Prenatal Phthalate Exposure Reduction Through an Integrated Intervention Strategy

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Abstract

Pregnancy represents a sensitive susceptibility window to phthalate esters (PAEs). In this study, we develop an intervention strategy for reducing the exposure of pregnant women to phthalates. Thirty-five pregnant women, who initially underwent maternity examination, were recruited from an ongoing longitudinal prospective prenatal cohort study. The intervention strategy integrates diet, lifestyle, and environmental factors. Participants were encouraged to modify their behaviors and habits according to the intervention strategy at three different periods. Urine samples were collected from the participants after antenatal examination every month, for 8 months, to measure ten PAEs metabolites. The mono-(2-ethyl-5-hydroxyhexyl) (MEHHP), mono-*n*-butyl phthalate (MnBP), and mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) declined significantly after the 1st intervention, while mono-isobutyl phthalate (MiBP) and mono-methyl phthalate (MMP) noticeably reduced after 2nd intervention. The sum of the molar concentrations of MEHP, MEHHP, MEOHP, and MECPP reduced by 20% to 40% during subsequent intervention. In addition, the sum of the molar concentrations of MEP, MnBP, MMP, and MiBP) as well as the sum of the molar concentrations of the ten metabolites also reduced. Our findings suggest that intervention through written recommendations can effectively reduce the burden of phthalates during pregnancy.

Introduction

Phthalate esters (PAEs), the diesters of 1,2-benzenedicarboxylic acid, represent a class of synthetic chemicals widely employed in manufacturing processes (Wang et al., 2016). In general, high molecular weight phthalates (HMWPs; ≥250 Da, such as di-2-ethylhexyl phthalate (DEHP), benzylbutyl phthalate (BzBP), butyl benzyl phthalate (BBP), and di-*n*-octyl phthalate (DnOP), are primarily utilized as softeners in the production of polyvinyl chloride (PVC). Conversely, low molecular weight phthalates (LMWPs), such as dimethyl phthalate (DMP), diethyl phthalate (DEP), and di-*n*-butyl phthalate (DBP), are common components of personal care products and pharmaceuticals (Koch et al., 2013).

Considering that phthalate plasticizers are not chemically bound to PVC, for instance, these can leach, migrate, or evaporate to indoor air and the atmosphere, food, and other materials (Wu et al., 2018a). Therefore, humans are threatened by these chemicals through ingestion, inhalation, and dermal exposure during their lifetime, including intrauterine development (Radke et al., 2019). Several studies from around the world report detectable levels of phthalate metabolites in pregnancy urine, and further associated with impaired neurodevelopment, altered genital development, and respiratory problems in infants (Ferguson et al., 2015; Minatoya et al., 2017; Qian et al., 2019). Therefore, prenventing exposure to phthalates in daily life is important, especially for pregnant women, who are more susceptible to endocrine-disrupting chemicals.

Intervention studies for reducing exposure to PAEs are available. Rudel and his colleges indicated that limiting the consumption of food prepared or stored in plastic containers reduced phthalates in urine (Rudel et al., 2011). In addition, Sathyanarayana et al. demonstrated that Bisphenol A and DEHP can be

reduced through dietary intervention (Sathyanarayana et al., 2013). Harley et al. suggested that limited use of personal care products can reduce the urine concentrations of MnBP and MiBP in adolescent girls (Harley et al., 2016). However, these studies were either simple (focused on dietary), short-term (< two weeks), or involved few participants (4 families or less). Moreover, the participants in these studies were either children or adults, and thus, the results may inadequately reflect pregnancy. Therefore, studies aimed at reducing the exposure of pregnant women to PAEs is urgently need.

In this study, we developed an intervention strategy to reduce the exposure of pregnant women to phthalates. Considering that eliminating these chemicals is not feasible (Barrett et al., 2015), the intervention were executed through written recommendation. The concentrations of phthalate metabolites in their urine samples were measured monthly during pregnancy, while the participants received written recommendations in the 1st, 2nd, and 3rd trimesters. We hypothesized that intervention through written recommendations effectively reduces the exposure of pregnant women to PAEs.

