VYTAUTAS MAGNUS UNIVERSITY
LITHUANIAN RESEARCH CENTRE FOR AGRICULTURE AND FORESTRY

Asta Danilevičiūtė

Effects of exposures to drinking water trihalomethanes and tobacco smoke on adverse pregnancy outcomes in relation to glutathione S-transferase T1 and M1 gene polymorphism

Summary of Doctoral Dissertation

Biomedical Sciences, Ecology and Environmental Sciences (03 B)

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LIETUVOS AGRARINIŲ IR MIŠKŲ MOKSLŲ CENTRAS

Asta Danilevičiūtė

Trihalometanų geriamajame vandenyje ir tabako dūmų įtaka nepalankioms nėštumo baigtims, esant glutationo S-transferazės T1 ir M1 genetiniam polimorfizmui

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INTRODUCTION

Adverse pregnancy outcomes cause a large public-health burden because of its high prevalence, leading cause of neonatal morbidity and mortality, and environmental hazards is considered to be potential risk factors. The main causes of many adverse pregnancy outcomes are not known, but there is increasing evidence that the greatest impact may have the environment (Bove et al., 2002; Aggazzotti et al., 2004; Gehring et al., 2011).

Fetal development likely depends on a number of interacting factors, including genetic, epigenetic, and environmental risk factors (Windham et al., 2000; Plunkett & Muglia, 2008). Given different environmental exposures and individual genetic variations of pregnant women, the study may reveal women group susceptible to environmental hazards and may explain the differences in risk of adverse pregnancy outcomes among individuals exposed to a particular environmental toxicant (Rothman et al., 2001). Furthermore, enhanced understanding of pathologic mechanisms may allow the development of interventions that can be used to prevent adverse pregnancy outcomes.

Experimental and epidemiologic studies provide evidence that a number of drinking water disinfection by-products (DBPs), including trihalomethanes (THM), may be associated with adverse pregnancy outcomes. Epidemiological studies suggested that pregnant women exposed to water containing elevated THM concentrations may be at greater risk for adverse pregnancy outcomes, including fetal growth, but findings of the studies to date have been inconsistent (Nieuwenhuijsen et al., 2000; Graves et al., 2001; Bove et al., 2002; Grazuleviciene et al., 2011).

Numerous studies have shown that maternal cigarette smoking during pregnancy is associated with reduced birth weight or increased other adverse pregnancy outcomes (Windham et al., 2000; Savitz et al., 2001; Sasaki et al., 2008). However, not all women who smoke cigarettes during pregnancy have low birth infants. The reason for this variability is largely unknown, but may be related to maternal genetic susceptibility, since genes involved in metabolic detoxification processes may be associated with pregnancy outcomes (Wang et al., 2000, 2002; Nukui et al., 2000; Infante-Rivard, 2004).

In human detoxification processes Glutathione S-transferase (GSTs) catalyse the conjugation of glutathione to toxic compounds that may be excreted (Raijmakers et al., 2001; Infante–Rivard, 2004). The polymorphic GST could be characterised as a class theta enzyme (GSTT1) by means of molecular biology. "Conjugator" and "non-conjugator" phenotypes are coincident with the presence (*GSTT1-1*) and absence (*GSTT1-0*) of the gene activity that may lead to altered individual susceptibility to environmental exposures (Infante–Rivard et al., 2002; Thier et al., 2003).

Research related to individual susceptibility to environmental toxins and identification of gene-environmental interaction studies is part of modern molecular epidemiology (Thier et al., 2003). Using molecular epidemiology methods in environmental epidemiological studies we can establish the increased susceptibility of human groups and explain individual differences in response to the same environmental factor.

The objective of the research was to evaluate the effects of drinking water THM exposure and tobacco smoking on adverse pregnancy outcomes in relation to Glutathione S-transferase T1 and M1 gene polymorphism.

To achieve this objective the following tasks were set up:

- To determine individual THM internal dose (mg/d) during pregnancy.
- To assess *GSTT1–0* and *GSTM1–0* genotype prevalence among Lithuanian reproductive age women.
- To assess trihalomethanes exposure and *GSTM1* and *GSTT1* polymorphisms effect on adverse pregnancy outcomes.
- To evaluate exposure to tobacco smoke effect on newborn birth weight in relation to maternal *GSTT1–0* and *GSTM1–0* genotypes.
- To evaluate gene-environment interaction effect on adverse birth outcomes controlling for the main confounding variables.

The research was based on the hypothesis:

- metabolic genes *GSTM1* and *GSTT1* polymorphisms and trihalomethanes exposure interaction has adverse effect on pregnancy outcomes;
- maternal smoking effect on birth weight is modified by *GSTM1* and *GSTT1* gene polymorphism.

Scientific novelty and significance. To date, it is not clear what substances forming THMs and what their doses influence human fetal development. Our findings provide additional insight into the biological determinants of response to environmental exposure based on the combination of genes and individual characteristics of a previously unstudied ethnic group of Lithuanians. The present study suggests the prevalence of *GSTT1–0* and *GSTM1–0* genotypes in Lithuanian population, which characterises the altered individual susceptibility to environmental exposures. the effects of drinking water THM exposure and tobacco smoking on adverse pregnancy outcomes in relation to Glutathione S-transferase T1 and M1 gene polymorphism were evaluated for the first time in Lithuania. Furthermore, we found an association between exposure to high THM internal dose during pregnancy and the presence of the *GSTM1 null* and *GSTT1 null* genotypes for the risk of low birth weight, providing evidence that both genetic and environmental factors determine complex traits such as adverse pregnancy outcomes. Genes involved in metabolic detoxification processes such as *GSTM1* and *GSTT1* should be treated as candidate risk factors for low birth weight.

The results of this study stress the need for appropriate policy and programs aimed at cessation of tobacco smoking and chlorinated drinking water use during pregnancy. Improved understanding of etiological mechanisms of adverse pregnancy outcomes should allow clinicians to design appropriate interventions so that the incidence of low birth weight and related fetal and neonatal morbidity and mortality will be reduced.

Defending propositions.

1. THMs in drinking water increase the risk for adverse birth outcomes.
2. *GSTT1–0* and *GSTM1–0* genotypes polymorphism modifie the association between THM exposure and adverse birth outcomes.
3. Tobacco smoking in pregnancy increases the risk for low birth weight.

4. Tobacco smoking effect on birth weight is higher among carriers of *GSTT1–0* and *GSTM1–0* genotypes.
5. Women carriers of *GSTT1–0* and *GSTM1–0* genotypes should be treated as subjects with increased genetic susceptibility to chemical substances.

Approval of the research work. Research findings were published in 3 journals which are assessed by the Institute for Scientific Information database ISI Web of Science and 7 papers in the proceedings of the international conferences.

Volume and structure of the work. The dissertation is written in Lithuanian. It consists of Introduction, Literature review, Material and methods, Results, Discussion and References. The dissertation comprises of 94 pages, including 26 tables, 7 figures and 173 references.

MATERIAL AND METHODS

Participant characteristics and outcome assessment

This nested case-control study is a part of a prospective cohort study of pregnant women in Kaunas city, Lithuania, conducted as a part of the European Commission FP6 project Health impacts of long-term exposure to disinfection by-products in drinking water in Europe (HiWATE) (Nieuwenhuijsen et al., 2009). Details on study subjects and the methods have been reported elsewhere (Grazuleviciene et al., 2009).

During their first visit to a general practitioner, all pregnant women living in Kaunas between 2007 and 2008 were invited to join the cohort. We recruited these women for the prospective cohort study, enrolling them at first trimester of gestation at the four prenatal care clinics affiliated to the hospitals of the Kaunas University of Medicine. Participation was on a voluntary basis and the women were enrolled in the study only if they consented to participate in the cohort. The research protocol was approved by the Lithuanian Bioethics Committee and oral informed consent was obtained from all subjects.

In total 889 women were involved into nested case-control study. Pregnant women were asked to answer two questionnaires provided to them at the clinic. The first questionnaire was designed to determine gestational age, maternal social and demographic characteristics, diseases, and health behaviour. A special water consumption and water use habits questionnaire was used to interview the women who agreed to participate in the study. Women were interviewed before delivery at hospital and blood samples for genetic analysis were collected.

During the interview women were queried regarding demographics, residence and job characteristics, chronic diseases, reproductive history, including date of last menstrual period, previous preterm delivery. We also asked the women to report their age (less than 20 years, 20-29 years, 30 years, and more), educational level (primary, secondary, university), marital status (married not married), smoking (non-smoker, smoker at least one cigarette per day), alcohol consumption (0 drinks per week, at least one drink per week), blood pressure (<140/80 mm/Hg, ≥140 or ≥ 90 mm/Hg), body mass index (<25 kg/m2, 25-30 kg/m2, >30 kg/m2), and other potential risk factors for LBW.

Outcomes of interest related to low birth weight (LBW), small for gestational age (SGA), preterm birth (PB) and birth weight. Pregnancy outcomes were abstracted from

the medical records. LBW were defined as infant's birth weight less than 2,500 g. Infants were considered SGA if they were in the lowest 10th percentile of birth weight for each gestational week stratified by infant gender and maternal ethnic group. Preterm birth was defined as infant of gestational age of less than 37 weeks. Birth weight was abstracted from the birth certificate for all newborns. The reference group was defined as singleton term births (born at >37 weeks of gestation, >2,500 g).

Genetic analysis

The *GSTM1-null* and *GSTT1-null* genotypes were identified by the multiplex polymerase chain reaction (PCR) in peripheral blood DNA samples. This method allows the detection of the presence of the genotype (at least 1 allele present: AA or Aa) or its absence (complete deletion of both alleles: aa). Maternal blood samples were collected in vials containing EDTA and stored at a temperature of −20 °C. DNA was purified from the peripheral blood using DNA purification kits (MBI "Fermentas", Vilnius, Lithuania). DNA concentrations were quantified with a spectrophotometer (Eppendorrf BioPhotometer, 61310488, Hamburg, Germany). A PCR-based study of *GSTM1* and *GSTT1* polymorphism was carried out according to the method described previously (Arand et al., 1996).

The primers used for PCR were as follows:
GSTM1 forward 5′-GAA CTC CCT GAA AAG CTA AAG C-3′and
reverse 5′-GTT GGG CTC AAA TAT ACG GTG G-3′;
GSTT1 forward 5′-TTC CTT ACT GGT CCT CAC ATC TC-3′ and
reverse 5′-TCA CCG GAT CAT GGC CAG CA-3′.

As internal control, a 268-bp fragment of the human β-globin gene was co amplified with a second set of primers: (5′-CAA CTT CAT CCA CGT TCA CC-3′) and (5′-GAA GAG CCA AGG ACA GGT AC- 3′) (Biomers.net – the Biopolymer factory, Germany).

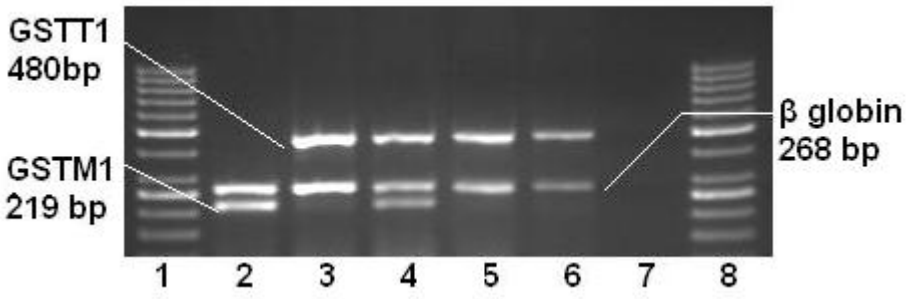

Fig.1. Multiplex PCR analysis of the GST polymorphisms. The analysis resulted in the unequivocal assignment of the following genotypes: samples 1 and 8 are DNA size marker, sample 2 is *GSTT1-0*/*GSTM1-1*, samples 3, 5 and 6 are *GSTT1-1*/ *GSTM1-0*, sample 4 is *GSTM1-1*/*GSTT1-1*, and sample 7 is water control.

PCR was carried out in a final volume of 25 μl. The procedure followed for PCR was: primary denaturation at 94 °C for 5 min, denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, extension at 72 °C for 1 min, 30 cycles were conducted. Final extension was at 72 °C for 10 min. The PCR products were electrophoresed in 2% agarose gels and stained in ethidium bromide. The DNA bands were visualised by UV

transillumination (EASY Win32, Herolab, Germany) (Fig.1). *GSTM1* and *GSTT1* genotypes were coded as present (*GSTM1-1* and *GSTT1-1*) or absent (*GSTM1-0* and *GSTT1-0*).

