Exposure Characteristics of Phthalate Metabolites Among The Zunyi Cohort of Pregnant Women in Southwest China

Juan Liao (✉ 630140512@qq.com)

Soochow University Medical College <https://orcid.org/0000-0002-3799-2272>

Derong Fang

Affiliated Hospital of Zunyi Medical University

Yijun Liu

Zunyi Medical University

Shimin Xiong

Zunyi Medical University

Xia Wang

Zunyi Medical University

Yingkuan Tian

Zunyi Medical University

Haonan Zhang

Zunyi Medical University

Songlin An

Zunyi Medical University

Caidie He

Zunyi Medical University

Wei Chen

Zunyi Medical University

Xiang Liu

Zunyi Medical University

Nian Wu

Zunyi Medical University

Kunming Tian

Zunyi Medical University

Linglu Wang

The Second Affiliated Hospital of Zunyi Medical University

Ya Zhang

Zunyi Medical University

Hongyu Yuan

Zunyi Medical University

Li Zhang

Zunyi Medical University

Quan Li

Affiliated Hospital of Zunyi Medical University

Xubo Shen

Zunyi Medical University

Yuanzhong Zhou

Zunyi Medical University

Research Article

Keywords: phthalate, pregnancy, urinary metabolites, exposure levels, sampling season, detectable rate

Posted Date: February 3rd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1190081/v1>

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Abstract

Reported evidence has increasingly indicated that exposure to phthalates can cause adverse pregnancy outcomes. However, phthalate exposure levels among pregnant women remains unclear. We aimed to evaluate the concentrations of phthalate metabolites in urine samples of the Zunyi cohort of pregnant women from Southwest China. The study was conducted between August 2020 and July 2021, and urine samples were collected from 1003 pregnant women during their third trimester of pregnancy. The concentrations of nine phthalate metabolites in urine samples were then determined. Data on socio-demographic profiles of the participants, lifestyle during pregnancy, parity, and sampling season were collected using questionnaires. The detectable rate of phthalate metabolites ranged from 76–100%. On average, mono-butyl phthalate exhibited the highest median concentration (84.24 µg/L), while mono-benzyl phthalate exhibited the lowest median concentration (0.08 µg/L). Urine concentrations of phthalate metabolites were significantly higher in older, multiparous, higher-body mass index, higher-income, and passive-smoking during pregnancy participants. The levels of low-molecular-weight phthalate metabolites were highest during the summer. In light of our findings, phthalate metabolites present a demographic and seasonal differential distribution among the study population.

Introduction

Phthalates are widely used in numerous consumer and food packaging products, resulting in the inevitable long-term exposure of the human body to phthalate metabolites (Benjamin et al., 2017; Calafat et al., 2015). The results of previously published studies suggest that some of these metabolites may adversely impact human health, particularly during sensitive periods of life, such as in utero (Ipapo et al., 2017; Bergman et al., 2013).

Published epidemiological data suggest that maternal prenatal exposure to phthalate metabolites endanger maternal and fetal health as well as birth outcomes. A prospective cohort study conducted in Denmark (Toft et al., 2012) found that the concentration of mono(2-ethylhexyl) phthalate (DEHP) in pregnant women's urine before pregnancy was correlated with the risk of spontaneous abortions. Several studies in United States (Messerlian et al., 2016) and China (Mu et al., 2015; Liao et al., 2018; Gao et al., 2017) have reached the same conclusion. Additionally, maternal exposure to phthalates can increase the odds of preterm birth (Ferguson et al., 2019; Broe et al., 2019; Gao et al., 2019), and it is related to fetal birth size (Guo et al., 2020; Polańska et al., 2016; De et al., 2015). Moreover, maternal exposure to phthalates was observed to lead to gestational hypertension and gestational diabetes mellitus (Werner et al., 2015; James-Todd et al., 2016). Finally, in vivo (Chen et al., 2020) and in vitro experimental studies (Zong et al., 2015) have reached the same conclusions as the population studies. Chen *et al.* found that exposure to di-n-butyl phthalate (DBP) during pregnancy promotes the insurgence of gestational diabetes mellitus by significantly increasing the expression of protein pSTAT1 and inhibiting that of FOXM1; the authors also found that the said exposure causes a decrease in islets β cell viability (Chen et al., 2020). Zong proposed that, in mice, exposure to DEHP during pregnancy administration disrupted labyrinth vascularization of placentas, and inhibited proliferation and induced apoptosis of placenta by the activation of caspase-3 and caspase-8, up-regulation of Bax and down-regulation of Bcl-2 mRNA and protein resulting in an increase in the probability of embryonic loss and a decrease in fetal body weight (Zong et al., 2015).

