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Article

ENVIRONMENTAL CHEMICAL CONTAMINANTS

GC-MS Analysis of Phthalates and Di-(2-thylhexyl) Adipate in Canadian Human Milk for Exposure Assessment of Infant Population

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Abstract

Background: Although more information has become available on the occurrence of phthalates and di(2-ethylhexyl) adipate (DEHA) in foods including cow's milk, information on their presence in human milk, the important and recommended sole diet for infants up to six months of age, is very limited, especially for DEHA.
Objective: To develop a GC-MS method for simultaneous analysis of DEHA and phthalates in human milk samples and generate occurrence data for exposure assessment.
Method: Human milk samples were extracted with acetonitrile followed by dispersive solid-phase extraction and GC-MS analysis.
Results: Among the 305 human milk samples collected from the Canadian Maternal-Infant Research on Environmental Chemicals Study, some phthalates (DHxP, BBzP, and DOP) were not detected in any of the samples, while DEHA and the other phthalates (DMP, DEP, DBP, DiBP, and DEHP) were detected at low frequencies with levels from 30.4–237 ng/g in up to 31 of the 305 human milk samples.
Conclusions: In general, DEHA and phthalates were detected at low frequencies and low levels in the 305 human milk samples.
Highlights: A GC-MS method based on dispersive solid phase extraction was developed for analysis of DEHA and eight phthalates in 305 human milk samples for exposure assessment.

Phthalates are a group of diesters of ortho-phthalic acid and primarily used as plasticizers to soften polyvinyl chloride (PVC) products or as solvents to hold color and scent in various cosmetics and personal care products (1) depending on their molecular weights. Some phthalates are also used in food contact materials, such as di-(2-ethylhexyl) phthalate (DEHP) which is used in PVC gaskets of lids for glass bottles and jars (2–5) and PVC cling film (6), and can migrate from food contact materials into foods, especially fatty foods, since they are not chemically bound to the polymer.

Di-(2-ethylhexyl) adipate (DEHA) is structurally different from the phthalates, but they are all lipophilic and thus have been analyzed together in some studies to investigate their occurrence in foods (7, 8). DEHA is also widely used as a plasticizer in food contact materials, such as PVC food wrapping or cling films (5, 6, 9–15) and PVC gaskets of lids for glass jars (2–4). Like

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other plasticizers, DEHA can also migrate from food contact materials to foods.

DEHA and various phthalates including DEHP were identified as priority chemicals for risk assessment under the Government of Canada's Chemicals Management Plan due to their potential for exposure from indoor air, dust, water, and food. In the 2011 Government of Canada screening assessment report for DEHA (16), food was estimated to be the highest contributor to the total intake for most age groups, however, a high level of uncertainty with the estimates of exposure from food was highlighted. More information has become available on the occurrence of DEHA and phthalates in foods including cow's milk, especially from recent studies (15–22). However, information on their presence in human milk, the important and recommended sole diet for infants up to six months of age, is very limited, especially for DEHA (23–26). The last Canadian study where human milk was analyzed for selected phthalates was in 2006 (27). In this study, a GC-MS method based on liquid and dispersive solid-phase extraction was developed and used to simultaneously determine DEHA and selected phthalates in human milk samples, generating recent Canadian occurrence data of both DEHA and selected phthalates in human milk for exposure assessment of the infant population.

Experimental

Sample Collection and Storage

Human milk samples were collected by the study participants, either by hand or using the breast pump provided, between the second and tenth week after delivery. Although human milk samples from 990 donors were collected from 10 cities (Vancouver, Edmonton, Winnipeg, Toronto, Hamilton, Sudbury, Kingston, Ottawa, Montreal, and Halifax) across Canada over a period of 2 years (2009–2011), aliquots of only 305 of them were analyzed for DEHA and phthalates based on the capacity of our laboratory. All sample aliquots were stored in 15 mL polypropylene vials in a freezer in our laboratory at −20°C until analysis. Both the breast pump (Medela, Mississauga, ON) and the 15 mL polypropylene vial were tested for migration of DEHA and the eight phthalates to water prior to use, and they were not detected.

