26; 0.42)
oxo-MINCH	0.27	0.15	0.04	−0.26	0.32 (0.24; 0.40)
∑DINCH[c]	0.28	0.19	0.01	−0.23	0.34 (0.25; 0.42)

Abbreviations: T2: second trimester of pregnancy, T3: third trimester of pregnancy, Mother: geometric mean pregnancy values (T2 and T3), M2: second month infants, M12: 12 month infants, MnBP: Mono-n-butyl phthalate, MiBP: Mono-iso-butyl phthalate, MBzP: Monobenzyl phthalate, MEP: Monoethyl phthalate, oh-MiNP: Mono-4-methyl-7-hydroxyoctyl phthalate, oxo-MiNP: Mono-4-methyl-7-oxooctyl phthalate, cx-MiNP: Mono-4-methyl-7-carboxyoctyl phthalate, oh-MINCH: 2-(((Hydroxy-4-methyloctyl) oxy) carbonyl) cyclohexanecarboxylic acid, oxo-MINCH: 2-(((4-Methyl-7-oxooctyl) oxy) carbonyl) cyclohexanecarboxylic acid, oh-MPHP: Mono-6-hydroxy-propylheptyl phthalate, MEOHP: Mono-2-ethyl-5oxohexyl phthalate, MECPP: Mono-2-ethyl-5-carboxypentyl phthalate, MEHP: Mono-2-ethylhexyl phthalate, MEHHP: Mono-2-ethyl-5-hydroxyhexyl phthalate, MMCHP: Mono-2-carboxymethyl hexyl phthalate, DEHP: Di (2-ethylhexyl) phthalate, DiNP: Di-isononyl phthalate, DINCH: 1,2-Cyclohexane dicarboxylic acid, diisononyl ester.

[a] Correlations were restricted to mother-child pairs with measures at all four time points.

[b] ICCs were computed for all mothers with a sample at both the second and third trimesters.

[c] Molar sum of all metabolites from the same parent compound.

3.4. Time trends

Maternal urinary concentrations of MiBP, MBzP, the five DEHP metabolites and oxo-MINP significantly decreased between 2014 and 2017 (Mann-Kendall p-values < 0.05), while concentrations of oh-MiNP and of the two DINCH metabolites significantly increased (Mann-Kendall p-values < 0.05, Fig. 2, Table S4). Maternal urinary concentrations of MEP, MnBP, oh-MPHP and cx-MiNP did not show a trend across years of collection (Mann-Kendall p-values > 0.1).

3.5. Intra-individual variability

When correlating urinary concentrations of the second and the third trimester of pregnancy we observed moderate Spearman correlation coefficients (rho between 0.50 and 0.65) for metabolites of low molecular weight phthalates (MEP, MnBP, MiBP) as well as for MBzP. Low correlation (rho < 0.42) was observed for the other metabolites. Using ICC instead of Spearman correlations led to similar conclusions (Table 2). Correlations between urinary concentrations at two and twelve months were all below 0.26. Finally, when relating maternal and infant concentrations, except for MBzP at two months, all Spearman correlation coefficients were lower than 0.40, with some even being negative (Table 2). For a given phthalate, using the molar sum lead to similar correlation coefficients than those observed for the individual metabolites.

3.6. Within-sample correlation across biomarkers

Within-sample correlation coefficients were high for metabolites of the same parent (Supplemental Material, Figure S1). As an example, correlation coefficients for oh-MINCH and oxo-MINCH, metabolites of DINCH was >0.95 at each sampling period. For DEHP, correlations between the oxidized metabolites (MEOHP, MECPP, MEHHP and MMCHP) were all above 0.71 at all sampling periods. However, MEHP, was only highly correlated to the DEHP oxidized metabolites in maternal samples (rho > 0.65). In infants, correlations between MEHP and the other DEHP metabolites were all below 0.19 at two months and below 0.39 at twelve months.

