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Measurement of 24 phthalate metabolites in 1st trimester urine samples: The MIREC study

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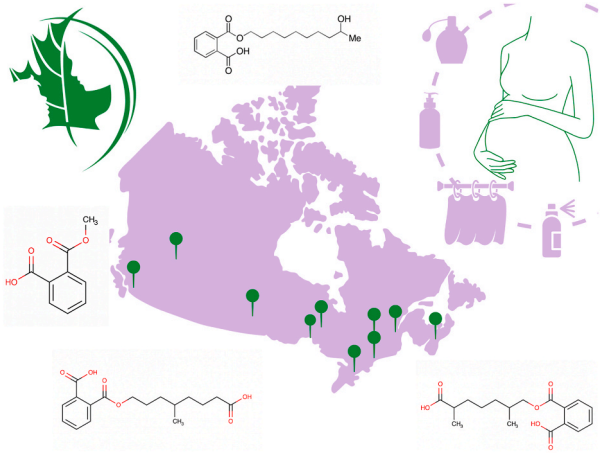
HIGHLIGHTS

- Methods developed to measure 24 urinary phthalate metabolites.
- Improved sensitivity for MMP.
- MCHP, MCHpP, MiDP, and MnOP rarely detected.
- Due to isomeric mixtures, MCiOP, MiNP and MCiNP reported semi-quantitatively.
- When comparing results, reporting algorithms and lab methods need to be considered.

GRAPHICAL ABSTRACT



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ABSTRACT

Phthalates are non-persistent chemicals measured as metabolites in urine. Over time, new metabolites have been identified. In the original Maternal-Infant Research on Environmental Chemicals (MIREC) study (2008–2011), we measured 11 phthalate metabolites in first trimester urine samples. The goal of the present study was to develop a method to measure new metabolites, to increase the sensitivity for some previously measured metabolites, and to measure these new metabolites in biobanked urine samples from MIREC participants.

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Biomonitoring
Cohort

Using Ultra Performance Liquid Chromatography with a tandem mass spectrometer, we developed a method to measure 24 metabolites from 10 different parent phthalates. Chromatographic interpretation of some of the di-iso-decyl phthalate metabolites (mono-(2-propyl-6oxoheptyl) phthalate (MOiDP), mono-(2,7-methyl-7-carboxyheptyl) phthalate (MCiNP), mono-(2-propyl-6-hydroxy-heptyl) phthalate (MHiDP)) and di-iso-nonyl phthalate metabolites (mono(oxo-isononyl) phthalate (MOiNP), mono(carboxy-isooctyl) phthalate (MCiOP), mono (hydroxy-isononyl) phthalate (MHiNP) and mono-isononyl phthalate (MiNP)) was challenging as these are complex isomeric mixtures.

To validate and confirm our quantitation peaks, an assay using a high-resolution detection technique was developed on a Quadrupole Time-of-Flight (QToF) system. This system has a mass resolution of at least 0.005 amu, compared to 0.5 amu for the MS/MS detector. Using the QToF system, the distinction between an isomer and possible interference was achieved with the use of the exact mass.

In about 1800 MIREC samples, mono-cyclo-hexyl phthalate (MCHP), mono-(7-carboxy-n-heptyl) phthalate (MCHpP), mono-iso-decyl phthalate (MiDP), and mono-n-octyl phthalate (MnOP) were rarely detected, while detection of MMP was improved. MCiOP, MiNP and MCiNP had to be reported semi-quantitatively.

Given the complexity of isomeric mixtures of some phthalates, researchers must be careful in their determination of the analytes and the approach used in their quantification when generating biomonitoring data.

This study produced biomonitoring data for a large population of pregnant people that can be used in risk assessment of phthalates. Future work will examine associations with birth and child outcomes.

1. Introduction

Phthalates are a large group of chemicals used in many consumer products such as vinyl flooring, lubricating oils, children's toys, personal-care products (e.g., fragrances, soaps, shampoos, and hair sprays), plastic packaging, garden hoses and medical tubing (Government of Canada, 2020). Phthalate metabolites have been measured in biomonitoring surveys of the general population conducted in Canada (Health Canada, 2021), the United States (CDC, 2022) and Germany (Schwedler et al., 2020), which have demonstrated their ubiquity in the environment. Analytical methods continue to be developed to identify new metabolites, as well as increase the sensitivity for detection.

We previously published results of an analysis of 11 phthalate metabolites (from DMP (dimethyl phthalate), DEP (diethyl phthalate), DnBP (di(n-butyl) phthalate), BBzP (butyl benzyl phthalate), DEHP (di (2-ethylhexyl) phthalate), DCHP (dicyclohexyl phthalate), DINP (Di-iso-nonyl phthalate), and DNOP (Di-n-octyl phthalate) in urine samples from pregnant people enrolled in the Maternal-Infant Research on Environmental Chemicals (MIREC) study (Arbuckle et al., 2014). Similarly, the same 11 metabolites were measured in urine from the general population in Canada during the same time period (Saravanabhavan et al., 2013). However, several of the major metabolites of DEHP and DnBP, as well as other common phthalates such as DiBP (di-iso-butyl phthalate) were not measured in these studies. Methods have since been developed to identify and quantify additional phthalate metabolites in urine and to improve the sensitivity of others such as those for DMP. Furthermore, results from a 2012 Canadian Environmental Protection Act section 71 survey determined that DINP, DIDP (Di-iso-decyl phthalate), and DEHP were manufactured in and/or imported into Canada in quantities greater than 10 million kg/year (Environment and Climate Change Canada and Health Canada, 2020); it was therefore important for human risk assessment to quantify the major identified metabolites of these compounds. Based on the metabolism of other high carbon side chain phthalic acid esters (like DNOP and DEHP), the DINP and DIDP hydrolytic monoester (MiNP and MiDP) and their corresponding carboxy (MCiOP and MCiNP), hydroxy (MHiNP and MHiDP) and oxo (MOiNP and MOiDP) monoester metabolites were chosen to evaluate our population's exposure.