Given the hazards to maternal and infant health associated with the exposure to phthalate metabolites, it is necessary to determine the exposure levels of phthalate metabolites in pregnant women and analyze the risk factors leading to that exposure. As described in the literature, following exposure to phthalates present in a variety of sources, such as water, air, soil, and food, these chemicals enter the human body through ingestion, inhalation, or dermal exposure (Dualde et al., 2020); as a result, different exposure routes and sources may be associated with different exposure levels for the human population. Previously published studies have discussed the distribution and predictors of the presence of phthalate metabolites in the urine of pregnant women in Eastern China (He et al., 2019; Gao et al., 2017) and other developed countries (Polinski et al., 2018; Wenzel et al., 2018; Wu et al., 2020). These population surveys conducted in economically developed geographical areas indicate that the exposure level of phthalates in pregnant women's urine is related to economic conditions, education, race, age, body mass index (BMI), dietary patterns, and lifestyle. Nevertheless, data on the level of phthalate pollution in areas characterized by underdeveloped economies, good ecological environmental protection, and relatively small chemical pollution are still missing, leading to no comprehensive assessment of maternal–fetal harm of phthalate metabolites.

Therefore, the goal of the present investigation was to conduct a population-based study aimed at determining the level of phthalate metabolites in pregnant women from undeveloped Southwest China as well as the correlation of this parameter with demographic characteristics and seasonal variations. The results of such a study could offer suggestions on suitable specific interventions to reduce the risk of high phthalate exposure during pregnancy.

Methods

Study population

Pregnant women hospitalized for childbirth in the third trimester were recruited from August 2020 to July 2021 at Xishui and Meitan County People's Hospital, which are located in Zunyi city, Southwest China. After informed consent, study participants signed the informed consent form and filled in the electronic questionnaire during face-to-face interviews. Study eligibility criteria included natural conception, singleton pregnancy, and live fetus. Patients were excluded if they were suffering from serious chronic diseases and infectious diseases, such as cancer, chronic cardiovascular and cerebrovascular diseases, chronic renal failure, and HIV infection. This study was ethically reviewed by the Affiliated Hospital of Zunyi Medical University (batch No.: KLL-2019-006).

Survey questionnaire

The questions to include in the questionnaire were discussed and decided by several epidemiological experts over many meetings. Demographic information was obtained through a questionnaire administered to women upon enrollment in the study; the questionnaire included queries on the demographic data of pregnant women: age, pre-pregnancy weight, height, education, annual household income, lifestyle during pregnancy (smoking, drinking, passive smoking, and drinking bottled water), parity, and sampling season. The participants' BMI (weight (kg)/height (m)²) was calculated based on physician-recorded height and weight at the time of enrollment. Based on maternal age, participants were divided into the following three groups: < 25 years, 25–29 years, and ≥ 30 years of age. Based on pre-pregnancy BMI, participants were divided into the following three groups: underweight (<18.5 kg/m²), normal (18.5–23.9 kg/m²), and overweight (≥ 24 kg/m²). Participants were divided into four groups based on annual household income: low income (< ¥100,000), middle income (¥100,000–¥150,000), high income (> ¥150,000), and don't know. Based on maximum education level reached, participants were divided into three ordinal groups: middle school or below, high school and middle special school, college degree or above. Parity afforded the definition of two participant groups: nulliparous (0) and multiparous (≥ 1). Passive smoking is defined as exposure to cigarette smoke for at least half an hour a day. The sampling seasons were defined as follows: spring (March–May), summer (June–August), fall (September–November), and winter (December–February). Maternal age and BMI were treated as continuous variables, and annual household income, maternal education, passive smoking, parity, drinking bottled water, and sample collection season were treated as categorical variables.

Measurement of phthalate metabolites

Urine samples from 1003 women were analyzed to determine the concentration of nine phthalate metabolites by high-performance gas chromatography mass spectrometry (GC-MS/MS, Agilent 7010b, Santa Clara, CA, USA); these metabolites are mono-methyl phthalate (MMP), mono-methyl phthalate (MEP), mono-ethyl phthalate (MiBP), mono-butyl phthalate (MBP), mono-octyl phthalate (MOP), mono-benzyl phthalate (MBzP), mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP). Spot urine specimens were collected in polypropylene tubes in the obstetrical clinics and transferred to the Zunyi Medical University Laboratory (Zunyi, Southwest China) for analysis; urine samples were divided into 1-mL aliquots and stored at –80 °C until analysis. Urinary creatinine concentration was determined by the laboratory of the Affiliated Hospital of Zunyi Medical University using an automatic urine analyzer (Beckman, AU5800, Kraemer Boulevard Brea, CA, USA). Details of the method for detecting the concentration of metabolites in urine can be found in the **Supplementary information**. Briefly, 1.5 mL of urine were added into a 10mL centrifuge tube; add 1 mL of sodium acetate solution buffer to urine sample and standard sample, 10 µL internal standard solution and 10 µL β-glucuronidase/sulphatase enzyme to fully hydrolyze the sample; excess MgSO₄·7H₂O was then added to reach solution supersaturation. To the tubes was then added 1 mL of n-hexane + diethyl ether (4:1); the tubes were then centrifuged, and the relevant supernatants were transferred to new 5-mL centrifuge tubes; this step was repeated twice and blow dry twice with nitrogen; the silylation reagent [N. O-bis (trimethylsilane) trifluoroacetamide: trimethylchlorosilane=99:1] was then added to the 5-mL centrifuge tubes containing the supernatants; after the complete derivatization and analyze them after allowing the bottle to cool to 37 °C.