Exposure Assessment

THM exposure assessment. The Kaunas city municipal drinking water is supplied by four water treatment plants. Groundwater sources were used for the water supply system. However, the four water treatment plants, which disinfected water with sodium hypochlorite, produced different concentrations of THMs in finished water. One treatment plant (Petrasiunai) supplied finished water with higher levels of THMs ("high level THM site"), and the three other plants supplied finished water with lower levels of all THMs ("low level THM site"). Water samples were collected four times per year and analyzed from each water zone. The 12 sites selected for sampling in the Kaunas cohort study were spatially distributed across the city water system (Fig.2). Tap water samples were collected over a three-year study period (2007–2009) in the morning at multiple locations. Samples were analysed at the University of the Aegean, Greece, by using gas chromatography with electron capture detection (Nikolaou et al., 2005). Measurements included specific values for the four regulated THMs (chloroform, bromoform, bromodichlormethane, and dibromochlormethane).

Fig.2 Kaunas districts, water treatment plants and sampling points

We used tap water THM concentration from the each distribution system, and geocoded maternal address at birth to assign the individual women residential exposure index. Integration of the information on residential THM levels (µg/L) and ingested amounts (L/day) yielded an estimate of ingested amount of THM (internal ingested dose), expressed in micrograms per day (µg/d). Finally, we addressed dermal absorption and inhalation by considering showering and bathing alone and combined with ingestion. We multiplied residential THM levels (µg/L) by frequency and average duration of bathing or showering per day (min/day) and derived indices of daily uptake from showering and bathing, respectively (Backer et al., 2000; Lynberg et al., 2005). The uptake factors of THMs individual constituents were assessed on the relative changes in blood levels after 10 minutes exposure (after versus before ingestion 1 L of tap water, 10 minutes showering, and 10 minutes bathing).

The actual algorithms of internal dose from showering and bathing were:
min/day showering × µg/l chloroform in water × 0.001536261 µg/min/µg/l;
min/day showering × µg/l brominated THM in water × 0.001352065 µg/min/µg/l;
min/day bathing × µg/l chloroform in water × 0.001320755 µg/min/µg/l;
min/day bathing × µg/l brominated THM in water × 0.00129571 µg/min/µg/l.

Finally, we combined this information with THM intake by ingestion, using an estimated uptake factor expressed in micrograms per day (Whitaker et al., 2003).

The actual algorithms of internal dose from ingestion were:
chloroform level (µg/l) × water consumption (l/day) × 0.00490196 µg/µg/l;
brominated THM level (µg/l) × water consumption (l/day) × 0.00111848 µg/µg/l.

Then we used average daily total uptakes in our analysis as categorised variables by median (below vs. above) of THM internal dose in different maternal genotypes subgroups.

Tobacco smoke exposure assesment**.** Women were asked to report their daily cigarette consumption before pregnancy as well as during pregnancy. We defined "smokers" as those who smoked any number of cigarettes during pregnancy. We compared non-smokers women with women who smoked during pregnancy.

Statistical Analysis

We first compared the LBW, SGA and PB of exposed and unexposed women without consideration of maternal genotypes and evaluated the possible relationship between increases in adverse birth outcomes risk for an increase in estimated total THM (TTHM) internal dose. The internal dose were categorised as binary variables: "high level" (above median) and "low level" (below median). We used logistic regression to estimate adjusted odds ratios (ORs) and 95-percent confidence intervals (CIs) for LBW, SGA and PB, and the various exposure indices. Then we investigated whether the association between maternal exposure to THM and birth outcomes was modified by maternal genotypes. The subgroups were defined by maternal genotype for *GSTT1* (present, absent) and *GSTM1* (present, absent) and maternal exposure to THM status during pregnancy (above median/below median). We run multivariate logistic regression models for the TTHMs, chloroform, dibromochlormethane, and

bromodichlormethane for total gestational and trimester-specific periods, while adjusting for potential confounders.

Subsequently, we tested the interaction effect of maternal THM exposure, *GSTT1* and *GSTM1* with LBW, SGA and PB by adding all the product terms in the regression models, while adjusting for potential confounders. We estimated the exposure effect by a multivariable analysis controlling for influence of major covariates that changed the adjusted ORs for THM by 10% or more. For the LBW analyses were adjusted for square gestational age, marital status, maternal education, maternal smoking, paternal smoking, alcohol consumption, body mass index, blood pressure, ethnic group, pregnancy history, infant gender, and birth year. For SGA analyses we adjusted for parity, marital status, maternal education, maternal smoking, body mass index, and birth year. For PB analyses we adjusted for family status, body mass index, maternal smoking, alcohol consumption, maternal education, infant birth year. Two-tailed statistical significance was evaluated by using a p value of 0.05. All statistical analyses were carried out using the SPSS software for Windows version 12.0.1.

Using personal data of the nested case-control sample, we first examined the association between smoking and birth outcomes without consideration of genotypes. Further, we examined the combined association of maternal cigarette smoking and maternal genotypes with birth outcomes controlling for effect of major covariates that changed the adjusted odds ratio for smoking by 10% or more. Comparisons of the associations between smoking and LBW risk factors were made by using Fisher's exact probability test (Agresti et al. 1979). The subgroups were defined for LBW by maternal smoking status during pregnancy (no vs. yes) and genotype for *GSTT1* (present vs. absent) and *GSTM1* (present vs. absent). We used chi-square tests to examine the association between genetic polymorphisms and individual susceptibility to tobacco smoking. The gene-cigarette smoke interaction was also tested by adding a product term to the regression models. All the analyses were adjusted for following potential effect modifiers: maternal age, BMI, education, and marital status.

RESULTS

Individual THM internal dose (mg/d) during pregnancy

The women under study tended to be highly educated (47.4% with a university degree). In general women were predominantly Lithuanian in ethnic origin (96.6%), 71.6% of woman were >20 age, 76.3% women were married, 80% of women up to the conception and during pregnancy has been constantly exposed to the same residential district of environmental factors and stable water supply system for drinking water source. The mean birth weight was 3323.3 g, the mean gestational age of newborns was 38.82 weeks.

THM exposure level

The mean TTHM level in the low level site from three water treatment plants was 1.3 µg/L and in the high level site (Petrasiunai) it was 21.3 µg/L (Table 1). Except for the March 2008 data, the other yearly or seasonal means were the same, especially when considering the standard deviations. Chloroform was the dominant THM species in this water, contributing ~80% of the mass of the TTHMs on average. The brominated THM species were significantly lower: dibromochlormethane ranged from 0.06 to 0.5 µg/L and bromodichlormethane ranged from 0.3 to 3.5 µg/L. Bromoform concentration was below the limit of detection.

Overall, women during pregnancy consumed an average of 0.79 L of cold tap water, 1.04 L of boiled water, and 1.09 L of bottled water per day (Table 2). In this study, women consumed an average of 0.33 L of tea and 0.18 L of coffee per day.

Table 1. THM levels (μg/L) by sampling site, water supply zone, year and season of sampling

Tap water sampling	TTHMs[a] Mean (SD[b])	TCM Mean (SD)	DBCM Mean (SD)	BDCM Mean (SD)
Sampling sites				
All	9.8 (12.4)	7.8 (10.2)	0.3 (0.5)	1.7 (2.2)
Low THM level[c]	1.3 (1.2)	0.9 (1.0)	0.1 (0.2)	0.3 (0.5)
High THM level[d]	21.3 (11.4)	17.3 (9.4)	0.5 (0.6)	3.5 (2.2)
Year of sampling				
2007	10.3 (13.5)	8.7 (12.0)	0 (0)[d]	1.5 (1.6)
Low THM level[c]	0.9 (1.3)	0.39 (1.0)	0 (0)	0.6 (0.5)
High THM level[d]	24.2 (11.0)	21.3 (9.6)	0 (0)	2.9 (1.7)
2008	6.2 (10.2)	4.4 (7.5)	0.3 (0.5)	1.5 (2.4)
Low THM level[c]	1.5 (1.1)	0.9 (0.6)	0.2 (0.3)	0.5 (0.5)
High THM level[d]	12.7 (13.5)	9.3 (9.8)	0.6 (0.6)	2.8 (3.3)
2009	11.8 (12.8)	9.5 (10.0)	0.4 (0.5)	1.9 (2.3)
Low THM level[c]	1.4 (1.1)	1.3 (1.0)	0.1 (0.2)	0.1 (0.2)
High THM level[d]	24.9 (7.1)	19.7 (5.6)	0.8 (0.5)	4.3 (1.3)
Season of sampling				
Spring	8.5 (12.1)	6.8 (9.7)	0.3 (0.4)	1.4 (2.1)
Low THM level[c]	1.5 (1.3)	1.2 (1.1)	0.1 (0.3)	0.2 (0.4)
High THM level[d]	17.2 (13.9)	13.8 (11.1)	0.5 (0.5)	2.9 (2.4)
Summer	9.9 (12.7)	8.3 (11.3)	0 (0)	1.6 (1.7)
Low THM level[c]	1.0 (1.4)	0.4 (1.0)	0 (0)	0.7 (0.5)
High THM level[d]	24.1 (8.3)	21.0 (7.0)	0 (0)	3.1 (2.0)
Autumn	11.1 (13.4)	8.8 (11.1)	0.2 (0.5)	2.0 (2.4)
Low THM level[c]	1.2 (1.1)	0.8 (0.9)	0 (0)	0.4 (0.5)
High THM level[d]	24.8 (9.7)	20.1 (8.6)	0.6 (0.6)	4.2 (2.4)
Winter	10.9 (12.1)	8.4 (9.3)	0.5 (0.6)	1.9 (9.3)
Low THM level[c]	1.1 (1.0)	0.9 (0.6)	0.1 (0.3)	0.1 (0.3)
High THM level[d]	24.5 (1.4)	18.9 (1.2)	1.1 (0.1)	4.5 (0.2)

[a]TTHMs = total trihalomethanes: the sum of TCM (chloroform), DBCM (dibromochlormethane), and BDCM (bromodichlormethane)
[b]SD = standard deviation
[c]Viciunai, Eiguliai, Kleboniskis, [d]Petrasiunai
[d]0 = below the limit of detection

Most of the study participants took showers or in combination with baths during the pregnancy (96%). Mean frequency of showering was 6.5 times per week, with a mean duration of 15.2 min per shower (Table 3). Average frequency of bathing was 1.8 times per week, with a mean duration of 33.5 min per bath. The percentage of participants who attended swimming pools was low (7%). THM integrated uptake included ingestion, showering, and bathing. Uptake via ingestion contributed 8%, whereas showering and bathing were 92% of the total internal dose.

Table 2. Summary of Kaunas cohort study subjects daily water intake (litres) for water users

Mean daily ingestion	Min.	Max.	Mean	SD
Consumption tap water				
At home	0	4.0	0.66	0.49
At work	0	2.0	0.10	0.25
Other	0	1.4	0.03	0.11
In total (60.4%)	0.2	5.2	0.79	0.59
Consumption bottled water				
At home	0	4.0	0.66	0.55
At work	0	3.0	0.37	0.40
Other	0	3.0	0.06	0.18
In total (97.3%)	0.2	8.0	1.09	0.74
Consumption tea				
At home	0	1.6	0.20	0.11
At work	0	1.0	0.11	0.12
Other	0	0.4	0.02	0.06
In total (100%)	0.1	2.0	0.33	0.22
Consumption coffee				
At home	0	0.7	0.11	0.06
At work	0	0.7	0.06	0.08
Other	0	0.6	0.01	0.04
In total (80.0%)	0.1	1.0	0.18	0.11
Other tap-water beverages				
At home	0	2.0	0.39	0.36
At work	0	1.6	0.07	0.16
Other	0	2.0	0.06	0.16
In total (15.4%)	0.2	2.4	0.53	0.41
Total tap water consumption (70.7%)	0.2	5.2	0.83	0.61
Total hot tap water consumption (100%)	0.2	4.0	0.85	0.46

The individual total uptake of TTHMs ranged between 0.0025 and 2.40 μg/d (Table 4). The total gestational chloroform uptake ranged between 0.0013 and 2.13 μg/d., daily uptake of bromodichlormethane ranged between 0.0001 and 0.34 μg/d and dibromochlormethane ranged between 0 and 0.064 μg/d.