The laboratory at the *Centre de Toxicologie du Québec (CTQ) Institut national de santé publique du Québec* (INSPQ) developed a new method to measure 24 phthalate metabolites, as well as to improve the sensitivity for some of the previously measured metabolites. Here, we report the details of that method as well as the descriptive statistics for the 24 phthalate metabolites measured in first trimester biobanked urine samples.

2. Methods

2.1. Study population

Between 2008 and 2011, approximately 2000 participants from 10 cities across Canada were recruited to participate in the MIREC Study (Arbuckle et al., 2013). Informed consent was obtained for participating in the study and storing data and excess biospecimens in the MIREC Biobank for future research throughout pregnancy and into childhood. The study was reviewed and approved by the Health Canada Research Ethics Board and ethics committees at all recruitment sites.

2.2. Analytical method for urinary phthalates

First trimester urine specimens were collected in 125 mL Nalgene® containers (Thermo-Fisher Scientific Inc., Rochester NY, USA), aliquoted into 2 mL Simport® tubes and stored at −20 °C in the MIREC Biobank. Samples were removed from cold storage and brought to room temperature on the day of analysis. The duration of this stage was usually less than 60 min.

Metabolites of 10 different parent phthalates were measured for a total of 24 phthalate metabolites (Table 1). The analytical method for phthalate metabolites was as follows: 500 μL of urine was enriched with labeled internal standards (MMP-$^{13}C_4$, MEP-$^{13}C_4$, MCPP-$^{13}C_4$, MiBP-d_4, MnBP–$^{13}C_4$, 2-OH-MiBP-d_4, MHBP-d_4, MCHP-$^{13}C_4$, MBzP-$^{13}C_4$, MECPP-$^{13}C_4$, MCMHP-d_4, MEHP-$^{13}C_4$, MEHHP-$^{13}C_4$, MEOHP-$^{13}C_4$, MiDP-d_4, MnOP–$^{13}C_4$ and MiNP-$^{13}C_4$). The urinary metabolites were then hydrolyzed with 100 μL of 2% β-glucuronidase enzyme solution in a 1 M acetate buffer at pH 6.5 for 75 min at 37 °C. Thereafter, the samples were acidified with a 50% H_3PO_4 solution and were extracted with a mixture of hexane:ethyl acetate (1:1) from the aqueous matrix using a liquid-liquid extraction. The extracts were evaporated to dryness and dissolved in 400 μL of 25% acetonitrile.

The samples were then analyzed by Ultra Performance Liquid Chromatography (UPLC Waters Acquity) with a tandem mass spectrometer (MS/MS Waters Xevo TQ-S) (Waters; Milford, MA, USA) in the Multiple Reaction Monitoring (MRM) mode with an electrospray ion source in the negative mode. The column used was an ACE EXCEL C-18-AR 50 mm × 2.1 mm x 2.0 μm (ACE; Aberdeen, Scotland). The chromatography conditions consisted of a linear gradient (including isocratic steps) of 0.1% acetic acid in methanol:0.1% acetic acid in water (10:90) to (95:5) in 12.5 min at a flow rate of 0.4 mL/min at 30 °C.

A calibration curve prepared in washed urine was used to measure the phthalate metabolites. The limits of detection (LOD) reported were between 0.065 and 0.76 μg/L depending on the analyte (Table 2). The LOD was calculated following the analysis of the samples containing the

Table 1
Phthalate metabolites measured in the MIREC Study, 2008–2011.

Parent Compound	Metabolite	Metabolite Abbreviation
Di-iso-butyl phthalate (DiBP)	2-hydroxy-mono-iso-butyl phthalate	2-OH-MiBP
	Mono-iso-butyl phthalate	MiBP
Butyl benzyl phthalate (BBzP)	Mono-benzyl phthalate	MBzP
Dicyclohexyl phthalate (DCHP)	Mono-cyclo-hexyl phthalate	MCHP
Di-n-octyl phthalate (DNOP)	Mono-(7-carboxy-n-heptyl) phthalate	MCHpP
	Mono-n-octyl phthalate	MnOP
	Mono-(3-carboxypropyl) phthalate	MCPP
Di-iso-decyl phthalate (DIDP)	Mono-(2,7-methyl-7-carboxyheptyl) phthalate OR Mono-carboxy-isononyl phthalate (cx-MiNP)	MCiNP
	Mono-(2-propyl-6-hydroxy-heptyl) phthalate OR Mono-hydroxy-isodecyl phthalate (OH-MiDP)	MHiDP
	Mono-iso-decyl phthalate	MiDP
	Mono-(2-propyl-6oxoheptyl) phthalate OR Mono-oxo-isodecyl phthalate (oxo-MiDP)	MOiDP
Di-iso-nonyl phthalate (DINP)	Mono(carboxy-isooctyl) phthalate	MCiOP
	Mono(hydroxy-isononyl) phthalate (OH-MiNP)	MHiNP
	Mono-isononyl phthalate	MiNP
	Mono(oxo-isononyl) phthalate OR 7-Oxo-(Mono-methyloctyl) phthalate (oxo-MiNP)	MOiNP
Di-(2-ethylhexyl) phthalate (DEHP)	Mono(2-carboxy-methylhexyl) phthalate	MCMHP
	Mono(2-ethyl-5-carboxy-pentyl) phthalate	MECPP
	Mono-(2-ethylhexyl) phthalate	MEHP
	Mono-(2-ethyl-5-hydroxy-hexyl) phthalate	MEHHP
	Mono-(2-ethyl-5-oxo-hexyl) phthalate	MEOHP
Diethyl phthalate (DEP)	Mono-ethyl phthalate	MEP
Dimethyl phthalate (DMP)	Mono-methyl phthalate	MMP
Di-n-butyl phthalate (DnBP)	Mono-3-hydroxy-n-butyl phthalate	MHBP
	Mono-n-butyl phthalate	MnBP