Statistical analyses

The limit of detection (LOD) of phthalate metabolites were assumed to correspond numerically to three times the signal-to-noise ratio; therefore, the LOD values for MMP, MEP, MiBP, MBP, MOP, MBzP, MEOHP, MEHHP, and MEHP were determined to be 0.038, 0.029, 0.002, 0.002, 0.063, 0.041, 1.071, 0.010, and 0.033 µg/L, respectively; notably, in our analyses, we replaced the phthalate metabolite concentrations that were below the LOD with the relevant LOD value divided by the square root of 2. The total concentration of low-molecular-weight (LMW) phthalates (ΣLMW) was calculated as the total sum of the molar concentrations of the three metabolites (MMP, MEP and MBP), and the formula is ΣLMW (nmol/L) = [MMP (µg/L)/180+ MEP (µg/L)/194 + MBP(µg/L)/222] × 1000. The total concentration of DEHP (ΣDEHP) was calculated as the total sum of the molar concentrations of the three metabolites (MEHP, MEOHP and MEHHP), and the formula is ΣDEHP (nmol/L) = [MEHP (µg/L)/278+ MEOHP (µg/L)/294 + MEHHP(µg/L)/292] × 1000. Urine creatinine concentration was used to correct for urine dilution employing to the following formula: urine creatinine-corrected phthalate metabolite concentration (mg/g crea) = phthalate metabolite concentration (mg/L) / [urine creatinine concentration (mg/L) × 113 (g/mol) × 10^{–6}]. Notably, we calculated both the unadjusted and creatinine-adjusted urine phthalate metabolite concentration distributions (geometric mean and percentile values). The distributions of nine phthalate metabolites as well as ΣLMW and ΣDEHP were skewed, thus we performed the natural logarithm transformation(ln) of creatinine-adjusted phthalate metabolite concentrations to produce more normal distributions for statistical analyses. Multivariable linear regression was employed

to determine the relationship between demographic variable category and season and metabolite concentrations corrected for urinary creatinine. Finally, Spearman’s rank correlation coefficient was used to test the correlation between different phthalate metabolite concentrations. All analyses were performed with SPSS version 25.0 (IBM Corp. Armonk, NY, USA); the applied threshold for statistical significance was $p \leq 0.05$ (two-tailed).

Results

Basic demographic characteristics

The basic demographic characteristics of the overall population are presented in Table 1. The average age was 26.7 ± 5.0 years old, and the age group comprising the largest proportion of participants (40.4%) was the 25–29 years old category. The average pre-pregnancy BMI value was $22.4 \pm 3.5 \text{ kg/m}^2$, and the BMI category comprising the largest proportion of participants (61.3%) was the normal weight group. Moreover, 59.4% of participants had an education level of middle school or below, 58% came from households in the annual middle-income bracket, and multiparous participants accounted for 60.4% of the cohort. In this study, none of the participants smoked or drank alcohol during pregnancy, but 11.7% were categorized as passive smokers. Approximately 7.6% of participants drank bottled water during pregnancy. The highest proportion of participants was enrolled in the study during the winter (44%), while the lowest proportion was enrolled during the summer (7.5%).

Table 1. Characteristics of study subjects (N =1003) in the study.

Characteristics	Mean ± SD or n (%)
Maternal age at delivery (years)	26.7±5.0
≤25	342 (34.1)
25-29	405 (40.4)
≥30	256 (25.5)
Pre-pregnancy BMI (kg/m²)	22.4±3.5
Underweight (<18.5)	105 (10.5)
Normal weight (18.5–23.9)	615 (61.3)
Overweight (≥24)	283 (28.2)
Annual household income (Yuan)	
≤100000	174(17.4)
100000–150000	582 (58.0)
≥150000	231 (23.0)
Don't know	16(16.0)
Education	
middle school or below	596(59.4)
High school and middle special school	266(26.5)
College degree or above	141(14.1)
Parity	
Nulliparous	397(39.6)
Multiparous	606(60.4)
Passive smoking	
Yes	117(11.7)
No	886 (88.3)
Drinking bottled water	
Yes	927(92.4)
No	76(7.6)
Sample collection season	
Spring	255 (25.4)
Summer	75 (7.5)
Fall	232 (23.1)
Winter	441 (44.0)

Abbreviations: BMI, body mass index; SD, standard deviation.

Phthalate metabolite distribution

The values for the detectable rate, geometric mean, and the quartile of both unadjusted and creatinine-adjusted concentrations of phthalate metabolites are listed in Table 2. The detectable rates of the phthalate metabolites MMP, MEP, MiBP, MBP, MOP, MB₂P, MEHP, MEOHP, and MEHHP in participants' urine samples were 76%, 97.8%, 99.6%, 100%, 87.1%, 77.5%, 93.7%, 87.4%, and 93.7%, respectively. On average, MBP exhibited the highest concentration in urine samples, and the median concentration value for this metabolite after creatinine correction was 84.24 mg/L. By

contrast, MB₂P exhibited on average the lowest concentration in urine samples, and the median concentration for this metabolite after creatinine correction was 0.08 mg/L.

Table 2. Distribution of urinary phthalate metabolite concentrations [unadjusted(µg/L) and creatinine-adjusted (µg/g creatinine)] among pregnant women (N = 1003).