Table 3. Showering and bathing during pregnancy

Water use habits	Min	Max	Mean	SD
Shower (96.0%)				
Times/week	1	21	6.49	3.20
Duration (min)	2	90	15.25	7.86
Time (min/week)	4	420	92.40	56.75
Bath (41.4%)				
Times/week	1	14	1.78	1.38
Duration (min)	1	120	33.48	17.18
Time (min/week)	7	360	57.19	48.61
Shower& Bath (37.5%)				
Shower (37.5%)				
Times/week	1	21	6.25	3.00
Duration (min)	2	60	14.25	7.20
Time (min/week)	4	420	84.75	53.40
Bath (37.5%)				
Times/week	1	14	1.61	1.16
Duration (min)	5	120	33.71	16.74
Time (min/week)	7	360	52.98	43.41
Swimming pool (8.6%)				
Times/week	1	8	1.77	0.96
Duration (min)	1	120	50.62	23.52
Time (min/week)	1	600	88.86	65.66

Table 4. The individual total uptake of trihalomethanes for trimester-specific and entire pregnancy

	Min	Max	Median	SD
TTHM[d]				
Entire pregnancy	0.0025	2.400	0.1733	0.3050
First trimester	0.0025	2.6231	0.1666	0.3169
Second trimester	0.0025	2.4079	0.1645	0.3090
Third trimester	0.0025	2.5142	0.1630	0.3134
Chloroform				
Entire pregnancy	0.0013	2.1300	0.1424	0.2619
First trimester	0.0013	2.3317	0.1360	0.2808
Second trimester	0.0013	2.1328	0.1333	0.2690
Third trimester	0.0013	2.1328	0.1298	0.2660
BDCM[d]				
Entire pregnancy	0.0001	0.3400	0.0280	0.0409
First trimester	0.0001	0.3244	0.0276	0.0382
Second trimester	0.0001	0.3728	0.0279	0.0407
Third trimester	0.0001	0.3777	0.0283	0.0450
DBCM[d]				
Entire pregnancy	0.0000	0.0640	0.0026	0.0073
First trimester	0.0000	0.0630	0.0012	0.0067
Second trimester	0.0000	0.0710	0.0021	0.0077
Third trimester	0.0000	0.0719	0.0033	0.0087

***GSTT1–0* and *GSTM1–0* genotype prevalence among Lithuanian reproductive age women**

The percentage of *GSTT1 null* genotype was 17% (Fig 3) and *GSTM1 null* genotype was 47% (Fig. 4).

Maternal genotype frequency distribution by THM internal dose median (below median vs. above median) and pregnancy outcome is shown in Fig. 5. Among women carrying *GSTT1–0* genotype and exposed to THM above median, LBW prevalence was 12.2%, and to compare to exposed to below median p was 0.432; for PB ─ 24.2% (p=0.024), for SGA ─12.2% (p=0.416). Among women carrying *GSTM1–0* genotype and exposed THM above median, LBW prevalence was 13.9% (p=0.003), PB ─ 14.6% (p=0.507), SGA ─ 19.2% (p=0.243).

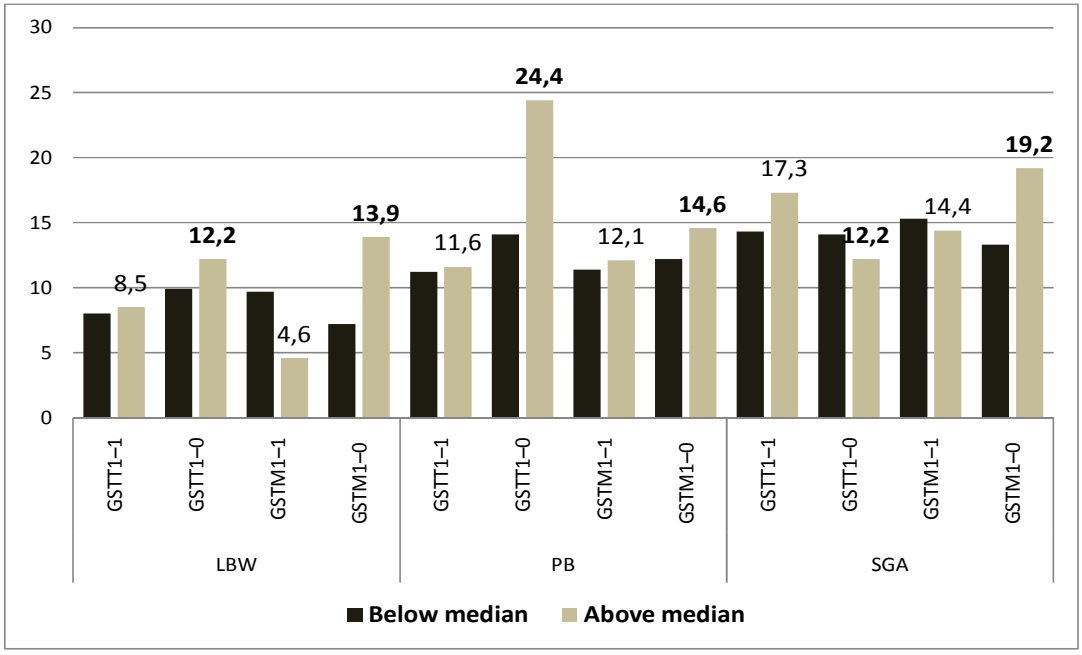

Fig. 5. Maternal genotype prevalence (in %) by THM internal dose median and pregnancy outcome.

Trihalomethanes exposure and *GSTM1* and *GSTT1* polymorphisms effect on adverse pregnancy outcomes

Using entire pregnancy and trimester-specific daily uptakes median of THM, we examined the association between internal dose and LBW, SGA and PB risk (Table 5).

Table 5. Low birth weight, small for gestation age and preterm birth adjusted odds (OR) ratios and 95% confidence intervals (CI) for trimester-specific and entire pregnancy exposure to internal dose THM

THM[d] exposure	LBW	SGA	PB

Above/below median	OR[a] (95% CI)	OR[b] (95% CI)	OR[c] (95% CI)
TTHM[e]			
Entire pregnancy	1.27 (0.59–2.74)	1.38 (0.88–2.16)	1.23 (0.76–2.02)
First trimester	1.03 (0.48–2.23)	1.27 (0.81–2.00)	1.29 (0.79–2.11)
Second trimester	1.15 (0.53–2.47)	1.24 (0.79–1.95)	1.33 (0.82–2.17)
Third trimester	1.33 (0.62–2.87)	1.38 (0.88–2.18)	1.16 (0.71–1.89)
Chloroform			
Entire pregnancy	1.24 (0.57–2.68)	1.37 (0.87–2.15)	1.23 (0.75–2.00)
First trimester	1.15 (0.54–2.48)	1.33 (0.84–2.08)	1.27 (0.78–2.07)
Second trimester	1.29 (0.60–2.76)	1.31 (0.83–2.06)	1.31 (0.80–2.13)
Third trimester	1.45 (0.67–3.13)	1.38 (0.87–2.16)	1.20 (0.74–1.96)
BDCM[e]			
Entire pregnancy	1.26 (0.58–2.72)	1.26 (0.81–1.98)	1.21 (0.74–1.96)
First trimester	1.28 (0.59–2.76)	1.27 (0.81–2.00)	1.16 (0.71–1.88)
Second trimester	1.26 (0.58–2.73)	1.22 (0.78–1.91)	1.23 (0.76–2.01)
Third trimester	1.27 (0.59–2.76)	1.27 (0.81–1.99)	1.21 (0.75–1.98)
DBCM[e]			
Entire pregnancy	3.00 (0.34–27.0)	1.66 (0.99–2.80)	1.66 (0.95–2.89)
First trimester	1.76 (0.66–4.69)	2.95 (1.63–5.36)*	3.70 (1.97–6.95)
Second trimester	1.46 (0.62–3.42)	1.63 (0.96–2.76)	2.40 (1.36–4.25)
Third trimester	1.54 (0.65–3.63)	1.91 (1.11–3.28)*	1.58 (0.89–2.780

[a]Adjusted for marital status, square gestational age, maternal education, maternal smoking, paternal smoking, alcohol consumption, body mass index, blood pressure, premature baby, infant gender, and birth year.
[b] Adjusted for parity, maternal status, maternal education, maternal smoking, body mass index, birth year.
[c] Adjusted for family status, body mass index, maternal smoking, alcohol consumption, maternal education, infant birth year.
[d] Referent group below median.
[e]TTHM, total trihalomethane; DBCM–dibromochlormethane, BDCM– bromodichlormethane.
*p<0,05.

Maternal exposure to TTHM and chloroform internal dose above median during the entire pregnancy was associated with a slight increase in OR for LBW, SGA and PB as compare to the referent group below median, after adjustment for potential confounding factors (Table 5). We observed a tendency of increasing LBW risk with increasing pregnancy duration for exposed to TTHM and chloroform. During the third trimester, the odds ratios for LBW were 1.33, 95% CI 0.62–2.87; and OR 1.45, 95% CI 0.67–3.13, respectively, for TTHM and chloroform. Similarly, third trimester TTHM and chloroform exposures slightly increased in risk for SGA (OR 1.33, 95% CI 0.84–2.13; and OR 1.31, 95% CI 0.82–2.08). For the DBCM and BDCM exposure we also observed slight elevated odds ratios for LBW and SGA. Adjusted OR for preterm birth among women exposed to TTHM above median during the second trimester pregnancy was 1.33 (95% CI 0.82–2.17) and to chloroform OR 1.31 (95% CI 0.80–2.13). For the DBCM, exposure we found statistically significant risk for the second trimester of pregnancy OR 2.40 (95% CI 1.36–4.25).

When *GSTT1*genotype was considered, the association between exposure to THM and LBW differed by genotype: OR for LBW among women exposed to TTHM during the entire pregnancy was 1.19 (95% CI 0.50–2.82) and 7.40 (95% CI 0.13–409) for the

present and absent genotypes, respectively (Table 6). The findings were similar for chloroform: in carriers of *GSTT1–0* genotype exposure was associated with higher OR than in carriers of *GSTT1–1* genotype for all three trimesters. However, these findings were not evident when the BDCM exposures were analyzed. The OR for LBW among women exposed to BDCM during entire pregnancy was 1.34 (95% CI, 0.57–3.16) and 0.89 (95% CI, 0.05–15.7) for the present and absent genotypes, respectively.

Table 6. Low birth weight adjusted odds ratios (OR) and 95% confidence intervals (CI) for gestational exposure to internal dose THMs according maternal polymorphisms in the *GSTT1* gene

THM Above/ below [a]	*GSTT1* genotype	Entire pregnancy OR (95% CI)	First trimester OR (95% CI)	Second trimester OR (95% CI)	Third trimester OR (95% CI)
TTHM[b]	*GSTT1–1*	1.19 (0.50–2.82)	0.97 (0.41–2.28)	1.10 (0.47–2.59)	1.23 (0.52–2.90)
	GSTT1–0	7.40 (0.13–409)	7.48 (0.13–441)	7.48 (0.13–441)	7.30 (0.14–391)
TCM[b]	*GSTT1–1*	1.19 (0.50–2.82)	1.12 (0.48–2.63)	1.25 (0.53–2.92)	1.35 (0.57–3.20)
	GSTT1–0	7.48 (0.13–441)	7.48 (0.13–441)	7.48 (0.13–441)	7.30 (0.14–391)
BDCM[b]	*GSTT1–1*	1.34 (0.57–3.16)	1.37 (0.58–3.23)	1.34 (0.57–3.16)	1.36 (0.58–3.22)
	GSTT1–0	0.89 (0.05–15.7)	0.89 (0.05–15.7)	0.89 (0.05–15.7)	0.89 (0.05–15.9)
DBCM[b]	*GSTT1–1*	1.16 (0.10–13.1)	1.22 (0.38–3.91)	1.16 (0.43–3.13)	1.41 (0.54–3.70)
	GSTT1–0	56.1 (0.00–$2*10^7$)	8.79 (0.21–377)	1.20 (0.06–25.3)	0.54 (0.02–12.51)

[a]Referent group below median.

[b]TTHM, total trihalomethane; DBCM– dibromochlormethane, BDCM– bromodichlormethane , TCM-chloroform

Adjusted for marital status, square gestational age, maternal education, maternal smoking, paternal smoking, alcohol consumption, body mass index, blood pressure, ethnic group, pregnancy history, infant gender, and birth year.

In the analysis of SGA, we found the largest association among exposed women for third trimester (Table 7). Women carries *GSTT1–0* genotype exposed to TTHM OR for third trimester were 1.51, 95% CI 0.43–5.29, while for *GSTT1–1* it were 1.22, 95% CI 0.73–2.03; and for exposed to chloroform OR were 1.75, 95% CI 0.50–6.10 and 1.18, 95% CI 0.71–1.97, respectively, for absent and present genotype. However, a test of interaction did not show statistically significant results.

