DOCTORAL THESIS

Human Exposure to Benzotriazoles and Benzothiazoles During Pregnancy and Health Implications

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ABSTRACT

Benzotriazoles (BTRs) and benzothiazoles (BTHs) are emerging contaminants with high production volume worldwide. The occurrence of BTRs and BTHs (collectively as BTs) in the environmental matrix has been widely documented. The toxicity of BTs reported *in vivo* and *in vitro* study has raised increasing concerns about the health risks of exposure to BTs. However, little is known about the exposure profile, health implications, and the underlying mechanism of toxicity, especially among susceptible population, such as pregnant women.

In the present study, we aimed to a) characterize the exposure profile of BTs during pregnancy, b) evaluate the health effects of gestational exposure to BTs on pregnant women and their offspring, c) identify the metabolic perturbation that were associated BTs exposure, and d) unveil the underlying mechanism of the health implications that were associated with BTs exposure *in vitro* analysis. To address these issues, the present study was nested on an ongoing birth cohort in Wuhan, China. The subjects were recruited between 2014 and 2015 at Wuhan Women and Children's Medical Care Center. Spot urine samples were collected at prenatal care to examine the body burden of BTs and identify the metabolic biomarkers. The most commonly reported BTRs (1-H-benzotriazole, 1-hydroxy-benzotriazole, xylyltriazole, tolyltriazole, 5-chloro-1-H-benzotriazole) and BTHs (benzothiazole, 2-hydroxy-benzothiazole, 2-methylthio-benzothiazole, 2-amino-benzothiazole, 2-thiocyanomethylthio-benzothiazole) were selected as targeted compound in the present study. Demographic characteristics were obtained via a face-to-face questionnaire

administered by well-trained nurses. The pregnancy complications and birth outcomes were retrieved from medical records.

We first characterized the exposure profiles, temporal variability, and potential predictors of urinary BTs during pregnancy. The concentrations of BTs were measured in urine samples that were collected at three trimesters (13.1 ± 1.1, 23.7 ± 3.2, and 35.7 ± 3.4 gestational weeks) among 856 pregnant women. Benzothiazole was the major derivative in urine samples with a geometric mean (GM) concentration of 1.6 ng/mL. Urinary concentrations of BTRs exhibited considerable within-subject variation [intra-class correlation coefficients (ICCs): 0.12-0.56] during pregnancy. Relatively high temporal reliability was observed for urinary concentrations of BTHs with ICCs ranging from 0.42 to 0.85. It was found that parity, household income, pregnancy occupational status, sampling season and menstrual cycle were associated with urinary concentrations of BTs in pregnant women ($p < 0.05$). The widespread BTs exposure among pregnant women has raised concerns about the health consequence that were associated with BTs exposure in this susceptible population.

We then evaluated the association between gestational exposure to BTs and pregnancy complication and fetal growth. BTs are known to exert potential insulin modulation activities. With pregnancy being a state of increasing insulin resistance, pregnant women exposure to BTs may perturb the homeostasis of glucose level. We prospectively investigated the associations of exposure to BTs at early pregnancy with the blood glucose levels and the risks of gestational diabetes mellitus (GDM). In a prospective cohort of 1770 pregnant women who were free of diabetes at baseline, 8.31% of them ($n = 147$) were diagnosed with

GDM based on a 75g oral glucose tolerance test (OGTT) conducted at 26.4 ± 2.4 weeks of gestation. It was found that urinary levels of benzothiazole and 2-hydroxy-benzothiazole (2-OH-BTH) were positively associated with 2-hour blood glucose (p for trend < 0.050). Comparing the high exposure group with the low exposure group of 2-OH-BTH, the adjusted relative risk (RR) of GDM was 1.79 (95% CI = 1.18 to 2.69, p for trend = 0.012). Sensitivity analysis indicated that the positive association of the urinary 2-OH-BTH level with the RR of GDM remained significant among pregnant women who had a male fetus (RR = 1.76, 95% CI = 1.02 to 3.03, p for trend = 0.041).

The occurrence of BTs detected in maternal urine and amniotic fluid indicated the widespread fetal exposure. We investigated the associations of prenatal BTs exposure with fetal and birth size and explored the window of susceptibility among the 856 mother-infant pairs. In boys, per doubling of averaged concentration of urinary benzothiazole was associated with decrement in fetal size z-scores [e.g., femur length (FL): β = -0.068, p < 0.001] and birth size z-scores (e.g., length: β = -0.055, p = 0.005). In girls, positive associations with FL z-score were observed for prenatal exposure to 1-H-benzotriazole (β = 0.028, p = 0.043), 1-hydroxy-benzotriazole (β = 0.033, p = 0.041) and 2-amino-benzothiazole (β = 0.066, p = 0.003). Furthermore, the significant associations between urinary BTH and decreased birth length z-score among boys were observed at 25-35 gestational weeks. The underlying mechanism for the health consequences associated with BTs exposure warrants investigation.

We used mass-spectrometry based high throughput targeted metabolic analysis to identify the urinary biomarkers that were associated with gestational

BTs exposure. In a subset of 159 pregnant women drawn from the prospective birth cohort, targeted metabolic profiling was analyzed in urine sample collected at the third trimester. A total of 44 metabolites were identified as perturbed biomarkers that were associated with urinary BTs concentration during pregnancy. After adjusting for false discovery rate, urinary benzothiazole (BTH) was negatively associated with 14 metabolites that were broadly involved in purine metabolism and tryptophan metabolism. The co-occurrence network indicated that urinary BTs have shared and unique metabolites. The findings indicated that prenatal exposure to BTs may pose neurotoxicity, as indicated by the down-regulation of the tryptophan metabolism, that is known to be involved in the neurotransmitter.

The toxicity of BTs was further evaluated in cell-based *in vitro* analysis. Benzothiazole (BTH) as the predominant BTs found in maternal urine sample, was found to be associated with the disturbed glucose level among pregnant women and the restricted fetal growth among male fetus. We applied high throughput targeted metabolomics to identify perturbed biomarkers that were associated with BTH exposure in MCF-7 breast cancer cells. Based on the variable importance in projection (VIP) value and *p* value in the multivariate model, a total of 45 metabolites were selected as biomarkers for BTH exposure including amino acids, redox substrates, and fatty acids. The upregulation of arginine biosynthesis pathway indicated that exposure to BTH may induce oxidative stress. The oxidative damage was further evidenced by the reduced biogenic amines including putrescine, spermine and spermidine. In addition, the upregulation of carnitines metabolism pathway including palmitoylcarnitine

(C16), tetradecanoylcarnitine (C14) and dodecanoylcarnitine (C12) indicated that exposure to BTH may cause mitochondrial dysfunction.

The present study evaluated the body burden of BTs among the susceptible population of pregnant women and provided preliminary health implications for prenatal exposure to BTs. Moreover, the present study also showed that mass-spectrometry-based high-throughput targeted metabolomics is a powerful tool in environmental science to unveil the toxicity of exposure to environmental contaminants. The results underscore the need of follow-up studies to validate the findings and elucidate the underlying biological mechanism. Future studies will need to consider the use of *in vivo* and *in vitro* study as a complimentary approach to identify the metabolic biomarkers that were associated with BTs exposure, potentially underpinning the maternal-fetal health outcomes.

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