analytes at a concentration varying from 7- to 10-fold the estimated LOD, on a minimum of 30 replicas. The calculated LOD was equal to 3 times the standard deviation (SD) of those replicas. The limit of quantification (LOQ) was equal to 10 times the SD. The intra-day precision ranged between 2.2 and 13% and the inter-day precision ranged between 3.2 and 12% (Table 2). The spiked recoveries ranged between 76 and 99% (Table 2).

The internal reference materials used to control the quality of the phthalate metabolite analyses were in-house reference materials prepared from a pool of urine of non-occupationally exposed people. The overall quality and accuracy of the analytical method were monitored by participation in the interlaboratory program of the German External Quality Assessment Scheme (G-EQUAS; Erlangen, Germany) for the following phthalate monoesters: mono-(2-ethyl-5-hydroxy-hexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxo-hexyl) phthalate (MEOHP), mono(2-ethyl-5-carboxy-pentyl) phthalate (MECPP), mono-(2-ethylhexyl) phthalate (MEHP), mono-n-butyl phthalate (MnBP), mono-isobutyl phthalate (MiBP) and mono-benzyl phthalate (MBzP).

To account for heterogeneity in urinary dilution, individual phthalate concentrations were standardized for urine specific gravity (SG), measured using a refractometer, according to the following formula: $P_c = P_i\ [(SG_m - 1)/(SG_i - 1)]$, where P_c = SG standardized metabolite concentration (μg/L), P_i = observed metabolite concentration, SG_i = specific gravity of the urine sample, and SG_m = median SG for the cohort (adapted from Hauser et al., 2004).

Based on the analysis of field and laboratory blanks, there was no evidence of contamination.

2.2.1. DINP and DIDP analysis

DINP (Di-iso-nonyl phthalate) and DIDP (Di-iso-decyl phthalate) are medium- and long-chain phthalates, respectively. DINP is a complex isomeric mixture containing mainly C8 and C9-branched isomers on its side chains (NICNAS, 2008). DIDP is a complex isomeric mixture containing mainly C10-branched isomers (Environment Canada and Health Canada, 2015). Consequently, the chromatographic interpretation of some of DIDP metabolites (mono-(2-propyl-6oxoheptyl) phthalate (MOiDP), mono-(2,7-methyl-7-carboxyheptyl) phthalate (MCiNP), mono-(2-propyl-6-hydroxy-heptyl) phthalate (MHiDP)) and DINP metabolites (mono(oxo-isononyl) phthalate (MOiNP), mono (carboxy-isooctyl) phthalate (MCiOP), mono(hydroxy-isononyl) phthalate (MHiNP) and mono-isononyl phthalate (MiNP)) was challenging.

Contrary to the DINP and DIDP metabolites in exposed participants, the source of DINP and DIDP metabolites used to build the calibration curve corresponded to only one of the possible isomers. Consequently, major differences were observed between the chromatography of calibrators (one peak) and the exposed participant (clustering of peaks). The possible differences in the fragmentation pattern among isomers were also something to consider for data interpretation. In fact, even if they have the same molar mass, isomers may produce different fragmentation patterns depending on how and where the C_8 to C_{10} branched chain phthalates were with regards to their molecular skeleton.

The following summarizes the interrogations we encountered during the peak integration process:

a) The participant sample ion ratios (IR) (quantitative transition/qualitative transition) were different than the calibrator IR. Was the quantitative transition selective?
b) Coelution was observed for several DINP and DIDP metabolites in participant chromatograms near the calibrator retention time (Rt). Which peak should be integrated? Were they all isomers?
c) There was no peak at the expected calibrator Rt, but several other peaks were found nearby. Were they isomers or interferences?

To validate and confirm our quantitation peaks, an assay using a high-resolution detection technique was developed on a Waters G2 Quadrupole Time-of-Flight (QtoF) system (Waters; Milford, MA, USA). This system has a mass resolution of at least 0.005 amu, compared to 0.5 amu for the MS/MS TQ-S detector. Using the QtoF system, the distinction between an isomer and a possible interference could be achievable with the use of the exact mass.