Table 7. Small for gestational age adjusted odds ratios (OR) and 95% confidence intervals (CI) for gestational exposure to internal dose THMs according maternal polymorphisms in the *GSTT1*gene

THM Above/ below [a]	*GSTT1* genotype	Entire pregnancy OR (95% CI)	First trimester OR (95% CI)	Second trimester OR (95% CI)	Third trimester OR (95% CI)
TTHM[b]	*GSTT1–1*	1.30 (0.78–2.17)	1.17 (0.70–1.94)	1.23 (0.68–1.88)	1.22 (0.73–2.03)
	GSTT1–0	1.04 (0.29–3.74)	0.99 (0.28–3.58)	0.99 (0.28–3.58)	1.51 (0.43–5.29)
TCM[b]	*GSTT1–1*	1.30 (0.78–2.17)	1.23 (0.74–2.06)	1.18 (0.71–1.98)	1.18 (0.71–1.97)
	GSTT1–0	0.99 (0.28–3.58)	0.99 (0.88–3.58)	1.15 (0.32–4.11)	1.75 (0.50–6.10)
BDCM[b]	*GSTT1–1*	1.28 (0.77–2.14)	1.30 (0.78–2.18)	1.28 (0.77–2.14)	1.29 (0.77–2.15)
	GSTT1–0	1.03 (0.29–3.69)	1.04 (0.30–3.67)	0.72 (0.19–2.71)	1.03 (0.29–3.69)
DBCM[b]	*GSTT1–1*	1.29 (0.71–2.34)	1.85 (0.93–3.67)	1.20 (0.65–2.20)	1.89 (1.01–3.54)
	GSTT1–0	1.43 (0.43–4.76)	3.79 (0.89–16.1)	2.36 (0.66–8.46)	1.04 (0.31–3.53)

[a]Referent group below median.
[b]TTHM, total trihalomethane; DBCM– dibromochlormethane, BDCM– bromodichlormethane , TCM-chloroform.
Adjusted for parity, maternal status, maternal education, maternal smoking, body mass index, birth year.

Table 8 and Table 9 show the association of maternal exposure to THMs above internal dose median in different *GSTM1* genotype with LBW and SGA.

Table 8. Low birth weight adjusted odds ratios (OR) and 95% confidence intervals (CI) for gestational exposure to internal dose THMs according maternal polymorphisms in the *GSTM1* gene

THM exposure[a]	*GSTM1* genotype	Entire pregnancy OR (95% CI)	First trimester OR (95% CI)	Second trimester OR (95% CI)	Third trimester OR (95% CI)
TTHM[b]	*GSTM1–1*	0.34 (0.09–1.22)	0.32 (0.09–1.14)	0.34 (0.09–1.23)	0.34 (0.09–1.24)
	GSTM1–0	4.23 (1.25–14.32) [c]	2.88 (0.90–9.22)	3.21 (1.01–10.2)[c]	4.37 (1.36–14.08)[c]
	Interaction	13.37 (2.36–75.8)[c]	9.29 (1.71–50.35)[c]	10.28 (1.88–56.23)[c]	13.35 (2.41–73.87)[c]
TCM[b]	*GSTM1–1*	0.34 (0.09–1.22)	0.43 (0.13–1.42)	0.48 (0.14–1.59)	0.35 (0.10–1.28)
	GSTM1–0	4.08 (1.20–13.9)[c]	2.81 (0.87–9.03)	3.08 (0.96–9.87)	5.06 (1.50–17.05)[c]
	Interaction	12.88 (2.27–73.2)[c]	6.70 (1.29–34.73)[c]	7.04 (1.34–37.0)[c]	15.86 (2.75–91.40)[c]
BDCM[b]	*GSTM1–1*	0.55 (0.16–1.89)	0.57 (0.17–1.95)	0.56 (0.16–1.90)	0.55 (0.16–1.89)
	GSTM1–0	2.65 (0.85–8.23)	2.63 (0.85–8.14)	2.65 (0.85–8.23)	2.74 (0.88–8.51)
	Interaction	5.16 (1.01–26.52)[c]	4.89 (0.96–25.0)	5.11 (1.00–26.24)[c]	5.29 (1.03–27.15)[c]
DBCM[b]	*GSTM1–1*	0.94 (0.07–12.14)	2.52 (0.54–11.7)	0.74 (0.19–2.90)	1.36 (0.36–5.11)
	GSTM1–0	11.97 (0.42–337)	1.47 (0.41–5.34)	2.13 (0.67–6.81)	1.78 (0.55–5.75)
	Interaction	13.75 (0.23–83.3)	0.88 (0.19–4.10)	3.05 (0.63–14.87)	1.95 (0.40–9.56)

[a]Referent group below median.
[b]TTHM, total trihalomethane; DBCM– dibromochlormethane, BDCM– bromodichlormethane , TCM-chloroform.
[c] $p<0.05$
Adjusted for marital status, square gestational age, maternal education, maternal smoking, paternal smoking, alcohol consumption, body mass index, blood pressure, ethnic group, pregnancy history, infant gender, and birth year.

The findings suggest that woman carriers *GSTM1–0* genotype and exposed to THM has an increased risk for LBW and SGA as compare to woman carriers *GSTM1–1* genotype. The highest risk for LBW was found during the third trimester among woman exposed to TTHM (OR 4.37, 95% CI 1.36–14.08) and chloroform (OR 5.06, 95% CI 1.50–17.05) (Table 8). Exposure to BDCM during the third trimester among woman carriers *GSTM1–0* genotype was associated with OR 1.43, 95% CI 0.73–2.81 and exposure to DBCM produced OR 1.55, 95% CI 0.72–3.36. A test of interaction between maternal exposure to THM and maternal GSTM1 genotypes shows statistically significant results for LBW of second and third trimesters for TTHM (OR 10.28 and 13.35), chloroform (OR 7.04 and 15.86), and BDCM (OR 5.11 and 5.29) exposure.
Adjusted analyses of SGA showed a consistent but small increase in ORs among woman carriers *GSTM1–0* genotype and exposed to THMs. In third trimester OR was 2.19, 95% CI 0.83–5.79 for TTHM, OR 1.98, 95% CI 0.76–5.20 for chloroform, and OR 1.43, 95% CI 0.73–2.81 for BDCM exposure (Table 9). These increases were more evident when interaction was examined. However, a test of interaction between GSTM1 genotypes and exposure to THMs did not show statistically significant results for SGA.

Table 9. Small for gestational age adjusted odds ratios (OR) and 95% confidence intervals (CI) for gestational exposure to internal dose THMs according to maternal polymorphisms in the *GSTM1* gene

THM exposure[a]	GSTM1 genotype	Entire pregnancy OR (95% CI)	First trimester OR (95% CI)	Second trimester OR (95% CI)	Third trimester OR (95% CI)
TTHM[b]	*GSTM1–1*	0.84 (0.42–1.68)	0.80 (0.40–1.61)	0.78 (0.39–1.57)	0.86 (0.43–1.74)
	GSTM1–0	1.80 (0.92–3.55)	1.60 (0.82–3.15)	1.54 (0.79–3.02)	1.81 (0.92–3.56)
	Interaction	2.26 (0.85–5.95)	2.18 (0.82–5.75)	2.07 (0.79–5.46)	2.19 (0.83–5.79)
TCM[b]	*GSTM1–1*	0.84 (0.42–1.68)	0.89 (0.45–1.78)	0.90 (0.45–1.80)	0.88 (0.44–1.78)
	GSTM1–0	1.78 (0.90–3.50)	1.59 (0.81–3.12)	1.52 (0.78–2.97)	1.74 (0.89–3.41)
	Interaction	2.12 (0.81–5.54)	1.87 (0.72–4.88)	1.71 (0.66–4.87)	1.98 (0.76–5.20)
BDCM[b]	*GSTM1–1*	1.05 (0.52–2.10)	1.00 (0.50–2.01)	0.96 (0.48–1.93)	1.05 (0.52–2.10)
	GSTM1–0	1.42 (0.72–2.79)	1.50 (0.77–2.95)	1.42 (0.72–2.79)	1.43 (0.73–2.81)
	Interaction	1.42 (0.55–3.71)	1.60 (0.61–4.16)	1.55 (0.59–4.03)	1.42 (0.55–3.71)
DBCM[b]	*GSTM1–1*	1.57 (0.72–3.40)	2.33 (0.91–5.95)	1.44 (0.67–3.12)	1.63 (0.73–3.64)
	GSTM1–0	1.09 (0.51–2.32)	1.74 (0.77–3.97)	1.23 (0.57–2.65)	1.55 (0.72–3.36)
	Interaction	0.61 (0.23–1.61)	0.43 (0.16–1.14)	0.58 (0.22–1.53)	0.87 (0.33–2.26)

[a]Referent group below median.
[b]TTHM, total trihalomethane; DBCM– dibromochlormethane, BDCM– bromodichlormethane , TCM- chloroform.
Adjusted for parity, maternal status, maternal education, maternal smoking, body mass index, birth year.

Table 10 shows association of maternal exposure to THMs above internal dose median in different *GSTT1* and *GSTM1* genotypes with preterm birth. When *GSTM1* genotype was considered, the association between exposure to THM and preterm birth differed by genotype: OR for preterm birth among women exposed to TTHM above median during the second trimester pregnancy was 1.03 (95% CI 0.52–2.06) and 2.07 (95% CI 1.00–4.35) for the present and absent genotype, respectively. The findings were similar for chloroform and bromodichlormethane: in carriers of *GSTM1-0* genotype exposure was associated with higher OR than in carriers of *GSTM1-1* genotype for all three trimesters. However, these findings were not evident when the dibromochlormethane exposures were analyzed. The OR for preterm birth among women exposed to dibromochlormethane during the second trimester was 4.33 (95% CI 1.69–11.10) and 1.69 (95% CI 0.0.78–3.64) for the present and absent genotypes, respectively.

The findings suggest that carriers of the *GSTT1–0* genotype and exposed to TTHM, chloroform and bromodichlormethane had an increased risk for preterm birth as compared to carriers of the *GSTT1–1* genotype: the ORs during the second trimester among woman *GSTT1–1* genotype carriers were 1.03–1.17, while among GSTT1–0 genotype carriers ORs were 2.46–3.08. Exposure to dibromochlormethane during the second trimester among carriers of the *GSTT1–1* genotype was associated with an OR of 2.89, 95% CI 1.46–5.69, and among carriers of the *GSTT1–0* genotype produced an OR of 1.42, 95% CI 0.43–4.64.

Table 10. Preterm birth adjusted OR and 95% confidence intervals for trimester–specific and entire pregnancy internal THM dose according to maternal polymorphisms in the *GST* gene

THM exposure[a]	*GSTM1–1* GS (95% CI)	*GSTM1–0* GS (95% CI)	*GSTT1–1* GS (95% CI)	*GSTT1–0* GS (95% CI)
TTHM[b]				
Entire pregnancy	1.00 (0.50–1.99)	1.86 (0.89–3.88)	1.06 (0.61–1.83)	2.55 (0.82–7.97)
First trimester	1.05 (0.53–2.10)	1.91 (0.91–4.01)	1.13 (0.65–1.96)	2.46 (0.79–7.67)

Second trimester	1.03 (0.52–2.06)	2.07 (1.00–4.35)	1.17 (0.67–2.03)	2.46 (0.80–7.68)
Third trimester	1.00 (0.50–2.00)	1.59 (0.77–3.28)	0.99 (0.87–1.72)	2.30 (0.76–6.99)
TCM[b]				
Entire pregnancy	1.00 (0.50–1.99)	1.83 (0.88–3.81)	1.05 (0.61–1.83)	2.46 (0.79–7.67)
First trimester	1.04 (0.52–2.07)	1.86 (0.88–3.91)	1.11 (0.64–1.92)	2.46 (0.79–7.67)
Second trimester	1.03 (0.52–2.06)	1.97 (0.94–4.15)	1.14 (0.65–1.97)	2.66 (0.85–8.29)
Third trimester	1.00 (0.50–2.01)	1.69 (0.82–3.49)	1.03 (0.59–1.78)	2.49 (0.82–7.60)
BDCM[b]				
Entire pregnancy	1.06 (0.53–2.13)	1.66 (0.80–3.45)	1.03 (0.59–1.79)	2.63 (0.85–8.09)
First trimester	1.01 (0.51–2.01)	1.56 (0.76–3.22)	0.98 (0.56–1.69)	2.52 (0.83–7.65)
Second trimester	1.11 (0.55–2.21)	1.66 (0.80–3.45)	1.03 (0.59–1.79)	3.08 (0.97–9.75)
Third trimester	1.06 (0.53–2.13)	1.69 (0.81–3.51)	1.04 (0.60–1.80)	2.63 (0.85–8.09)
DBCM[b]				
Entire pregnancy	2.02 (0.85–4.79)	1.61 (0.76–3.42)	1.76 (0.93–3.34)	1.37 (0.43–4.38)
First trimester	7.35 (2.62–20.6)	2.81 (1.21–6.52)	4.29 (2.06–8.93)	2.47 (0.68–8.95)
Second trimester	4.33 (1.69–11.1)	1.69 (0.78–3.64)	2.89 (1.46–5.69)	1.42 (0.43–4.64)
Third trimester	2.51 (0.99–6.39)	1.34 (0.62–2.89)	1.88 (0.97–3.66)	0.96 (0.29–3.11)

[b]Referent group bellow median.

Adjusted for: family status, smoking, education, stress, previous preterm birth, and infant birth year.