Materials And Methods

Study population

The study population is part of an ongoing longitudinal prospective prenatal cohort study in E-zhou city, Hubei province. Pregnant women were invited to participate in the study during their first antenatal examinations (< 8 weeks of gestation) at the E-zhou Medical & Healthcare Center for Women and Children in November 2018. The women were eligible for enrollment if the following criteria were satisfied: (1) resident in E-zhou city, (2) willing to adhere to the intervention procedure, (3) ready to participate in monthly antenatal examinations and donate urine samples, (4) planned to deliver in the Healthcare Center.

This study was approved by the Ethics Committee of Hubei University of Chinese Medicine. Written informed consent was obtained from all participants, and 35 women were recruited to participate in the baseline assessment (6.8 ± 2.2 weeks gestation).

Intervention strategy

Considering previous studies, we developed an strategy incorporating three components presented in **Table 1**. Owing to diet being a major exposure medium to HMWPs (Pacyga et al., 2019), the participants were reminded to restrict puffer, canned, and microwaved food consumption, and healthy eating was encouraged (e.g., organic food, folic acid supplement, and vegetarianism diets). Hsieh CJ and his colleges point out phthalate can stay on the skin for a long duration (Hsieh et al., 2019). Therefore, the intervention strategy also considered lifestlye, such as avoiding to touch decorated materials and plastic floors, restricting useage of personal-care products (e.g., body lotion, cosmetics, perfumes, and hair sprays), and limiting storage of prepared food in plastic containers including bags and cans. Owing to the ubiquity of phthalates contaminants in indoor dust and air inside vehicles (Zhang et al., 2014), the

participants were encouraged to avoid second-hand smoke, minimize transportation by cars, and exercise adequately.

The present study lasted until the ninth month of pregnancy. The participants were advised to alter their behaviors according to the intervention strategy in the 1st (first antenatal), 2nd (between the 4th and 5th month of pregnancy), and 3rd (between the 7th and 8th months pregnancy) trimesters. During the first visit, a counselor discussed the negative effects of PAEs on health with the participants, and asked them to follow written recommendations developed by ours team. In subsequent interventions, the women provided feedback from self-monitoring records, and they were encouraged to continue following the intervention strategy and avoid PAEs-containing products.

A flowchart highlighting the components of the study is shown in **Figure 1**.

Urine sampling and phthalate metabolite analyses

Spot urine samples were collected monthly during antenatal examinations. After collection, the spot urine samples were divided into aliquots and stored at -80 °C until analysis was performed. Ten metabolites belonging to six phthalate diesters were analyzed. These included the following: mono-isobutyl phthalate (MiBP) and mono-methyl phthalate (MMP) from dimethyl phthalate (DMP); mono-ethyl phthalate (MEP) from di-ethyl-phthalate (DEP); mono-*n*-butyl phthalate (MnBP) from di-n-butyl phthalate (DnBP); mono-*n*-octyl phthalate (MOP) from di-n-octyl-phthalate (DnOP); mono-benzyl phthalate (MBzP) from butyl benzyl phthalate (BBzP), and mono (2-ethylhexyl) phthalate (MEHP) from DEHP. The analyses also involved three secondary oxidation metabolites of DEHP: namely mono-(2-ethyl-5-hydroxyhexyl)-phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP). The term ΣDEHP represents the sum of the molar concentrations of MEHP, MEHHP, MEOHP, and MECPP, while ΣLMWP denotes the sum of the molar concentrations of MEP, MnBP, MMP and MiBP, and Σ_{10}PAEs is the sum of the 10 molar concentrations of the 10 metabolites.

