Science of the Total Environment 551–552 (2016) 344–356

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Maternal and early life exposure to phthalates: The Plastics and Personal-care Products use in Pregnancy (P4) study

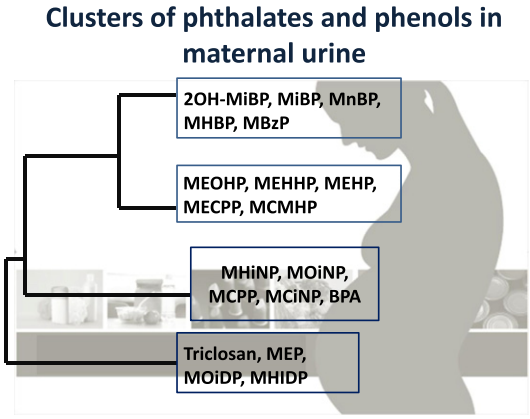
Tye E. Arbuckle [a,*], Mandy Fisher [a], Susan MacPherson [a], Carly Lang [a], Gilles Provencher [b], Alain LeBlanc [b], Russ Hauser [c], Mark Feeley [d], Pierre Ayotte [b,e], Angelica Neisa [a], Tim Ramsay [f,g], George Tawagi [h]

[a] Population Studies Division, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, ON, Canada
[b] Centre de toxicologie du Québec (CTQ), Institut national de santé publique du Québec (INSPQ), Québec, QC, Canada
[c] Department of Environmental Health and Epidemiology, Harvard School of Public Health, Boston, MA, United States
[d] Bureau of Chemical Safety, Health Products and Food Branch, Health Canada, Ottawa, ON, Canada
[e] Axe Santé des populations et pratiques optimales en santé, Centre de recherche du CHU Québec, Québec, QC, Canada
[f] Clinical Epidemiology Program, Ottawa Hospital Research Institute, Ottawa, ON, Canada
[g] Department of Obstetrics and Gynecology, University of Ottawa, Ottawa, ON, Canada
[h] Department of Obstetrics and Perinatal Medicine, The Ottawa Hospital, Ottawa, ON, Canada

HIGHLIGHTS

- Data are limited on levels of phthalates in various maternal-fetal matrices.
- Metabolites were measured in maternal and infant urine, meconium and breast milk.
- Maternal urinary levels in Ottawa Canada generally lower than in European studies.
- Postnatal maternal and infant urinary MBzP highly correlated
- Results support some maternal-fetal-infant transfer of phthalates.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 7 December 2015
Received in revised form 15 January 2016
Accepted 3 February 2016
Available online 13 February 2016

ABSTRACT

Phthalates are a group of chemicals found in a number of consumer products; some of these phthalates have been shown to possess estrogenic activity and display anti-androgenic effects. While a number of biomonitoring studies of phthalates in pregnant women and infants have been published, there is a paucity of data based on both multiple sampling periods and in different matrices. Phthalate metabolites were measured in 80 pregnant

Abbreviations: 2OH-MiBP, 2-hydroxy-mono-isobutyl phthalate; BBzP, butyl benzyl phthalate; BPA, bisphenol A; DCHP, dicyclohexyl phthalate; DEHP, di-(2-ethylhexyl) phthalate; DEP, diethyl phthalate; DiBP, di-isobutyl phthalate; DiDP, di-isodecyl phthalate; DiNP, di-isononyl phthalate; DMP, dimethyl phthalate; DnBP, di-*n*-butyl phthalate; DnOP, di-*n*-octyl phthalate; MBzP, mono-benzyl phthalate; MCHP, mono-cyclohexyl phthalate; MCiNP, mono(carboxy-isononyl) phthalate; MCiOP, mono(carboxy-isooctyl) phthalate; MCMHP, mono (2-carboxy-methylhexyl) phthalate; MCPP, mono-3-carboxypropyl phthalate; MECPP, mono(2-ethyl-5-carboxy-pentyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, mono-2-ethylhexyl phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEP, mono-ethyl phthalate; MHBP, mono-3-hydroxy-*n*-butyl phthalate; MHiDP, monohydroxyisodecyl phthalate; MHiNP, mono(hydroxy-isononyl) phthalate; MiBP, mono-isobutyl phthalate; MiNP, mono-isononyl phthalate; MMP, mono-methyl phthalate; MnBP, mono–*n*-butyl phthalate; MnOP, mono-*n*-octyl phthalate; MOiDP, mono-(2-propyl-6oxoheptyl) phthalate; MOiNP, mono(oxo-isononyl) phthalate.

* Corresponding author at: Population Studies Division, Healthy Environments and Consumer Safety Branch, Health Canada, 50 Colombine Dr., AL 0801A, Ottawa, ON K1A 0K9, Canada.
E-mail address: Tye.Arbuckle@hc-sc.gc.ca (T.E. Arbuckle).

http://dx.doi.org/10.1016/j.scitotenv.2016.02.022
0048-9697/Crown Copyright © 2016 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Editor: Adrian Covaci

Keywords:
Metabolites
Urine
Meconium
Infant
Pregnancy
Breast milk

women and their infants in Ottawa Canada (2009–2010) in urine, meconium and breast milk collected at various time periods pre- and post-parturition. At least 50% of the women had at least one urine sample greater than the limit of detection (LOD) for the various phthalate metabolites, with the exception of mono-*n*-octyl phthalate (MnOP), mono-isononyl phthalate (MiNP) and mono(carboxy-isooctyl) phthalate (MCiOP). Four major clusters of maternal urinary metabolites were identified. Among infants (n = 61), the following metabolites were rarely (< 10%) detected: mono-cyclohexyl phthalate (MCHP), mono-isononyl phthalate (MiNP), mono-methyl phthalate (MMP), and mono-*n*-octyl phthalate (MnOP). While mono-benzyl phthalate (MBzP), mono-3-carboxypropyl phthalate (MCPP), MEHHP, and MEOHP were frequently detected in maternal urines at any time point, these metabolites were rarely detected in breast milk. Maternal urinary concentrations of MEP and the DEHP metabolites were higher in samples collected during pregnancy than postnatally. No statistically significant differences were observed in infant's urinary phthalate concentrations between breast-fed and bottle-fed infants. Significant correlations were observed between maternal urinary MEHHP (r = 0.35), MEOHP (r = 0.35) and MEP (r = 0.37) collected at <20 weeks gestation with levels in meconium and between MBzP (r = 0.78) and MEP (r = 0.56) in maternal and infant urine collected 2–3 months after birth. These results suggest at least some maternal-fetal-infant transfer of phthalates and that meconium may be a useful matrix for measuring in utero exposure to phthalates.
Crown Copyright © 2016 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Prenatal and early post-natal exposures to elevated levels of certain environmental chemicals may impact the health of the fetus and child and have long lasting effects into adulthood. The development of sensitive and specific biomarkers of exposure which can be measured in multiple matrices and at increasingly lower limits of detection has facilitated research on potential health effects of chemicals that we are exposed to in our daily life such as phthalates. Phthalates are a family of chemicals found in a number of consumer products ranging from intravenous tubing to vinyl flooring. In Canada (Health Canada, 2011), the allowable concentrations of certain phthalates are restricted in soft vinyl toys and child care articles. Diet, dust ingestion, inhalation, dermal absorption and direct dermal uptake from air are potential contributors to human exposure (Larsson et al., 2014; Serrano et al., 2014; Xu et al., 2015; Bekö et al., 2013; Weschler et al., 2015). In Europe, the major sources of exposure to dimethyl (DMP), diethyl (DEP), butylbenzyl (BBzP), diisononyl (DiNP), and diisodecyl (DiDP) phthalates are from the use of consumer products and indoor sources, whereas food is a major source of exposure to diisobutyl (DiBP), di-*n*-butyl (DnBP), and di-2-ethylhexyl (DEHP) phthalates (Wormuth et al., 2006). Phthalates do not bioaccumulate and are mainly excreted in urine with phthalate mono esters generally having shorter elimination half-lives than the oxidized metabolites. For example, for DiBP, the estimated half-life for mono-isobutyl phthalate (MiBP) is 3.9 h, in contrast to 4.1 and 4.2 h for the oxidized metabolites 3- and 2-hydroxy MiBP, respectively (Koch et al., 2012).

Numerous phthalates have been shown to possess estrogenic activity and show anti-androgenic effects (reviewed in Kiyama and Wada-Kiyama, 2015; Marie et al., 2015). Although there is inconsistency in the literature, a wide range of potential human health effects have been associated with prenatal exposure to various phthalates including preterm birth (Ferguson et al., 2014a, 2014b), adverse effects on child behavior, intellectual and motor development (Whyatt et al., 2012; Kim et al., 2011; Engel et al., 2009; Factor-Litvak et al., 2014), and reduced anogenital distance in male infants (Bornehag et al., 2015; Bustamante-Montes et al., 2013; Suzuki et al., 2012; Swan et al., 2015). A systematic review of the toxicological literature has concluded that there is sufficient evidence to suggest that phthalates are reproductive and developmental toxicants, albeit at concentrations higher than those observed in contemporary human biomonitoring studies (Kay et al., 2013). The European Union (2015) has classified DEHP, DnBP, DiBP and BBzP as reproductive toxicants.

While several biomonitoring studies of phthalates in pregnant women that measured exposure at multiple time points (Valvi et al., 2015; Cantonwine et al., 2014; Braun et al., 2012; Adibi et al., 2008) have been published, there is a paucity of data that has examined the correlations between maternal and infant exposures in different matrices. The P4 Study (Plastics and Personal-care Product use in Pregnancy) was designed to measure exposure to phthalates throughout pregnancy and in the early post-natal period in a healthy Canadian population. Measuring phthalate metabolites in various matrices will assist with estimating exposure for the fetus and young infant.

2. Methods

2.1. Study population

The P4 Study has been described in detail elsewhere (Fisher et al., 2015; Arbuckle et al., 2015). Pregnant women (<20 weeks gestation) were recruited at early prenatal clinics in Ottawa Canada between December 2009 and December 2010. In addition, posters and pamphlets were distributed in the obstetrical and ultrasound clinics of The Ottawa Hospital and physician offices. To be eligible, women had to be able to communicate in English or French, be 18 years or older and planning on delivering locally. Excluded were women with major medical conditions such as renal disease, epilepsy, heart disease and cancer or with known fetal abnormalities or major malformations, and women already participating in 2 or more research studies. The women were followed prospectively through pregnancy and up to 2–3 months postnatally. The study was approved by human studies ethics committees at Health Canada and all participating hospitals; all participants signed informed consent forms.

2.2. Maternal urine collection

Women collected serial urine samples over a 24-h period at <20 weeks gestation on a weekday (T1a) and/or weekend day (T1b), as well as spot urine voids during the 2nd (24–28 weeks) (T2) and 3rd trimesters (32–36 weeks) (T3) and 2–3 months post-partum (T5). Women were asked to collect and record the dates and times of all urine voids over the 24-h periods. For the single spot urine voids (T2, T3 and T5), the time of the void was noted. Urine was collected in prescreened urine cups (polypropylene) and kept cool (4 °C) to avoid degradation of the chemical until aliquoted within 36 h of collection and then stored at −80 °C.

2.3. Infant urine collection

Infant urine was collected within the first month of life (T4) and 2–3 months post-partum (T5) in prescreened newborn urine-bags (U-bags) (Hollister Inc. Libertyville, IL and Mabis Healthcare, Waukegan, IL). The genital area was cleansed using only warm water and a washcloth, allowed to air dry and then the U-bag was attached (maximum 4 h at a time). Multiple voids on the same day were combined in the same sterile 30 mL Nalgene® container to obtain sufficient volume. The date and time were noted and the urine refrigerated. Within 24 h

of collection, the urine was delivered to the lab for processing and aliquoting and then frozen at −80 °C.

2.4. Meconium collection

Prescreened Mère Hélène® bioliners (Mère Hélène, Quebec Canada) were inserted into the diapers to facilitate collection of the meconium. A wooden spatula was used to transfer the meconium collected within the first two days after delivery to a 50 mL Sarstedt® tube and frozen within 72 h of collection at −20 °C. Staff was asked to note if the diaper was wet with urine and if any lotions, powders, wipes or creams had been applied to the baby's bottom.