Exposure to tobacco smoke effect on newborn birth weight in relation to maternal *GSTT1–0* and *GSTM1–0* genotypes

Among the pregnant women with smoking and pregnancy outcome data, 71.1% never smoked, 21.5% smoked before but not during pregnancy. Among the women who smoked during pregnancy, light smokers (mean 4.8 cigarettes/day) predominated (92.3% of smokers) and only 7.7% of smokers smoked 10 or more cigarettes per day. Table 11 presents maternal characteristics by tobacco smoke exposure status. This is the overall low-risk population, with the majority of women at their optimal reproductive ages, high education, most having the ideal BMI, blood pressure, and most woman are non-smokers. Smoking during pregnancy was associated with maternal age, education, marital status, and smoking history before pregnancy: the P value of exact test was $p < 0.05$. Infants of active smokers revealed non-significant reduction in mean birth weight: among non-smokers, the birth weight was 3445 ± 25 g, and light smokers – 3365 ± 59, $p = 0.2$.

Table 11. Percent distribution of subjects by smoking for various characteristic

Maternal characteristics	**Total**	**Smoking during pregnancy (%)**		**Exact test**
Variables	**N**	**None**	**Yes**	**p**
Age:				
≤ 20 y	28	71.4	28.6	
21 – 30 y	402	86.8	13.2	
>30 y	216	92.1	7.9	0.004
Education:				
university	309	96.8	3.2	
college and ≤ 12 y	337	79.8	20.2	< 0.001
Marital status:				
married	493	92.3	7.7	

not married	153	73.9	26.1	< 0.001
Parity:				
1st	320	89.1	10.9	
2rd and more	326	86.8	13.2	0.38
Pregnancy history:				
no prior	517	87.2	12.8	
losses	129	90.7	9.3	0.28
Gestational age:				
≥ 37 weeks	600	87.5	12.5	
< 37 weeks	46	93.5	6.5	0.23
Blood pressure:				
≤ 140 – 90 mm/Hg	558	87.8	12.2	
> 140/90 mm/Hg	88	88.6	11.4	0.83
Stress:				
no	523	88.5	11.5	
yes	123	85.4	14.6	0.33
Mother diseases:				
no	474	88.8	11.2	
yes	172	85.5	14.5	0.25
Body mass index (BMI):				
normal - overweight (25.1 –30)	558	87.8	12.2	
obesity (> 30)	88	88.6	11.4	0.83
Smoking before pregnancy:				
none	461	100.0	0.0	
1 – 9 cigs./d.	169	60.4	39.6	
> 9 cigs./d.	16	31.3	68.8	< 0.001
Smoking duration before pregnancy:				
non smoker	461	100.0	0.0	
1 – 5 y	122	66.4	33.6	
6 – 10 y	47	44.7	55.3	
> 10 y	16	31.3	68.7	< 0.001
GSTT1, n (%)				
GSTT1–*1*	450	84.6	73.5	0.018
GSTT1–*0*	93	15.4	26.5	
GSTM1, n (%)				
GSTM1–*1*	293	53.0	59.0	0.340
GSTM1–*0*	250	47.0	41.0	
Mean birth weight (g), ± SD	3,436 ± 24	3,445 ± 25	3,365 ± 59	0.21

The percentage of *GSTT1-0* genotype was 16.9% and that of *GSTM1* was 46.6%. In terms of the frequency of the *GSTM1–0* genotype, women in the group exposed to tobacco smoke and the group that was not exposed were similar (41.0% and 47.0%, P = 0.340), whereas the *GSTT1–0* genotype was found in 26.5% of the smokers and in 15.4% of the non-smokers (P = 0.018).

Table 12 presents the combined association of maternal cigarette smoking and maternal genotypes with LBW controlling for effect of major covariates. Without consideration of genotype, maternal smoking during pregnancy was associated with an adjusted OR of 1.21 (95% CI 0.44 – 3.31) for LBW compared with the non – smokers. When *GSTT1* genotype was considered, the association between maternal smoking and LBW increased and the adjusted OR was 2.06 (95% CI 0.67 – 6.37) among mothers with genotype present, but we could not assess the association among mothers with absent genotype because of 0 LBW cases in the smokers group.

Table 12. Crude and adjusted associations as odds ratios (OR) maternal smoking during pregnancy with low birth weight by maternal genotypes.

Genotype	Smoking status	N	LBW, %	Crude OR	Crude 95% CI	Adjusted* OR	Adjusted* 95% CI
Total sample	Never	342	8.8				
	Quitter	86	10.5	1.22	0.55–2.67	1.18	0.53–2.62
	Smoking	52	11.5	1.36	0.54–3.44	1.21	0.44–3.31
GSTT1–*1*	Never	289	9.0				
	Smoking	38	15.8	1.90	0.73–4.96	2.06	0.67–6.37
GSTM1–0	Never	53	7.5				
	Smoking	14	0				
GSTM1–*1*	Never	168	8.9				
	Smoking	31	9.7	1.09	0.30–4.0	1.11	0.26–4.76
GSTM1–0	Never	174	8.6				
	Smoking	21	14.3	1.77	0.47–6.69	1.91	0.43–8.47
[a]Interaction: smoking x GSTM1–*0*		OR 1.62 (0.25–10.4), p = 0.60; OR* 1.54 (0.25–9.91), p = 0.59					
GSTT1–*1* & GSTM1–*1*	Never	145	9.7				
	Smoking	22	13.6	1.48	0.39–5.62	1.49	0.33–6.79
GSTM1–1 & *GSTM1–0*	Never	144	8.3				
	Smoking	16	18.8	2.54	0.63 – 10.2	3.31	0.60–18.4
[b]Interaction: smoking x GSTT1–*1* & *GSTM1–0*		OR 1.72 (0.25–11.8), p = 0.58; OR* 1.45 (0.22–10.1), p = 0.66					

[a]Logistic regression model: women BMI ≤ 30, age ≥ 20 years, adjustment for maternal education and marital status.
[b]Test of interaction: a P value is presented for testing the null hypothesis, odds ratio = 1.0 in logistic regression models for the product term, smoking x genotypes.

When *GSTM1* genotypes were considered, the association between maternal smoking and LBW differed: the adjusted OR was 1.11 (95% CI 0.26–4.76) among mothers with *GSTM1–1* but adjusted OR was 1.91 (95% CI 0.43–8.47) among mothers with *GSTM1–0* genotypes. However, a test of interaction between smoking and the *GSTM1–0* genotype showed that there was no statistically significant evidence for an effect modification adjusted OR 1.54; 95% CI 0.25–9.91, p=0.59. The presence of both GSTT1 and *GSTM1* genotypes tended to increase the smoking effect by 1.49, while the *GSTM1–1* genotype and *GSTM1–0* genotype were associated with 3.31 times higher risk among smokers (OR 3.31 95% CI 0.60–18.4). A test of interaction between maternal smoking and two studied genotypes did not confer a significant adverse effect on LBW risk, adjusted OR 1.45; 95% CI 0.22–10.1, p = 0.66.

Table 13 demonstrate the influence of maternal characteristics on the birth weight of the infants as the difference in mean birth weight in relation to the maternal characteristics listed in each row.

Table 13. Influence of maternal characteristics on infants birth weight

Maternal characteristics continuous and binary	ß	SE	P-value	ß[a]	SE	P-value
BMI[b]	42.7	6.0	<0.001			
Gestational age, week[b]	191.0	9.2	<0.001			
Age < 20 or > 30 years	-34.2	56.7	0.547	-11.43	41.3	0.783
Low education, ≤ 12 years	-279.7	91.7	0.002	-155.3	67.2	0.021
Not married	-148.9	64.7	0.022	-87.1	47.2	0.066

Parity 2nd and more	49.5	55.0	0.368	90.8	39.9	0.023
Pregnancy complications	15.2	69.7	0.828	4.0	50.8	0.937
Fetus complications	-400.1	135.2	0.003	82.5	100.0	0.409
Stress	-94.7	70.7	0.181	5.4	51.6	0.916
Hypertension > 120/80 mm/Hg	189.4	76.6	0.014	87.5	59.0	0.139
Maternal smoking	-114.9	76.1	0.132	-137.0	55.2	0.013
Parental smoking	-86.9	55.0	0.115	-93.3	40.1	0.020
GSTT1–1/*GSTT1–0*	104.1	72.7	0.153	72.7	52.7	0.170
GSTM1–1/*GSTM1–0*	25.3	55.1	0.646	26.0	40.0	0.516
GSTT1–0 & smoking	-207.0	138.9	0.137	-211.8	100.8	0.036
GSTM1–0 & smoking	-60.4	113.3	0.594	-150.2	82.4	0.069
GSTT1 & *GSTM1–0* and smoking	-294.0	194.4	0.131	-340.4	141.2	0.016

ß represent the difference in mean birth weight for maternal characteristics in each row
ß[a]adjusted for body mass index, gestational age and loss outlier.
[b]Continuous variable
SE standard error of the difference between the means

The characteristics that positively affected the crude mean birth weight were increased BMI, hypertension and gestational age. Low levels of education, not married status and foetal complications were associated with reduction in the mean birth weight. In terms of the *GSTM1*- and *GSTT1*-genotype frequencies, there was no significant influence on the crude birth weight of infants. After adjustment for the BMI, gestational age and loss outliers, the maternal characteristics that affected the reduction in birth weight were as follows: low education levels (−155.3 g), maternal smoking during pregnancy (−137.0 g), parental smoking (−93.3 g) and *GSTT1−0* genotype in smokers (−211.8 g, p = 0.036). When both *GSTT1−0* and *GSTM1−0* genotypes were considered, continuous maternal smoking during pregnancy was associated with a mean reduction of 340.4 g (p = 0.016) in birth weight of infants.

Table 14 shows the crude and adjusted combined associations of continuous maternal smoking during pregnancy and maternal *GSTT1* and *GSTM1* genotypes with reference to infant birth weight, where β represents the difference in mean birth weight between each subgroup and the reference group.

After complete adjustment for gestational age, BMI, education, family status, parity and hypertension, the reduction in birth weight (analysed as a continuous variable) for continuous smokers was 83.4 g (p = 0.073). Among non-smoking mothers, the *GSTT1−0* genotype alone did not confer a significant adverse effect on birth weight (−22.6 g, p = 0.345).

Table 14. Associations of maternal smoking during pregnancy with infant birth weight by maternal GSTT1 and GSTM1 genotype

Genotype	Smoking status	Birth weight, g	Birth weight, g+ β (SE)	P	Birth weight, g++ β (SE)	P	Birth weight, g++ β (SE)	P
Total sample	Non-smoking (n = 456)	3390.3	Referent		Referent		Referent	
	Smoking (n = 83)	3284.1	-86.5 (57.5)	0.066	-83.4 (57.1)	0.073	-83.4 (57.1)	0.073
GSTT1–1	Non-smoking (n = 385)	3401.4	Referent		Referent		Referent	
GSTT1–1	Smoking (n = 61)	3320.7	-70.4 (65.7)	0.143	-72.3 (65.1)	0.134	-38.8 (57.6)	0.250
GSTT1–0	Non-smoking (n = 71)	3330.0	Referent		Referent		-22.6 (57.6)	0.345
GSTT1–0	Smoking (n = 22)	3182.9	-115.9 (129)	0.186	-123.7 (131)	0.175	-162.9 (93.0)	0.041
*Interaction: smoking × GSTT1-null			-111.9 (123.1)	0.182		-96.8 (122.5)	0.215	
GSTM1								
GSTM1–1	Non-smoking (n = 242)	3413.6	Referent		Referent		Referent	
GSTM1–1	Smoking (n = 49)	3255.9	-87.2 (73.9)	0.119	-85.1 (74.2)	0.126	-58.8 (66.1)	0.187
GSTM1–0	Non-smoking (n = 214)	3363.9	Referent		Referent		-32.4 (41.2)	0.216
GSTM1–0	Smoking (n = 34)	3324.8	-97.1 (92.0)	0.146	-100.6 (90.2)	0.133	-118.7 (79.6)	0.069
*Interaction: smoking ×GSTM1-null			-17.0 (106.1)	0.437		-26.6 (105.6)	0.400	
GSTT1 & GSTM1								
GSTM1–1	Non-smoking (n = 207)	3429.4	Referent		Referent		Referent	
GSTM1–1	Smoking (n = 38)	3251.2	-137.9 (82.3)	0.048	-135.5 (82.6)	0.051	-84.7 (71.2)	0.118
GSTM1–0	Non-smoking (n = 36)	3339.7	Referent		Referent		10.1 (76.1)	0.447
GSTM1–0	Smoking (n = 11)	3093.5	-318.0 (198)	0.058	-320.8 (203)	0.061	-311.2 (128)	0.008
*Interaction: smoking ×GSTT1-null ×GSTM1-null			- 240.3 (164)	0.072		-234.5 (164.3)	0.078	

ß represent the difference in mean birth weight for cigarette smoking between the variant genotype

+ ß crude

++ ß after adjustment for the covariates: gestational age, body mass index, education, family status, parity and hypertension

*Test of interaction: a P value is presented for testing the null hypothesis, ß = 0 in multiple linear regression models for the product term, smoking x genotypes

Continuous maternal smoking was associated with a mean reduction of 162.9 g, p = 0.041 in birth weight for the *GSTT1−0* genotype. Maternal smoking was associated with a mean reduction of 58.8 g in birth weight for the *GSTM1−1* and 118.7 g for the *GSTM1−0* genotypes; nevertheless, there was no statistically significant difference. We found that the frequency of carriers both *GSTT1−0* and *GSTM1−0* genotypes was 8.7% in the total population studied. Double-null genotypes (*GSTT1−0* and *GSTM1−0*) among smokers showed a synergistic effect and was associated with a 311.2 g reduction in mean birth weight (p = 0.008). However, a test of the interaction between maternal smoking, *GSTT1* and *GSTM1* genotypes does not show a statistically significant reduction in birth weight (−234.5 g, p = 0.078).