Materials and Reagents

Di-methyl phthalate (DMP, >99%), di-ethyl phthalate (DEP, 99.5%), di-(2-ethylhexyl) phthalate (DEHP, 99%), n-hexane (99%), Na_2SO_4 (anhydrous, ACS grade), and the pre-packaged 15 mL centrifuge tube with 900 mg $MgSO_4$ and 150 mg PSA were purchased from Sigma–Aldrich (Mississauga, ON, Canada). Di-iso-butyl phthalate (DiBP, 99%), di-n-butyl phthalate (DBP, >99%), di-n-hexyl phthalate (DHxP, 98%), *n*-butyl benzyl phthalate (BBzP, 98%), di-(2-ethylhexyl) adipate (DEHA, 99%), and di-n-octyl phthalate (DOP) were purchased from Alfa Aesar (Ward Hill, MA). The following labelled chemicals were all purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada): di-methyl phthalate-3,4,5,6-d4 (DMP-d4, 99 atom% D), di-ethyl phthalate-3,4,5,6-d4 (DEP-d4, 98 atom% D), di-iso-butyl phthalate-3,4,5,6-d4 (DiBP-d4, 99 atom% D), di-n-butyl phthalate-3,4,5,6-d4 (DBP-d4, 99 atom% D), n-butyl benzyl phthalate-3,4,5,6-d4 (BBzP-d4, 99 atom% D), di-n-hexyl phthalate-3,4,5,6-d4 (DHxP-d4, 99 atom% D), di-(2-ethylhexyl) adipate-d8 (DEHA-d8, 98 atom% D), di-(2-ethyhexyl) phthalate-3,4,5,6-d4 (DEHP-d4, 98 atom% D), and di-n-octyl phthalate-3,4,5,6-d4 (DOP-d4, 99 atom% D). Acetonitrile (HPLC grade) was purchased from J.T. Baker (Phillipsburg, NJ, USA). Sodium chloride (>99%) was obtained from VWR (Mississauga, ON, Canada). The human milk used for method validation and quality control was purchased from Innovative Research (www.innov-research.com).

Six composite standard solutions containing DEHA and phthalates, with concentrations from 0.02 to 1 ng/μL, were prepared in hexane. The solution containing deuterated DEHA and the phthalates, used for spiking samples, was prepared in hexane with concentrations from 2.7 to 3.5 ng/μL. All solutions were stored at 4 °C.

Sample Extraction and Analysis

About 0.5 g of each human milk sample was weighed into a 15 mL glass centrifuge tube with 0.5 g of NaCl, and spiked with deuterated internal standard solution. The sample was extracted with 5 mL of acetonitrile for about 30 min at room temperature, and the acetonitrile extract was transferred to a pre-packaged 15 mL centrifuge tube containing 900 mg $MgSO_4$ and 150 mg PSA for dispersive solid-phase extraction. After centrifugation for 15 min, the acetonitrile extract was filtered through Na_2SO_4 packed in a 16 × 100 mm glass tube and concentrated to about 0.5 mL under nitrogen flow for analysis.

An Agilent 7890 gas chromatograph (GC) coupled to a 5975 mass selective detector (MSD) was used for analysis. The injector temperature was 280°C. The GC column used was DB-5MS capillary column (30 m × 0.25 mm × 1.0 μm, Agilent Technologies). The GC oven temperature program was set at 50°C for 2 min, then raised to 280°C for 6 min at 15°C/min. The flow rate of the helium carrier gas was 1.2 mL/min. The MSD was operated with electron impact ionization in selected ion monitoring (SIM) mode. Table 1 shows the list of ions used for GC-MS analysis of DEHA and phthalates in SIM mode. The dwell time was 35 ms for each ion. The GC-MSD interface temperature and MSD source temperature were 280 and 230°C, respectively.