2.5. Breast milk and/or formula collection

The women were provided with instructions to collect a sample of breast milk in a 150 mL glass jar 2–3 months post-partum. Either the Medela® (Medela International, Zug, Switzerland) manual breast pump provided or hand expression could be used to collect the milk. Women were asked not to use any creams or cleansers on her breast prior to pumping or expressing. Women who were not breastfeeding or who were supplementing breastfeeding with formula were asked to provide a sample of infant formula in the additional glass jar provided. All samples were kept refrigerated until delivered to the hospital where samples were aliquoted into 30 mL Nalgene® containers and then frozen at −20 °C, prior to shipping to the laboratory.

2.6. Screening for potential contamination

All materials used to collect or store biological samples were prescreened for potential phthalate contamination. Field blanks (Steril.O reagent grade deionized distilled water) were included as part of the protocol to assess potential risks of contamination at the collection and aliquoting premises and during processing and storage of the specimens prior to analysis.

For the home collection of maternal urine over the 24-h periods (T1a, T1b), in addition to the urine collection cups and freezer packs, each participant's cooler bag also contained a field blank. This blank was appropriately labeled and underwent the same conditions as the urine samples (i.e. kept in the cooler bag and then delivered to the hospital lab with the urine samples, aliquoted, frozen and shipped to the lab). A glass jar containing 50 mL of deionized water was included with the empty containers in the participant kit for the breast milk collection and underwent similar handling and processing to the breast milk or formula collection.

To prepare field blanks for the infant urines, 5 mL of deionized water was added to sample U-bags, jostled to simulate infant movement while wearing the bag and left to sit at room temperature for at least 30 min, then transferred from the U-bag into sterile 30 mL Nalgene® containers. In addition, a supplemental study was conducted on the infant urine bags (Mabis® Healthcare U-Bag newborn urine collector REF 7535, Lot # 2E13 and 1A06) after specimen collections were completed. Five milliliters of deionized water was added to the U-bag, using a disposable glass pipette previously rinsed with 5 mL of methanol. The U-bag was placed in an incubator with an agitator (Innova 4230) set to 50 RPM at a temperature of 35 °C for 4 h and then refrigerated until analysis. The contents of the bag were transferred into a 30 mL Nalgene® container and a 1 mL sample was extracted. The previous steps were repeated with two other U-bags. These steps were also repeated using low phthalate concentration urine (previously assayed to know the urine concentrations of phthalates) instead of deionized water.

2.7. Data collection

Participants completed a short questionnaire at recruitment and at each contact throughout the study. The questionnaire collected information on occupation, socio-economic status, obstetrical history, smoking and the current pregnancy. Data on infant feeding and care practices were also collected post-partum. Additional information on the pregnancy and the birth were abstracted from medical charts.

2.8. Laboratory chemical analysis for phthalate metabolites

Samples were shipped on dry ice to the laboratory where they were stored frozen until analysis. The Centre de toxicologie du Québec, Institut national de santé publique du Québec conducted all the biospecimen analyses for phthalate metabolites. Specific gravity was measured using a refractometer (Refractometer UG-1, Atago # 3461) on urine that had undergone a freeze-thaw cycle. Specific gravity was measured as correcting for urine dilution using creatinine is likely problematic for populations undergoing physiological changes in renal function such as pregnant women (Abduljalil et al., 2012; Gordon, 2012) and young infants (Matos et al., 1999; Quigley, 2012) and for chemicals that are rapidly metabolized and where exposure patterns may not be continuous and ongoing such as DEHP (Lorber et al., 2011).

Initially the biospecimens were analyzed for 11 phthalate metabolites (group 1) for which the laboratory had previously developed methods: MnBP, MCPP, MEP, MBzP, mono-methyl phthalate (MMP), mono-cyclohexyl phthalate (MCHP), mono-isononyl phthalate (MiNP), mono-*n*-octyl phthalate (MnOP), mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-oxo-hexyl) phthalate Mono-(2-ethyl-5-oxo-hexyl) phthalate (MEOHP), and mono-(2-ethyl-5-hydroxy-hexyl) phthalate (MEHHP). Subsequently, new methods were developed for additional important phthalate metabolites: mono-3-hydroxy-*n*-butyl phthalate (MHBP), MCiOP, mono(hydroxy-isononyl) phthalate (MHiNP), mono (2-carboxy-methylhexyl) phthalate (MCMHP), mono(2-ethyl-5-carboxy-pentyl) phthalate (MECPP), MiBP, 2-hydroxy-mono-isobutyl phthalate (2OH-MiBP), mono(oxo-isononyl) phthalate (MOiNP), monohydroxyisodecyl phthalate (MHiDP), mono(carboxy-isononyl) phthalate (MCiNP),and mono-(2-propyl-6oxoheptyl) phthalate (MOiDP). However, these latter metabolites (group 2) were only measured in the serial urine samples of women who had contributed sufficient urine samples in both the T1a and T1b collection periods (n = 31 women). Due to an issue with the accuracy of several commercial phthalate metabolite standards (Langlois et al., 2012), correction factors were developed and applied to the phthalate results (Langlois et al., 2014).

2.8.1. Urine

For the first group of phthalate metabolites, following enrichment with analogues of phthalates isotopically labeled with carbon 13 and enzymatic deconjugation using β-glucuronidase, the phthalate monoester compounds were extracted by solid phase extraction with anion exchange media using the Janus robotic system. The extracts were brought to dryness, solubilized in water and analyzed by LC–MS–MS in MRM mode with an electrospray ion source in negative mode (Waters Acquity UPLC; tandem mass detector Waters Quattro Premier Xe). The limits of detection for the phthalate metabolites ranged from 0.2 to 7.0 μg/L. As there was a contamination problem in several analytical sequences, no infant urine MEHP results were reported.

For the phthalate metabolites of the second group, the urine sample was first enriched with analogues of phthalates isotopically labeled with carbon 13 (or deuterium) and diluted with water. Then an enzymatic deconjugation using β-glucuronidase was performed. After liquid-liquid extraction with a hexane/ethyl acetate mixture, the organic phase was evaporated. The extract was then solubilized in an water: acetonitrile solution and analyzed by LC-MS-MS in MRM mode with an electrospray ion source in negative mode (Waters Acquity UPLC; tandem mass detector Waters TQ-S or Quattro Premier Xe). The limits of detection for this group of phthalate metabolites ranged from 0.056 to 0.3 μg/L.

2.8.2. Breast milk and infant formula

Breast milk contains active esterases which can hydrolyze phthalate diesters into their respective monoesters. It is recommended that milk be pre-treated to denature the milk enzymes and avoid overestimating the concentration of phthalate metabolites present from diester contamination during collection, storage or processing (Calafat et al., 2004b). For the breast milk, the esterases in the milk were first de-activated by adding 125 μL of 1 M H3PO4 to 0.5 mL of milk prior to extraction. Following enzymatic deconjugation using β-glucuronidase and enrichment with analogues of phthalates isotopically labeled with carbon 13, the analytes were extracted on a solid phase extraction cartridge with an anion exchange support. The extracts are brought to dryness, dissolved in ammonium acetate buffer and analyzed by LC-MS-MS in MRM mode with an electrospray ion source in negative mode (Waters Acquity UPLC; tandem mass detector Waters TQ-S). The limits of detection for this group of phthalate metabolites (group 1) ranged from 9.4 to 91 ng/L.

2.8.3. Meconium

For the meconium method, 125 μL of 1 M H3PO4 per g of meconium was added to denature the endogenous esterases prior to extraction. Following enzymatic deconjugation using β-glucuronidase and enrichment with analogues of phthalates isotopically labeled with carbon 13, the analytes were extracted by a liquid-liquid extraction with ethyl acetate as organic solvent. The extracts were brought to dryness, dissolved in ammonium acetate buffer and analyzed by LC-MS-MS in MRM mode with an electrospray ion source in negative mode (Waters Acquity UPLC; tandem mass detector Waters TQ-S). The limits of detection for this group of phthalate metabolites (group 1) ranged from 0.075 to 0.58 ng/g.

2.8.4. Quality control/quality assurance

For all the above methods and at each sequence performed, 2 reagent blanks were extracted and injected to ensure that no exogenous interference interfered with the reported analyte concentrations. The exactitude of the reported concentrations was ensured by monitoring 3 levels (low, mid, high) of quality controls (prepared in the same matrix as the participant).

2.9. Statistical analysis

Descriptive statistics, such as geometric mean (GM) and 95% confidence intervals (CI), median, and percentiles were calculated for unadjusted and SG-adjusted urinary concentrations, breast milk, infant formula and meconium. SG-adjusted metabolite concentrations were calculated using the following formula (Hauser et al., 2004):

$P_c = P_i\,[(SG_m - 1)/(SG_i - 1)]$where P_c is the *SG*-adjusted metabolite concentration (ng per mL), P_i is the observed metabolite concentration, SG_i is the specific gravity of the urine sample and SG_m is the median SG for the cohort (calculated separately for maternal versus infant urines). Any machine reading value below the limit of detection was used in the calculations and zero values were replaced by 0.0001.

Only those metabolites with at least 30% of the samples exceeding the LOD were examined further. As the data were right skewed, the natural log transformed phthalate concentration was the outcome in the models. Maternal urinary collection information (season, time of day, collection period, and weekend/weekday) as well as infant characteristics including gender, collection period and feeding practices were examined in relation to urinary concentrations using linear mixed models. A separate model was run for each predictor variable, which was included as a fixed effect with a random subject effect to account for potential correlations of measurements within an individual. Specific gravity was included in all models as a covariate. Estimates were produced using Restricted Maximum Likelihood (REML) estimation and p-values were constructed using the Kenward Roger degrees of freedom method.

Spearman correlations were calculated between T1 maternal urine concentrations of phthalates. The Fisher z transformation was used to approximate the 95% confidence limits. Using 1 minus the Spearman correlation as the distance measure, an agglomerative hierarchical clustering technique was used to group the metabolites into clusters. To show a visual correlation between phthalates, heatmaps were plotted using the Spearman correlation coefficients, including a dendrogram which illustrates clusters that have been joined and the distance between clusters at the time of joining. Spearman correlations were also calculated between each time point and matrix.

Data were analyzed using SAS Enterprise Guide (version 4.2; SAS Institute, Cary, NC, USA) and R (version 3.1.1; Vienna, Austria).

3. Results

While our initial objective was to recruit women before the 14th week of pregnancy, some women were hesitant to participate so early in their pregnancy because they did not want others to know of their pregnancy. Therefore the eligibility window was expanded to 19 weeks, 6 days gestation in the winter of 2010, which helped to significantly increase our participation rates. Table 1 presents characteristics of the study participants who provided urine, breast milk, meconium and infant urine samples. Most of the women were well educated, married, had a household income exceeding $100,000 Canadian, were primiparous or this was their second pregnancy, had never smoked, were in their early 30's, were born in Canada, had a normal pre-pregnancy BMI, and were employed. Although the numbers are small, there did not appear to be any noticeable differences between the extents of a woman's participation in the various segments of the study.

No phthalate metabolites were detected during pre-screening of the diaper liners, urine containers, Sarstedt® tubes or the Medela® breast milk pump; however, trace contamination from the urine bags with

Table 1
Characteristics of study participants with available biospecimens.

Characteristic	Maternal urine	Breast milk	Meconium	Infant urine
Number of participants providing biospecimens	80	56	54	61
Maternal education				
High school	3.75%	3.57%	5.56%	3.28%
Some college or university	7.50	3.57	1.85	6.56
College	17.50	10.71	20.37	14.75
University	45.00	48.21	42.59	42.62
Graduate degree	26.25	33.93	29.63	32.79
Marital status				
Married	78.75%	80.36%	77.78%	80.33%
Common-law	20.00	19.64	22.22	18.03
Single	1.25	0	0	1.64
Household income (n = 5 missing)				
<$70,000	8.75%	5.36%	7.41%	6.56%
$70,000–100,000	30.00	28.57	25.93	27.87
>$100,000	55.00	62.50	61.11	59.02
Parity				
0	46.25%	50.00%	48.15%	49.18%
1	42.50	41.07	40.74	40.98
2+	11.3	8.9	11.1	9.8
Mean maternal age (years)	32.42	33.13	32.77	32.67
Maternal smoking status (early pregnancy) (n = 2 missing)				
Never	66.25%	69.64%	66.67%	63.93%
Ever	31.25	26.79	29.63	32.79
Maternal pre-pregnancy BMI (kg/m^2)				
Underweight (<18.50)	2.50%	3.57%	1.85%	1.64%
Normal (18.50–24.99)	60.00	62.50	62.96	59.02
Overweight (25.00–29.99)	18.75	17.86	16.67	21.31
Obese (>29.99)	7.50	8.93	9.26	8.20
Maternal country of birth				
Canada	78.75%	78.57%	81.48%	78.69%
Outside Canada	21.25	21.43	18.52	21.31
Employed at Visit 1 (n = 1 missing)	81.25%	82.14%	83.33%	80.33%

the mono metabolites such as MEP, MnBP and MEHP is possible (Supplemental Material Table S1). The results of the field blank analysis are presented in the Supplemental Material Table S2. While there is a possibility that some of the biospecimen samples may have been contaminated with phthalates, the maximum value measured was generally below the 10th percentile of the metabolite distribution in the biospecimens.