DISCUSSION

Trihalomethanes exposure and GSTM1 and GSTT1 polymorphisms effect on adverse pregnancy outcomes

Our study contribute to the findings of researchers who studied genetic polymorphism along with environmental toxicants (Nukui et al., 2004; Suh et al., 2008) in showing effect modification between mothers with and without *GSTT1*- and *GSTM1*-genotype variant and response to the environmental hazards. The results of this study suggest that prenatal THM exposure, at the elevated internal dose levels of THM, have a slight impact on LBW or SGA risk and that the polymorphisms of metabolic gene *GSTM1* affect the association of maternal exposure to THM with LBW and SGA risk. When we considered both individual THM exposure and maternal genotypes, we were able to demonstrate a consistent, statistically significant effect on LBW associated with TTHM, chloroform and BDCM as compared to unexposed women. The largest associations for LBW were found for third trimester among TTHM and chloroform exposed women with the *GSTM1–0* genotype (OR 4.37, 95% CI, 1.36–14.08 and OR 5.06, 95% CI 1.50–17.05, respectively). The results were more evident when the interaction of genotype and THM exposure were examined. The adjusted ORs for TTHM were 15.86, 95% CI 1.36–14.08; for chloroform ORs were 5.06, 95% CI 1.50–17.05; and for BDCM it were 5.29, 95% CI 1.03–27.15. We find an insignificant elevated risk for SGA for those exposed to TTHMs during the three trimesters with highest ORs during the third trimester among women carriers of *GSTM1–0* genotype (OR 1.81, 95% CI 0.92–3.56) and *GSTT1–0* genotype (OR 1.51, 95% CI 0.43–5.29). These data suggest that women with an absence of the genotype activity appear to be susceptible to the adverse effects of THMs, such as increased risk of LBW.

At present, no other published study has evaluated risk for LBW, SGA or PB and THM constituents as individual internal dose in association with *GSTT1* and *GSTM1* genotypes polymorphism.

Our results are consistent with previous studies, which suggested that exposure to THMs in the third trimester has a greater adverse effect on LBW and SGA than exposure early in pregnancy (Hoffman et al., 2008). Some authors presented that term LBW risk mostly increase during the second trimester (Lewis et al., 2006) (OR 1.50, 95% CI 1.07 to 2.10). Our results show that highest SGA risk associated with TTHM exposure was found during the third trimester (OR 1.33, 95% CI 0.84 to 2.13), while Wright et al (Wright et al., 2003) found an increased risk of SGA for second trimester (OR 1.13; 95% CI 1.03 to 1.24). Some studies results provide no evidence of an increased risk of LBW, TLBW, and preterm delivery at the relatively low concentration

of TTHMs (Jaakkola et al., 2001; Yang et al., 2007). Data of Sweden study shows that exposure to sodium hypochlorite increase LBW (OR 1.15, 95% CI 1.05 to 1.26) (Kallen and Robert 2000). Kramer et al (1992) concluded that chloroform concentrations greater than or equal to 10 micrograms/litter were associated with the increased risk for intrauterine growth retardation (OR 1.8, 95% CI 1.1 to 2.9).

Relative to previous epidemiologic studies of this issue, this study has the advantage of seeking to overcome the exposure assessment drawbacks by using individual internal dose estimation based on different routes, detailed water use behaviours, studying individual THMs, to examine relationships between the exposure and fetal growth in genetically susceptible women. The major strength of our study is the concurrent measurement of THM concentrations that we used for internal dose estimation, over the course of pregnancy. As a result, the assignment of trimester average residential THM concentrations and estimation of individual THM uptake through drinking, showering and bathing should be more accurate than in previous studies. Another advantage of this study was the extensive control for confounding variables estimated for studying population. We estimated the association between THM internal dose levels and LBW controlling for gestational age, family status, gestational age, education, maternal and paternal smoking, alcohol consumption, body mass index, blood pressure, ethnic group, pregnancy history, infant gender, and birth year, for which we were able to adjust. The lack of information regarding the validity of the internal dose assessment models that we used is one of the limitations of this study.

However, not all women exposed to THM during pregnancy have adverse reproductive outcomes, and several studies have suggested that potential reasons lie in genotoxicity, oxidative stress, disruption of foliate metabolism (Nieuwenhuijsen et al., 2009), and maternal genetic susceptibility (Wang et al., 2002; Perera et al., 2004; Chen et al., 2005).

Adjusted ORs for preterm birth among women exposed to TTHM above median internal dose during the second trimester pregnancy was 1.03 (95% CI 0.52–2.06) and 2.07 (95% CI 1.00–4.35) for the present and absent *GSTM1* genotype, respectively. The findings suggest that carriers of the *GSTT1–0* genotype and exposed to TTHM, chloroform and bromodichlormethane had an increased risk for preterm birth compared to carriers of the *GSTT1–1* genotype: the ORs during the second trimester among woman *GSTT1–1* genotype carriers were 1.03–1.17, while among *GSTT1–0* genotype carriers ORs were 2.46–3.08. Our data indicated that individuals with *GSTT1–0* and *GSTM1–0* genotypes tended to be more susceptible to THM exposure.

The single study which analyzed drinking water contaminants, fetal growth and *CYP2E1* genetic polymorphisms, was conducted in Canada (Infante–Rivard, 2004). The adjusted odds ratio for intrauterine growth restriction associated with exposure to average TTHM above the 29.4 μg/L was 13.20 (95% CI 1.19–146.72). These findings suggest that exposure to THM at the highest levels can affect fetal growth in genetically susceptible newborns.

The metabolism of environmental toxicants includes the several allelic variants of the polymorphic GST group which shows impaired enzyme activities and increase the susceptibility to both environmental xenobiotics and adverse birth outcomes (Hayes et al., 2000; Infante-Rivard et al., 2006). *GSTM1* polymorphism is found to be present in 40 to 60% of most populations. Among Kaunas pregnant women *GSTM1–0* genotype is present in 47% subjects. The deficiency of *GSTM1* has been shown to increase DNA-adduct formation and cytogenic damage (Nukui et al., 2004). The frequency of the

GSTT1–0 allele was reported to be 30 to 40% in Germany (Peter et al., 1989), in Lithuania is 17.0%. It is possible that GST induction represents part of an adaptive response mechanism to chemical stress (Hayes et al., 2000), therefore genetic polymorphism of *GSTM1* and *GSTT1* may modify the oxidative stress caused by maternal exposure to THM and lead to adverse pregnancy outcomes.

While interpreting the results of this study, a few conditions should be considered. This is a low-risk population with low-level THM exposure and low prevalence of *GSTT1–0* genotype; these factors may limit the extrapolation of these results to other populations. The THM exposure classification was based on median internal dose level, and consecuently the possibility of bias in exposure classification exists. However, in this study, we controlled for the main variables that might confound the association between THM, genetic polymorphism and fetal growth; therefore, the residual confounding of the results by exposure is expected to be small.

Previous studies have suggested several plausible gene-environment interaction explanations. First, chemical substances could disturb fetal and placental cellular regulation via elevated PAH-DNA adducts due to the increased activity of enzymes that metabolize toxins (e.g. *CYP1A1*) and lower or absent activity of enzymes that detoxify these compounds (e.g. *GSTT1* and *GSTM1–0* genotypes) (Sasaki et al., 2008). Second, gene-xenobiotic interactions may exert their synergistic effects through oxidative stress that occurs upon chemical exposure. In response to this stress various inflammatory cytokines are produced in lung tissue increasing inflammatory responses and immune responses (Tsai et al., 2008). Finaly, other environmental factors and genetic polymorphism of *GSTM1* and *GSTT1* may modify the response to oxidative stress and lead to adverse pregnancy outcomes (Sable et al., 2008).

Since there has been only a single epidemiological study that included the association between GST genes polymorphism, human susceptibility to THMs and adverse birth outcomes, further study is required to clarify the role of the GST polymorphism in fetal development.

Exposure to tobacco smoke effect on newborn birth weight in relation to maternal GSTT1–0 and GSTM1–0 genotypes

In this molecular epidemiological study on maternal cigarette smoking and genetic determinants of xenobiotic metabolism, we found some evidence that the effects of maternal smoking on LBW and infant birth weight were modified by the maternal *GSTT1* and *GSTM1* genotypes.

Consistent with previous studies, we found that maternal cigarette smoking was associated with increased risk of LBW risk (Pollack et al., 2000; Wang et al., 2002). Our findings are consistent with a number of other studies that LBW risk may vary in relation to maternal age, BMI, parity, and other variables of the population in the study (Agresti et al., 1979; Luke et al., 2007; Oberg et al., 2007). Some other investigators who have examined the issue revealed dose-response gradients in relation to the amount smoked (Windham et al., 2000; Savitz et al., 2001).

Our present findings show the greater LBW risk among light-smoking mothers with *GSTM1–0* genotype as compared to those with *GSTM1–1* genotype, however the findings do not show statistically significant results. These results are consistent with previous studies which analysed genetic susceptibility to cigarette smoke in the context of LBW risk.

Wang et al. reported that pregnant women with certain genotypes are susceptible to the adverse pregnancy effects of tobacco smoking, such as an increased risk of LBW (Wang et al., 2002). Without consideration of genotype, maternal smoking during pregnancy was associated with reduction in birth weight and elevated risk of LBW. When *GSTT1* genotype was considered, the reduction in birth weight increased and 1.7 (0.9 – 3.2) - fold elevated risk of LBW for those with the genotype present, and 3.5 (1.5 – 8.3) - fold elevated risk of LBW for *GSTT1* genotype absent was found among smoking mothers.

It has been reported that an individual difference in metabolic activation and detoxification xenobiotics partly depends on the genetic polymorphisms associated with *GSTT1* and *GSTM1* enzymes (Savitz et al., 2001). The interactive effect of exposure to tobacco smoke and the presence of the *GSTT1* polymorphism on infant birth weight was found to be significant by multivariate analysis, whereas the interactive effect of the presence the *GSTM1* polymorphism did not reach statistical significance ($p = 0.21$) (Infante-Rivard et al., 2006).

Sasaki *et al.* also reported combined effects between maternal genetic polymorphisms and smoking during pregnancy (Sasaki et al. 2006). The effects on reduction birth weight were not observed among women with *GSTM1–0* genotype who had never smoked. The authors conclude that maternal smoking in combination with maternal genetic susceptibility may adversely affect infant birth weight. However, results presented here do not show a statistically significant association between infant birth size and maternal smoking as linked to the *GSTT1* genotype, while birth weight and length were significantly lower in subjects with *GSTM1–0* genotype.

Sram *et al.* found that the risk of LBW and prematurity was significantly increased by the genotypes of *GSTM1–0* and a genotype combination with the *CYP1A1*2A* genotype (Sram et al., 2006). A survey among pregnant women has showed that a combination of the *GSTM1–0* and the *GSTT1–0* genotypes exacerbate the effect of maternal exposure to tobacco smoke on birth weight more than the presence of either genotype one (Hong et al., 2003).

In this study, we demonstrate that there is increase in LBW risk among smoking women even after adjusting for maternal age, education, BMI, and marital status; however, these findings suggest that there was no statistically significant association between the *GSTT1* and *GSTM1* polymorphism with low-level maternal smoking during pregnancy. The reason may be that the size of our nested case-control study and the proportion of women who smoked during pregnancy were too small to detect any significant difference.

Consistent with previous studies, we found that the effect of tobacco smoke increased LBW risk in the women's group with combination of *GSTT1–1* and *GSTM1–0* alleles was more than 3 times greater as compared with the non-smokers group (OR 3.31; 95% CI 0.6 – 18.4).