		Selected percentiles					
phthalate metabolites		DR (%)	GM	25th	50th	75th	95th
MMP	UA ^a	76	0.86	0.15	1.63	4.47	17.71
	CA ^b		1.13	0.17	1.79	6.28	36.38
MEP	UA ^a	97.8	8.49	4.01	8.64	20.78	82.26
	CA ^b		11.09	4.17	11.85	30.22	148.24
MiBP	UA ^a	99.6	24.07	12.57	24.61	47.28	140.93
	CA ^b		31.44	14.77	32.34	71.07	240.08
MBP	UA ^a	100	64.64	30.45	62.45	130.80	427.30
	CA ^b		84.42	34.64	84.24	202.32	698.81
MOP	UA ^a	87.1	0.14	0.04	0.13	0.28	0.93
	CA ^b		0.18	0.08	0.17	0.40	1.38
MB ₂ P	UA ^a	77.5	0.08	0.03	0.04	0.16	0.78
	CA ^b		0.10	0.04	0.08	0.23	1.25
MEHP	UA ^a	93.7	7.18	2.46	6.78	37.84	244.35
	CA ^b		9.38	2.95	9.60	50.25	276.0
MEOHP	UA ^a	87.4	5.47	2.24	6.31	11.92	44.58
	CA ^b		7.14	2.57	7.24	18.99	78.60
MEHHP	UA ^a	93.7	4.96	3.51	7.63	14.63	40.67
	CA ^b		6.48	3.72	9.14	20.09	72.28
ΣLMW	UA ^a		379.76	177.81	355.32	758.45	2449.65
	CA ^b		495.99	208.27	497.86	1182.32	4199.32
ΣDEHP	UA ^a		115.01	47.65	106.16	272.97	1040.99
	CA ^b		150.22	58.07	140.40	359.23	1622.26

Abbreviations: UA, unadjusted; CA, creatinine-adjusted; DR, detectable rate; GM, geometric mean; MMP, mono-methy phthalate; MEP, mono-ethyl phthalate; MiBP, mono-isobutyl phthalate MBP, mono-butyl phthalate; MOP, mono-octyl phthalate; MB₂P, mono-benzyl phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, mono-(2-ethyl-5-carboxypentyl) phthalate; ΣLMW, the molar sum of three metabolites (MMP, MEP, and MBP); ΣDEHP, the molar sum of three metabolites (MEHP, MEHHP, and MEOHP); a The unit was µg/L; b The unit was µg/g creatinine.

Socio-demographic variable and sampling season analyses

In Tables 3 and 4 are listed data reflecting the effects that different demographic variables and sampling seasons have on the concentrations of LMW metabolites and high-molecular-weight (HMW) metabolites in participants' urine. Data reflecting the correlation between sampling season

and phthalate metabolite concentration, is reported in Figures 1. The correlations between other demographic variables (maternal age, BMI, annual household income, education, passive smoking, drinking bottled water, and parity) and phthalate metabolite concentrations are reflected by data reported in Figures S1–7. The results of multivariable linear regression analyses indicated that the exposure level to phthalate metabolites during pregnancy was mainly related to maternal age, BMI, annual household income, passive smoking during pregnancy, parity, and sampling season. Notably, the concentration of MEHHP in the urine samples from pregnant women aged ≥ 30 years was the highest. MEP, MEHP, Σ LMW, and Σ DEHP were the most abundant metabolites present in the urine samples collected from study participants in the overweight BMI group. Compared with their high-annual-income counterparts, low-annual-income participants exhibited substantially lower concentrations of MiBP, MBP, MOP, MB₂P, MEHP, and Σ DEHP, whereas middle-annual-income participants had lower concentrations of MiBP, MB₂P, MEHP, and Σ DEHP. The concentration of MEHP in urine samples from participants in the passive smoking group was higher than that in the no passive smoking group. Multiparous participants exhibited higher concentrations of MEHP and lower concentrations of MEHHP than nulliparous participants. Phthalate metabolite concentration was observed to be related to the sampling season. LMW metabolites (i.e., MMP, MEP, MiBP, MBP, and Σ LMW) exhibited the highest concentrations in urine samples collected in participants enrolled during the summer period. By contrast, MEHHP concentration in urine samples was lowest for participants enrolled during the summer, and MOP concentration in urine samples was lowest for participants enrolled during the spring. In this study, no statistically significant correlation was observed between phthalate metabolite exposure level and maternal education or drinking bottled water during pregnancy.

Table 3. Associations between LMW metabolite concentrations and demographic/season categories by multivariable linear regression (ln-transformed, $\mu\text{g/g}$ creatinine) (N = 1003).