The metabolites were analyzed in the School of Laboratory Medicine, Hubei University of Chinese Medicine by liquid chromatography-mass spectrometry (LC-MS/MS; Agilent, USA), according to the method for urine phthalates measurement described in Specht et al (Specht et al., 2015). This method was also validated in our previous study (Wu et al., 2018b). The stored urine sample were thawed, and approximatedly 200 μL of each sample was vortexd, sonicated for 5 min, and buffered using ammonium acetate. The samples were then spiked with 40 μL of a labeled isotope mixture (500 ng/mL), followed by addition of 5 μL of β-glucuronidase to eliminate glucuronic acid. After incubation, the urine samples were diluted using 1 mL of a phosphate buffer (0.14 M NaH_2PO_4 in 0.85% phosphoric acid) before loading onto a solid-phase extraction cartridge. After sequential equilibration using 1 mL of acetonitrile (ACN), 1 mL of H_2O, and 1 mL of the phosphate buffer, the solutions were transferred to glass vials before analyses by LC-MS/MS. Good separation was achieved for all analyses, with retention times on the column varying between 6.68–27.20 min. The calibration curve covered the range 0.100–200 ng/m, and

each batch of samples analyzed involved blanks and quality control (QC) samples. The intra- and inter-day relative standard deviations (RSD) were below 11.7% and 13.2%, respectively.

The metabolite concentrations were corrected for the urine dilution using specific gravity (SG) as follows: $Pc = P\ [(SG_M - 1)/(SG - 1)]$, where Pc is the SG-adjusted urine concentration (ng/mL), P is the measured metabolite concentration, SG is the specific gravity of the urine sample, and SG_M is the median SG of the sample for the studied population (Upson et al., 2013). The SG of each sample was measured using a handheld refractometer (PAL10-S; Atago, Tokyo, Japan) at room temperature. For PAEs concentrations below the limit of detection (LOD), an value equal to th LOD$\sqrt{2}$ was imputed.

Urine concentrations change

The urine concentration change (%) was calculated using the following equation: Urine concentration change = [(urine phthalate concentration during a visit – baseline urine phthalate concentration)/(baseline urine phthalate concentration)] ×100%.

Statistical analyses

Descriptive statistics were employed to characterize the study population with values expressed as a percentage or using the mean ± standard deviation (SD). The detection frequency, geometric mean (GM), and median SG-adjusted concentrations of urine phthalate metabolites for various antenatal examinations were calculated and used to characterize exposure. The Mann-Whitney U-tests was utilized for comparing the significance of the urine concentration differences for each visit and the baseline. The Spearman's rank correlation test was employed for assessment of correlations among the urine phthalate metabolite levels, with the significance was set at $p < 0.05$. All statistical analyses were performed using R (version 3.5.3; http://www.r-project.org).

Results

Demographics

The basic characteristics of the 35 participants in this study are presented in **Table 2**. Most of the pregnant woman are 26-34 years old (n = 23, 65.7%), lived in an urban area (n=20, 57.1%), are workers (n = 18, 51.4%), completed high school education (n = 18， 51.4%), are pregnant with a first child (n = 19, 54.3%), did not use alcohol in the 3 months prior to testing (n = 28, 80.0%), and live in households with incomes between 5,000 and 20,000 US dollars (n =19, 54.2%).

Intervention strategy

The intervention strategy used for the participants involved three periods of written recommendations. This involved a first intervention representing the baseline during the first antenatal, a second intervention during the fourth months of pregnancy (2^{nd} trimester), and a third intervention in the seventh month (3^{rd}

trimester). At least 77% (27/35) of the participants underwent antenatal examination each month. Finally, 241 urine samples were collected from the baseline to the endpoint.

Distribution and variability of phthalate metabolites

All metabolites, except MOP (< 40%), were detected in > 85% of the samples. The distributions of nine phthalate metabolites (excluding MOP), involving three groups, for each visit are presented in **Figure 2**. Most of the phthalate metabolites decreased relative to the baseline after each visit.