The association between metabolic GST genes and maternal smoking has shown (Kishi et al., 2008). When the *GSTT1* genotype is considered in smoking pregnant women, the reduction in birth weight among the *GSTT1–1* and *GSTT1–0* groups was as follows: 43 g ($p = 0.48$) (Sasaki et al., 2006), 222 g ($p < 0.05$) (Sram et al., 2006) and 642 g ($p < 0.001$) (Wang et al., 2002). When the GSTM1 genotype is considered, the estimated reduction in birth weight between *GSTM1–1* and *GSTM1–0* groups is 171 g ($p = 0.04$) (Sasaki et al., 2006) and 222 g ($p < 0.05$) (Sram et al., 2006), respectively. The effects on the reduction of birth weight are not observed among women with

GSTM1–0 or *GSTT1–0* genotypes who had never smoked and the data have been adjusted to the main confounding factors. A combination of the *GSTM1–0* and the *GSTT1–0* genotypes has been found to exacerbate the effect of maternal exposure to environmental tobacco-smoking on the birth weight of infants more than the presence of either genotype alone. These data indicate that the effect can be modified by the maternal metabolic genotypes, *GSTM1* and *GSTT1* (Hong et al., 2003).

We can postulate that the significant differences among the publicised studies, which are devoted to the effects of tobacco-smoke exposure on birth weight, could be attributed to the diverse ethnic composition of the populations considered in the studies, resulting in different distributions of the GST allelic frequency and different levels of cigarette-smoke exposure, because dose–response gradients in relation to the number of cigarettes smoked do exist (Nukui et al., 2004). Furthermore, these results may be affected by the residual uncontrolled confounding variables, such as prepregnancy BMI, hypertension, gestational age and others, which are negatively or positively associated with birth weight.

The main factors influencing birth-weight reduction are gestational age and the organism's response to toxicity from environmental xenobiotics, such as tobacco PAHs. Birth-weight reduction may be a consequence of DNA damage resulting from the activation of metabolites or the absence of detoxification of the reactive intermediates formed in the system due to the reduced activities of *GSTT1* or *GSTM1* (Perera et al., 2004). As reported by some authors, maternal exposure to tobacco smoke induces oxidative stress. Furthermore, maternal genetic polymorphisms related to *GSTM1* and *GSTT1* may modify the oxidative stress caused by maternal exposure to tobacco smoke and lead to adverse pregnancy outcomes (Hudson et al., 2009)

Previous studies have suggested several plausible gene-smoking interaction explanations. First, tobacco smoke could disturb fetal and placental cellular regulation via elevated PAH-DNA adducts due to the increased activity of enzymes that metabolize cigarette toxins (e.g. *CYP1A1*) and lower or absent activity of enzymes that detoxify these compounds (e.g. *GSTT1–0* and *GSTM1–0* genotypes) (Sasaki et a., 2008). Second, gene-smoking interactions may exert their synergistic effects through oxidative stress that occurs upon tobacco smoke exposure. In response to this stress, various inflammatory cytokines are produced in lung tissue increasing inflammatory responses and immune responses (Tsai et al., 2008). Moreover, as reported by some authors, maternal exposure to tobacco smoke affects the fetal urine cotinine concentration and also induces production of oxidative stress (Park et al., 2008). Furthemore, other environmental factors and genetic polymorphism of *GSTM1* and *GSTT1* may modify the response to oxidative stress and lead to adverse pregnancy outcomes (Sable et al., 2000).

This study has the advantage of being the first to show that even light maternal smoking, in association with double-null *GSTT1* and *GSTM1* genotypes, might significantly decrease the infant birth weight. In this study, we estimated that among Lithuanian women the percentage of *GSTT1–0* genotype was 17.0% and that of *GSTM1–0* was 46.0%. The carriers of double-null genotypes were 8.7% of the total population studied.

Our results show a statistically significant association between the reduction in birth weight and maternal smoking when linked to the single polymorphism of *GSTT1* after adjusting for gestational age, education, BMI, and marital status. Among non-smoking mothers, genotype alone did not confer any significant adverse effect on the

birth weight. However, tests of interaction do not show a statistically significant effect. This might be due to the relatively low frequency of *GSTT1–0* genotype in the Lithuanian population and low-level exposure to tobacco smoke (mean number of cigarettes smoked per day was 4.8); under such conditions, the moderate size of our epidemiological study was too small to detect any significant interaction effect. The estimated effect of smoking tended to be higher among mothers with the *GSTT1–0* genotype as compared to the *GSTM1–0* genotype: continuous maternal smoking was associated with a mean reduction of 162.9 g in birth weight for the *GSTT1–0* genotype and a mean reduction of 118.7 g for the *GSTM1–0* genotype. We found that the effect of tobacco smoking was significantly higher in the group of women with a combination of *GSTT1–* and *GSTM1–0* alleles. The double-null genotype for both *GSTT1* and *GSTM1* among light smokers showed a synergistic effect and the combination of these genes was associated with a 311.2-g reduction in birth weight ($p = 0.008$). These data present evidence that subjects with the double-null genotype for both *GSTT1* and *GSTM1* have a greater risk of being affected by toxic tobacco smoke and should hence be treated as an increased susceptibility group for adverse pregnancy outcomes.

While interpreting the results of this study, a few conditions should be considered. This is a low-risk population with low-level tobacco smoke exposure and low prevalence of *GSTT1–0* genotype; these factors may limit the extrapolation of these results to other populations. The evaluation of exposure to tobacco smoke was indirect; we used self-reported information on smoking during and before pregnancy, and thus the possibility of bias in both reporting and exposure classification exists. However, in this study, we controlled for the main variables that might confound the association between maternal smoking, genetic polymorphism and birth weight; therefore, the residual confounding of the results by smoking is expected to be small.

Our findings provide additional insight into the biological determinants of response to environmental exposure based on the combination of genes and individual characteristics to a previously unstudied ethnic group of Lithuanians. We have shown that tobacco smoke exposure, even at a low-level, is associated with the reduction in birth weight of infants. Such an association, however, is modified by the genotype of an individual. We provide coherent evidence proving that smoking women carrying two null genotypes of *GSTT1* and *GSTM1* are at a significantly increased risk of infant birth-weight reduction. Our data also show that identification of a susceptible-subject group should be based on both environmental exposure and gene polymorphism.

CONCLUSIONS

1. Among Lithuanian women the percentage of *GSTT1–0* genotype was 17% and of *GSTM1–0* genotype was 47.0%.

2. During pregnancy the individual total uptake of TTHMs from drinking water ranged between 0.0025 and 2.40 (median 0.1733) μg/d. The total chloroform uptake ranged between 0.0013 and 2.13 (median 0.1424) μg/d. Daily uptake of bromodichlormethane ranged between 0.0001 and 0.34 (median 0.0280) μg/d and dibromochlormethane ranged between 0 and 0.064 (median 0.0026) μg/d.

3. The risk for LBW is smaller for woman carriers *GSTM1–1* and *GSTT1–1* genotypes as compare to *GSTM1–0* and *GSTT1–0* genotypes carriers. The highest THM and chloroform impact on LBW was during third trimester of pregnancy: OR 1.33, 95% CI 0.62–2.87 and OR 1.45 (0.67–3.13), respectively. The woman carriers *GSTM1–0* genotype OR 4.37 (1.36–14.08) and 5.06 (1.50–17.05), respectively and carriers *GSTT1–0* genotype OR 7.30 (0.14–391) and 7.30 (0.14–391).

4. The risk for PB is smaller for woman carriers *GSTM1–1* and *GSTT1–1* genotypes. The highest THM and chloroform impact on LBW was during second trimester of pregnancy: OR 1.33 (0.82–2.17) and OR 1.31 (0.80–2.13), respectively. The woman carriers *GSTM1–0* genotype OR 2.07 (1.00–4.35) and 1.97 (0.94–4.15), while carriers *GSTT1–0* genotype OR 2.46 (0.80–7.68) and 2.66 (0.85–8.29).

5. The highest THM and chloroform impact on SGA was during third trimestre of pregnancy: OR 1.38 (0.88–2.18) and 1.38 (0.87–2.16), respectively. The woman carriers *GSTM1–0* genotype OR 1.91 (1.00–3.66) and 1.85 (0.97–3.53) and carriers *GSTT1–0* genotype OR 1.42 (0.41–5.00) and 1.67 (0.47–5.95).

6. The major effects of maternal smoking on adverse pregnancy outcomes are for woman carriers *GSTM1–0* genotype: 5 and more cigarettes per day slightly increase the risk of LBW, OR 1.11, 95% CI 0.26 –4.47. The woman carriers *GSTM1–1* genotype OR 1.91 (0.43–8.47); a mean reduction birth weight was 118.7 g, while among smoking woman carriers *GSTT1–0* genotype a mean reduction birth weight was 162,9 g. *GSTM1–0* and *GSTT1–0* genotypes interaction resulted in reduction birth weight to 234.5 ($p = 0.078$); *GSTT1–1* and *GSTM1–0* genotypes interaction reduced birth weight by 311,2 g ($p = 0,008$).

7. The effects of maternal smoking and THM exposure on adverse pregnancy outcomes were modified by the maternal *GSTT1* and *GSTM1* gene polymorphism. The LBW, SGA and PB risk increase among woman carriers *GSTM1–0* and *GSTT1–0* genotypes.

LIST OF PUBLICATIONS

Publications which are assessed by the Institute for Scientific Information database ISI Web of Science, and with a citation index

1. Gražulevičienė, R., Nieuwenhuijsen, MJ, Venclovienė, J., Kostopoulou-Karadanelli, M., Krasner, SW., Danilevičiutė, A., Balčius, G., Kapustinskienė V. 2011. Individual exposures to drinking water trihalomethanes, low birth weight and small for gestational age risk: a prospective Kaunas cohort study. Environmental health 10 (32):1-11.
2. Gražulevičienė, R., Nieuwenhuijsen, MJ, Danilevičiutė, A., Nadišauskienė, R., Buinauskienė, J. 2010. Gene - environment interaction maternal smoking and contribution of GSTT1 and GSTM1 polymorphisms to infant birth-weight reduction in a Kaunas cohort study. Journal of epidemiology and community health. 64(7):648-648.

Other publications which are assessed by the Institute for Scientific Information database ISI Web of Science

1. Gražulevičienė, R., Danilevičiutė, A., Nadišauskienė, R., Venclovienė, J. 2009. Maternal Smoking, GSTM1 and GSTT1 Polymorphism and Susceptibility to Adverse Pregnancy Outcomes. International Journal of Environmental Research and Public Health. 6(3):1282-1297.

In conference proceedings and other publications

1. Danilevičiutė, Asta; Gražulevičienė, Regina. Geriamojo vandens taršos trihalometanais įtaka per anksti gimusių naujagimių rizikai, esant motinos genetiniam jautrumui // Žmogaus ir gamtos sauga : 17-osios tarptautinės mokslinės-praktinės konferencijos medžiaga, 2011 m. gegužės 11-13 d, birželio 16-18 d, Kaunas = Human and nature safety : proceedings of the international scientific conference. Kauno raj. : Akademija. ISSN 1822-1823. 2011, D. 1, p. 91-94.
2. Danilevičiutė, Asta; Gražulevičienė, Regina. Genetinio jautrumo ir tabako dūmų įtaka nepalankioms nėštumo baigtims // Žmogaus ir gamtos sauga : 15-osios tarptautinės mokslinės-praktinės konferencijos medžiaga, 2009 m. gegužės 13-15 d, birželio 11-12 d, Kaunas = Human and nature safety : proceedings of the international scientific conference. Kauno raj. : Akademija, 2009. ISSN 1822-1823. D. 1, p. 123-126.
3. Kogevinas, Manolis; Patelarou, Evridiki; Villanueva, Cristina M.; Bustamante, Mariona; Cordier, Sylvaine; Gražulevičienė, Regina; Smith, Rachel B.; Stephanou, Evridips G.; Kargaki, Sophia; Gracia, Esther; Coster, Nathalie; Danilevičiutė, Asta; Toledano, Mireille; Nieuwenhuijsen, Mark J.. Disinfection Byproducts, Polymorphism, and Susceptibility to Adverse Pregnancy Outcomes // Epidemiology. Baltimore : Lippincott Williams & Wilkins. ISSN 1044-3983. 2011, Vol. 22, no. 1, suppl, p. 67-68.
4. Gražulevičienė, Regina; Danilevičiutė, Asta; Venclovienė, Jonė; Balčius, Gediminas; Gražulevičius, Tomas. Exposure to drinking water trihalomethanes through different routes and low birth weight risk in genetically susceptible women // Epidemiology. Baltimore : Lippincott Williams & Wilkins. ISSN 1044-3983. 2011, Vol. 22, no. 1, suppl, p. 168-168. Prieiga per internetą:

<http://journals.lww.com/epidem/Fulltext/2011/01001/Exposure_to_Drinking_Water_Trihalomethanes_Through.498.aspx#>.
5. Gražulevičienė, Regina; Dėdelė, Audrius; Danilevičiutė, Asta; Gražulevičius, Tomas. The association between environmental exposure to nitrogen dioxide and preterm birth in genetically susceptible women // Towards improved coordination of birth cohort research in Europe, Barcelona, 11-12 April 2011 : workshop. [Barcelona: CHICOS project], 2011. p. 23-23. Prieiga per internetą: <http://www.chicosproject.eu/assets/86/Delegate_Booklet_CHICOS_April2011_final.pdf>.
6. Gražulevičienė, Regina; Danilevičiutė, Asta. Maternal Smoking, GSTT1 and GSTM1 Polymorphisms and Infant Birth-Weight Reduction in a Kaunas Cohort Study // Epidemiology. [ISEE 21st Annual Conference, Dublin, Ireland, August 25-29, 2009: symposium abstracts]. Hagerstown, MD : Lippincott Williams & Wilkins. ISSN 1531-5487. Vol. 20, iss. 6, suppl. (2009), p. 108-108.
7. Gražulevičienė, Regina; Danilevičiutė, Asta; Nadišauskienė, Rūta; Venclovienė, Jonė; Buinauskienė, Jūratė; Gražulevičius, Tomas. Trihalomethanes exposure through drinking water and low birth weight risk in Kaunas cohort // Epidemiology. [ISEE 21st Annual Conference, Dublin, Ireland, August 25-29, 2009: symposium abstracts]. Hagerstown, MD : Lippincott Williams & Wilkins. ISSN 1531-5487. Vol. 20, iss. 6, suppl. (2009), p. 105-105. Prieiga per internetą: <https://isee.conference-services.net/programme.asp?conferenceID=1651&action=prog_presenters>.

