

DOCTORAL THESIS

Human Exposure to Benzotriazoles and Benzothiazoles During Pregnancy and Health Implications

ZHOU, Yanqiu

Date of Award:
2021

[Link to publication](#)

General rights

Copyright and intellectual property rights for the publications made accessible in HKBU Scholars are retained by the authors and/or other copyright owners. In addition to the restrictions prescribed by the Copyright Ordinance of Hong Kong, all users and readers must also observe the following terms of use:

- Users may download and print one copy of any publication from HKBU Scholars for the purpose of private study or research
- Users cannot further distribute the material or use it for any profit-making activity or commercial gain
- To share publications in HKBU Scholars with others, users are welcome to freely distribute the permanent URL assigned to the publication

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): $\beta = -0.068$, $p < 0.001$] and birth size z-scores (e.g., length: $\beta = -0.055$, $p = 0.005$). In girls, positive associations with FL z-score were observed for prenatal exposure to 1-H-benzotriazole ($\beta = 0.028$, $p = 0.043$), 1-hydroxy-benzotriazole ($\beta = 0.033$, $p = 0.041$) and 2-amino-benzothiazole ($\beta = 0.066$, $p = 0.003$). 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.

Table of Contents

DECLARATION	i
ABSTRACT	ii
ACKNOWLEDGEMENTS	vii
Table of Contents	ix
List of Tables	xvi
List of Figures	xix
List of Abbreviations	xxi
Chapter 1. Introduction	1
1.1. Disease burden of exposure to environmental pollutants	1
1.1.1. Exposure to environmental pollutants among susceptible population	2
1.1.2. Prioritizing emerging endocrine-disrupting compound (EDC) for biomonitoring and health-related research	2
1.2. Emerging EDCs of benzotriazoles (BTRs) and benzothiazoles (BTHs) ..	4
1.2.1. Characteristics of BTRs and BTHs (collectively as BTs)	4
1.2.2. Environmental occurrence and human exposure of BTs	5
1.2.3. Environmental transformation of BTs	10
1.2.4. Toxicity of BTs	12
1.3. Epidemiology study of exposure profile and health risk assessment.....	15

1.4. Mass spectrometry based method for human biomonitoring and metabolomics study in environmental science	17
1.5. Objectives of the present study	20
Chapter 2. Occurrence, variability and predictors of benzotriazoles and benzothiazoles among pregnant women	21
2.1. Introduction	21
2.2. Methods.....	23
2.2.1. Study population	23
2.2.2. Data and sample collection	26
2.2.3. Chemicals and reagents.....	26
2.2.4. Sample pretreatment	27
2.2.5. Instrumental analysis	28
2.2.7. Quality control and quality assurance.....	32
2.2.8. Statistical analysis	35
2.3. Results.....	36
2.3.1. Population characteristics	36
2.3.2. Profiles of urinary BTs among pregnant women.....	38
2.3.3. Correlations and exposure patterns of urinary BTs during pregnancy.....	42
2.3.4. Variability of urinary BTs during pregnancy.....	45
2.3.5. Predictors of urinary BTs among pregnant women	47

2.4. Discussion	50
2.4.1. Exposure profiles of BTs during pregnancy	50
2.4.2. ICCs and implications for sampling scheme in etiological studies	55
2.4.3. Predictors of urinary BTs during pregnancy	57
2.4.4. Strengths and limitations of the study	59
2.5. Conclusion	60
Chapter 3. Early pregnancy exposure to benzotriazoles and benzothiazoles in relation to gestational diabetes mellitus.....	61
3.1. Introduction	61
3.2. Methods	63
3.2.1. Study population	63
3.2.2. GDM diagnosis	65
3.2.3. Data collection	65
3.2.4. Urinary BTs measurement	65
3.2.5. Quality control and quality assurance	66
3.2.6. Covariates	66
3.2.7. Statistical analysis	67
3.3. Results	68
3.3.1. Demographic characteristics	69
3.3.2. Urinary concentrations of BTs	72
3.3.3. Urinary concentrations of BTs associated with glucose levels	74