Regarding correlations between metabolites from different phthalates, at both two and twelve months, oxydized DEHP metabolites were highly correlated (rho > 0.6) with the two DiNP metabolites as well as with oh-MPHP in the twelve-month urine samples (Supplemental Material, Figure S1). These correlations were not as strong in the pregnancy samples (rho between 0.34 and 0.61).

4. Discussion

In our study population recruited in France between 2014 and 2017, we detected phthalate and DINCH metabolites in most pooled urine samples, suggesting widespread exposure in both pregnant women and infants. Maternal urinary concentrations of several metabolites (MiBP, MBzP, DEHP metabolites, oxo-MINP) significantly decreased with time, while a few others (oh-MiNP, DINCH metabolites) increased with time. Regarding differences across time points, DINCH metabolite medians were higher for twelve month infants compared to during pregnancy, suggesting higher circulating levels in infants. For both DEHP and DiNP, we observed differences in the relative distribution of the monoester and oxidative metabolites, with some ratios being higher at two months compared to the other time points. This suggests that metabolism for these phthalates may differ with age. Despite the fact that we relied on pools of many repeated urine samples, within-subject correlations were not very high, except for low molecular weight phthalate metabolites (MEP, MnBP, MiBP), MBzP and MEHP during pregnancy.

4.1. Strengths and limitations

This study is one of the first relying on multiple urine specimens to assess exposure during both pregnancy and infancy. The high number of

samples collected (median 21 samples at each pregnancy time point and 6 at each infancy time point) is expected to improve accuracy compared to previous studies relying on spot samples (Perrier et al., 2016; Vernet et al., 2019). Assessing exposure biomarker concentrations in pools of samples collected over seven consecutive days allowed us to assess variability between periods but prevented us to explore within-day and within-week variability. In addition to the metabolites classically assessed, we measured urinary concentrations of DINCH and DPHP metabolites. The parent compounds of these two metabolites are used in replacement of high molecular weight phthalates such as DEHP (Koch et al., 2013; Schmidtkunz et al., 2019; Schutze et al., 2014), and no data on exposure to these chemicals was available so far in the French population. While their medians were among the lowest observed in our study population, the high frequencies of detection for these metabolites (99–100% in the pregnancy and twelve-month infant samples, and up to 64% at two months of age) suggested widespread exposure to these new plasticizers.

4.2. Exposure to phthalates during pregnancy

Urinary concentrations were of similar range than those reported in two pregnancy subcohorts recruited at the same time in Barcelona (Spain, N = 52) and Oslo (Norway, N = 55 (Warembourg et al., 2019)) and that relied on a similar design for urine collection during pregnancy (Supplemental Material, Figure S2). Compared to the EDEN cohort, recruiting French pregnant women in 2003–2006, median urinary concentrations were lower in our cohort for most metabolites except cx-MINP, a metabolite of DiNP. In line with these findings, we observed a negative time trend between 2014 and 2017 in maternal urinary concentration of several phthalate metabolites (DEHP, BBzP and DiBP), while maternal urinary concentrations of DINCH metabolites increased during this period. Positive time trends have been reported for DINCH metabolites in Sweden (Gyllenhammar et al., 2017), Germany (Kasper-Sonnenberg et al., 2019) and the USA (Silva et al., 2013) and are consistent with the increase in use of this plasticizer reported in Europe (European Chemicals Agency, 2013; HBM4EU, 2018). This, along with the higher urinary median concentrations observed at twelve months for DINCH metabolites in our cohort, highlights the need of detailed investigations to warrant its safety.

Surprisingly, in our cohort we observed opposite time trends for two DiNP metabolites. Concentrations of oxo-MiNP decreased between 2014 and 2017 while concentrations of oh-MiNP increased. This might results from changes in DiNP uses over time since DiNP metabolism might differ according to routes of exposure (i.e, dermal versus oral (Koch et al., 2012)).