Variables	β estimates and 95% confidence intervals					
		MMP	MEP	MiBP	MBP	ΣLMW
Maternal age						
≤25	342	-0.01(-0.36,0.35)	-0.05(-0.29,0.20)	0.10(-0.09,0.30)	0.04(-0.14,0.22)	0.03(-0.15,0.21)
25-29	405	Ref				
≥30	256	0.12(-0.25,0.50)	0.17(-0.08,0.43)	0.14(-0.06,0.35)	0.07(-0.12,0.26)	0.11(-0.07,0.30)
BMI (kg/m²)						
≤18.4	105	0.23(-0.26,0.72)	0.01(-0.33,0.35)	-0.12(-0.39,0.15)	-0.07(-0.32,0.17)	-0.04(-0.29,0.20)
18.5-23.9	615	Ref				
≥24	283	0.01(-0.33,0.34)	0.45(0.23,0.68)	0.16(-0.03,0.34)	0.15(-0.02,0.32)	0.19 (0.02,0.36)
Household income						
≤100000	174	0.01(-0.38,0.59)	0.02(-0.31,0.36)	-0.63(-0.89,-0.36)	-0.30(-0.54,-0.05)	-0.22(-0.46,0.03)
100000-150000	582	-0.14(-0.51,0.23)	-0.04(-0.30,0.21)	-0.25(-0.45,-0.05)	-0.12(-0.31,0.07)	-0.10(-0.28,0.08)
≥150000	231	Ref				
Don't know	16	0.06(-0.14,2.31)	-0.02(-1.03,0.64)	-0.19(-0.86,0.47)	0.09(-0.52,0.71)	0.07(-0.53,0.68)
Education						
middle school or below	596	0.22(-0.28,0.71)	0.03(-0.28,0.34)	0.07(-0.17,0.32)	0.10(-0.13,0.32)	0.11(-0.12,0.33)
High school and middle special school	266	0.22(-0.38,0.59)	0.21(-0.12,0.55)	0.04(-0.23,0.31)	0.11(-0.14,0.36)	0.12(-0.12,0.36)
College degree or above	141	Ref				
Passive smoking						
Yes	117	0.07(-0.40,0.54)	0.00(-0.32,0.32)	-0.01(-0.27,0.25)	0.08(-0.16,0.31)	0.08(-0.15,0.31)
NO	886	Ref				
Drinking bottled water						
Yes	927	-0.19(-0.75,0.38)	0.16(-0.23,0.54)	-0.17(-0.48,0.13)	-0.06(-0.34,0.23)	-0.05(-0.33,0.23)
NO	76	Ref				
Parity						
Nulliparous	397	Ref				
Multiparous	606	0.02(-0.32,0.35)	-0.06(-0.29,0.16)	0.04(-0.14,0.22)	0.03(-0.13,0.20)	0.01(-0.16,0.17)
Sampling season						
Spring	255	-0.07(-0.43,0.30)	0.50(0.25,0.75)	0.14(0.22,0.62)	0.77(0.58,0.95)	0.76(0.58,0.94)
Summer	75	0.95(0.37,1.53)	1.37(0.98,1.77)	0.18(0.60,1.23)	1.74(1.45,2.03)	1.78(1.50,2.07)
Fall	232	0.93(0.54,1.32)	0.70(0.44,0.97)	0.17(0.32,0.74)	0.66 (0.46,0.85)	0.67(0.48,0.87)
Winter	441	Ref				

LMW metabolite including MMP, MEP MiBP, MBP and ΣLMW. Bold font indicates p value ≤ 0.05.

Table 4. Associations between HMW metabolite concentrations and demographic/season categories by multivariable linear regression (ln-transformed, µg/g creatinine) (N = 1003).

Variables	β estimates and 95% confidence intervals						
	N	MOP	MB ₂ P	MEHP	MEOHP	MEHHP	ΣDEHP
Maternal age							
≤25	342	-0.05(-0.23,0.13)	-0.04(-0.24,0.16)	-0.25(-0.60,0.09)	0.15(-0.07,0.37)	0.24(-0.06,0.55)	-0.03(-0.23,0.17)
25-29	405	Ref					
≥30	256	0.15(-0.04,0.34)	-0.01(-0.22,0.20)	0.14(-0.22,0.51)	0.01(-0.22,0.25)	0.40(0.08,0.72)	0.15(-0.07,0.36)
BMI (kg/m²)							
≤18.5	105	-0.15(-0.40,0.10)	-0.22(-0.49,0.06)	0.19(-0.29,0.66)	-0.18(-0.49,0.13)	-0.24(-0.66,0.18)	-0.05(-0.33,0.23)
18.5-23.9	615	Ref					
≥24	283	0.07(-0.10,0.24)	-0.02(-0.21,0.16)	0.67(0.34,0.99)	-0.01(-0.22,0.20)	0.21 (-0.08,0.49)	0.26(0.07,0.45)
Household income							
≤100000	174	-0.42(-0.67,-0.18)	-0.49(-0.76,-0.22)	-0.49(-0.96,-0.02)	-0.17(-0.47,0.14)	-0.23(-0.65,0.19)	-0.41(-0.68,-0.13)
100000-150000	582	-0.21(-0.40,-0.02)	-0.21(-0.42,-0.01)	-0.48(-0.84,-0.12)	-0.17(-0.40,0.06)	-0.22(-0.54,0.10)	-0.30(-0.51,-0.09)
≥150000	231	Ref					
Don't know	16	0.11(-0.51,0.73)	0.29(-0.39,0.97)	-0.42(-1.61,0.77)	0.43(-0.33,1.20)	0.49(-0.55,1.53)	-0.07(-0.77,0.62)
Education							
middle school or below	596	-0.02(-0.25,0.20)	-0.13(-0.38,0.12)	-0.10(-0.54,0.33)	-0.22(-0.50,0.06)	0.19(-0.20,0.57)	-0.11(-0.36,0.15)
High school and middle special school	266	0.19(-0.06,0.44)	-0.20 (-0.47,0.08)	-0.04(-0.51,0.44)	-0.21(-0.51,0.10)	0.17(-0.25,0.59)	-0.08(-0.36,0.20)
College degree or above	141	Ref					
Passive smoking							
Yes	117	-0.04(-0.28,0.20)	-0.09(-0.35,0.18)	0.49(0.03,0.95)	-0.25(-0.55,0.04)	-0.03(-0.43,0.37)	0.09(-0.18,0.36)
NO	886	Ref					
Drinking bottled water							
Yes	927	0.17(-0.12,0.45)	-0.05(-0.37,0.26)	-0.04(-0.59,0.51)	0.01(-0.34,0.36)	-0.17(-0.65,0.31)	-0.10(-0.42,0.22)
NO	76	Ref					
Parity							
Nulliparous	397	Ref					
Multiparous	606	0.03(-0.14,0.20)	0.05(-0.14,0.23)	0.37(0.05,0.69)	0.13(-0.08,0.34)	-0.46(-0.75,-0.18)	0.17(-0.02,0.36)
Sampling season							
Spring	255	-0.30(-0.48,-0.11)	-0.87(-1.08,-0.67)	0.14(-0.21,0.50)	-0.05(-0.28,0.18)	0.60(0.28,0.91)	0.06(-0.15,0.27)
Summer	75	-0.28(-0.57,0.01)	-0.69(-1.01,-0.36)	-0.37 (-0.93,0.20)	0.33(-0.04,0.69)	-0.73(-1.22,-0.23)	0.10(-0.23,0.43)
Fall	232	0.00(-0.20,0.20)	-0.46(-0.68,-0.25)	0.24(-0.14,0.62)	-0.37(-0.62,-0.13)	0.41(0.08,0.75)	0.10(-0.12,0.32)
Winter	441	Ref					