3.3.4. Urinary concentrations of BTs and the risk of GDM.....	77
3.4. Discussion	88
3.5. Conclusion	93
Chapter 4. Prenatal exposure to benzotriazoles and benzothiazoles in relation to fetal and birth size	94
4.1. Introduction	94
4.2. Methods.....	96
4.2.1. Study population	96
4.2.2. Exposure measurement	96
4.2.3. Fetal size and birth size assessment	97
4.2.4. Covariates	98
4.2.5. Statistical analysis	100
4.3. Results.....	102
4.3.1. Characteristics of the study population.....	103
4.3.2. Maternal urinary concentrations of BTs	105
4.3.3. Associations between averaged concentrations of urinary BTs and fetal size z-scores	108
4.3.4. Associations between averaged concentrations of urinary BTs and birth size z-scores	110
4.3.5. Identifying window of exposure susceptibility	120
4.4. Discussion	123

4.5. Conclusion	128
Chapter 5. Urinary metabolomics signatures related to prenatal benzotriazoles and benzothiazoles exposure	129
5.1. Introduction	129
5.2. Methods.....	131
5.2.1. Study population	131
5.2.2. Urinary BTs measurement	131
5.2.3. Urinary metabolites measurement	132
5.2.4. Statistical analysis	133
5.3. Results	135
5.3.1. Demographic characteristics	135
5.3.2. BTs profiles in urine	137
5.3.3. Combined effects and perturbed metabolic pathway	138
5.3.4. Individual effects and co-occurrence network.....	142
5.4. Discussion	144
5.4.1. Urinary metabolic footprint of BTs exposure during pregnancy ...	144
5.4.2. Network of co-occurrence.....	146
5.4.3. Health implications	147
5.4.4. Strengths and limitations.....	148
5.5. Conclusion	149

Chapter 6. Metabolomics study of benzothiazole exposure on MCF-7 cells	151
6.1. Introduction	151
6.2. Methods.....	153
6.2.1. Chemicals and materials	153
6.2.2. Cell culture and cell viability assay	153
6.2.3. Metabolomics sample preparation and metabolite extraction.....	154
6.2.4. Instrumental analysis	155
6.2.5. Data processing and analysis	156
6.3. Results	156
6.3.1. Cytotoxicity assay	157
6.3.2. Potential metabolite biomarkers.....	159
6.3.3. Perturbed metabolic pathways	163
6.4. Discussion	165
6.4.1. BTH exposure associates with the disruption of oxidative stress..	166
6.4.2. BTH exposure associates with the disruption of biogenic amines.	166
6.4.3. BTH exposure associates with fatty acid oxidation	167
6.4.4. Health implication for BTH exposure.....	168
6.5. Conclusion	169
Chapter 7. Conclusions and future perspectives.....	171
REFERENCES	177

List of Publications.....204

CURRICULUM VITAE.....205

List of Tables

Table 1-1. Selected physicochemical properties of the targeted analyte.	9
Table 2-1. Comparisons of the demographic characteristics between the study population and the excluded population [<i>n</i> , %].	25
Table 2-2. Selected MRM transitions and optimized Tandem MS parameters for the analysis of BTs.	31
Table 2-3. The linear range, correlation coefficient, LOD, LOQ, accuracy and recovery of the method.	34
Table 2-4. Demographic characteristics of the participants (<i>n</i> = 856) from Wuhan, China (2014-2015).	37
Table 2-5. Distribution profiles of urinary BTs concentrations (ng/mL) (<i>n</i> = 2568) among 856 study participants.	39
Table 2-6. Intraclass correlation coefficients (ICCs) and 95% confidence intervals (95% CIs) for concentrations of urinary BTs during pregnancy.	46
Table 2-7. Associations between ln-transformed concentrations of urinary BTs and demographic categories (<i>n</i> = 2568).	48
Table 2-8. Comparisons with other studies conducted on exposure assessment of BTs.	53
Table 3-1. Comparisons of the demographic characteristics between the study population and the excluded population [<i>n</i> , %].	64
Table 3-2. Demographic characteristics of the pregnant women (<i>n</i> = 1770) from Wuhan, China (2013-2015).	70

