DOCTORAL THESIS

Determination and evaluation of endocrine disrupting chemicals in urine samples of pregnant women by liquid chromatography-tandem mass spectrometry

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ABSTRACT

Endocrine disrupting chemicals (EDCs) are emerging contaminants that can interfere with the hormone system and may cause cancers, birth defects and reproductive system disorders. Prevalence of endocrine-related dysfunction and disease has increased steadily over the past decades. Although accumulating data suggest that these diseases have fetal origins, associations of EDC exposure during pregnancy and adverse health effects on both mothers and fetuses have not been thoroughly evaluated, particularly at multiple points in time.

We firstly developed an analytical method for quantification of 28 EDCs (9 phthalates, 8 bisphenols, 5 parabens, 5 benzophenones and triclosan) in urine samples using ultra high performance liquid chromatography coupled with triple quadrupole mass spectrometer. The method was applied to measure targeted compounds in a total of 5220 urine samples collected from 951 pregnant women at three trimesters and 1501 pregnant women at one or two trimesters in Wuhan, China between 2014 and 2015. Based on the quantification results, exposure patterns and health risks of 28 EDCs on participants were evaluated and discussed in detail below.

Among these samples, bisphenol A (BPA), bisphenol S (BPS), bisphenol F (BPF), methylparaben (MeP), ethylparaben (EtP), propylparaben (PrP), 4-hydroxybenzophenone (4-OH-BP), 2,4-dihydroxybenzophenone (BP-1), 2-hydroxy-4-methoxybenzophenone (BP-3), triclosan, mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethylhexyl) phthalate (MEHHP), monobenzyl phthalate (MBzP), mono-n-butyl phthalate (MnBP), monoisobutyl phthalate (MiBP) were determined with detection rates exceeding

50%, in which BPA, BP-3, MeP and MnBP were the predominant compounds. We found the U-shaped trends of urinary concentrations of phthalate metabolites over trimesters. Parabens, benzophenones and triclosan displayed a downward trend over three visits. We also found the levels of targeted compounds varied by exposure-related factors, such as sampling seasons, physical activities, computer using time and decoration information. In addition, multiple EDCs were mostly determined at low doses over trimesters, indicating that real-world exposure of pollutants were dominated by low-dose mixtures.

We then evaluated the combined health hazards induced by EDC exposure via calculating the estimated daily intakes on the basis of average urinary concentrations at three trimesters. It was found that 24.9% of participants had potential health risks caused by exposure to phthalate mixtures. The most frequency of cumulative risks occurred in women who were exposed to a high dose of one specific phthalate, di-n-butyl phthalate (DnBP) or di(2-ethylhexyl) phthalate (DEHP). We also evaluated the cumulative health risks of BPA and its alternatives and found that about 1.6% of participants were at risks induced by bisphenol exposure. Combined health hazards were mainly driven by one specific bisphenol (BPS or BPA). Our findings suggested that regional interventions of DnBP, DEHP, BPA and BPS in application and production should be tighten and/or taken.

Considering the low-dose effects of BPA, we further investigated the associations of BPA and three major natural estrogens, including estrone (E1), estradiol (E2) and estriol (E3), at three trimesters of pregnancy. We observed non-monotonic dose-response relationships of BPA to E1, E2 and E3 over trimesters even when BPA concentrations were below the current safety

thresholds. In the gender-stratified models, we found significant negative relationships ($\beta < 0$, $p < 0.05$) between BPA and E2 among mothers with male fetuses in the first trimester. However, we found that no significant relationship between BPA and E2 among mothers with female fetuses over three trimesters. Significant non-monotonic associations (from significant negative to positive associations) between BPA and E3 were observed among mothers with female fetuses in the second trimester. The above mentioned findings suggested the gender-specific and trimester-specific effects of BPA on estrogens. Our findings also indicated that the current tolerance daily intake value maybe not safe enough to evaluate the potential health risks induced by BPA exposure.

We next investigated the effects of maternal exposure to phthalates on both mothers and fetuses. Associations of phthalate exposure with the risks of gestational diabetes mellitus (GDM) and plasma glucose levels were evaluated based on a nested case-control study design. It was found that the levels of phthalate metabolites in women with GDM were significantly higher than those without GDM. Meanwhile, positive associations between urinary concentrations of phthalate metabolites and the risks of GDM were obvious, indicating that phthalate exposure may be a risk factor for GDM. In addition, phthalate levels were related to the increased plasma glucose levels after 75 g oral glucose tolerance test. Our findings suggested that phthalates might disturb the glucose homeostasis and increase GDM risks.

Furthermore, we assessed the trimester-specific and gender-specific effects of DEHP exposure on fetal growth, birth size and postnatal growth at 6, 12 and 24 months. We found that among male offspring, 1st-trimester DEHP was negatively related to fetal growth ($\beta < 0$, $p < 0.05$), but positively related to 24-month body

mass index (BMI). 2nd-trimester DEHP was negatively related to fetal growth, birth weight and birth length, but positively related to the weight gain rates from birth to 12 months old. 3rd-trimester DEHP was positively ($\beta > 0$, $p < 0.05$) associated with birth weight, BMI at 6 and 12 months. However, among females, 1st-trimester DEHP was associated with increased birth length, while 2nd-trimester DEHP was negatively associated with BMI at 6 and 12 months. A negative association between DEHP and weight gain rates at 6 months was noted among females. Our findings indicated the second trimester maybe the sensitive window of DEHP exposure for offspring growth since 2nd-trimester DEHP levels were related to the decreased fetal growth, decreased birth size, but increased weight gain rates in early childhood age among male offspring.

To investigate the mechanism underlying the associations of DEHP exposure with glucose and lipid metabolism, we investigated the biotransformation of DEHP and the disturbed metabolisms induced by MEHP, the putative toxic metabolite of DEHP, in human normal liver cell L02 using metabolomics and lipidomics. We found that MEHP was the major metabolite of DEHP. Decreased uptake of glucose and accumulation of glucose in liver cells were obvious after MEHP exposure. Phospholipid remodeling, incomplete fatty acid β-oxidation, inhibition of purine metabolism and glycolysis, and increased oxidative stress were noted in MEHP-exposed L02 cells, which were related to insulin resistance.

In this work, we measured 28 EDCs in a total of 5220 urine samples provided by 951 pregnant women (three trimesters) and 1501 pregnant women (one or two trimesters) and then evaluated the exposure levels, exposure patterns (variations, variability and correlations), health risks and health effects of these compounds on pregnant women and fetuses. Our data suggested that participants had potential

health risks induced by exposure to phthalates or bisphenols. Phthalate exposure was related with the increased plasma glucose levels and risks of GDM. Prenatal DEHP exposure may induce the intrauterine growth restriction and catch-up growth among males, which supported the evidence of fetal origin. To explore the underlying mechanisms of MEHP on glucose and lipid metabolic disorders, we exposed the human normal hepatic L02 cells with MEHP, and applied metabolomic and lipidomic approaches for finding potential biomarkers and disturbed pathways. We found that MEHP exposure inhibited glucose uptake, caused phospholipid remodeling and increased oxidative stress in L02. These findings suggest that the usage of products containing EDCs, particularly phthalates, in pregnant women should be limited in China, intervention of BPS should be considered, and threshold values of BPA are called for reevaluation.

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