3.1. Maternal urine results

Descriptive statistics and limits of detection for the phthalate metabolite concentrations from various matrices are shown in Supplemental Table S3. Over the course of the study, all women had at least one urine sample with detectable levels of the following metabolites (SG-adjusted median, μg/L): MEP (26.90), MnBP (18.37), MHBP (1.49), MiBP (7.49), 2OH-MiBP (4.42), MBzP (9.13), MCPP (2.08), MEHP (2.77), MEHHP (13.08), MEOHP (8.34), MCMHP (2.97), MECPP (10.73), MHiNP (2.02), MOiNP (1.57), MCiNP (0.94) and MOiDP (0.24). At least 50% of the women had at least one urine sample with detectable levels for: MMP, MCHP, and MHiDP. MnOP, MiNP and MCiOP were rarely detected in maternal urine. However, for MCiOP there was too much uncertainty in quantification as the retention times of peaks from the samples significantly deviated from those from the calibration standards. Therefore the non-detects do not necessarily imply that MCiOP was not present.

Analysis of potential predictors of maternal urinary concentrations including specific gravity as a covariate (Tables 2 and 3) showed that compared to winter collections, levels were higher in the spring for MBzP, MEHHP, MEHP and MOiDP, lower in the fall for MBzP and MEHHP, and higher in the summer for MCPP, MEHP and MCMHP. In regards to time of day when the urine was collected, compared to urine collected between 4 pm and midnight, urine collected at other times were significantly lower for all metabolites except for MnBP, MBzP, 2OH-MiBP, and MiBP. Maternal urinary MEP concentrations were highest when collected between 9 am and 4 pm. The DEHP metabolites and MEP were significantly lower in urine collected after pregnancy. MnBP concentrations were higher in urine collected on a weekend, while MBzP levels were lower, compared to a weekday collection.

To examine clustering of chemicals, maternal urinary concentrations of bisphenol A and triclosan (Arbuckle et al., 2015) from the same cohort were combined with the phthalate data. As expected, the DEHP metabolites were highly correlated in maternal urine ($r = 0.60–0.96$) and none of the phthalates were strongly correlated with triclosan or bisphenol A concentrations (Fig. 1). An examination of how the T1 maternal urinary metabolites clustered showed: a primary cluster of triclosan, MEP, MOiDP and MHiDP; another of BPA, MCiNP, MCPP, MOiNP, and MHiNP; a third cluster of the DEHP metabolites (MEOHP, MEHHP, MEHP, MECPP and MCMHP) and the final cluster of the DnBP and DiBP metabolites and MBzP.

3.2. Breast milk and infant formula results

While MEHP, MnBP, MEP, and MMP were detected in all 56 breast milk samples, MCPP, MCHP, and MiNP were not detected in any of the samples (Supplemental Table S3). Most of the 23 infant formula samples had no detectable concentrations of phthalate metabolites with the exception of MEHP, MnBP, MEP, and MMP. However, given the results of the field blank analysis (see Supplemental Material), these latter results may be due to contamination.

3.3. Infant biospecimen results

All infants had at least one urine sample above the LOD for MnBP (SG-adjusted median 4.37 μg/L) and MEP (SG-adjusted median 4.58 μg/L), with over 80% detected for MCPP, MBzP, MEHHP, and

Table 2
Bivariate mixed model analysis of predictors of urinary phthalates in all maternal urine samples, with specific gravity as a covariate. Specific gravity-adjusted geometric means (SG-GM) and 95% CI (μg/L) and p-values generated from mixed models.

Characteristic	# Urines	MnBP		MBzP		MCPP		MEHHP		MEHP		MEOHP		MEP	
		SG-GM	p-Value	SG-GM	p-Value	SG-GM	p-Value	SG-GM	p-Value	SG-GM	p-Value	SG-GM	p-Value	SG-GM	p-Value
Season urine collected															
Spring	299	19.59 (17.80, 21.56)	0.39	16.83 (14.55, 19.46)	0.0009	1.93 (1.47, 2.53)	0.08	16.05 (14.40, 17.88)	0.02	3.42 (2.98, 3.94)	0.003	9.43 (8.48, 10.49)	0.07	33.92 (29.89, 38.48)	0.08
Summer	297	20.23 (18.32, 22.35)	0.11	9.50 (8.47, 10.67)	0.33	2.47 (2.01, 3.04)	0.03	14.16 (12.82, 15.65)	0.12	2.97 (2.67, 3.31)	0.004	9.00 (8.13, 9.95)	0.06	34.45 (29.62, 40.07)	0.98
Fall	387	21.52 (19.67, 23.55)	0.12	5.79 (5.14, 6.52)	<0.0001	1.70 (1.53, 1.89)	0.71	10.67 (9.83, 11.59)	0.04	2.43 (2.23, 2.66)	0.15	7.31 (6.74, 7.93)	0.66	32.31 (28.51, 36.63)	0.30
Winter	277	16.46 (15.01, 18.04)	Ref	13.40 (11.77, 15.26)	Ref	1.56 (1.08, 2.23)	Ref	14.04 (12.74, 15.47)	Ref	1.85 (1.42, 2.42)	Ref	7.76 (7.05, 8.54)	Ref	34.69 (30.12, 39.96)	Ref
Time of day															
00:00–08:59	379	19.28 (17.65, 21.08)	0.71	10.19 (8.99, 11.54)	0.11	1.71 (1.36, 2.15)	0.0004	12.18 (11.11, 13.34)	<0.0001	2.03 (1.74, 2.39)	<0.0001	7.74 (7.06, 8.48)	<0.0001	32.33 (28.81, 36.28)	0.15
09:00–15:59	440	18.18 (16.68, 19.82)	0.06	9.50 (8.51, 10.61)	0.36	1.31 (1.06, 1.63)	<0.0001	11.11 (10.29, 11.99)	<0.0001	2.20 (1.92, 2.53)	<0.0001	6.85 (6.35, 7.38)	<0.0001	36.47 (32.24, 41.26)	0.03
16:00–23.59	412	20.99 (19.48, 22.62)	Ref	10.01 (8.88, 11.28)	Ref	2.98 (2.56, 3.47)	Ref	17.53 (16.15, 19.02)	Ref	3.84 (3.48, 4.25)	Ref	10.65 (9.83, 11.53)	Ref	31.04 (27.67, 34.82)	Ref
Collection period															
T1a	512	18.05 (16.71, 19.49)	Ref	11.55 (10.29, 12.96)	Ref	1.51 (1.19, 1.91)	Ref	14.80 (13.59, 16.12)	Ref	2.49 (2.11, 2.94)	Ref	8.82 (8.11, 9.59)	Ref	34.90 (31.29, 38.92)	Ref
T1b	544	20.61 (19.18, 22.15)	0.0001	8.80 (8.01, 9.68)	<0.0001	2.31 (2.03, 2.63)	0.0007	12.54 (11.74, 13.39)	0.0004	2.90 (2.70, 3.12)	0.01	7.90 (7.41, 8.42)	0.01	32.61 (29.53, 36.01)	0.93
T2	70	20.88 (16.81, 25.95)	0.06	9.60 (7.17, 12.84)	0.14	1.41 (0.90, 2.21)	0.94	10.49 (8.29, 13.27)	0.0007	2.28 (1.81, 2.87)	0.87	7.19 (5.64, 9.17)	0.05	37.40 (28.46, 49.15)	0.22
T3	71	22.64 (19.18, 26.73)	0.006	10.67 (8.25, 13.79)	0.40	1.85 (1.19, 2.87)	0.34	13.49 (11.29, 16.12)	0.40	2.25 (1.83, 2.77)	0.58	9.72 (8.15, 11.59)	0.26	42.23 (30.38, 58.70)	0.01
T5	63	18.78 (15.58, 22.62)	0.23	10.66 (8.00, 14.20)	0.08	2.58 (1.73, 3.86)	0.56	12.81 (10.59, 15.50)	0.002	1.98 (1.66, 2.36)	0.01	6.95 (5.79, 8.33)	<0.0001	23.36 (17.41, 31.34)	0.0001
Collection day															
Weekend	574	20.54 (19.15, 22.04)	0.004	8.93 (8.14, 9.80)	<0.0001	2.32 (2.04, 2.63)	0.0009	12.63 (11.82, 13.48)	0.02	2.90 (2.70, 3.11)	0.002	7.96 (7.46, 8.49)	0.09	31.99 (29.03, 35.24)	0.90
Weekday	686	18.76 (17.59, 20.01)		11.15 (10.13, 12.28)		1.57 (1.30, 1.90)		13.99 (13.03, 15.01)		2.39, (2.10, 2.71)		8.53 (7.95, 9.14)		35.22 (32.05, 38.70)	

Ref: referent group for statistical comparisons.

Table 3
Bivariate mixed model analysis of predictors of urinary phthalates in subsample of T1 maternal urine samples, with specific gravity as a covariate. Specific gravity-adjusted geometric means (SG-GM) and 95% CI (μg/L) and p-values generated from mixed models.

Characteristic	# Urines	C_2OH_MiBP		MiBP		MCMHP		MECPP		MHiDP		MOiDP	
		SG-GM	p-Value	SG-GM	p-Value	SG-GM	p-Value	SG-GM	p-Value	SG-GM	p-Value	SG-GM	p-Value
Season urine collected													
Spring	138	4.12 (3.70, 4.58)	0.90	7.01 (6.30, 7.81)	0.36	2.84 (2.57, 3.13)	0.73	10.63 (9.48, 11.91)	0.20	0.004 (0.002, 0.006)	0.08	0.20 (0.16, 0.24)	0.01
Summer	101	6.97 (5.78, 8.41)	0.76	11.99 (9.54, 15.07)	0.66	4.18 (3.58, 4.89)	0.05	15.20 (13.22, 17.48)	0.66	0.004 (0.002, 0.009)	0.22	0.18 (0.12, 0.29)	0.18
Fall	170	4.34 (4.06, 4.64)	0.75	7.52 (6.98, 8.09)	0.65	2.76 (2.50, 3.05)	0.42	9.72 (8.74, 10.82)	0.10	0.005 (0.003, 0.009)	0.08	0.26 (0.21, 0.33)	0.06
Winter	133	5.00 (4.36, 5.72)	Ref	6.93 (6.15, 7.81)	Ref	2.84 (2.13, 3.79)	Ref	10.60 (9.34, 12.03)	Ref	0.001 (0.0007, 0.002)	Ref	0.08 (0.05, 0.11)	Ref
Time of day													
00:00–08:59	145	4.94 (4.35, 5.62)	0.13	8.33 (7.15, 9.70)	0.10	3.59 (3.15, 4.08)	0.144	10.37 (9.03, 11.91)	0.001	0.002 (0.001, 0.003)	0.006	0.11 (0.08, 0.16)	0.0002
09:00–15:59	180	4.85 (4.33, 5.43)	0.18	7.57 (6.75, 8.49)	0.48	2.47 (2.04, 2.99)	0.003	9.30 (8.52, 10.14)	<0.0001	0.002 (0.001, 0.004)	0.01	0.15 (0.11, 0.20)	0.005
16:00–23:59	209	4.74 (4.34, 5.17)	Ref	7.88 (7.24, 8.57)	Ref	3.21 (2.90, 3.55)	Ref	13.31 (12.13, 14.61)	Ref	0.006 (0.004, 0.01)	Ref	0.24 (0.20, 0.31)	Ref

Ref: referent group for statistical comparisons

MEOHP (Supplemental Table S3). MEHP, MCPP, MBzP, MnBP, MEP, MMP, MEHHP, and MEOHP were detected in at least 60% of the meconium samples (Supplemental Table S3). Among those metabolites with sufficient detection, all were higher in the older infants, with urinary concentrations of MEOHP, MCPP and MEHHP significantly higher in the older infants than in the neonates (Table 4). No differences in infant urinary or meconium concentrations were observed by gender or by infant feeding practices. Breast milk concentrations of MEP were significantly higher when the milk was collected by manual pump than by hand.