During each visit, MnBP, MEHHP, and MEOHP were found to be the most abundant PAEs in the urine samples, with corresponding mean value of 83.5, 88.0, and 85.0 ng/mL for the baseline, and 39.8 , 49.1, 51.0 ng/mL, for the last visit, respectively. Conversely, MBzP and MEHP are the least abundant PAEs, with mean baseline value of 2.41 and 5.78 ng/mL, and 1.96 and 6.05 for the 9^{th} visit, respectively. The ΣmLPAEs, ΣmDEHP, Σ_{10}mPAEs, representing mean values are 189, 196, and 386 ng/mL for the baseline, and 101, 112, and 213 ng/mL for the last visit, respectively. Detailed information on the absolute and on creatinine-adjusted phthalate metabolites distribution is presented in the Supplementary Table 1.

Strong correlations were observed between MMP, MiBP, and MEP (all r > 0.75). MECPP, MEHHP, MEOHP were moderately correlated with MEHP (r = 0.44—0.70), which are second-oxidative metabolite of DEHP (Supplementary figure 1).

Urinary concentration change

The urine concentraion changes associated with the participants of this study are presented in **Figure 3.** Most of the phthalate metabolites declined after each visit, expect for MBzP and MEHP. Compared with the baseline concentration, MEHHP, MnBP and MEOHP decreased by more than 20% after the first intervention. Relatedly, MiBP, and MMP reduced by almost 30% in the fifth moth, after the second intervention. In fact, the ΣmLPAEs, ΣmDEHP, Σ_{10}mPAEs decreased by 20% after the first intervention and reduced by 40% after the final visit.

Discussion

In this study, we developed an intervention strategy comprising dietary, lifestyle habit, and environmental components. This intervention strategy involved repeated and voluntary self-restraining components aimed at reducing urine phthalate concentrations during pregnancy. The decline in the urinary concentrations of the 10 phthalate metabolites measured implied that our intervention approach efficiently reduced the exposure of the participants to PAEs.

Dietary is the primary route for intake of phthalates, and studies conducted in Canada (Pacyga et al., 2019) and the US (Koch et al., 2013) showed detectable concentrations of phthalates in fast foods such as french fries, hamburgers, and sandwiches). Among these fast foods, hamburgers exhibited the highest PAEs concentration. According to previous study, the consumption of more fresh vegetables, fruits, nuts

and fish is associated with low exposure to phthalates (Bai et al., 2015). Therefore, our diet in the intervention strategy restricted participants from eating fast-foods and encouraged them to consume healthy foods. Our results demonstrated that the concentrations of MnBP, MEHHP and MEOHP significantly declined after the 1st intervention, with the decrease reaching 40% after the 3rd intervention. These findings are consistent with those of Rudel's (Rudel et al., 2011) and Sathyanarayana's (Sathyanarayana et al., 2013) studies, in which phthalate metabolites were significantly lowered after the diet of participants was restricted to foods involving limited packaging. However, these studies involved complete diet replacement. In contrast, in our study, the participants were advised to pursue a self-guided intervention. Therefore, although our study did not involve a complete replacement intervention, the repeated remind through written recommendation appeared to exhibit similar effectiveness.

According to an intervention study by Barrett (Barrett et al., 2015), the MMP and MiBP concentrations showed no appreciable change across three time periods. In fact, after a 3-day dietary intervention, the mean concentrations of MiBP were 15.9, 24.0, and 23.7 ng/mL for the pre, mid, and post-intervention periods, respectively. The corresponding MMP concentrations for the three periods were 19.4, 24.6, and 27.5 ng/mL. However, in a study involving a school for girls in Taiwan reported reduction in MiBP, MEP and MMP through an intervention strategy (Chen et al., 2015). A possible reason for the inconsistency of results from different studies is that most interventions involve a simple dietary change, whereas the underlying intervention strategy requires avoiding foods in plastic containers and using less personal care products such as shampoo and shower lotion. Many studies have also demonstrated that besides ingesting of contaminated foods, dermal absorption from personal care products is another route for human intake of phthalates (Valvi et al., 2015; Wenzel et al., 2018). In the present study, after the pregnant women decreased their usage of personal care products such as hair dye, shampoo, perfumes, body lotions, and nail polish, the concentration of MiBP and MMP significantly declined after the 2nd intervention, reaching 50% after the 3rd intervention. These results suggest that some LMWPs could be reduced through our intervention strategy.