To prove the validity of the analyte peaks, participant samples that showed DINP and DIDP metabolite concentrations greater than 1 μg/L were selected to make sure that all peaks were sensitive enough on the UPLC-QtoF system and to confirm the masses found. Eight out of 250 of the first participant samples were chosen because they matched this concentration criterion. A 200 μg/L calibrator was added to confirm the retention time and the exact mass of the metabolites. The extraction and injection conditions were the same as described in section 2.2.

We show typical data used to achieve our conclusion in Figs. 1–4.

To be considered selective, the participant peak(s) chosen and the calibrator peak must respect the following conditions:

1 Having the same Rt
2 Mass difference must be less than 0.0025 amu
3 No interference mass present.

For the above example (Fig. 4), all the peaks showed presence of calibrator mass (307.1554), but only Peak 3 did not show any presence of interference mass (307.1194). Consequently, only peak 3 was

integrated among the clustering of peaks present in the MHiNP MS/MS chromatogram and this compound was reported quantitatively. The same approach was applied for the six other DINP and DIDP metabolites.

For the analytes MCiOP, MiNP and MCiNP, a cluster of peaks was also present in the participant samples. After analysis, the chosen participant peaks showed mass related to the calibrator and no interference mass was present, but their retention times (participant versus calibrator) were different. Considering this difference in retention times, MCiOP, MiNP and MCiNP have been reported semi-quantitatively. All other analytes have been reported quantitatively.

3. Results

First trimester urinary concentrations of 24 phthalate metabolites were measured in about 1800 pregnant participants (Tables 3 and 4). In addition to being able to measure 13 new metabolites, this analytical method substantially improved the sensitivity for MMP from 5.0 (Arbuckle et al., 2014) to 0.21 μg/L and the percentage of non-detects was lowered from 85% to 4%. While the new method had a higher LOD (was less sensitive) for some other metabolites, these metabolites were still highly detected (e.g., mono-ethyl phthalate (MEP) and MnBP).

The following metabolites were rarely detected (<30%) in first trimester urine samples: the metabolite of dicyclohexyl phthalate (MCHP), the metabolites of di-n-octyl phthalate – (MCHpP and MnOP), and some of the metabolites of di-iso-decyl phthalate - (MiDP, MHiDP) and MOiDP) (Tables 3 and 4). The DIDP metabolite MCiNP was detected in 97% of the sample but based on a semi-quantitative analysis. Among the DINP metabolites, MCiOP and MiNP were detected in about 70% of the samples, but are reported as semi-quantitative, whereas MHiNP and MOiNP were found in about 90% and 80% respectively of the first trimester urines.

Spearman correlation coefficients identified moderate to high correlations among several of the metabolites, especially those from parent phthalates (Fig. S1, Supplemental material).

4. Discussion and conclusions

Using this new method, we measured 24 phthalate metabolites in biobanked first trimester urine samples. The rarely detected analytes were the metabolites of dicyclohexyl phthalate (MCHP), di-n-octyl phthalate – (MCHpP and MnOP), and some of the metabolites of di-iso-decyl phthalate - (MiDP, MHiDP and MOiDP). Based on their limited sources of exposure in the general population, observing low detection rates for these phthalates is not surprising. The main routes of exposure to DCHP are inhalation or ingestion through product use via transfer from hand to mouth or via through mouthing of articles containing dicyclohexyl phthalate (e.g., g adhesives and sealants, arts, crafts and hobby materials, fabric, textile and leather products, paper products and toys, playground and sporting equipment) (EPA 2020). DNOP can be found in products such as carpetback coating, packaging films, medical tubing and blood storage bags, floor tiles, wire, cables, and adhesives, as well as in cosmetics and pesticides (ATSDR, 1992), but has had limited use in Canada (Government of Canada, 1993). DIDP is a plasticizer used as a polyvinylchloride (PVC) liner to package aqueous, acidic and low alcohol food products; for the general population, indirect exposure (e.g., off-gassing) is considered a relevant source; however, young children likely have the highest daily intake from food and beverages (Environment Canada and Health Canada, 2015). Those phthalates with the most frequently detected metabolites are primarily used in products that consumers are frequently exposed to such as nail polish and cosmetics, some food packaging, printing inks, pharmaceutical coatings, textiles and insecticides (e.g., DiBP, DnBP, DEP, DEHP, BBzP). For example, in a biomonitoring study of 80 pregnant people, MEP concentrations were significantly higher when women reported using makeup or body lotion in the last 24 h compared to those who hadn't used these products and was highest when the usage occurred within 0–6 h before the urine sample collection (Fisher et al., 2019). Elimination of different phthalates in urine and feces tends to be lower after dermal exposure, compared to the total elimination (sum of the median eliminations in urine and feces) of these compounds after oral or intravenous exposure (Domínguez-Romero and Scheringer, 2019). In addition to the variability of exposure due to different sources and

Table 2
Limits of detection (LOD) and quantification (LOQ), spiked recoveries, intra-day, and inter-day precision (reproducibility) for measurement of phthalate metabolites.