Table 1. List of ions used for GC-MS analysis of phthalates in SIM mode

Phthalates	CAS no.	Quantification ion	Qualifier ion
DMP	131-11-3	163	194
DEP	84-66-2	149	177
DiBP	84-69-5	149	223
DBP	84-74-2	149	223
DHxP	84-75-3	149	251
BBzP	85-68-7	149	206
DEHA	103-23-1	129	147
DEHP	117-81-7	149	167
DOP	117-84-0	149	279
DMP-d4	93951-89-4	167	
DEP-d4	93952-12-6	153	
DiBP-d4	358730-88-8	153	
DBP-d4	93952-11-5	153	
DHxP-d4	1015854-55-3	153	
BBzP-d4	93951-88-3	153	
DEHA-d8	1214718-98-5	137	
DEHP-d4	93951-87-2	153	
DOP-d4	93952-13-7	153	

Confirmation of the identities of DEHA and phthalates was based on the retention time and the ion ratio observed for analytical standards. The calculation of DEHA and phthalate concentrations in samples was based on isotope dilution method.

Results and Discussion

Linearity of the instrument and the method was demonstrated using six standard solutions of DEHA and phthalates with concentrations from 0.02 to 1 ng/μL. R^2 values of >0.999 were observed for DEHA and phthalate calibration curves, plotted with peak areas normalized to the internal standard versus concentrations. The method was validated for human milk at three spiking levels from 50 to 400 ng/g and the results are shown in Table 2. The average recovery for all analytes was 107.7%, with relative standard deviations from 0.64 to 9.9% (mean: 3.2%).

The Maternal–Infant Research on Environmental Chemicals (MIREC) Study was one of the human biomonitoring initiatives under the Chemicals Management Plan launched by the Government of Canada in 2006. It was a multi-year research study that recruited about 2000 women from major cities across Canada (28, 29). One of the goals of the MIREC Study was to measure the concentrations of various environmental chemicals in human milk, such as dioxins/furans and PCBs (30), 2- and 3-monochloropropanediol fatty acid esters (31), perchlorate (32), and bisphenol A (33).

In this study, a total of 305 human milk samples were analyzed for DEHA and the eight phthalates. Each batch of analysis included five method blanks, three unspiked commercial human milk samples, and three spiked commercial milk samples as quality control (QC). All samples were analyzed in duplicate. Method detection limits (MDLs) were estimated for every batch of analysis due to variation of background contamination. MDLs for the analytes which were not detected in the method blanks (DMP, DHxP, and DOP) were estimated as 10 times the signal-to-noise ratios from the spiked QC samples, and the average MDLs ranged from 1.01 ng/g for DMP to 9.97 ng/g for DOP (Table 3). For those analytes which were detected in the method blanks (DEP, DiBP, DBP, BBzP, DEHA, and DEHP), MDLs were calculated as three times the standard deviation of the method blanks, and the average MDLs varied from 2.85 ng/g for DEP to 66.8 ng/g for DEHP (Table 3).

Among the eight phthalates, DMP, DHxP, BBzP, and DOP were not detected in any of the 305 human milk samples. DEHA was detected in only one human milk sample at 31.4 ng/g. The detection frequencies of the other four phthalates were also low; DEP was detected in 31 samples (10.2%) with a maximum concentration of 87.7 ng/g and an average of 7.35 ng/g, DiBP was detected in 27 samples (8.85%) with a maximum of 85.4 ng/g and an average of 33.3 ng/g, DBP was detected in 21 samples (6.89%) with a maximum of 30.4 ng/g and an average of 12.9 ng/g, while DEHP was detected in 23 samples (7.54%) with a maximum of 237 ng/g and an average of 97.7 ng/g.

Sources of phthalates in human milk may vary depending on the applications of each phthalate. For example, the presence of DEHP and DEHA in human milk could be from dietary exposure due to migration from food packaging, while the presence of lower molecular weight phthalates, such as DEP and DBP, in human milk is more likely from dermal exposures by the mothers due to use of various personal care products, especially with the use of leave-on personal care products (34, 35).

Compared to the results from limited studies from other countries (Table 4), results of DEP and DiBP from our study are higher by about 10-fold. It should be mentioned that the averages of our results were calculated with the positives only, that is, non-detect results were not included in the calculation of averages. However, it is not always clear how the averages of results from the other studies (23–27) were calculated, that is, if non-detects were included and what values were used for non-detects (0, ½ detection limit, or detection limit). Results of DBP from our study are also higher than those from Zhu et al. (27), Fromme et al. (25), Zimmermann et al. (26), and Hogberg et al. (24), but much lower than those from the Chinese study (22). For DEHP, our results are higher than those from the German studies (25, 26), but much lower than those from the previous Canadian study by Zhu et al. (27). The maximum DEHP concentration from our study (237 ng/g) is close to the result (305 ng/g) from the Swedish study by Hogberg et al. (24), much lower than the European Union specific migration limit (SML) of 1.5 mg/kg