3.4. Correlations between matrices

There were weak correlations between SG-adjusted maternal T1 concentrations and meconium for MEHHP ($r = 0.35$), MEOHP ($r = 0.35$) and MEP ($r = 0.37$), and between maternal T5 and breast milk concentrations for MnBP ($r = 0.43$) (Table 5). Moderate to strong positives correlations were observed between maternal and infant urine concentrations at T5 for MBzP ($r = 0.78$), MnBP ($r = 0.40$), MCPP ($r = 0.41$) and for MEP ($r = 0.56$).

4. Discussion

This study is among the first to measure exposure to phthalates across pregnancy and into infancy combined with the analysis of multiple matrices to demonstrate maternal-infant transfer for at least some of the phthalates.

One of the main concerns with biomonitoring studies of ubiquitous chemicals such as phthalates is potential external contamination of the biospecimens by the collection, processing and transporting materials, laboratory reagents, sampling equipment, and analytical apparatus. One approach to limit misinterpretation of biomonitoring results for these chemicals is to routinely measure biomarkers that cannot be formed in the environment, such as the oxidized metabolites of phthalates (Koch and Calafat, 2009). Potential external contamination is a particular problem for the monoesters and especially in matrices containing lipase activity such as breast milk and meconium, where the lipases can cleave the contaminating phthalate into its monoester, making them indistinguishable from the monoesters formed during the body's metabolism of the phthalate (Koch and Angerer, 2012). Additional approaches commonly used include using field blanks and reagent or quality control blanks and quality control samples (Ye et al., 2013). In our study, while efforts to minimize contamination were employed (e.g., pre-screening of biospecimen collection materials, inclusion of field blanks, addition of phosphoric acid to milk and meconium), the possibility of contamination exists.

4.1. Maternal urine

It is difficult to draw any conclusions on whether pregnancy status affects urinary phthalate concentrations as comparisons in the same woman while pregnant and not pregnant are rare and inconsistent. In our study, MEP, MEHHP, MEOHP, and MEHP urinary concentrations were consistently higher in samples collected during pregnancy than postnatally. A German study found median MEP concentrations were higher during pregnancy (54.1 μg/L) than 2 months after delivery (34 μg/L), whereas MBzP, MEHP, MEOHP and MEHHP were higher post-pregnancy (W. Völkel, personal communication, 2015-07-13). In contrast, Braun et al. (2012) measured phthalates in urine prior to and during pregnancy and reported somewhat higher geometric mean urinary concentrations of MEP prior to pregnancy (61 vs. 55 μg/L during pregnancy); these levels were higher than in our P4 Study (where GM was 33–42 μg/L during pregnancy and 23 μg/L post pregnancy).

A comparison of median urinary concentrations across pregnancy and into the post-partum period between studies in Germany (Völkel et al., 2014; Enke et al., 2013) and our study showed that for almost

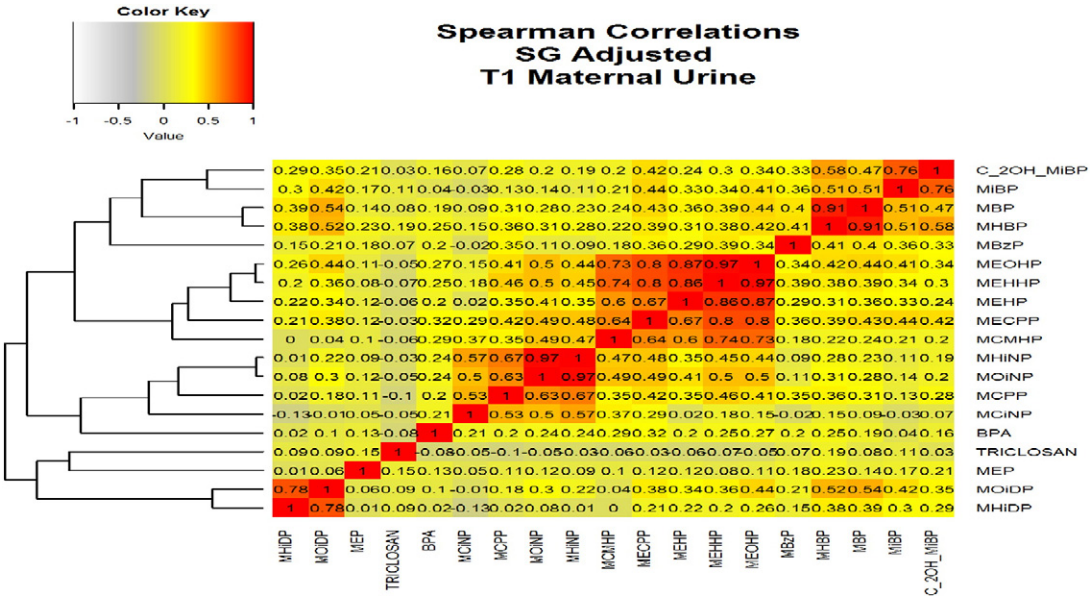


Fig. 1. Cluster analysis and Spearman correlations between maternal urinary phthalates and phenols at T1 (<20 weeks gestation).

every metabolite examined, except MMP and MCPP, concentrations in our study were lower. This was especially true for MiBP, where median concentrations were up to 10-fold higher in Germany (63.6 μg/L) (Völkel et al., 2014) compared to our P4 data (6.6 μg/L. Other European countries (Tefre de Renzy-Martin et al., 2014; Ye et al., 2008; Valvi et al., 2015) also had higher MiBP concentrations during pregnancy (medians ranging from 25.2 to 42.1 μg/L) than American studies (4.0–4.4 μg/L) (Guo et al., 2014; Swan et al., 2015), suggesting DiBP exposure from diet or non-dietary sources (Sakhi et al., 2014; Koch et al., 2013; Wormuth et al., 2006) between the two continents may differ. Similar to our results, Völkel et al. (2014) reported that nearly all maternal urines were below the limit of detection for MHiNP (7OH-MiNP), MCiNP (cx-MiDP), MHiDP (OH-MiDP), MiNP, MHPP, and MnOP, while MEOHP (5oxo-MEHP), MECPP (5cx-MEPP), MnBP and MiBP were found in all maternal samples.

The proportion of urinary concentrations of DEHP metabolites appear to be similar between those observed in non-pregnant adults (MEHP 6.6%, MEHHP 29.7%, MEOHP 15.4%, MECPP 31.8%, MCMHP 10.3%) (Silva et al., 2006) and our study using a similar statistical approach for T1 urines (MEHP 7%, MEHHP 35%, MEOHP 22%, MECPP 28%, MCMHP 8%) (data not shown).

In comparison with other Canadian studies conducted by the same laboratory, maternal phthalate concentrations in the P4 study were similar to or somewhat higher than those from a larger cohort of pregnant women (Arbuckle et al., 2014). For MEP, P4 unadjusted geometric mean concentrations were lower (29.9 μg/L at T1) than those reported in a population-based national survey of women of reproductive age (43 μg/L) in Canada (Health Canada, 2013). Differences between the socio-economic status of the study populations and the urine sampling protocol (multiple versus single void) may have accounted for differences in the levels observed.

Our bivariate modeling suggested that even after adjustment for specific gravity, maternal urinary concentrations were significantly associated with variables related to the urine collection such as season and especially time of day when the urine was collected. The timing of the sample relative to food consumption has a direct impact on the estimated exposure concentrations in spot urines (Janjua et al., 2008; Ye et al., 2011). For most of the phthalates measured in our study, urine collected any time between early morning and 4 pm had significantly lower concentrations than those collected after 4 pm. This is in general agreement with other studies (Cantonwine et al., 2014; Preau et al., 2010; Valvi et al., 2015). These findings suggest that time of day should be considered when designing a biomonitoring study, especially when measuring multiple phthalates that will have different sources, frequency and timing of use.

For a few phthalates we found that season was a significant predictor. There were higher maternal levels of MBzP and MEHHP in the spring and lower levels in the fall, compared to winter. Summer urine collections were significantly higher in MCPP, MEHP and MCMHP than those in the winter. In Germany, Hildenbrand et al. (2009) found a significant trend towards higher levels of the DEHP metabolite MEHHP in January ($r = 0.64$), while an American study found no significant difference for any phthalate measured by season (Peck et al., 2010).

A number of factors may be responsible for observed differences in maternal urinary concentrations and factors associated with these concentrations, including: study populations that differ in size, ethnicity and socio-economic status; frequency and timing of urine collection; availability of different types of consumer products and food packaging materials; and potential contamination of the urine samples.

Our cluster analysis identified four major clusters of maternal urinary metabolites during early pregnancy: (1) the DEHP metabolites; (2) triclosan, MEP, and 2 of the DiDP metabolites (oxo and OH); (3) bisphenol A, 2 of the high molecular weight DiNP (oxo and OH) and 1 of the DiDP (cx) metabolites plus MCPP, the non-specific metabolite of high molecular weight phthalates; and (4) the DiBP and DnBP metabolites plus MBzP. As there is the potential for mixtures of endocrine disrupting chemicals to enhance the toxicity of the individual chemicals (Sobolewski et al., 2014; Christiansen et al., 2012; Christen et al., 2012), identifying clusters is important for risk assessments. Other studies have reported positive correlations between various phthalates and phenols in Danish (Tefre de Renzy-Martin et al., 2014) and US pregnant women (LaRocca et al., 2014), Greek mothers and children (Myridakis et al., 2015) and in Flemish adolescents (Geens et al., 2014).

4.2. Breast milk

Although the metabolites are more frequently detected in breast milk, both the parent phthalate compounds and their metabolites can be measured, indicating that both can be transferred from the woman to her infant; the esterases in the milk could also cleave the diesters into the metabolites (Fromme et al., 2011). As the addition of the inhibitor (phosphoric acid) to stop the esterase activity in breast milk was not added until the sample was thawed in the laboratory, we cannot rule

Table 4
Univariate associations between major phthalate metabolites in infant urine (all samples, specific gravity-adjusted) and meconium with infant characteristics.