Except for MEP, median urinary concentrations observed in our cohort were higher than those reported among ELFE, the French national cohort recruited in 2011 (Supplemental Material, Figure S2), for which urine had been sampled in the maternity just before delivery, possibly after several hours without eating or using any cosmetic product. This difference was unexpected and may reflect the differences in timing of urine collection, as our maternal samples were collected several weeks before delivery.

4.3. Exposure to phthalates during infancy

Frequencies of detection were high for most metabolites, suggesting widespread exposure to phthalates among young infants. The lower medians observed for all metabolites, except MEHP, at two months compared to twelve months and pregnancy may reflect age differences in exposure, in urine dilution, since median SG was also markedly lower at two months compared to the other periods as well as in metabolism.

For DEHP, we observed high ratios of monoester to oxidized metabolites at two months. MEHP results from human metabolism but can also be generated by other processes such as enzymatical and microbiological hydrolysis (Koch et al., 2003), making this metabolite at risk of external contamination. However, the fact that the monoester to oxidized metabolite ratio was elevated only at two months and not at twelve months – when the exact same protocol was used for urine collection – led us to conclude that the high ratios at two months were unlikely to result of external contamination. Interestingly, the distribution of each oxidized metabolite also differed by period. High MECPP/MEOHP and cx-MiNP/oxo-MinP ratios have also been reported in newborns (Enke et al., 2013), suggesting different DEHP and DiNP metabolism in the first months of life. These differences may attenuate with advanced child age as suggested by the values of the twelve-month ratios, which for DEHP metabolites, were closer to those observed during pregnancy than at two months. This was supported by another study that shows decreased monoester to oxidized metabolite ratio with increased child age (Navaranjan et al., 2019).

Compared to studies in other countries among children aged between one and six months, median urinary concentrations observed in our cohort at two months were overall lower than those reported in Germany (Völkel et al., 2014), Canada (Arbuckle et al., 2016; Navaranjan et al., 2019) and Sweden (Carlstedt et al., 2013), except for MEHP for which urinary median was higher in our cohort compared to Germany (Völkel et al., 2014) and Canada (Navaranjan et al., 2019) (Supplemental Material, Figure S3). Similarly, compared to other cohorts collecting urine samples between one and two years of age (Fromme et al., 2013; Navaranjan et al., 2019; Watkins et al., 2014), urinary levels were overall lower in our cohort, except again for MEHP for which slightly higher concentrations were observed (medians of 2.81 μ/L compared to 2.10 and 1.80 in this paper, Fromme et al. (2013) and Navaranjan et al. (2019)).

4.4. Temporal variability

The highest correlation coefficients and ICCs between both pregnancy samples were observed for MEP, MnBP, MiBP, MBzP and MEHP. This is in line with results of another study relying on pooled samples (Casas et al., 2018), and suggested more stable sources of exposure or less varying toxicokinetics during pregnancy for these metabolites than for the other assessed in the study. Compared to previous studies relying on repeated spot urine samples collected during pregnancy (reviewed by Casas et al., 2018), ICCs were overall higher in our study, likely because we relied on pools of repeated urine samples collected over a week, instead of spots. While improved compared to studies relying on spots, most of the ICCs were below 0.40 suggesting high variability during pregnancy for most metabolites assessed.

Correlation coefficients between samples collected in infancy were lower than those observed during pregnancy, and were all below 0.30. This high variability likely reflected the major changes occurring between two and twelve months that can be related to phthalate exposure (e.g., food diversification, increased hand to mouth behavior and increased time spent on the floor) as well as difference in phthalate metabolism with age.

The low correlation coefficients observed within-individual for most phthalates and between mother and their infants highlight the difficulties to assess exposure over long time windows for phthalates. Consequently, detailed knowledge about sensitive windows of exposure are needed to efficiently design urine sampling protocols in epidemiological studies exploring health effects; without such a priori information, collecting biospecimens at different time windows and pooling them within-period instead of doing a unique pool of all the samples collected at different time points, appears to be a safe option. Even though this increases analytical cost, it reduces measurement error especially if the sensitive window is a specific trimester of the pregnancy.