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REZIUMĖ

Nepalankios nėštumo baigtys kelia didelį visuomenės sveikatos specialistų rūpestį dėl didelio jų paplitimo, didelio neišnešiotų naujagimių ir mažos kūno masės naujagimių sergamumo ir mirtingumo, o kenksmingi aplinkos veiksniai yra laikomi potencialiais nepalankių nėštumo baigčių rizikos veiksniais. Pagrindinės nepalankių nėštumo baigčių priežastys nėra gerai žinomos, tačiau daugėja įrodymų, kad didelės įtakos gali turėti aplinka (Bove et al., 2002; Aggazzotti et al., 2004; Gehring et al., 2011).

Vaisiaus raida priklauso nuo daugelio tarpusavyje susijusių veiksnių, įskaitant genetinius, epigenetinius ir aplinkos rizikos veiksnius (Windham et al., 2000; Plunkett & Muglia, 2008). Tyrimai, kurie nagrinėja įvairių aplinkos veiksnių poveikį ir nėščių moterų individualius genetinius ypatumus, gali išaiškinti padidinto jautrumo kenksmingiems aplinkos veiksniams asmenis ir paaiškinti, kodėl skiriasi nepalankių nėštumo baigčių rizika, esant vienodo dydžio ekspozicijai (kenksmingo aplinkos veiksnio poveikiui) (Rothman et al., 2001). Be to, geresnis patologinių mechanizmų suvokimas sudaro tikslinių intervencijų, skirtų nepalankių nėštumo baigčių profilaktikai, pagrindą.

Eksperimentiniai ir epidemiologiniai tyrimai pateikė duomenų, kad daugelis geriamojo vandens dezinfekcijos pašalinių produktų (DPP), įskaitant trihalometanus (THM), gali būti susiję su nepalankia vaisiaus raida. Epidemiologinių tyrimų duomenimis, nėščioms moterims, vartojusioms geriamąjį vandenį, kuriame yra padidėjusi THM koncentracija, gali sutrikti vaisiaus augimas, tačiau tyrimų rezultatai yra nevienareikšmiai (Nieuwenhuijsen et al., 2000; Graves et al., 2001; Bove et al., 2002; Grazuleviciene et al., 2011).

Daugelis tyrėjų nustatė, kad tabako rūkymas nėštumo metu yra susijęs su naujagimių svorio sumažėjimu ar kitomis nepalankiomis nėštumo baigtimis (Windham et al., 2000; Savitz et al., 2001; Sasaki et al., 2008). Tačiau ne visos moterys, kurios rūkė nėštumo metu, susilaukė mažos kūno masės naujagimio. Skirtingos rūkymo pasekmės priežastys nėra gerai žinomos. tačiau tai gali būti susiję su motinos genetiniu jautrumu, kadangi genai, dalyvaujantys cheminių medžiagų detoksikacijos procese, gali turėti įtakos nepalankioms nėštumo baigtimis (Wang et al., 2000, 2002, Nukui et al., 2000, Infante-Rivard, 2004).

Žmogaus organizmo detoksikacijos procese glutationo S–transferazė (GST) katalizuoja glutationo junginius su toksinėmis medžiagomis, kurie gali būti pašalinti iš organizmo (Raijmakers et al., 2001; Infante–Rivard, 2004). Molekulinėje biologijoje polimorfinė GST apibūdinama kaip *teta* klasės fermentas (GSTT1), o "sujungiantis" ir "nesujungiantis" fenotipai atitinka esančio aktyvaus (*GSTT1–1*) ir nesančio (*GSTT1–0*) geno aktyvumą, kurie gali padidintį individualų jautrumą toksiškų medžiagų poveikiui (Infante–Rivard et al., 2002; Thier et al., 2003). Aplinkos epidemiologiniuose tyrimuose pasitelkus molekulinės epidemiologijos metodus galima nustatyti padidinto jautrumo žmonių grupes ir paaiškinti individualaus atsako skirtumus į tą patį aplinkos veiksnį.

Iki šiol nėra aišku, kokios THM sudarančios medžiagos ir kokios jų dozės įtakoja žmogaus vaisiaus vystymąsi. Šis tyrimas teikia naujų duomenų apie organizmo atsaką į aplinkos teršalų ekspoziciją esant genetiniam polimorfizmui iki šiol netyrinėtoje Lietuvos moterų populiacijoje.

Šio darbo tikslas - nustatyti geriamojo vandens dezinfekcijos pašalinių produktų trihalometanų (THM) ir tabako dūmų poveikį nepalankioms nėštumo baigtims, esant glutationo *S*-transferazės (GST) genetiniam polimorfizmui.

Tiriamos hipotezės: metabolinių genų *GSTT1* ir *GSTM1* polimorfizmo ir trihalometanų ekspozicijos sąveika turi įtakos nepalankioms nėštumo baigtims; tabako rūkymas, esant genetiniam jautrumui, turi įtakos vaisiaus raidai.

Hipotezėms patikrinti, Kaune buvo atliktas lizdinis atvejo-kontrolės tyrimas, kuris yra Europos Sąjungos 6-sios Bendrosios Programos projekto HiWATE dalis. Tyrimo objektu buvo 889 nėščios moterys, kurios buvo apklaustos naudojant klausimynus. Naudojant duomenis apie motinos vandens vartojimo įpročius ir THM patekimo į kraują koeficientus, buvo askaičiuota motinų gauta THM vidinė dozė. Ryšiui tarp vidinės THM dozės ir rūkymo nėštumo metu, esant motinos genetiniam jautrumui, ir nepalankių nėštumo baigčių nustatyti buvo naudojama daugiaveiksnė logistinė regresija ir kontroliuojami ryšį iškreipiantieji veiksniai.

Nustatyta, kad tarp Lietuvos 19–45m. amžiaus moterų *GSTT1–0* genotipo paplitimas sudaro 17 %, o *GSTM1–0* genotipo – 47 %. Vidutinė individuali moterų ekspozicija THM viso nėštumo metu buvo: THM 0,0025–2,40 (mediana 0.1733), chloroformo 0,0013–2,13 (mediana 0,1424), BDCM 0,0001–0,34 µg/d (mediana 0,0280), o DBCM 0–0,064 (mediana 0,0026) µg/d.

Didžiausias THM ir chloroformo poveikis mažos kūno masės naujagimių rizikai buvo III–me nėštumo trimestre: GS 1.33, 95% PI 0.62–2.87 ir GS 1.45, 95% PI 0.67–3.13, atatinkamai. Mažos kūno masės naujagimių rizika yra mažesnė moterims, turinčioms *GSTM1–1* ir *GSTT1–1* genotipus. Turinčioms *GSTM1–0* genotipą, GS 4,37, 95% PI 1,36–14,08 ir GS 5,06; 95% PI 1,50–17,05. Turinčioms *GSTT1–0* genotipą, GS 7,30; 95% PI 0,14–391 ir GS 7,30; 95% PI 0,14–391.

Didžiausias THM ir chloroformo poveikis neišnešiotų naujagimių rizikai buvo II–me nėštumo trimestre: GS 1,33; 95% PI 0,82–2,17 ir GS 1,31; 95% PI 0,80–2,13, atatinkamai. Neišnešiotų naujagimių rizika yra mažesnė moterims, turinčioms GSTM1–1 ir GSTT1–1 genotipus. Turinčioms *GSTM1–0* genotipą, GS 2,07; 95% PI 1,00–4,35 ir GS 1,97; 95% PI 0,94–4,15. Turinčioms *GSTT1–0* genotipą, GS 2,46; 95 % PI % 0,80–7,68 GS 2,66; 95% PI 0,85–8,29.

Didžiausias THM ir chloroformo poveikis mažiems pagal gestacijos amžių naujagimių rizikai buvo III–me nėštumo trimestre: GS 1,38; PI 0,88–2,18 ir 1,38; PI 0,87–2,16, atatinkamai. Mažų pagal gestacijos amžių naujagimių rizika yra mažesnė moterims, turinčioms *GSTM1–1* ir *GSTT1–1* genotipus. Turinčioms *GSTM1–0* genotipą, GS 1,91; 95 % PI 1,00–3,66 ir GS 1,85; 95 % PI 0,97–3,53. Turinčioms *GSTT1–0* genotipą, GS 1,42; 95% PI 0,41-5,00 ir GS 1,67; 95 % PI 0,47–5,95.

Tabako dūmų poveikis nepalankioms nėštumo baigtims yra didesnis rūkančioms moterims, turinčioms *GSTM1–0* genotipą: 5 ir daugiau cigarečių per dieną didina mažos kūno masės naujagimių riziką, GS 1,11; 95% PI 0,26 –4,47. Turinčioms *GSTM1–1* genotipą, GS 1,91; 95% PI 0,43 –8,47, lyginant su turinčiomis GSTM1–0 genotipą; vidutinis naujagimių gimimo svoris sumažėja 118,7 g. Turinčioms *GSTT1–0* genotipą, rūkančių moterų vidutinis naujagimių gimimo svoris sumažėja 162,9 g, o turinčioms *GSTM1–0* ir *GSTT1–0* genotipus, tarpusavio sąveikos pasekoje gimimo svoris sumažėja 234,5 g (p=0,078). Turinčioms *GSTT1–1* genotipą ir *GSTM1–0* genotipą, gimimo svoris sumažėja 311,2 g (p = 0,008).

Tabako ir trihalometanų poveikio dydį nepalankioms nėštumo baigtims modifikuoja motinos *GSTT1* ir *GSTM1* genų polimorfizmas. Moterims, turinčioms

GSTM1–0 ar *GSTT1-0* genotipus, didėja naujagimių mažos kūno masės, per anksti gimusių (neišnešiotų) ir mažų pagal gestacijos amžių naujagimių rizika.

Mokslinis naujumas ir praktinė svarba. Tyrimo metu buvo nustatytas *GSTT1–0* ir *GSTM1–0* genotipų, turinčių įtakos individualaus atsako į aplinkos teršalus, paplitimas Lietuvos moterų populiacijoje. Pirmą kartą Lietuvoje nustatyta THM ir tabako dūmų individualios ekspozicijos ir Glutatione S-transferazės T1 ir M1 genų polimorfizmo įtaka nepalankioms nštumo baigtims. Be to, nustėme, kad egzistuoja ryšys tarp nėštumo metu gautos THM vidinės dozės, *GSTT1–0* ir *GSTM1–0* genotipų ir mažos naujagimių kūno masės rizikos, kuris pagrindžia, kad genetiniai ir aplikos veiksniai turi įtakos nepalankioms nėštumo baigtims. Todėl *GSTM1* ir *GSTT1* genus, dalyvaujantčius metabolitų detoksikacijos procese, reikia vertinti kaip potencialius mažos naujagimių kūno masės rizikos veiksnius.

Šio tyrimo rezultatai byloja apie kryptingos politikos ir prevencinių programų, skirtų nutraukti rūkymą ir chloruoto vandens vartojimą nėštumo metu, reikalingumą. Nepalankių nėštumo baigčių etiologinių mechanizmų supratimo gerinimas skatins taikyti tinkamas priemones, skirtas mažos naujagimių kūno masės rizikos prevencijai ir naujagimių sergamumui ir mirtingumui mažinti.

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