Covariates	MEHP		MnBP		MEP		MEOHP		MCPP		MBzP		MEHHP		MMP	
	GM (95% CI)	p-Value	GM (95% CI)	p-Value	GM (95% CI)	p-Value	GM (95% CI)	p-Value	GM (95% CI)	p-Value	GM (95% CI)	p-Value	GM (95% CI)	p-Value	GM (95% CI)	p-Value
Infant urine																
Visit																
T4 (n = 45)	N/A		3.76 (2.94, 4.82)	0.14	4.80 (3.10, 7.45)	0.35	0.72 (0.57, 0.91)	0.02	0.62 (0.46, 0.83)	0.04	1.32 (0.88, 2.00)	0.055	0.68 (0.53, 0.86)	0.0007	N/A	
T5 (n = 55)	N/A		4.79 (3.85, 5.95)		5.95 (4.73, 7.49)		0.98 (0.76, 1.27)		1.00 (0.72, 1.39)		2.29 (1.57, 3.35)		1.03 (0.81, 1.31)			
Gender																
Female (n = 47)	N/A		4.34 (3.41, 5.52)	0.70	5.27 (3.50, 7.94)	0.82	0.91 (0.68, 1.22)	0.62	0.73 (0.52, 1.01)	0.46	1.89 (1.20, 2.97)	0.64	0.88 (0.65, 1.19)	0.76	N/A	
Male (n = 46)	N/A		4.00 (3.11, 5.13)		5.58 (4.24, 7.32)		0.79 (0.62, 1.00)		0.88 (0.61, 1.27)		1.64 (1.09, 2.47)		0.80 (0.63, 1.01)			
Infant feeding[a]																
Exclusively breastfed (n = 21)	N/A		5.54 (4.08, 7.51)	Ref	6.33 (4.27, 9.39)	Ref	0.84 (0.64, 1.11)	Ref	1.41 (0.82, 2.41)	Ref	3.13 (1.82, 5.37)	Ref	0.84 (0.64, 1.10)	Ref	N/A	
Exclusively formula (n = 6)	N/A		5.05 (2.44, 10.46)	0.80	6.98 (3.19, 15.27)	0.81	1.02 (0.38, 2.75)	0.65	0.94 (0.27, 3.22)	0.47	2.74 (0.43, 17.45)	0.84	1.14 (0.49, 2.65)	0.45	N/A	
Combination of the two (n = 26)	N/A		4.51 (3.11, 6.53)	0.408	5.55 (3.85, 8.00)	0.61	1.07 (0.68, 1.66)	0.40	0.83 (0.49, 1.41)	0.16	1.82 (1.01, 3.27)	0.20	1.13 (0.74, 1.73)	0.27	N/A	
Meconium																
Infant gender																
Female (n = 27)	0.76 (0.49, 1.19)	0.61	2.01 (1.32, 3.05)	0.82	1.31 (0.88, 1.97)	0.72	0.13 (0.06, 0.26)	0.92	1.36 (1.04, 1.77)	0.52	0.45 (0.28, 0.71)	0.89	0.45 (0.31, 0.65)	0.25	0.06 (0.01, 0.27)	0.39
Male (n = 24)	0.93 (0.46, 1.90)		2.14 (1.43, 3.21)		1.46 (0.94, 2.28)		0.13 (0.10, 0.18)		1.19 (0.87, 1.65)		0.42 (0.23, 0.79)		0.34 (0.26, 0.46)		0.02 (0.00, 0.13)	
Breast milk																
Collected by																
Hand (n = 16)	1.36 (0.97, 1.89)	0.97	0.58 (0.41, 0.83)	0.50	0.17 (0.11, 0.27)	0.001	N/A		N/A		N/A		N/A		0.47 (0.33, 0.67)	0.16
Manual pump (n = 20)	1.37 (1.00, 1.88)	Ref	0.71 (0.46, 1.09)	Ref	0.44 (0.30, 0.65)	Ref	N/A		N/A		N/A		N/A		0.66 (0.51, 0.86)	Ref
Electrical pump (n = 17)	1.66 (1.11, 2.50)	0.41	0.94 (0.59, 1.49)	0.31	0.28 (0.19, 0.43)	0.10	N/A		N/A		N/A		N/A		0.52 (0.33, 0.81)	0.30

N/A: not available due to contamination problems.
Ref: referent group for statistical comparisons.
[a] Based on T5 infant urine.

Table 5
Significant ($p < 0.05$) Spearman correlations (r) (with 95% confidence intervals) between specific gravity adjusted maternal and infant urinary concentrations, breast milk and meconium concentrations of phthalate metabolites.

Phthalate metabolite	MT1 maternal urine (<20 weeks)	MT2 maternal urine (24–28 weeks)	MT3 maternal urine (32–36 weeks)	MT5 maternal urine (2–3 mo post-partum)	IT4 infant urine (<1 mo)	IT5 infant urine (2–3 mo)
MEHP	MT2 r = 0.47 (0.27, 0.64) MT3 r = 0.26 (0.02, 0.47)	MT3 r = 0.29 (0.04, 0.50)	T5 r = 0.40 (0.13, 0.62)			
MEHHP	MT2 r = 0.33 (0.10, 0.52) Mec r = 0.35 (0.09, 0.57)				IT5 r = 0.56 (0.27, 0.76)	
MEOHP	MT2 r = 0.35 (0.13, 0.54) Mec r = 0.35 (0.08, 0.57)	MT3 r = 0.26 (0.02, 0.47) MT5 r = 0.30 (0.05, 0.52)	MT5 r = 0.28 (0.03, 0.50)		IT5 r = 0.50 (0.19, 0.72)	
MnBP	MT2 r = 0.52 (0.33, 0.67) MT3 r = 0.37 (0.15, 0.56) MT5 r = 0.28 (0.04, 0.49) IT4 r = 0.49 (0.20, 0.70) IT5 r = 0.28 (0.01, 0.52)	MT3 r = 0.39 (0.16, 0.57) MT5 r = 0.29 (0.04, 0.51)	MT5 r = 0.54 (0.32, 0.70) IT4 r = 0.36 (0.03, 0.62) IT5 r = 0.33 (0.05, 0.56) For r = −0.71 (−0.88, −0.38)	IT5 r = 0.40 (0.14, 0.61) BM r = 0.43 (0.18, 0.62)	IT5 r = 0.39 (0.05, 0.64)	
MBzP	MT2 r = 0.46 (0.25, 0.63) MT3 r = 0.34 (0.11, 0.53) IT5 r = −0.30 (−0.54, −0.02)	IT4 r = 0.41 (0.09, 0.70)	MT5 r = 0.30 (0.04, 0.51) IT4 r = 0.39 (0.05, 0.64) BM r = 0.31 (0.04, 0.53)	IT5 r = 0.78 (0.64, 0.87)		
MCPP	MT2 r = 0.26 (0.03, 0.47)	IT4 r = − −0.34 (−0.60, −0.01)		IT5 r = 0.41 (0.13, 0.62)		Mec r = 0.37 (0.04, 0.63)
MEP	MT2 r = 0.71 (0.57, 0.81) MT3 r = 0.41 (0.19, 0.58) MT5 r = 0.50 (0.29, 0.66) Mec r = 0.37 (0.11, 0.59) BM r = 0.27 (0.01, 0.50)	MT3 r = 0.51 (0.30, 0.67) MT5 r = 0.40 (0.16, 0.59) IT4 r = 0.37 (0.05, 0.62) Mec r = 0.34 (0.06, 0.57) BM r = 0.36 (0.10, 0.58)	MT5 r = 0.47 (0.24, 0.65) IT5 r = 0.29 (0.01, 0.53) Mec r = 0.34 (0.07, 0.56) BM r = 0.27 (0.01, 0.51)	IT5 r = 0.56 (0.33, 0.72) Mec r = 0.30 (0.01, 0.55) BM r = 0.28 (0.02, 0.50)	IT5 r = 0.52 (0.21, 0.73) BM r = 0.35 (0.01, 0.61)	BM r = 0.34 (0.04, 0.58)

out the hydrolysis of phthalate diesters into monoesters in our study. So an unknown amount of the monoesters measured may have arisen from enzyme-induced hydrolysis of diesters or contaminants from the process of collection and handling.

The long-branched and/or hydrophobic phthalates such as DnBP, DEHP and DiNP may be excreted unmetabolized or as the primary monoesters in breast milk, which may signal an alternative metabolic pathway for phthalates in breast-feeding women (Frederiksen et al., 2007). In our study, MEHP, MnBP, MEP, and MMP were detected in all breast milk samples, while MCPP, MCHP, and MiNP were not detected. Due to problems with contamination, MEHP is likely an unreliable measurement of DEHP exposure (de Cock et al., 2014); however, the oxidized metabolites MEHHP and MEOHP were detected in over 80% of our breast milk samples indicating DEHP transfer to the breast milk.

Median concentrations of MEP, MEHP, MnBP, MBzP, MEHHP, MEOHP and MiNP in breast milk were lower in our study compared to samples collected in Europe (Main et al., 2006; Schlumpf et al., 2010; Latini et al., 2009; Fromme et al., 2011) and Asia (Kim et al., 2015; Lin et al., 2011) (Supplemental Table S4).

The only phthalate concentration in breast milk that differed by how it was collected was MEP, where hand expression resulted in significantly lower concentrations (GM: 0.17 μg/L) compared to the manual pump (GM: 0.44 μg/L). Our screening did not suggest that the manual pump provided would be a source of MEP contamination, so the reason for this difference is unknown. Mortensen et al. (2005) reported no significant differences between concentrations of MMP, MBzP, MEHP or MiNP in breast milk samples collected with or without a breast pump, but did find significantly higher levels of MEP and MnBP in samples collected with a pump.

An earlier US study has reported that high urinary concentrations of phthalate metabolites did not predict concentrations in breast milk (Hines et al., 2009). However, we observed some correlation ($r = 0.43$) between maternal urine and breast milk MnBP collected at the same time and between all maternal and infant urines and breast milk for MEP.

Median MEP concentrations in breast milk were 100-fold lower (0.25 μg/L) compared to all maternal urinary concentrations (27 μg/L). MnBP concentrations were also lower (0.66 μg/L) in breast milk than in maternal urine collected at the same time (19 μg/L), as was MMP (breast milk 0.56 μg/L; post-parturition maternal urine 5.0 μg/L). Given that MEP is generally the phthalate with the highest urinary concentration but one of the lowest in breast milk, these results suggest different rates of maternal-breast milk transfer for these phthalates.

4.3. Infant formula

While MEHP, MnBP, MEP, and MMP were detected in some of the infant formula samples, there were no significant correlations with infant urine concentrations. In Denmark, higher concentrations of MnBP and MEHP were detected in infant formula than in our study, but MMP, MEP, and MBzP were not detected (Mortensen et al., 2005).

4.4. Infant urine

In our study MnBP and MEP were detected in all infant urines. In the younger infants (less than one month of age), MEHHP and MEOHP were present in the majority of urine samples. The presence of highly oxidized phthalate metabolites in neonatal urine supports the hypothesis

that the placenta is not an effective barrier for these metabolites (Enke et al., 2013). Our finding of MEHHP and MEOHP in the meconium would also lend support to this hypothesis.

Comparison of urinary concentrations of phthalates in young infants suggest that levels in Canadian infants are substantially lower than those reported in Germany (Enke et al., 2013; Völkel et al., 2014) and Finland (Frederiksen et al., 2014) (Supplemental Table S5). In a German study, only MiBP was quantified in every infant urine sample with the highest levels of all metabolites observed at 5 months of age and DEHP metabolites showing a continuous increase in concentration between 1 and 5 months of age (Völkel et al., 2014). However another study reported that infant urinary concentrations were relatively consistent between 1 and 6 months of age (Frederiksen et al., 2014). Similar to the German study (Enke et al., 2013), most phthalate metabolite concentrations in our study were higher in the older infants and no significant differences were observed between male and female urinary concentrations.

Although the geometric mean concentrations of MEHP, MnBP, MEP and MMP were higher in breast milk than infant formula, the results of our study would suggest that infant exposure to phthalates from breast milk or infant formula would be low as we did not find any statistically significant differences in the infant's urinary phthalate concentrations between breast-fed and bottle-fed infants. A Finnish study has reported that gender and breastfeeding versus bottle-feeding were not associated with infant urinary concentrations of phthalates, which suggested that diet was not the primary source of exposure for the young infants (Frederiksen et al., 2014). In addition to diet, other sources of phthalate exposure include consumer products (Sathyanarayana et al., 2008), indoor dust and air (Fromme et al., 2013; Bekö et al., 2013; Kubwabo et al., 2013) and PVC flooring (Carlstedt et al., 2013).

4.5. Ratio of MEHHP to MEOHP in urine

Examining the ratio of MEHHP to MEOHP concentrations in urine may provide some insight into differences in the metabolism of DEHP by various sub-populations. In our study, the ratio of medians of MEHHP/MEOHP for all maternal urines was 1.5, similar to the mean ratio of 1.4 reported in US (Barr et al., 2003) and German (Koch et al., 2003) studies of adults and children. The ratio of medians for MEHHP/MEOHP in our infants increased as they aged (0.78 vs. 1.04) and was even higher in meconium (3.08). A Finish study also reported changes in the proportion of oxidized metabolites of DEHP in infants from birth to 14 months of age consistent with maturation of infant metabolic pathways with the hydroxylated metabolite MEHHP increasing from on average of 18% to 39% but less so for the oxo metabolite MEOHP which increased from 13% to 20% (Frederiksen et al., 2014). Among six premature infants in neonatal intensive care units, the MEHHP/MEOHP ratio varied from 0.9 to 1.7 (Calafat et al., 2004a), while in children 3–14 years of age the ratio was 1.3 and did not vary by age (Becker et al., 2004). The change in the ratios of hydroxyl to oxo metabolites of DEHP for these populations may be related to the ability of infants to metabolize DEHP compared to adults. In an adult oral dosing study, 21% of the applied dose was excreted in urine as MEHHP within the first 10 h compared to 13% for MEOHP, indicating differences in the rate of elimination of these two metabolites (Koch et al., 2004). Differences between adults and infants in toxicokinetics and metabolism have been well documented (Anderson and Holford, 2013), especially for some phthalates (Enke et al., 2013).