5. Conclusion

Exposure to phthalates and their substitutes such as DINCH is widespread among French pregnant women and infants. Further

biomonitoring studies should make an effort to collect biospecimens in infancy since we reported higher urinary concentrations during this period than pregnancy for some metabolites. The low to moderate correlations observed across time points illustrates the need of repeated biospecimens collected in the assumed sensitive time windows in etiological studies aiming at studying the associations between these short half-life chemicals and health outcomes.

Author statements

Matthieu Rolland: Statistical analysis, writing original draft, conceptualization, Sarah Lyon-Caen: Data collection, reviewing & editing draft. Amrit K. Sakhi: assessment of phthalate biomarkers in urine, reviewing & editing draft. Azemira Sabaredzovic: assessment of phthalate biomarkers in urine, reviewing & editing draft. Cathrine Thomsen: assessment of phthalate biomarkers in urine, reviewing & editing draft. Isabelle Pin: Investigation, Rémy Slama: Investigation, reviewing & editing draft, Claire Philippat: Investigation, writing original draft, funding acquisition, conceptualization.

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Declaration of competing interest

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

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Appendix A. Supplementary data

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References

Arbuckle, T.E., Fisher, M., MacPherson, S., Lang, C., Provencher, G., LeBlanc, A., et al., 2016. Maternal and early life exposure to phthalates: the Plastics and Personal-care Products use in Pregnancy (P4) study. Sci. Total Environ. 551–552, 344–356. https://doi.org/10.1016/j.scitotenv.2016.02.022.

Carlstedt, F., Jönsson, B.a.G., Bornehag, C.-G., 2013. PVC flooring is related to human uptake of phthalates in infants. Indoor Air 23, 32–39. https://doi.org/10.1111/j.1600-0668.2012.00788.x.

Casas, M., Basagaña, X., Sakhi, A.K., Haug, L.S., Philippat, C., Granum, B., et al., 2018. Variability of urinary concentrations of non-persistent chemicals in pregnant women and school-aged children. Environ. Int. 121, 561–573. https://doi.org/10.1016/j.envint.2018.09.046.

Enke, U., Schleussner, E., Palmke, C., Seyfarth, L., Koch, H.M., 2013. Phthalate exposure in pregnant women and newborns - the urinary metabolite excretion pattern differs distinctly. Int. J. Hyg Environ. Health 216, 735–742. https://doi.org/10.1016/j.ijheh.2013.01.006.

European Chemicals Agency, 2013. Evaluation of New Scientific Evidence Concerning DINP and DIDP in Relation to Entry 52 of Annex XVII to REACH Regulation (EC) No 1907/2006.

Frederiksen, H., Kuiri-Hanninen, T., Main, K.M., Dunkel, L., Sankilampi, U., 2014. A longitudinal study of urinary phthalate excretion in 58 full-term and 67 preterm infants from birth through 14 months. Environ. Health Perspect. 122, 998–1005. https://doi.org/10.1289/ehp.1307569.

Fromme, H., Gruber, L., Schuster, R., Schlummer, M., Kiranoglu, M., Bolte, G., et al., 2013. Phthalate and di-(2-ethylhexyl) adipate (DEHA) intake by German infants based on the results of a duplicate diet study and biomonitoring data (INES 2). Food Chem. Toxicol. 53, 272–280. https://doi.org/10.1016/j.fct.2012.12.004.

Gyllenhammar, I., Glynn, A., Jönsson, B.A.G., Lindh, C.H., Darnerud, P.O., Svensson, K., et al., 2017. Diverging temporal trends of human exposure to bisphenols and plastizisers, such as phthalates, caused by substitution of legacy EDCs? Environ. Res. 153, 48–54. https://doi.org/10.1016/j.envres.2016.11.012.