Analyte	LOD (μg/L)	LOQ (μg/L)	Spiked Recovery (%)	Intra-day precision (%)	Inter-day precision (%)		
					Low QC[c]	Medium QC[c]	High QC[c]
MBzP	0.14 (0.2)[b]	0.46	98	7.7	8.3	3.5	4.1
MCHP	0.25 (0.2)[b]	0.84	98	6.8	4.7	3.8	4.0
MCHpP	0.083	0.28	98	5.5	6.9	3.6	5.1
MCiNP[a]	0.075	0.25	98	7.8	8.6	5.1	7.2
MCiOP[a]	0.13	0.45	99	5.6	7.1	5.7	6.5
MCMHP	0.27	0.89	98	10	6.1	4.5	5.5
MCPP	0.14 (0.2)[b]	0.45	77	7.3	5.8	3.5	5.2
MECPP	0.28	0.95	97	2.2	4.2	3.2	3.2
MEHHP	0.22 (0.4)[b]	0.73	99	6.1	6.1	3.9	5.4
MEHP	0.077 (0.2)[b]	0.26	96	3.6	6.4	3.7	4.7
MEOHP	0.17 (0.2)[b]	0.57	98	3.2	5.8	3.7	4.7
MEP	0.76 (0.5)[b]	2.5	90	7.2	7.7	3.9	4.0
MHBP	0.068	0.23	76	7.6	7.5	6.5	7.6
MHiDP	0.065	0.22	97	9.4	9.1	6.3	6.4
MHiNP	0.065	0.21	95	9.5	7.5	4.2	5.3
MiBP	0.57	1.9	94	8.7	6.7	5.4	5.0
MiDP	0.16	0.52	91	13	12	7.0	7.4
MiNP[a]	0.15 (0.4)[b]	0.49	96	6.0	7.3	5.3	6.4
MMP	0.21 (5.0)[b]	0.71	85	8.0	7.1	5.7	4.6
MnBP	0.60 (0.2)[b]	2.0	95	4.1	4.8	3.6	3.9
MnOP	0.16 (0.7)[b]	0.54	96	5.0	7.3	3.8	4.8
MOiDP	0.097	0.32	97	6.2	8.1	5.4	5.1
MOiNP	0.15	0.50	99	7.0	6.3	4.1	4.6
2-OH-MiBP	0.27	0.90	80	9.5	8.1	5.1	6.1

[a] Semi-quantitative analysis.
[b] The values in brackets are LODs from the original method (Arbuckle et al., 2014).
[c] QC: Quality Control.

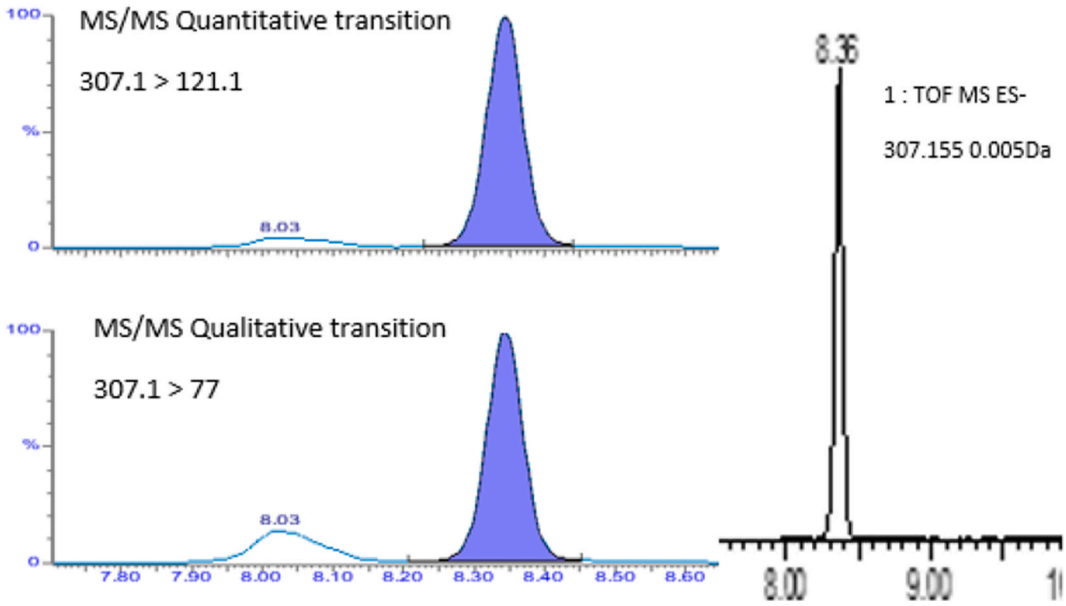

Fig. 1. MS/MS (left) and QtoF (right) MHiNP Calibrator chromatograms.