HMW metabolite including MOP, MB₂P, MEHP, MEOHP, MEHHP and ΣDEHP. Bold font indicates p value < 0.05.

Correlations between phthalate metabolite concentrations

In order to assess the variations in the potential sources of phthalate metabolite exposure, the correlations between urinary concentrations of individual phthalate metabolites in 1003 pregnant women were identified by Spearman's rank correlation coefficient analysis. A significant correlation among phthalate metabolites was recognized; specifically, there was a strong correlation between the concentrations of the four LMW phthalates, MMP, MEP, MiBP, and MBP, (the relevant Spearman's coefficients ranged between 0.534 and 0.855). On the contrary, the correlation between the three DEHP, MEHP, MEOHP, and MEHHP, was observed to be weak (the relevant Spearman's coefficients ranged between 0.228 and 0.444). The results of the mentioned analyses are listed in Table S3.

Comparison of uncorrected median concentrations of phthalate metabolites in different studies

As can be evinced from the data reported in Figure S8, a comparison was made between the results obtained herein and those obtained in several international studies; in the present study, the exposure levels of MBP were observed to be the highest, while those of MB₂P were the lowest; the MEP exposure level measured here in is similar to that reported in other studies conducted in Japan and Korea (Suzuki et al., 2010; Lee et al., 2020), but it is lower than that reported in studies conducted in Denmark, Spain, United States, and Canada (Tefre et al., 2014; Casas et al., 2011; Cantonwine et al., 2014; Arbuckle et al., 2014); the MiBP exposure level measured in this study is higher than that reported in Taiwan, China, Korea, and Thailand (Lin et al., 2010; Lee et al., 2020) and lower than that measured in Denmark and Spain (Tefre et al., 2014; Casas et al., 2011); the MEHP exposure level determined herein agrees with that reported in other studies conducted in Japan and Korea (Suzuki et al., 2010; Lee et al., 2020), but it is higher than that reported in studies conducted in Denmark, Spain, United States and Canada (Tefre et al., 2014; Casas et al., 2011; Cantonwine et al., 2014; Arbuckle et al., 2014); the exposure levels measured in the present study for MEOHP and MEHHP are lower than those reported in studies conducted in Taiwan, China, Spain, United States and Canada (Lin et al., 2010; Casas et al., 2011; Cantonwine et al., 2014; Arbuckle et al., 2014), but they are consistent with those reported in studies conducted in Japan and Korea (Suzuki et al., 2010; Lee et al., 2020).

Discussion

In this study, the concentrations of nine phthalate metabolites were measured in the urine samples of 1003 Chinese pregnant women during their third trimester of pregnancy. Notably, the detectable rates of the various analytes were observed to range between 76% and 100%; in particular, the detectable rates of MBP, MEP, MiBP, MEHHP, and MEHP were all > 90%, which are values similar to those reported in studies conducted in Mexico (Wu et al., 2020), Japan (Suzuki et al., 2010)–United States (Shin et al., 2020), Taiwan, China (Hsieh et al., 2020), Iran (Darvishmotevalli et al., 2019) and Israel (Berman et al., 2009); by contrast, the detectable rates measured herein for MMP, MB₂P, and MEOHP are significantly lower than those reported in the studies just mentioned. Remarkably, the detectable rate of MOP was observed to vary greatly between studies: the lowest reported values are 0.5%, from a study conducted in Denmark (Tefre et al., 2014), and 63%, from a study conducted in Poland (Polańska et al., 2016), which are significantly lower values than that measured herein, 87.1%. The difference in detectable rates between studies conducted in different geographic areas may be related to differences in eating and living habits of the study populations as well as differences in the industrialization and economic levels between geographic areas.