Hauser, R., Calafat, A.M., 2005. Phthalates and human health. Occup. Environ. Med. 62, 806–818. https://doi.org/10.1136/oem.2004.017590.

Hbm4Eu, 2018. Phthalates and Hexamoll DINCH ®Currently available HBM-evidence in Europe and ongoing work in HBM4EU in relation to identified policy. HBM4EU Workshop of Task 5.5 –phased Action Plan.

Kasper-Sonnenberg, M., Koch, H.M., Apel, P., Rüther, M., Pälmke, C., Brüning, T., et al., 2019. Time trend of exposure to the phthalate plasticizer substitute DINCH in Germany from 1999 to 2017: biomonitoring data on young adults from the Environmental Specimen Bank (ESB). Int. J. Hyg Environ. Health. https://doi.org/10.1016/j.ijheh.2019.07.011.

Koch, H.M., Haller, A., Weiss, T., Käfferlein, H.-U., Stork, J., Brüning, T., 2012. Phthalate exposure during cold plastisol application–a human biomonitoring study. Toxicol. Lett. 213, 100–106. https://doi.org/10.1016/j.toxlet.2011.06.010.

Koch, H.M., Rossbach, B., Drexler, H., Angerer, J., 2003. Internal exposure of the general population to DEHP and other phthalates - determination of secondary and primary phthalate monoester metabolites in urine. Environ. Res. 93, 177–185. https://doi.org/10.1016/s0013-9351(03)00083-3.

Koch, H.M., Schutze, A., Palmke, C., Angerer, J., Bruning, T., 2013. Metabolism of the plasticizer and phthalate substitute diisononyl-cyclohexane-1,2-dicarboxylate (DINCH((R))) in humans after single oral doses. Arch. Toxicol. 87, 799–806. https://doi.org/10.1007/s00204-012-0990-4.

LaKind, J.S., Idri, F., Naiman, D.Q., Verner, M.-A., 2019. Biomonitoring and nonpersistent chemicals-understanding and addressing variability and exposure misclassification. Curr Environ Health Rep 6, 16–21. https://doi.org/10.1007/s40572-019-0227-2.

Lyon-Caen, S., Siroux, V., Lepeule, J., Lorimier, P., Hainaut, P., Mossuz, P., et al., 2019. Deciphering the impact of early-life exposures to highly variable environmental factors on foetal and child health: design of SEPAGES couple-child cohort. Int. J. Environ. Res. Publ. Health 16. https://doi.org/10.3390/ijerph16203888.

Nakiwala, D., Vernet, C., Lyon-Caen, S., Lavorel, A., Rolland, M., Cracowski, C., et al., 2020. Use of personal care products during pregnancy in relation to urinary concentrations of select phenols: a longitudinal analysis from the SEPAGES feasibility study. Int. J. Hyg Environ. Health 227, 113518. https://doi.org/10.1016/j.ijheh.2020.113518.

Navaranjan, G., Takaro, T.K., Wheeler, A.J., Diamond, M.L., Shu, H., Azad, M.B., et al., 2019. Early life exposure to phthalates in the Canadian Healthy Infant Longitudinal Development (CHILD) study: a multi-city birth cohort. J. Expo. Sci. Environ. Epidemiol. https://doi.org/10.1038/s41370-019-0182-x.

Perrier, F., Giorgis-Allemand, L., Slama, R., Philippat, C., 2016. Within-subject pooling of biological samples to reduce exposure misclassification in biomarker-based studies. Epidemiology 27, 378–388. https://doi.org/10.1097/ede.0000000000000460.

Philippat, C., Calafat, A.M., 2020. Comparison of strategies to efficiently combine repeated urine samples in biomarker-based studies. Environ. Res. 110275 https://doi.org/10.1016/j.envres.2020.110275.