4.6. Meconium

Meconium is the early feces passed by the newborn and begins to form as early as the 12-13th week of gestation. It can be a cumulative repository of the chemicals that the fetus is exposed to throughout pregnancy and may be a better matrix to measure prenatal exposure to short-lived chemicals such as phthalates.

Published reports of phthalate levels in meconium are very limited. MEHP has been measured in three Chinese studies that all reported very high median concentrations of approximately 3800 μg/g (Li et al., 2013), 2.9 mg/g (Zhang et al., 2009), and 163.8 μg/g (Xie et al., 2015), compared to our study (median 0.64 ng/g). Median MnBP concentrations were also elevated in the Zhang et al. (2009) (1.7 mg/g) and Xie et al. (2015) studies (101.70 μg/g), versus the P4 study (2.09 ng/g). Among 5 American meconium samples, the average concentrations of MEOHP and MEHHP were 3.26 and 3.76 ng/g, respectively (Kato et al., 2006), higher than median concentrations reported here (0.12 and 0.37 ng/g for MEOHP and MEHHP). Another study of 5 meconium samples reported that only MECPP and a few other DEHP metabolites were found in meconium (Frederiksen et al., 2007).

The endogenous esterase activity in meconium has been measured in one study, which suggested that the esterases in meconium could hydrolyze phthalate diesters into monoesters (Kato et al., 2006) and explain the higher MEHP levels measured in some studies ((Li et al., 2013; Zhang et al., 2009; Xie et al., 2015). Meconium specimens should be treated to deactivate the enzymes after collection or collected in phthalate-free containers (both approaches were adopted in our study) (Kato et al., 2006). As the addition of the inhibitor to stop the esterase activity in meconium was not added until the sample was thawed in the laboratory, we cannot rule out the hydrolysis of phthalate diesters into monoesters. However, our very low results compared to other studies would suggest that this was not an issue in our analysis.

There is some concern that meconium may be contaminated by infant urine which would impact interpretation of these results. We were unable to confirm whether there was urine in the diaper when the meconium was collected. If cross-contamination of the meconium sample with urine occurred, this would reflect exposure during gestation as well as after birth (Calafat and Needham, 2009). However, we found no correlation between phthalate concentrations measured in meconium and infant urine at T4.

Lotions, wipes or powders used on the baby or the diaper itself may also be a source of contamination. In a few cases in our study, the provided diaper liner was not used. Vaseline® and Pampers® wipes were commonly used in the Ottawa hospitals and it is unlikely that they were a source of phthalates (Health Canada, 2014). Although detection frequencies and concentrations were low, US studies have reported DMP, DEP, DBP and DEHP in baby care products such as diaper cream and powder (Guo and Kannan, 2013), and body wash and moisturizer (Lampel and Jacob, 2011). Similarly in Canada, detection frequencies were very low for baby oils and diaper creams (Koniecki et al., 2011). In addition, loss of moisture from meconium to the diaper may affect the concentration later measured.

Statistically significant positive correlations ($r = 0.35–0.37$) were observed between T1 maternal urinary and infant meconium concentrations of MEHHP, MEOHP, and MEP, suggesting in utero exposure for the fetus.

5. Strengths and limitations

The strengths of this study include: (1) the collection of multiple maternal urine voids within a day and over the course of pregnancy and into the early post-partum period; (2) analysis of infant meconium, infant urine and breast milk from the same cohort; (3) urinary data on 22 phthalate metabolites during pregnancy; and (4) extensive use of field blanks to identify major sources of contamination. The major limitations of this study are the small and highly educated study population which will limit the generalizability of these results to other populations, as well as no data on the group 2 metabolites in the infant urine, meconium and breast milk.

6. Conclusions

Although a number of phthalates were detected in maternal and infant urine and breast milk from this study, concentrations tended to be lower than those reported in other international studies, particularly from Europe. The lower levels observed in this study compared to other regions, may be due to differences in study populations, food packaging, diet and consumer products. Meconium shows some promise as a matrix for evaluating fetal exposure to phthalates. Some significant correlations were observed between MEHHP, MEOHP and MEP metabolites in maternal urine at T1 with levels in meconium, and between MnBP and MEP levels in post-natal maternal urine and breast milk. Maternal and infant urinary concentrations of MBzP, MCPP, MnBP and MEP collected at T5 were also correlated. These results suggest at least some maternal-fetal-infant transfer of phthalates. Extensive incorporation of field blanks into phthalate biomonitoring studies is critical to identify and consider potential sources of contamination. Given the significant associations observed between maternal urinary concentrations and time of day of urine collection, this is an important factor to consider when designing and analyzing biomonitoring studies.

Acknowledgments

The work of Ruth White and Pauline Shields on recruitment and data and biospecimen collection is acknowledged. We especially thank Branka Jovic for her tireless efforts in setting up the P4 database for data entry and analysis. We are grateful for the advice of Antonia Calafat on specimen collection. Special thanks to the women who took the time and effort to participate in this demanding study. This work was funded by Health Canada's Chemicals Management Plan.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.02.022.

References

Abduljalil, K., Furness, P., Johnson, T.N., Rostami-Hodjegan, A., Soltani, H., 2012. Anatomical, physiological and metabolic changes with gestational age during normal pregnancy: a database for parameters required in physiologically based pharmacokinetic modelling. Clin. Pharmacokinet. 51 (6), 365–396. http://dx.doi.org/10.2165/11597440-000000000-00000 Jun 1.

Adibi, J.J., Whyatt, R.M., Williams, P.L., Calafat, A.M., Camann, D., Herrick, R., Nelson, H., Bhat, H.K., Perera, F.P., Silva, M.J., Hauser, R., 2008. Characterization of phthalate exposure among pregnant women assessed by repeat air and urine samples. Environ. Health Perspect. 116 (4), 467–473. http://dx.doi.org/10.1289/ehp.10749 Apr.

Anderson, B.J., Holford, N.H., 2013. Understanding dosing: children are small adults, neonates are immature children. Arch. Dis. Child. 98 (9), 737–744. http://dx.doi.org/10.1136/archdischild-2013-303720 Epub 2013 Jul 5; Sep.

Arbuckle, T.E., Davis, K., Marro, L., Fisher, M., Legrand, M., Leblanc, A., Gaudreau, E., Foster, W.G., Choeurng, V., Fraser, W.D., the MIREC Study Group, 2014. Phthalate and bisphenol A exposure among pregnant women in Canada — results from the MIREC study. Environ. Int. 68C, 55–65. http://dx.doi.org/10.1016/j.envint.2014.02.010 Apr 3.

Arbuckle, T.E., Weiss, L., Fisher, M., Hauser, R., Dumas, P., Bérubé, R., Neisa, A., LeBlanc, A., Lang, C., Ayotte, P., Walker, M., Feeley, M., Koniecki, D., Tawagi, G., 2015. Maternal and infant exposure to environmental phenols as measured in multiple biological matrices. Sci. Total Environ. 508, 575–584. http://dx.doi.org/10.1016/j.scitotenv.2014.10.107 Mar 1.

Barr, D.B., Silva, M.J., Kato, K., Reidy, J.A., Malek, N.A., Hurtz, D., Sadowski, M., Needham, L.L., Calafat, A.M., 2003 Jul. Assessing human exposure to phthalates using monoesters and their oxidized metabolites as biomarkers. Environ. Health Perspect. 111 (9), 1148–1151.

Becker, K., Seiwert, M., Angerer, J., Heger, W., Koch, H.M., Nagorka, R., Rosskamp, E., Schlüter, C., Seifert, B., Ullrich, D., 2004. DEHP metabolites in urine of children and DEHP in house dust. Int. J. Hyg. Environ. Health 207 (5), 409–417 Oct.

Bekö, G., Weschler, C.J., Langer, S., Callesen, M., Toftum, J., Clausen, G., 2013. Children's phthalate intakes and resultant cumulative exposures estimated from urine compared with estimates from dust ingestion, inhalation and dermal absorption in their homes and daycare centers. PLoS ONE 8 (4), e62442. http://dx.doi.org/10.1371/journal.pone.0062442 Apr 23.

Bornehag, C.G., Carlstedt, F., Jönsson, B.A., Lindh, C.H., Jensen, T.K., Bodin, A., Jonsson, C., Janson, S., Swan, S.H., 2015. Prenatal phthalate exposures and anogenital distance in Swedish boys. Environ. Health Perspect. 123 (1), 101–107. http://dx.doi.org/10.1289/ehp.1408163 Epub 2014 Oct 29; Jan.

Braun, J.M., Smith, K.W., Williams, P.L., Calafat, A.M., Berry, K., Ehrlich, S., Hauser, R., 2012. Variability of urinary phthalate metabolite and bisphenol A concentrations before and during pregnancy. Environ. Health Perspect. 120 (5), 739–745 Epub 2012 Jan 19; May.

Bustamante-Montes, L., Hernández-Valero, M., Flores-Pimentel, D., García-Fábila, M., Amaya-Chávez, A., Barr, D., Borja-Aburto, V., 2013. Prenatal exposure to phthalates is associated with decreased anogenital distance and penile size in male newborns. J Dev Orig Health Dis. 4 (4). http://dx.doi.org/10.1017/S2040174413000172 Aug.

Calafat, A.M., Needham, L.L., 2009 Oct. What additional factors beyond state-of-the-art analytical methods are needed for optimal generation and interpretation of biomonitoring data? Environ. Health Perspect. 117 (10), 1481–1485. http://dx.doi.org/10.1289/ehp.0901108 Epub 2009 Jun 24.

Calafat, A.M., Needham, L.L., Silva, M.J., Lambert, G., 2004a. Exposure to di-(2-ethylhexyl) phthalate among premature neonates in a neonatal intensive care unit. Pediatrics 113 (5), e429–e434 May.

Calafat, A.M., Slakman, A.R., Silva, M.J., Herbert, A.R., Needham, L.L., 2004b. Automated solid phase extraction and quantitative analysis of human milk for 13 phthalate metabolites. J Chromatogr B Analyt Technol Biomed Life Sci. 805 (1), 49–56 Jun 5.

Cantonwine, D.E., Cordero, J.F., Rivera-González, L.O., Anzalota Del Toro, L.V., Ferguson, K.K., Mukherjee, B., Calafat, A.M., Crespo, N., Jiménez-Vélez, B., Padilla, I.Y., Alshawabkeh, A.N., Meeker, J.D., 2014. Urinary phthalate metabolite concentrations among pregnant women in Northern Puerto Rico: distribution, temporal variability, and predictors. Environ. Int. 62, 1–11. http://dx.doi.org/10.1016/j.envint.2013.09.014 Epub 2013 Oct 24; Jan.

Carlstedt, F., Jönsson, B.A., Bornehag, C.G., 2013. PVC flooring is related to human uptake of phthalates in infants. Indoor Air 23 (1), 32–39. http://dx.doi.org/10.1111/j.1600-0668.2012.00788.x Epub 2012 Jun 18; Feb.

Christen V, Crettaz P, Oberli-Schrämmli A, Fent K. Antiandrogenic activity of phthalate mixtures: validity of concentration addition. Toxicol Appl Pharmacol. 2012 1;259 (2):169-76. doi: http://dx.doi.org/10.1016/j.taap.2011.12.021. Epub 2012 Jan 8. Erratum in: Toxicol Appl Pharmacol. 2012 Sep 15;263(3):402–3; Mar.

Christiansen, S., Kortenkamp, A., Axelstad, M., Boberg, J., Scholze, M., Jacobsen, P.R., Faust, M., Lichtensteiger, W., Schlumpf, M., Burdorf, A., Hass, U., 2012. Mixtures of endocrine disrupting contaminants modelled on human high end exposures: an exploratory study in rats. Int. J. Androl. 35 (3), 303–316. http://dx.doi.org/10.1111/j.1365-2605.2011.01242.x Jun.

de Cock, M., de Boer, M.R., Lamoree, M., Legler, J., van de Bor, M., 2014. First year growth in relation to prenatal exposure to endocrine disruptors — a Dutch prospective cohort study. Int J Environ Res Public Health. 11 (7), 7001–7021. http://dx.doi.org/10.3390/ijerph110707001 Jul 10.