The median concentrations of the nine phthalate metabolites measured in urine samples collected from the participants of our study are as follows: MBP, 62.45 mg/L; MiBP, 24.61 mg/L; MEP, 8.64 mg/L; MEHHP, 7.63 mg/L; MEHP, 6.78 mg/L; MEOHP, 6.31 mg/L; MMP, 1.63 mg/L; MOP, 0.13 mg/L; and MB₂P, 0.04 mg/L. Our above results are compared with published international studies conducted in Japan (Suzuki et al., 2010), Taiwan, China (Lin et al., 2010), Korea and Thailand (Lee et al., 2020), Denmark (Tefre et al., 2014), Spain (Casas et al., 2011), United States (Cantonwine et al., 2014), and Canada (Arbuckle et al., 2014). Remarkably, the herein-measured median concentration of MEP is similar to that reported for the rest of the Asian region, but it is significantly lower than that reported in studies conducted in the European and American regions. In particular, MEP concentrations up to 324 mg/L have been measured in Spain (Casas et al., 2011) and up to 99.2 mg/L in United States (Cantonwine et al., 2014), which are 11 to 36 times as large as the median concentration measured for the said metabolite in our survey. These striking differences may be related to the more extensive use that women in European and American countries make of personal care products (PCPs), such as perfumes, nail polish, and cosmetics (Parlett et al., 2013). Indeed, these products contain as a component diethyl phthalate (DEP), which is the parent compound of MEP. Although China is one of the three largest consumer countries of PCPs in the world (Wang et al., 2018), people living in China's rural areas have less access to PCPs than people living in urban areas, resulting in low MEP levels detected in people living in the rural areas of China (Cheng et al., 2021); in fact, almost all participants of our study were from rural China. Similar to the results of a previously published study on a Chinese pregnant women cohort, median concentrations of total MBP were the highest (Gao et al., 2017; Zhu et al., 2016), and the concentration of MBP is two to six times higher than that measured in studies conducted in South Korea, Thailand (Lee et al., 2020), and European and American countries (Tefre et al., 2014; Casas et al., 2011; Cantonwine et al., 2014; Arbuckle et al., 2014); this difference may be related to the pollution caused by China's heavy use of DBP in industrial production (Guo et al., 2011). The median

concentration of MB_zP determined in our survey is clearly significantly lower than the level of this phthalate metabolite measured in all other countries and regions (Suzuki et al., 2010; Lin et al., 2010; Lee et al., 2020; Tefre et al., 2014; Casas et al., 2011; Cantonwine et al., 2014; Arbuckle et al., 2014). Similarly, the results of previous investigations (He et al., 2019; Guo et al., 2020) also suggested that the exposure to MB_zP in South China is extremely low. Limited evidence exists that MB_zP exposure in China is mainly associated with exposure to cereal, seafood, and cooking oil, so exposure to MB_zP differs between countries and foods (Wang et al., 2018). Therefore, the low level of MB_zP exposure measured herein may be related to the dietary structure and dietary sources of the participants of our study.

We found that participants with BMI ≥ 24 kg/m² had higher levels of MEP, MEHP, Σ LMW and Σ DEHP in urine samples; the existence of this phenomenon is also supported by previous research data (Yaghjyan et al., 2015; Hatch et al., 2008). The compelling reason for this trend may be that phthalates exhibit a degree of lipophilicity, so they can be stored in the adipose tissue; therefore, people with higher BMI may exhibit higher concentrations of urinary phthalate metabolites (Philips et al., 2017). In addition, foods are an important source of phthalate metabolites exposure (Dualde et al., 2020), indeed, study participants with high BMI values tend to have higher food intake than those with low BMI values, which may account for the observed difference in exposure to phthalate metabolites. Compared with their high-annual-income peers, lower-annual-income participants exhibit lower urine levels of MiBP, MBP, MOP, MB_zP, MEHP, and Σ DEHP, a result that is inconsistent with those reported in previously published studies (Wenzel et al., 2018; Wang et al., 2018). The reasons for this inconsistency may have to do with the different income levels among different countries. In addition, people with lower incomes may be less exposed to cosmetics and food packaging products, which may also contribute to the mentioned observation.