However, after the 3rd intervention, MBzP showed no statistically significant changes, although this may be related to its concentration of less than 10 ng/mL from the baseline to the end. We also observed that MEHP changed sharply, reducing by approximately 30% in the fifth month of antenatal examination, and reducing a 60% decline in the seventh month antenatal. These results are similar to those of a two-week randomized dietary trial by Sathyanarayana, with MEHP geometric means of 3.9, 4.1, and 4.2 ng/mL for the baseline, during the intervention, and post intervention periods (Sathyanarayana et al., 2013), This response is possibly because of the complex, dynamic, multidimensional characteristics of the DEHP metabolite. The DEHP can initially be metabolized to MEHP, followed by MEHP metabolizing to the secondary mono-phthalate easters including MEHHP, MECPP and MEOHP (Minatoya et al., 2017). Notably, in our study, the ΣDEHP, ΣLMWP, andΣ_{10}PAEs significantly declined after the 1st and subsequent interventions. This is consistent with (Ackerman et al., 2014)), who reported that through a dietary intervention, the GM concentrations of BPA reduced by 66%, while those of DEHP metabolites decreased by 53–56% (Ackerman et al., 2014).

The environment is another factor associated with PAEs exposure. According to previous studies, MEOHP and MEHPP were higher among women who do smoke compared to non-smokers (Cantonwine et al., 2014). In the present study, after 1st intervention, MEOHP and MEHPP declined significantly, and this may be partially attributed to the avoidance of second-hand smoke. We also encouraged the participants to undertake limited transportation by car. Owing to the elevated internal temperature of the cabin of a car or truck, the vinyl interior trim of some vehicles can deteriorate and decompose, thereby releasing of phthalate particles (Rakkestad et al., 2007).

In addition, we recommended adequate exercise, such as walking and yoga, to the pregnant women. According to a study in Australian, slightly higher total phthalate metabolite concentrations were associated with insufficient activity (Bai et al., 2015) . Further investigation is warranted to better understand the link between physical activity and low phthalate ester levels biologically.

In this study, an intervention strategy for reducing the PAE concentrations in pregnant women is introduced. This strategy comprises eight components associated with diet, lifestyle and the environment. This approach closely parallels real life compared to studies focusing on intervention involving just one component. Moreover, repeated intervention and measures are better for evaluating the efficiency of the intervention strategy. However, the present study involves several limitations that require attention. First, the intervention components were not distinguished to clarify their impact on the phthalate reduction efficiency. Considering that some intervention components are clustered, determining the contribution of each component to the phthalate concentrations reduction was not achieved. Second, some findings may be biased because of the relatively small sample size. However, the longitudinal sampling involved in the present study allowed individuals to serve as their controls, thereby avoiding the many sources of confounding information that limit cross-sectional studies. Finally, because the participants in this study are from a moderate economic city in Hubei province, China, generalizing the study efficiency to other countries and socioeconomic groups may be limited.

Conclusion

The present study demonstrated that intervention through written recommendations can effectively reduce the burden of phthalates on the body. These findings suggest that our intervention strategy is valuable for establishing specific PAEs-limiting factors for improving pregnancy and fetal outcomes.

Declarations

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Hubei University of Chinese Medicine (No. 2018-IEC-010). Written informed consent was obtained from individual or guardian participants.