Table 3-3. Detection rate (DR, %) and specific gravity (SG)-adjusted concentrations (ng/mL) of urinary BTs among pregnant women ($n = 1770$) in Wuhan, China (2013-2015).	73
Table 3-4. Adjusted relative risk (95% CI) of GDM with increasing concentrations of urinary BTs (ng/mL).	78
Table 3-5. Adjusted relative risk (95% CI) of GDM with urinary BTs concentrations stratified by infant sex.	82
Table 3-6. Adjusted relative risk (95% CI) of GDM with urinary BTs concentrations stratified by pre-pregnancy BMI (kg/m^2).	85
Table 4-1. Demographic characteristics of the 856 pregnant women by infant sex from Wuhan, China (2014-2015).	104
Table 4-2. Specific gravity-adjusted concentrations of urinary BTs (ng/mL) stratified by infant sex.	107
Table 4-3. Adjusted associations (95% CI) between a 2-fold increase in averaged concentrations of urinary BTs and birth size z-scores in sensitivity analysis of multifactor composite models to control for targeted compounds simultaneously ($n = 856$).	113
Table 4-4. Adjusted associations (95% CI) between a 2-fold increase of averaged concentrations of urinary BTs and birth size z-scores in sensitivity analysis restricted to term babies ($n = 835$).	115
Table 4-5. Adjusted associations (95% CI) between a 2-fold increase of averaged concentrations of urinary BTs and birth size z-scores in contrastive analysis between mothers with ($n = 65$) and without GDM ($n = 791$).	117

Table 5-1. Demographic characteristics of the participants ($n = 159$) from Wuhan, China (2014-2015).....	136
Table 5-2. Specific gravity-adjusted concentrations of urinary BTs (unit: ng/mL).	137
Table 5-3. Identified biomarkers (VIP > 1.2) that were associated with urinary BTs.....	140
Table 6-1. Identified biomarkers of BTH exposure on MCF-7 cells.....	161

List of Figures

Figure 1-1. Chemical structure of targeted compounds.....	8
Figure 2-1. ESI-MS/MS chromatogram for separation of BTs in a calibration standard solution (50 ng/mL) using the Thermo Hypersil GOLD (2.1×100 mm).....	30
Figure 2-2. Boxplots comparing urinary concentrations (ng/mL) of BTs across three trimesters.....	41
Figure 2-3. Heatmap of Spearman correlations between SG-adjusted concentrations of urinary BTs.....	43
Figure 2-4. Exposure patterns of BTs during pregnancy: (A) co-exposure pattern of all targeted BTs at each trimester (T1, T2, T3 for Trimester 1,2,3, respectively); (B) exposure to specific BTs across pregnancy.....	44
Figure 3-1. Adjusted associations (95% CI) of urinary BTs concentrations with fasting, 1-hour and 2-hour glucose levels (mmol/L), respectively.....	75
Figure 3-2. Restricted cubic spline association between log ₂ -transformed concentrations of urinary BTs (ng/mL) and relative risks of GDM.....	80
Figure 4-1. Correlations between SG-adjusted concentrations of urinary BTs and gestational weeks of sampling.....	106
Figure 4-2. Adjusted associations (95% CI) between a 2-fold increase in averaged concentrations of urinary BTs (ng/mL) and fetal size z-scores (Red: girl; blue: boy).....	109
Figure 4-3. Adjusted associations (95% CI) between a 2-fold increase in averaged concentrations of urinary BTs (ng/mL) and birth size z-scores (Red: girl; blue: boy).....	112

Figure 4-4. Adjusted associations (95% CI) between a 2-fold increase in urinary concentrations of BTs and birth size z-scores by gestational ages of sample collection (Red: girl; blue: boy).121

Figure 5-1. O2PLS analysis of the relationship between urinary metabolites (A) and urinary BTs (B) 139

Figure 5-2. Co-occurrence network of the shared metabolites between urinary BTs. Solid lines: positive associations; dash lines: negative associations.143

Figure 6-1. Cell viability of MCF-7 after BTH exposure.158

Figure 6-2. OPLS-DA score plots of metabolomics data in BTH-treated MCF-7 cells.160

Figure 6-3. Comparisons of the potential biomarkers between groups162

Figure 6-4. Enriched pathway analysis for the identified biomarkers that were associated with BTH treatment (20 μ M) in MCF-7 cells.163

Figure 6-5. Perturbed arginine metabolism in response to BTH exposure in MCF-7 cells.164