Engel, S.M., Zhu, C., Berkowitz, G.S., Calafat, A.M., Silva, M.J., Miodovnik, A., Wolff, M.S., 2009. Prenatal phthalate exposure and performance on the Neonatal Behavioral Assessment Scale in a multiethnic birth cohort. Neurotoxicology 30 (4), 522–528 Jul. Epub 2009 Apr 16.

Enke, U., Schleussner, E., Pälmke, 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 (6), 735–742. http://dx.doi.org/10.1016/j.ijheh.2013.01.006 Epub 2013 Mar 7; Nov.

European Union. EUR-LEX. Communication from the Commission on the finalisation of the restriction process on the four phthalates (DEHP, DBP, BBP and DIBP) under Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Text with EEA relevance. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52014XC0809(01) (accessed September 14, 2015).

Factor-Litvak, P., Insel, B., Calafat, A.M., Liu, X., Perera, F., Rauh, V.A., Whyatt, R.M., 2014. Persistent associations between maternal prenatal exposure to phthalates on child IQ at age 7 years. PLoS ONE 9 (12), e114003. http://dx.doi.org/10.1371/journal.pone.0114003. eCollection 2014 Dec 10.

Ferguson, K.K., McElrath, T.F., Ko, Y.A., Mukherjee, B., Meeker, J.D., 2014a. Variability in urinary phthalate metabolite levels across pregnancy and sensitive windows of exposure for the risk of preterm birth. Environ. Int. 70C, 118–124. http://dx.doi.org/10.1016/j.envint.2014.05.016 Jun 12.

Ferguson, K.K., McElrath, T.F., Meeker, J.D., 2014b. Environmental phthalate exposure and preterm birth. JAMA Pediatr. 168 (1), 61–67. http://dx.doi.org/10.1001/jamapediatrics.2013.3699 Erratum in: JAMA Pediatr. 2014 Jul;168(7):684; Jan.

Fisher, M., Arbuckle, T.E., Mallick, R., LeBlanc, A., Hauser, R., Feeley, M., Koniecki, D., Ramsay, T., Provencher, G., Bérubé, R., Walker, M., 2015. Bisphenol A and phthalate metabolite urinary concentrations: daily and across pregnancy variability. J Expo Sci Environ Epidemiol. 25 (3), 231–239. http://dx.doi.org/10.1038/jes.2014.65 May.

Frederiksen, H., Skakkebaek, N.E., Andersson, A.M., 2007. Metabolism of phthalates in humans. Mol. Nutr. Food Res. 51 (7), 899–911 Review; Jul.

Frederiksen, H., Kuiri-Hänninen, 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 (9), 998–1005. http://dx.doi.org/10.1289/ehp.1307569 Epub 2014 May 30; Sep.

Fromme H, Gruber L, Seckin E, Raab U, Zimmermann S, Kiranoglu M, Schlummer M, Schwegler U, Smolic S, Völkel W; HBMnet. Phthalates and their metabolites in breast milk–results from the Bavarian Monitoring of Breast Milk (BAMBI). Environ. Int. 2011;37(4):715–22. doi: http://dx.doi.org/10.1016/j.envint.2011.02.008.May

Fromme, H., Lahrz, T., Kraft, M., Fembacher, L., Dietrich, S., Sievering, S., Burghardt, R., Schuster, R., Bolte, G., Völkel, W., 2013. Phthalates in German daycare centers: occurrence in air and dust and the excretion of their metabolites by children (LUPE 3). Environ. Int. 61, 64–72. http://dx.doi.org/10.1016/j.envint.2013.09.006 Nov.

Geens, T., Bruckers, L., Covaci, A., Schoeters, G., Fierens, T., Sioen, I., Vanermen, G., Baeyens, W., Morrens, B., Loots, I., Nelen, V., de Bellevaux, B.N., Larebeke, N.V., Hond, E.D., 2014. Determinants of bisphenol A and phthalate metabolites in urine of Flemish adolescents. Environ Res. 134C, 110–117. http://dx.doi.org/10.1016/j.envres.2014.07.020 [Epub ahead of print] Aug 12.

Gordon MC. Maternal physiology, chapter 3, IN Obstetrics: Normal and Problem Pregnancies, Sixth Edition. Gabbe, SG, Landon, MB, Niebyl, JR, Galan, HL, Simpson, JL, Jauniaux, ERM, Driscoll, DA (eds). Elsevier Saunders: Philadelphia PA 2012.

Guo, Y., Kannan, K., 2013. A survey of phthalates and parabens in personal care products from the United States and its implications for human exposure. Environ Sci Technol. 47 (24), 14442–14449. http://dx.doi.org/10.1021/es4042034 Epub 2013 Nov 27; Dec 17.

Guo, Y., Weck, J., Sundaram, R., Goldstone, A.E., Buck Louis, G., Kannan, K., 2014. Urinary concentrations of phthalates in couples planning pregnancy and its association with 8-hydroxy-2′-deoxyguanosine, a biomarker of oxidative stress: Longitudinal Investigation of Fertility and the Environment Study. Environ Sci Technol. 48 (16), 9804–9811. http://dx.doi.org/10.1021/es5024898 Epub 2014 Aug 8; Aug 19.

Hauser, R., Meeker, J.D., Park, S., Silva, M.J., Calafat, A.M., 2004. Temporal variability of urinary phthalate metabolite levels in men of reproductive age. Environ Health Perspect. 112 (17), 1734–1740 Dec. Erratum in: Environ Health Perspect. 2004 Dec;112(17): 1740.

Health Canada. 2011. Phthalates Regulations: Fact Sheet. January 2011. http://www.hc-sc.gc.ca/ahc-asc/media/nr-cp/_2011/2011_07fs-eng.php. Accessed August 18, 2014.

Health Canada. 2013. Second Report on Human Biomonitoring of Environmental Chemicals in Canada: Results of the Canadian Health Measures Survey Cycle 2 (2009–2011). April 2013. HC Pub.: 130019; Cat.: H128-1/10-601-1E-PDF; ISBN: 978–1-100-22140-3.

Health Canada. 2014. Cosmetics and Personal Care. Health Canada (HC), Consumer Product Safety Directorate — proprietary database. Accessed June 27, 2014. [http://hc-sc.gc.ca/cps-spc/person/cosmetic/index-eng.php]

Hildenbrand, S., Wodarz, R., Gabrio, T., Volland, G., 2009. Biomonitoring of the di(2-ethylhexyl) phthalate metabolites mono(2-ethyl-5-hydroxyhexyl) phthalate and mono(2-ethyl-5-oxohexyl) phthalate in children and adults during the course of time and seasons. Int. J. Hyg. Environ. Health 212 (6), 679–684. http://dx.doi.org/10.1016/j.ijheh.2009.06.003 Epub 2009 Jul 17; Nov.

Hines, E.P., Calafat, A.M., Silva, M.J., Mendola, P., Fenton, S.E., 2009. Concentrations of phthalate metabolites in milk, urine, saliva, and serum of lactating North Carolina women. Environ. Health Perspect. 117 (1), 86–92. http://dx.doi.org/10.1289/ehp.11610 Jan.

Janjua, N.R., Frederiksen, H., Skakkebaek, N.E., Wulf, H.C., Andersson, A.M., 2008. Urinary excretion of phthalates and paraben after repeated whole-body topical application in humans. Int. J. Androl. 31 (2), 118–130. http://dx.doi.org/10.1111/j.1365-2605.2007.00841.x Apr.

Kato, K., Silva, M.J., Needham, L.L., Calafat, A.M., 2006. Quantifying phthalate metabolites in human meconium and semen using automated off-line solid-phase extraction coupled with on-line SPE and isotope-dilution high-performance liquid chromatography–tandem mass spectrometry. Anal. Chem. 78 (18), 6651–6655 Sep 15.

Kay, V.R., Chambers, C., Foster, W.G., 2013. Reproductive and developmental effects of phthalate diesters in females. Crit. Rev. Toxicol. 43 (3), 200–219. http://dx.doi.org/10.3109/10408444.2013.766149 Mar.

Kim, Y., Ha, E.H., Kim, E.J., Park, H., Ha, M., Kim, J.H., Hong, Y.C., Chang, N., Kim, B.N., 2011. Prenatal exposure to phthalates and infant development at 6 months: prospective Mothers and Children's Environmental Health (MOCEH) study. Environ. Health Perspect. 119 (10), 1495–1500 Oct.

Kim, S., Lee, J., Park, J., Kim, H.J., Cho, G., Kim, G.H., Eun, S.H., Lee, J.J., Choi, G., Suh, E., Choi, S., Kim, S., Kim, Y.D., Kim, S.K., Kim, S.Y., Kim, S., Eom, S., Moon, H.B., Kim, S., Choi, K., 2015. Concentrations of phthalate metabolites in breast milk in Korea: estimating exposure to phthalates and potential risks among breast-fed infants. Sci. Total Environ. 508, 13–19. http://dx.doi.org/10.1016/j.scitotenv.2014.11.019 Mar 1.

Kiyama, R., Wada-Kiyama, Y., 2015. Estrogenic endocrine disruptors: molecular mechanisms of action. Environ. Int. 83, 11–40. http://dx.doi.org/10.1016/j.envint.2015.05.012 Jun 11.

Koch, H.M., Angerer, J., 2012. Phthalates: biomarkers and human biomonitoring. Chapter 3A, pp. 179–233. In: LE, Knudsen, DF, Merlo (Eds.), Issues in Toxicology No. 9. Royal Society of Chemistry.

Koch, H.M., Calafat, A.M., 2009. Human body burdens of chemicals used in plastic manufacture. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 364 (1526), 2063–2078. http://dx.doi.org/10.1098/rstb.2008.0208 Jul 27.

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 (2), 177–185 Oct.

Koch, H.M., Bolt, H.M., Angerer, J., 2004. Di(2-ethylhexyl)phthalate (DEHP) metabolites in human urine and serum after a single oral dose of deuterium-labelled DEHP. Arch. Toxicol. 78 (3), 123–130 Mar.

Koch, H.M., Christensen, K.L., Harth, V., Lorber, M., Brüning, T., 2012. Di-n-butyl phthalate (DnBP) and diisobutyl phthalate (DiBP) metabolism in a human volunteer after single oral doses. Arch. Toxicol. 86 (12), 1829–1839. http://dx.doi.org/10.1007/s00204-012-0908-1 Epub 2012 Jul 22; Dec.

Koch, H.M., Lorber, M., Christensen, K.L., Pälmke, C., Koslitz, S., Brüning, T., 2013. Identifying sources of phthalate exposure with human biomonitoring: results of a 48 h fasting study with urine collection and personal activity patterns. Int. J. Hyg. Environ. Health 216 (6), 672–681. http://dx.doi.org/10.1016/j.ijheh.2012.12.002 Epub 2013 Jan 18; Nov.

Koniecki, D., Wang, R., Moody, R.P., Zhu, J., 2011. Phthalates in cosmetic and personal care products: concentrations and possible dermal exposure. Environ. Res. 111 (3), 329–336. http://dx.doi.org/10.1016/j.envres.2011.01.013 Epub 2011 Feb 18; Apr.

Kubwabo, C., Rasmussen, P.E., Fan, X., Kosarac, I., Wu, F., Zidek, A., Kuchta, S.L., 2013. Analysis of selected phthalates in Canadian indoor dust collected using household vacuum and standardized sampling techniques. Indoor Air 23 (6), 506–514. http://dx.doi.org/10.1111/ina.12048 Epub 2013 Jun 7; Dec.

Lampel, H.P., Jacob, S.E., 2011. Phthalates in baby skin care products. Dermatitis 22 (5), 272–276. http://dx.doi.org/10.2310/6620.2011.11065 Sep–Oct.

Langlois, E., LeBlanc, A., Simard, Y., Thellen, C., 2012. Accuracy investigation of phthalate metabolite standards. J. Anal. Toxicol. 36, 270–279.