Preau, J.L., Wong, L.-Y., Silva, M.J., Needham, L.L., Calafat, A.M., 2010. Variability over 1 Week in the urinary concentrations of metabolites of diethyl phthalate and di(2-ethylhexyl) phthalate among eight adults: an observational study. Environ. Health Perspect. 118, 1748–1754. https://doi.org/10.1289/ehp.1002231.

Sabaredzovic, A., Sakhi, A.K., Brantsaeter, A.L., Thomsen, C., 2015. Determination of 12 urinary phthalate metabolites in Norwegian pregnant women by core-shell high performance liquid chromatography with on-line solid-phase extraction, column switching and tandem mass spectrometry. Journal of chromatography B, Analytical technologies in the biomedical and life sciences 1002, 343–352. https://doi.org/10.1016/j.jchromb.2015.08.040.

Schmidtkunz, C., Gries, W., Weber, T., Leng, G., Kolossa-Gehring, M., 2019. Internal exposure of young German adults to di(2-propylheptyl) phthalate (DPHP): trends in 24-h urine samples from the German Environmental Specimen Bank 1999-2017. Int. J. Hyg Environ. Health 222, 419–424. https://doi.org/10.1016/j.ijheh.2018.12.008.

Schutze, A., Kolossa-Gehring, M., Apel, P., Bruning, T., Koch, H.M., 2014. Entering markets and bodies: increasing levels of the novel plasticizer Hexamoll(R) DINCH(R) in 24 h urine samples from the German Environmental Specimen Bank. Int. J. Hyg Environ. Health 217, 421–426. https://doi.org/10.1016/j.ijheh.2013.08.004.

Silva, M.J., Jia, T., Samandar, E., Preau, J.L., Calafat, A.M., 2013. Environmental exposure to the plasticizer 1,2-cyclohexane dicarboxylic acid, diisononyl ester (DINCH) in U.S. adults (2000-2012). - PubMed - NCBI. Environ. Res. 126, 159:63.

Urbancova, K., Lankova, D., Sram, R.J., Hajslova, J., Pulkrabova, J., 2019. Urinary metabolites of phthalates and di-iso-nonyl cyclohexane-1,2-dicarboxylate (DINCH)-Czech mothers' and newborns' exposure biomarkers. Environ. Res. 173, 342–348. https://doi.org/10.1016/j.envres.2019.03.067.

Vernet, C., Philippat, C., Agier, L., Calafat, A.M., Ye, X., Lyon-Caen, S., et al., 2019. An empirical validation of the within-subject biospecimens pooling approach to minimize exposure misclassification in biomarker-based studies. Epidemiology 30, 756–767. https://doi.org/10.1097/EDE.0000000000001056.

Vernet, C., Philippat, C., Calafat, A.M., Ye, X., Lyon-Caen, S., Siroux, V., et al., 2018. Within-day, between-day, and between-week variability of urinary concentrations of phenol biomarkers in pregnant women. Environ. Health Perspect. 126, 037005 https://doi.org/10.1289/EHP1994.

Völkel, W., Kiranoglu, M., Schuster, R., Fromme, H., HBMnet, 2014. Phthalate intake by infants calculated from biomonitoring data. Toxicol. Lett. 225, 222–229. https://doi.org/10.1016/j.toxlet.2013.12.012.

Warembourg, C., Basagaña, X., Seminati, C., de Bont, J., Granum, B., Lyon-Caen, S., et al., 2019. Exposure to phthalate metabolites, phenols and organophosphate pesticide metabolites and blood pressure during pregnancy. Int. J. Hyg Environ. Health 222, 446–454. https://doi.org/10.1016/j.ijheh.2018.12.011.

Watkins, D.J., Eliot, M., Sathyanarayana, S., Calafat, A.M., Yolton, K., Lanphear, B.P., et al., 2014. Variability and predictors of urinary concentrations of phthalate metabolites during early childhood. Environmental science & technology. https://doi.org/10.1021/es501744v.