Consent to participate

Not applicable

Consent to Publish

Not applicable

Availability of data and material

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that there is no conflict of interest in this manuscript.

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Authors Contributions

Wei Wu: Editing and Supervision. Liu Cao: Original draft preparation. Ting-Ting Zheng: Investigation. Shu-Yu Feng: Software and Methodology. Guan-Wei Ma: Visualization. Ying-Ying He: Provision of study materials. Ping Wu: Reviewing. All authors read and approved the final manuscript.

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Tables

Table 1. Intervention strategy used in this study

Recommended Intervention items	Detail of items
Nutrition and diet	·Restricted eating fast-food, puffer food, canned food, microwaved food
	·Eating healthier food (e.g., organic/grown/raised/ caught food, folic acid supplements, vegetarianism)
Lifestyle habit	·Restricted use hair dye, shampoo, perfumes, skin lotions, nail polish, spraying perfumes
	·Restricted wrapping food in plastic containers, plastic bag, cans
	·Avoid touch plastic floors and decorating materials
Environment	·Faraway second-hand smoking
	·Limited transport with car
	·Proper exercise (e.g., walking, yoga)

Table 2. Demographic characteristics of all 35 participants at baseline		
Character		N (%)
Maternal age	20- 25	8 (22.9)
	26-34	23 (65.7)
	≥35	4 (11.4)
Residential area	Urban	20 (57.1)
	Suburb	10 (28.6)
	Rural	5 (14.3)
Occupation	Farmer	13 (34.2)
	Worker	18 (51.4)
	Housewife	4 (11.4)
Maternal pre-pregnancy BMI	< 18.5	6 (17.2)
	18.5-23.9	22 (62.9)
	≥ 24.0	7 (20.2)
Education levels	≤ Middle school	9 (25.6)
	High school	18 (51.4)
	≥ College degree	8 (22.9)
Ever fatered a child	Yes	16 (45.7)
	No	19 (54.3)
Second-hand smoking	Never	11 (31.4)
before 3 months	Former	18 (51.4)
	Current	6 (17.1)
Alcohol use before 3 months	Yes	7 (20.0)
	No	28 (80.0)
Household income ($/year)	<5,000	8 (22.9)
	5,000 −20,000	19 (54.2)
	>20,000	8 (22.9)