Langlois, É., Saravanabhavan, G., Arbuckle, T.E., Giroux, S., 2014. Correction and comparability of phthalate metabolite measurements of Canadian biomonitoring studies (2007-2012). Environ. Int. 64, 129–133. http://dx.doi.org/10.1016/j.envint.2013.12.002 Epub 2014 Feb 8; Mar.

LaRocca, J., Binder, A.M., McElrath, T.F., Michels, K.B., 2014. The impact of first trimester phthalate and phenol exposure on IGF2/H19 genomic imprinting and birth outcomes. Environ. Res. 133, 396–406. http://dx.doi.org/10.1016/j.envres.2014.04.032 Epub 2014 Jun 25; Aug.

Larsson, K., Ljung Björklund, K., Palm, B., Wennberg, M., Kaj, L., Lindh, C.H., Jönsson, B.A., Berglund, M., 2014. Exposure determinants of phthalates, parabens, bisphenol A and triclosan in Swedish mothers and their children. Environ. Int. 73, 323–333. http://dx.doi.org/10.1016/j.envint.2014.08.014 Epub 2014 Sep 16; Dec.

Latini, G., Wittassek, M., Del Vecchio, A., Presta, G., De Felice, C., Angerer, J., 2009. Lactational exposure to phthalates in Southern Italy. Environ. Int. 35 (2), 236–239. http://dx.doi.org/10.1016/j.envint.2008.06.002 Epub 2008 Aug 5; Feb.

Li, L.X., Chen, L., Meng, X.Z., Chen, B.H., Chen, S.Q., Zhao, Y., Zhao, L.F., Liang, Y., Zhang, Y.H., 2013. Exposure levels of environmental endocrine disruptors in mother–newborn pairs in China and their placental transfer characteristics. PLoS ONE 8 (5), e62526. http://dx.doi.org/10.1371/journal.pone.0062526. Print 2013 May 7.

Lin, S., Ku, H.Y., Su, P.H., Chen, J.W., Huang, P.C., Angerer, J., Wang, S.L., 2011. Phthalate exposure in pregnant women and their children in central Taiwan. Chemosphere 82 (7), 947–955 Epub 2010 Nov 13; Feb.

Lorber, M., Koch, H.M., Angerer, J., 2011. A critical evaluation of the creatinine correction approach: can it underestimate intakes of phthalates? A case study with di-2-ethylhexyl phthalate. J Expo Sci Environ Epidemiol. 21 (6), 576–586. http://dx.doi.org/10.1038/jes.2010.43 Epub 2010 Sep 8; Nov–Dec.

Main, K.M., Mortensen, G.K., Kaleva, M.M., Boisen, K.A., Damgaard, I.N., Chellakooty, M., Schmidt, I.M., Suomi, A.M., Virtanen, H.E., Petersen, D.V., Andersson, A.M., Toppari, J., Skakkebaek, N.E., 2006. Human breast milk contamination with phthalates and alterations of endogenous reproductive hormones in infants three months of age. Environ. Health Perspect. 114 (2), 270–276 Feb.

Marie, C., Vendittelli, F., Sauvant-Rochat, M.P., 2015. Obstetrical outcomes and biomarkers to assess exposure to phthalates: a review. Environ. Int. 83, 116–136. http://dx.doi.org/10.1016/j.envint.2015.06.003 Jun 25.

Matos, V., Drukker, A., Guignard, J.P., 1999. Spot urine samples for evaluating solute excretion in the first week of life. Arch. Dis. Child. Fetal Neonatal Ed. 80 (3), F240–F242 May.

Mortensen, G.K., Main, K.M., Andersson, A.M., Leffers, H., Skakkebaek, N.E., 2005. Determination of phthalate monoesters in human milk, consumer milk, and infant formula by tandem mass spectrometry (LC–MS–MS). Anal. Bioanal. Chem. 382 (4), 1084–1092 Jun. Epub 2005 Jun 3.

Myridakis, A., Fthenou, E., Balaska, E., Vakinti, M., Kogevinas, M., Stephanou, E.G., 2015. Phthalate esters, parabens and bisphenol-A exposure among mothers and their children in Greece (Rhea cohort). Environ. Int. 83, 1–10. http://dx.doi.org/10.1016/j.envint.2015.05.014 Oct.

Peck, J.D., Sweeney, A.M., Symanski, E., Gardiner, J., Silva, M.J., Calafat, A.M., Schantz, S.L., 2010. Intra- and inter-individual variability of urinary phthalate metabolite concentrations in Hmong women of reproductive age. J Expo Sci Environ Epidemiol. 20 (1), 90–100. http://dx.doi.org/10.1038/jes.2009.4 Jan.

Preau Jr., 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 (12), 1748–1754. http://dx.doi.org/10.1289/ehp.1002231 Epub 2010 Aug 25; Dec.

Quigley, R., 2012. Developmental changes in renal function. Curr. Opin. Pediatr. 24 (2), 184–190. http://dx.doi.org/10.1097/MOP.0b013e32834fe863 Apr.

Sakhi, A.K., Lillegaard, I.T., Voorspoels, S., Carlsen, M.H., Løken, E.B., Brantsæter, A.L., Haugen, M., Meltzer, H.M., Thomsen, C., 2014. Concentrations of phthalates and bisphenol A in Norwegian foods and beverages and estimated dietary exposure in adults. Environ. Int. 73, 259–269. http://dx.doi.org/10.1016/j.envint.2014.08.005 Epub 2014 Aug 28. Dec.

Sathyanarayana, S., Karr, C.J., Lozano, P., Brown, E., Calafat, A.M., Liu, F., Swan, S.H., 2008. Baby care products: possible sources of infant phthalate exposure. Pediatrics 121 (2), e260–e268. http://dx.doi.org/10.1542/peds.2006-3766 Feb.

Schlumpf, M., Kypke, K., Wittassek, M., Angerer, J., Mascher, H., Mascher, D., Vökt, C., Birchler, M., Lichtensteiger, W., 2010. Exposure patterns of UV filters, fragrances, parabens, phthalates, organochlor pesticides, PBDEs, and PCBs in human milk: correlation of UV filters with use of cosmetics. Chemosphere 81 (10), 1171–1183. http://dx.doi.org/10.1016/j.chemosphere.2010.09.079 Epub 2010 Oct 27; Nov.

Serrano, S.E., Braun, J., Trasande, L., Dills, R., Sathyanarayana, S., 2014. Phthalates and diet: a review of the food monitoring and epidemiology data. Environ. Heal. 13 (1), 43. http://dx.doi.org/10.1186/1476-069X-13-43 Jun 2.

Silva, M.J., Reidy, J.A., Preau, J.L., Samandar, E., Needham, L.L., Calafat, A.M., 2006. Measurement of eight urinary metabolites of di(2-ethylhexyl) phthalate as biomarkers for human exposure assessment. Biomarkers 11 (1), 1–13 Jan–Feb.

Sobolewski, M., Conrad, K., Allen, J.L., Weston, H., Martin, K., Lawrence, B.P., Cory-Slechta, D.A., 2014. Sex-specific enhanced behavioral toxicity induced by maternal exposure to a mixture of low dose endocrine-disrupting chemicals. Neurotoxicology 45, 121–130. http://dx.doi.org/10.1016/j.neuro.2014.09.008 Dec.

Suzuki, Y., Yoshinaga, J., Mizumoto, Y., Serizawa, S., Shiraishi, H., 2012. Foetal exposure to phthalate esters and anogenital distance in male newborns. Int. J. Androl. 35 (3), 236–244. http://dx.doi.org/10.1111/j.1365-2605.2011.01190.x Epub 2011 Jun 22; Jun.

Swan, S.H., Sathyanarayana, S., Barrett, E.S., Janssen, S., Liu, F., Nguyen, R.H., JB, Redmon, TIDES Study Team, 2015. First trimester phthalate exposure and anogenital distance in newborns. Hum. Reprod. 30 (4), 963–972. http://dx.doi.org/10.1093/humrep/deu363 Epub 2015 Feb 18; Apr.

Tefre de Renzy-Martin, K., Frederiksen, H., Christensen, J., Boye Kyhl, H., Andersson, A.M., Husby, S., Barington, T., Main, K.M., Jensen, T.K., 2014. Current exposure of 200 pregnant Danish women to phthalates, parabens and phenols. Reproduction 147 (4), 443–453. http://dx.doi.org/10.1530/REP-13-0461 Mar 2.

Valvi, D., Monfort, N., Ventura, R., Casas, M., Casas, L., Sunyer, J., Vrijheid, M., 2015. Variability and predictors of urinary phthalate Metabolites in Spanish pregnant women. Int. J. Hyg. Environ. Health 218 (2), 220–231. http://dx.doi.org/10.1016/j.ijheh.2014.11.003. Epub 2014 Mar.

Völkel, W., Kiranoglu, M., Schuster, R., Fromme, H., HBMnet, 2014. Phthalate intake by infants calculated from biomonitoring data. Toxicol. Lett. 225 (2), 222–229. http://dx.doi.org/10.1016/j.toxlet.2013.12.012 Epub 2013 Dec 24; Mar 3.

Weschler, C.J., Bekö, G., Koch, H.M., Salthammer, T., Schripp, T., Toftum, J., Clausen, G., 2015. Transdermal uptake of diethyl phthalate and Di(n-butyl) phthalate directly from air: experimental verification. Environ Health Perspect. Apr 7. [Epub ahead of print].

Whyatt, R.M., Liu, X., Rauh, V.A., Calafat, A.M., Just, A.C., Hoepner, L., Diaz, D., Quinn, J., Adibi, J., Perera, F.P., Factor-Litvak, P., 2012. Maternal prenatal urinary phthalate metabolite concentrations and child mental, psychomotor, and behavioral development at 3 years of age. Environ. Health Perspect. 120 (2), 290–295 Epub 2011 Aug 31; Feb.

Wormuth, M., Scheringer, M., Vollenweider, M., Hungerbühler, K., 2006. What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? Risk Anal. 26 (3), 803–824 Jun.

Xie, C., Jin, R., Zhao, Y., Lin, L., Li, L., Chen, J., Zhang, Y., 2015. Paraoxonase 2 gene polymorphisms and prenatal phthalates' exposure in Chinese newborns. Environ. Res. 140, 354–359. http://dx.doi.org/10.1016/j.envres.2015.03.028 Epub 2015 Apr 24; Jul.

Xu, Y., Liang, Y., Urquidi, J.R., Siegel, J.A., 2015. Semi-volatile organic compounds in heating, ventilation, and air-conditioning filter dust in retail stores. Indoor Air 25 (1), 79–92. http://dx.doi.org/10.1111/ina.12123 Epub 2014 Jun 10; Feb.

Ye, X., Pierik, F.H., Hauser, R., Duty, S., Angerer, J., Park, M.M., Burdorf, A., Hofman, A., Jaddoe, V.W., Mackenbach, J.P., Steegers, E.A., Tiemeier, H., Longnecker, M.P., 2008. Urinary metabolite concentrations of organophosphorous pesticides, bisphenol A, and phthalates among pregnant women in Rotterdam, the Netherlands: the Generation R study. Environ. Res. 108 (2), 260–267 Epub 2008 Sep 5; Oct.

Ye, X., Wong, L.Y., Bishop, A.M., Calafat, A.M., 2011. Variability of urinary concentrations of bisphenol A in spot samples, first morning voids, and 24-hour collections. Environ. Health Perspect. 119 (7), 983–988. http://dx.doi.org/10.1289/ehp.1002701 Epub 2011 Mar 15; Jul.

Ye, X., Zhou, X., Hennings, R., Kramer, J., Calafat, A.M., 2013. Potential external contamination with bisphenol A and other ubiquitous organic environmental chemicals during biomonitoring analysis: an elusive laboratory challenge. Environ. Health Perspect. 121 (3), 283–286. http://dx.doi.org/10.1289/ehp.1206093 Epub 2013 Jan 15; Mar.

Zhang, Y., Lin, L., Cao, Y., Chen, B., Zheng, L., Ge, R.S., 2009. Phthalate levels and low birth weight: a nested case-control study of Chinese newborns. J. Pediatr. 155 (4), 500–504. http://dx.doi.org/10.1016/j.jpeds.2009.04.007 Oct.