The results of previously published studies indicated that the distribution of some phthalate metabolites in the environment exhibits seasonal characteristics (Chen et al., 2018; Zhang et al., 2019). Indeed, higher levels of the LMW phthalate metabolites (i.e., MMP, MEP, MiBP, MBP, and Σ LMW) were measured in our study in participants recruited during the summer. Similar results were obtained for pregnant women, young children and general population in China (Guo et al., 2020; Liao et al., 2018; Yang et al., 2021). The reasons for the observed seasonal effects on metabolite concentrations remain unclear. Research results indicate that LMW phthalate metabolites, such as DEP and DBP, are mainly used in cosmetics and PCPs, and use of these products will lead to high exposure to phthalate metabolites (Hsieh et al., 2019; Koniecki et al., 2011). During the summer, women may use PCPs like perfumes, shampoos, nail polish, and sunscreen more frequently than in other seasons, and phthalate metabolites absorbed by skin is more frequent, resulting in higher exposure to LMW phthalate metabolites during the summer. Moreover, Gao et al. discovered that Chinese pregnant women stayed longer indoor daily in summer than in winter (Gao et al., 2017), cosmetics and PCPs have been suggested to be important sources of phthalates in indoor dusts in China (Zhang et al., 2013), and the higher temperature would cause an increase in the release of these phthalates due to their volatility (Chen et al., 2018; Zhang et al., 2019), resulting in the human body being exposed to increased amounts of LMW phthalate metabolites indoors. However, different phthalates are affected differently by the temperature (Kashyap et al., 2018). These differences may in part explain the different exposure levels of different metabolites in different seasons, although there may be other reasons for them that we don't yet understand. Further research in this field is thus necessary.

Our research has some limitations. First, a single urine sample was collected from each study participant for testing, so the measured phthalate metabolite content does not represent the average level during late pregnancy. Second, humans are exposed to phthalates from a variety of sources and pathways, and we only analyzed demographic variables and sampling season; by this approach, we may be missing other potentially important predictive variables, such as dietary and PCPs use habits. Considering, however, that our sample size is relatively large and the phthalate metabolites are examined according to the same standard, using the same instrumentation, and the same testing personnel, and the quality control of the data is reliable, we have reason to believe that our test data closely reflect the “real” situation of the study population.

Conclusion

To the best of our knowledge, this is the first study to investigate phthalate metabolite exposure characteristics among pregnant women in Southwest China. Phthalate metabolites present a demographic and seasonal differential distribution among the study population, an observation that will help inform the development of targeted interventions to reduce exposure to these potentially harmful chemicals during pregnancy.

Abbreviations

DEHP - mono(2-ethylhexyl) phthalate

DBP - di-n-butyl phthalate

BMI - body mass index

GC-MS/MS - high-performance gas chromatography mass spectrometry

MMP - mono-methyl phthalate

MEP - mono-methyl phthalate

MiBP - mono-ethyl phthalate

MBP - mono-butyl phthalate

MOP - mono-octyl phthalate

MBzP - mono-benzyl phthalate

MEHP - mono(2-ethylhexyl) phthalate

MEOHP - mono(2-ethyl-5-oxohexyl) phthalate

MEHHP - mono(2-ethyl-5-hydroxyhexyl) phthalate

LOD - the limit of detection

LMW - low-molecular-weight

Σ LMW - the total sum of low-molecular-weight phthalates (MMP, MEP and MBP)

Σ DEHP - the total sum of the three metabolites (MEHP, MEOHP and MEHHP)

SD - standard deviation

UA - unadjusted

CA - creatinine-adjusted

DR - detectable rate

GM - geometric mean

HMW - high-molecular-weight

CI - confidence interval

PCPs - personal care products

DEP - diethyl phthalat

Declarations

Acknowledgments

The authors thank all the participants and investigators

Ethical Approval

This study was ethically reviewed by the Affiliated Hospital of Zunyi Medical University (batch No.: KLL-2019-006).

Consent to Participate

All participants agreed to participate in this study and signed the informed consents.

Consent to Publish

The authors declare that this manuscript does not contain any individual person's data and material in any form.

Competing interests

The authors declare no competing interests.

Funding

This work was supported by National Key R&D Program of China (2018YFC1004300, 2018YFC1004302) and Science & Technology Program of Guizhou Province, China (QKHHBZ [2020]3002).

Availability of data and materials

Data associated with the present study can be accessed on request to the author (630140512@qq.com).

Author Contributions

Juan Liao: Formal analysis, Investigation, Validation, Data curation, Writing-original draft. **Derong Fang:** Investigation, Data curation. **Yijun Liu:** Data curation, Validation. **Shimin Xiong:** Data curation, Validation. **Xia Wang:** Methodology, Validation. **Yingkuan Tian:** Methodology, Validation. **Haonan Zhang:** Methodology, Validation. **Songlin An:** Investigation, Conceptualization. **Caidie He:** Investigation, Conceptualization. **Wei Chen:** Investigation, Conceptualization. **Xiang Liu:** Investigation, Conceptualization. **Nian Wu:** Data curation, Conceptualization. **Kunming Tian:** Investigation, Validation. **Linglu Wang:** Investigation, Validation. **Ya Zhang:** Investigation, Data curation. **Hongyu Yuan:** Investigation, Data curation. **Li Zhang:** Investigation, Data curation. **Quan Li:** Project administration, Investigation, Validation, Resources. **Xubo Shen:** Project administration, Investigation, Validation, Resources. **Yuanzhong Zhou:** Project administration, Investigation, Validation, Resources, Writing-review & editing, Funding acquisition.

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Figures

Figure 1

Correlation between sampling season and phthalate metabolites

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