Figures

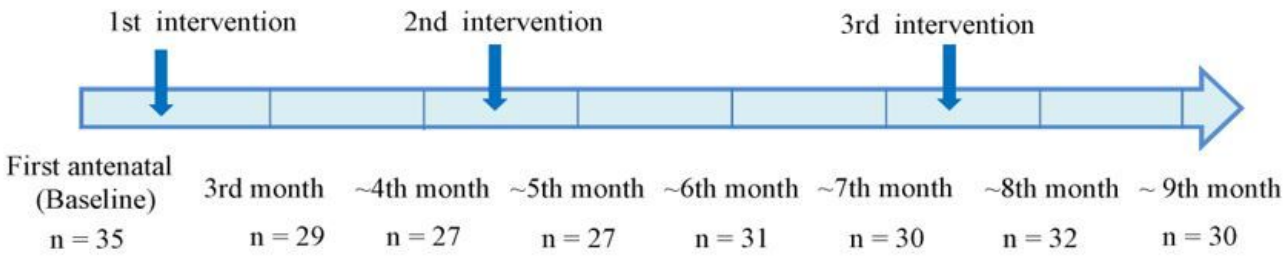

Figure 1

Intervention design and timing of sample collection in the present study

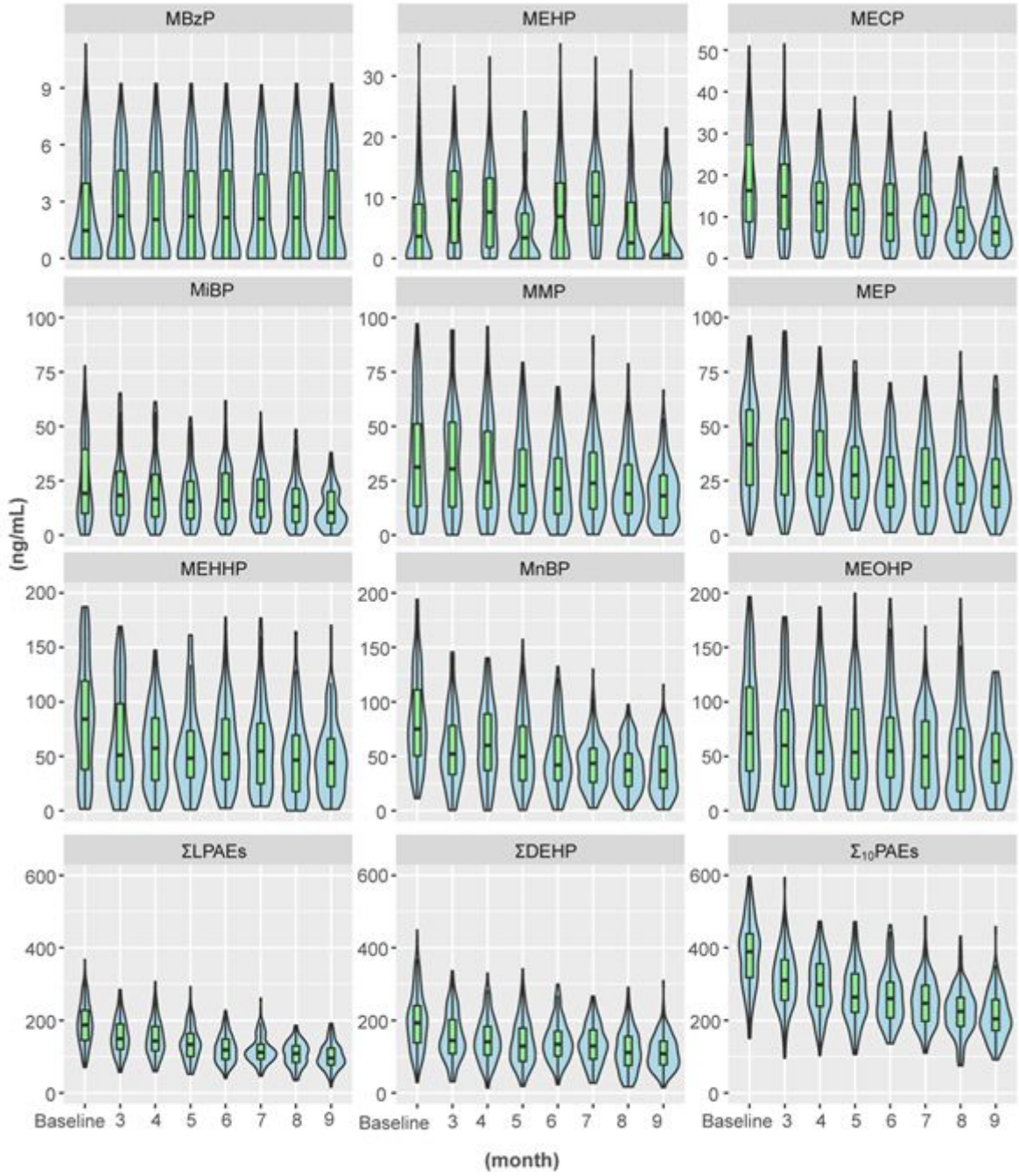

Figure 2

Distributions of nine phthalate metabolites and three combined groups in different month. (As the detection rate of MOP < 40%, we not presented it)