Table 2. Method accuracy and precision

Phthalates	Mean recovery, % ± SD ($n = 5$)								
	50 ng/g			100 ng/g			400 ng/g		
DMP	108	±	1.2	109	±	0.7	102	±	2.2
DEP	109	±	1.5	109	±	0.9	99.7	±	0.9
DiBP	115	±	9.6	113	±	5.3	99.1	±	5.1
DBP	106	±	6.0	115	±	4.5	85.3	±	3.5
DHxP	109	±	1.2	110	±	1.0	103	±	2.3
BBzP	114	±	2.5	114	±	1.7	104	±	2.0
DEHA	112	±	7.4	113	±	2.5	106	±	2.1
DEHP	110	±	9.3	113	±	5.6	107	±	10.6
DOP	110	±	1.3	112	±	0.9	99.3	±	1.6

Table 3. Summary of concentrations of DEHA and phthalates in human milk samples

Phthalate	Number of samples (no. of positives)	MDL, ng/g (mean, range)	Concentration, ng/g	
			Mean (positives)	Range
DMP	305 (0)	1.01 (0.16–2.40)	–	–
DEP	305 (31)	2.85 (0.94–7.67)	7.35	<MDL - 87.7
DiBP	305 (27)	44.6 (6.18–208)	33.3	<MDL - 85.4
DBP	305 (21)	14.9 (4.06–56.1)	12.9	<MDL - 30.4
DHxP	305 (0)	3.56 (0.92–11.5)	–	–
BBzP	305 (0)	7.41 (1.32–26.5)	–	–
DEHA	305 (1)	17.7 (4.24–40.0)	31.4	31.4
DEHP	305 (23)	66.8 (10.4–195)	97.7	<MDL - 237
DOP	305 (0)	9.97 (1.57–31.1)	–	–

Table 4. Comparison of average DEHA and phthalate concentrationsin human milk samples from different studies

Country	Sample size	DEP, ng/g (range)	DiBP, ng/g (range)	DBP, ng/g (range)	BBzP, ng/g (range)	DEHP, ng/g (range)	DEHA, ng/g (range)	References
Canada	86	0.2 (0.2–8.1)	–	0.5 (0.1–11)	–	116 (1.2–2920)	–	(26)
Canada	305	7.35 (<2.85–87.7)	33.3 (<44.6–85.4)	12.9 (<14.9–30.4)	–	97.7 (<66.8–237)	31.4	This study[a]
China	40	<0.05	–	53.5 (0.6–174)	–	–	–	(22)
Germany	78	<3	1.2 (<0.1–5.3)	0.8 (<0.1–7.4)	<10	3.9 (<0.5–23.5)	(<20.0–27.0)	(24)
Germany	30	–	1.0 (<LOQ–5.8)	0.6 (<LOQ–3.6)	<5	2.3 (<LOQ–7.6)	–	(25)
Sweden	42	0.2 (0.2–1.5)	–	1.5 (1.5–20)	0.5 (0.1–4.4)	9.0 (0.5–305)	–	(23)

[a] Average of positives detected.

for DEHP according to Directive 2007/19/EC (36), while the average DEHP concentration from our study is higher, 97.7 ng/g versus 9.0 ng/g. The result of DEHA from our study (31.4 ng/g) is very close to the results (<20.0–27.0 ng/g) from the German study (25), and much lower than the SML of 18 mg/kg (37).

In summary, a GC-MS method based on liquid and dispersive solid-phase extraction was developed and used for simultaneous analysis of DEHA and eight selected phthalates in 305 human milk samples collected from the Canadian MIREC Study. Some phthalates (DHxP, BBzP, and DOP) were not detected in any of the samples, while DEHA and the other phthalates (DMP, DEP, DBP, DiBP, and DEHP) were detected at low frequencies with the highest levels from 30.4 to 237 ng/g in up to 31 of the 305 human milk samples.

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Conflict of Interest

The authors declares there is no conflict of interest for this work.

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