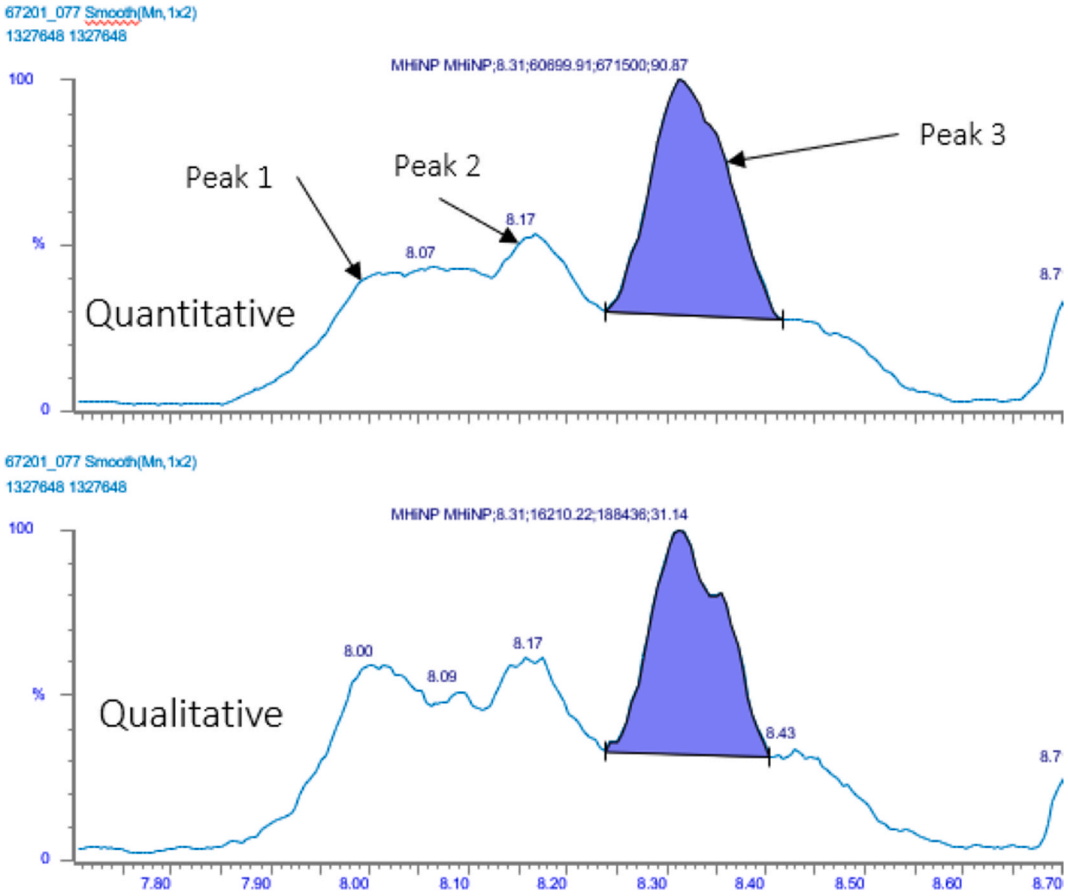

Fig. 2. Representative participant's MHiNP MS/MS chromatogram (quantitative and qualitative transitions). Peak 1 (7.95 min), Peak 2 (8.17 min) and Peak 3 at 8.36 min.

routes, urinary concentrations are influenced by metabolism, renal excretion rates and chemical structure. The proportion of the dose that is excreted via urine as the monoester and its hydroxy, oxo and carboxy products decreases with increasing alkyl chain length, whereas with short chain phthalates such as BBzP and DnBP, the simple monoesters seem to be the major metabolites (Wittassek and Angerer, 2008).

The German Environmental Survey GerES V measured several DINP and DIDP metabolites in the urine of 2200 children and adolescents and reported geometric mean concentrations approximately 10 times higher (Schwedler et al., 2020) than those observed in pregnant MIREC

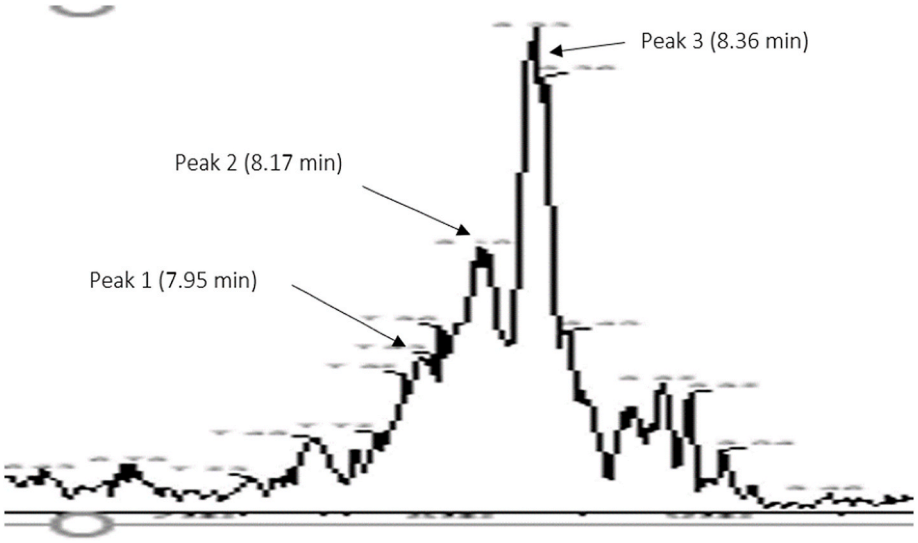

Fig. 3. QtoF chromatogram of the same participant as Fig. 2.