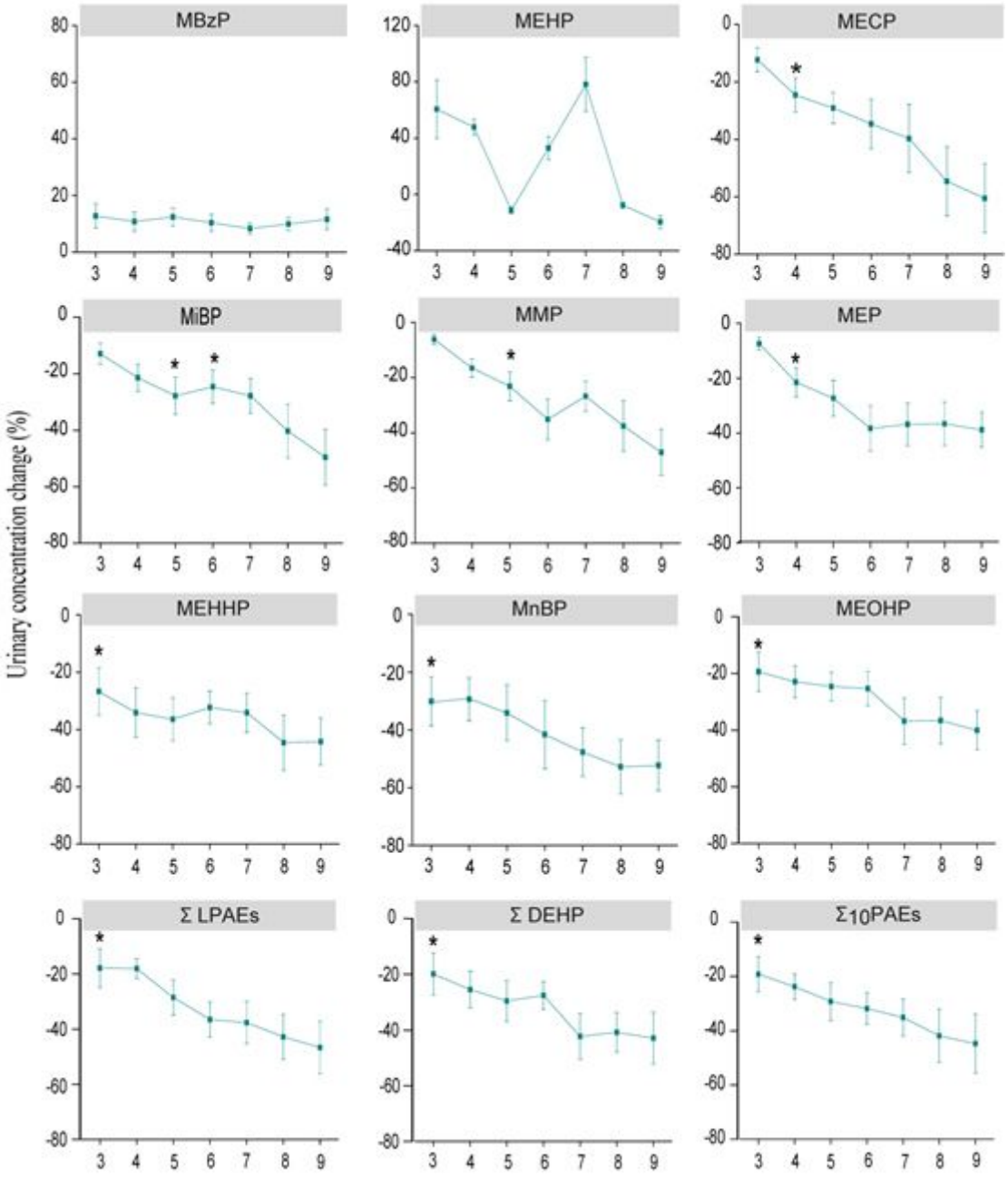

Figure 3

Change in urinary concentration Urine concentration change = [(urine phthalate concentration during a visit –baseline urine phthalate concentration)/(baseline urine phthalate concentration)] ×100%
*Statistically significant decline compare to baseline concentration

Supplementary Files

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