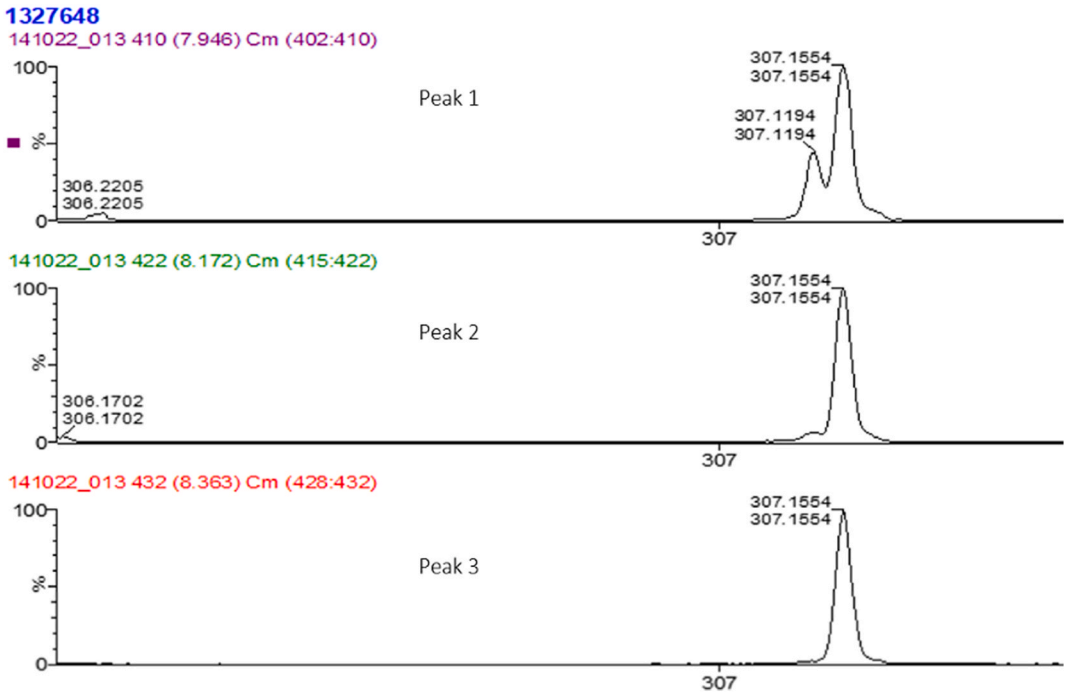

Fig. 4. Extracted mass (307.1545 ± 0.0050 amu) of the participant QtoF chromatogram for peak 1, 2 and 3. Theoretical exact mass of MHiNP ($C_{17}O_5H_{23}$) is 307.1545. The calibrator exact mass found was 307.1554 (data not shown).

participants. The years of sampling (2014–2017 for Germany and 2008–2011 for Canada) may be one reason for these differences as DINP exposure has increased over the past decade (Wang et al., 2019). Additionally, phthalate levels in children tend to be higher than in adults (Health Canada, 2021).

Some phthalate metabolites (MCHpP, the Di-iso-nonyl phthalate metabolites (MCiOP, MHiNP and MOiNP) and the Di-iso-decyl metabolites (MCiNP, MHiDP, MOiDP and MiDP)) were not reported in the US National Health and Nutrition Examination Survey (NHANES) 1999–2018 (CDC, 2022), but were measured in the Canadian Health Measure Survey (2018–2019) (Health Canada, 2021). However, metabolites of Di-iso-nonyl phthalate (MCiOP, MiNP) and Di-iso-decyl phthalate (MCiNP) had to be reported semi-quantitatively, a limitation of this study. This creates an issue when comparing phthalate metabolite results from one study with those from another laboratory if different reporting algorithms were used. Importantly, in this study we demonstrated the non-specificities of the reported concentrations of the metabolites (MCiOP, MiNP and MCiNP). Additional strengths of this study include the large sample size (over 1800 women) and the measurement of these metabolites in a susceptible population in early pregnancy.

Using state-of-the-art technology and having a wide array of different analytical technologies at our disposal, we were able to design an innovative and specific approach that provided sufficient information to allow us to decide whether an analyte should be reported as semi or fully quantitatively. Our approach could be applied to other similar analyses where there is a cluster of peaks due to the presence of isomers for a specific analyte. The main objective of the analysis is to avoid integrating interferences in the quantification peaks. This information

Table 3
Descriptive statistics of 24 phthalate metabolites in 1st trimester urine samples (μg/L), MIREC Study (2008–2011).
Note 1: non-positive machine reading results substituted by ½ of next smallest positive value. Note 2: If detection rate was lower than 50%, geometric mean and 95% CI were not calculated.

			Percentiles							
	n	% < LOD	25th	50th	75th	95th	Max	GM	Lower 95% CI	Upper 95% CI
2-OH-MiBP	1855	1.8	1.63	3.87	9.07	24.68	257.06	3.64	3.43	3.85
MBzP	1840	4.6	1.08	3.09	7.62	27.56	807.18	2.89	2.70	3.09
MCHP	1864	93.9	0.01	0.01	0.01	0.32	68.52			
MCHpP	1851	99.8	0.01	0.01	0.01	0.01	1.11			
MCiNP[1]	1695	2.6	0.35	0.78	1.74	6.65	495.72	0.80	0.75	0.85
MCiOP[1]	1871	22.8	0.21	0.63	1.74	9.44	562.14	0.46	0.42	0.51
MCMHP	1861	4.2	0.95	2.24	4.94	15.03	353.30	2.14	2.02	2.26
MCPP	1775	12.9	0.28	0.74	1.70	7.90	283.06	0.64	0.59	0.70
MECPP	1845	0.6	2.68	6.58	14.16	47.74	929.01	6.34	5.98	6.71
MEHHP	1867	0.6	2.00	5.19	12.92	43.98	812.94	5.10	4.80	5.42
MEHP	1831	1.6	0.59	1.47	3.65	12.55	256.20	1.47	1.38	1.56
MEOHP	1857	1.3	1.43	3.85	9.56	31.50	724.03	3.63	3.41	3.87
MEP	1871	1.0	9.12	24.28	70.58	429.13	11907.10	26.47	24.62	28.47
MHBP	1774	3.9	0.54	1.46	3.55	11.26	6952.54	1.24	1.14	1.34
MHiDP	1837	58.2	0.00	0.02	0.17	0.70	31.22			
MHiNP	1781	11.2	0.27	0.80	2.27	17.05	281.35	0.57	0.51	0.64
MiBP	1846	3.5	1.99	5.01	11.62	30.93	340.06	4.67	4.41	4.95
MiDP	1865	98.8	0.00	0.00	0.00	0.06	13.02			
MiNP[1]	1857	27.7	0.20	0.53	1.55	14.30	298.04	0.36	0.31	0.40
MMP	1790	4.3	0.84	1.84	3.51	8.32	784.40	1.54	1.44	1.64
MnBP	1822	2.1	3.11	8.28	19.82	60.90	51119.49	7.95	7.47	8.46
MnOP	1870	98.3	0.00	0.00	0.00	0.01	4.61			
MOiDP	1823	57.5	0.00	0.06	0.21	0.82	23.80			
MOiNP	1857	20.6	0.18	0.52	1.62	11.76	228.28	0.38	0.34	0.42

LOD limit of detection, GM geometric mean.
[1] Semi-quantitative analysis.

Table 4
Descriptive statistics of specific gravity standardized phthalate metabolites in 1st trimester urine samples (μg/L), MIREC Study (2008–2011).
Note 1: non-positive machine reading results substituted by ½ of next smallest positive value. His Majesty the King in Right of Canada, as represented by the Minister of Health, 2023. Note 2: If detection rate was lower than 50%, geometric mean and 95% CI were not calculated.

		Percentiles					Geometric mean		
	% < LOD	25th	50th	75th	95th	max	GM	Lower 95% CI	Upper 95% CI
2-OH-MiBP	1.8	2.39	4.11	6.78	16.20	111.39	4.07	3.91	4.23
MBzP	4.6	1.53	3.04	6.79	20.72	476.97	3.24	3.07	3.41
MCHP	93.9	0.00	0.01	0.02	0.27	36.91			
MCHpP	99.8	0.00	0.01	0.01	0.03	0.56			
MCiNP[a]	2.6	0.48	0.77	1.37	4.83	381.87	0.87	0.83	0.91
MCiOP[a]	22.8	0.32	0.66	1.45	7.31	304.49	0.52	0.48	0.57
MCMHP	4.2	1.44	2.24	3.78	10.25	208.77	2.41	2.32	2.51
MCPP	12.9	0.41	0.72	1.30	5.55	159.99	0.71	0.67	0.76
MECPP	0.6	4.01	6.55	10.84	33.33	606.54	7.06	6.78	7.35
MEHHP	0.6	3.00	5.56	9.66	29.91	557.72	5.74	5.49	6.00
MEHP	1.6	0.83	1.57	2.90	8.57	222.20	1.64	1.56	1.71
MEOHP	1.3	2.12	3.98	7.07	21.40	427.84	4.07	3.89	4.26
MEP	1.0	11.18	24.24	66.56	375.29	14347.67	29.88	28.08	31.79
MHBP	3.9	0.79	1.46	2.77	7.89	3929.69	1.36	1.28	1.45
MHiDP	58.2	0.00	0.02	0.15	0.48	17.65			
MHiNP	11.2	0.36	0.79	1.89	12.39	415.16	0.63	0.57	0.70
MiBP	3.5	2.96	5.40	9.12	19.15	144.53	5.24	5.04	5.44
MiDP	98.8	0.00	0.00	0.00	0.05	11.76			
MiNP[a]	27.7	0.25	0.59	1.40	10.90	265.90	0.40	0.36	0.45
MMP	4.3	1.20	1.80	2.80	6.13	424.88	1.71	1.63	1.79
MnBP	2.1	4.69	8.69	15.32	41.40	28893.62	8.81	8.42	9.21
MnOP	98.3	0.00	0.00	0.00	0.02	2.85			
MOiDP	57.5	0.00	0.08	0.18	0.55	23.55			
MOiNP	20.6	0.24	0.54	1.24	8.60	357.71	0.43	0.39	0.47

[a] Semi-quantitative analysis.

increased our method specificity and validated the peak masses of the measured analytes. Moreover, our method showed excellent precision, reproducibility and sensitivity.

Researchers should carefully assess the accuracy of the phthalate metabolites data by considering the complexity in the analytical determination and the approach used in the quantification.

Future research using these data will investigate potential associations with health indicators in MIREC participants.

CRediT authorship contribution statement

Tye E. Arbuckle: Conceptualization, Resources, Writing – original

draft, Funding acquisition. **Éric Gaudreau:** Investigation, Methodology, Writing – original draft. **Susan MacPherson:** Data curation, Formal analysis, Writing – review & editing. **Muzeyyen Kabasakal:** Data curation, Formal analysis, Writing – review & editing. **Michael M. Borghese:** Writing – review & editing. **Mandy Fisher:** Writing – review & editing. **Maryse F. Bouchard:** Writing – review & editing. **Warren Foster:** Writing – review & editing. **Jillian Ashley-Martin:** Supervision, Writing – review & editing. **Gilles Provencher:** Investigation, Methodology, Writing – original draft.

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.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

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