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Environmental Exposures, Genetic Susceptibility and Preterm Birth

Regina Grazuleviciene, Jone Vencloviene, Asta Danileviciute, Audrius Dedele and Gediminas Balcius
Vytautas Magnus University
Lithuania

1. Introduction

Preterm births 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 a potential risk factors (Adams et al., 2000; Bloom et al., 2001; Tucker & McGuire, 2004; Colvin et al. 2004; Fraser et al. 2004; Murphy et al. 2004). The frequency of preterm births is about 12–13% in the USA and 5–9% in many other developed countries; however, the rate of preterm birth has increased in many locations (Goldenberg et al., 2008). Thus, to elicit of risk factors that could predict high risk of preterm birth represents a challenge to practitioners and researchers. The increasing rate of preterm birth in recent decades, despite improvements in health care, creates an impetus to better understand and prevent this disorder. The identification of women at increased risk of preterm delivery is an important challenge. Preterm birth likely depends on a number of interacting factors, including genetic, epigenetic, and environmental risk factors (Windham et al., 2000; Plunkett & Muglia, 2008). The epidemiological data suggested that both genetic factors and socioenvironmental factors may influence preterm birth (Wang et al., 2000; Nukui et al., 2004; Lewis et al., 2006; Suh et al., 2008).

Genetic studies may identify stable over time markers, which can predict preterm birth in genetically susceptible subjects and the gene-environment studies may explain how the variations in the human genome (polymorphisms) can modify the effects of exposures to environmental health hazards (Kelada et al., 2003). Given individual genetic variations of pregnant women and different environmental exposures, the study may reveal women group susceptible to environmental hazards and may explain the differences in risk of preterm birth 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 or treat preterm birth.

To date, however, only a relatively few studies on the association of gene-environment interactions with preterm birth have been published (Wang et al., 2002; Genc et al., 2004; Macones et al., 2004).

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). The relationship between DBPs exposure and reproductive health outcomes remains unclear, mainly owing to limitations in the crude exposure assessment in most studies (Tardiff et al., 2006; Yang et al., 2007; Nieuwenhuijsen et al., 2009).

A recent meta-analysis of epidemiologic studies data on the association of THMs concentration in water and birth outcome, without taking into account exposure routes, concluded that there was little or no evidence for associations between THM concentration and prematurity and fetal growth, and that the uncertainties-relating particularly to exposure that may have affected them (Grellier et al., 2010). In addition, some epidemiological studies show little evidence for an association between THM and preterm birth, term low birth weight and other adverse birth outcomes (Bove et al., 2002; Hinckley et al., 2005; Nieuwenhuijsen et al., 2009). These epidemiological studies of reproductive outcomes had relied on different methods of assessing exposure, which presents difficulties in making comparisons between investigations and in generalizing results. The inconsistency of the association between exposure to THM and birth outcomes also could be related to both – qualitative and quantitative differences in exposure between the compared studies (Jaakkola et al., 2001). So, a major challenge in studies that examine the association between DBPs in drinking water and pregnancy outcomes is the accurate representation of a subject's exposure (King et al., 2004).

Seeking to improve the exposure assessment, studies have begun to incorporate behavioural determinants of different routes of exposure to DBPs such as dermal absorption and inhalation during bathing and showering, and ingestion of drinking water (Savitz et al. 2006; Hoffman et al., 2008; MacLehose et al., 2008). In our previous prospective Kaunas cohort study, which incorporated of different routes of exposure to THMs, we found dose–response relationships for entire pregnancy and trimester-specific gestational THMs and chloroform internal dose for low birth weight and reduction in birth weight (Grazuleviciene et al., 2011).

The epidemiological studies concluded that, while there appears to be suggestive evidence associating elevated total THM levels with some adverse reproductive outcomes, evidence for relationships with preterm birth and fetal growth is inconclusive and inconsistent (Richardson et al., 2003; Lewis et al., 2006; Grellier et al., 2010).

Most of the previous investigations have evaluated crude THM exposure; these studies differed on control of maternal characteristics that could also to be associated with adverse pregnancy outcomes; these studies did not evaluated genetic susceptibility to individual THM in relation to adverse pregnancy outcomes. Polymorphic variation in metabolic genes involved in detoxification of xenobiotics may explain some of the variation in individual susceptibility to the adverse effects of pollutants on preterm birth.

There is now some evidence concerning adverse effects of traffic-related air pollution on pregnancy outcomes and infant health. The evidence is suggestive of causality for the association of birth weight with air pollution, although for preterm birth and fetal growth, the current evidence is insufficient to infer a causal relationship and effects were not always consistent between studies Maisonet et al., 2001**;** Maroziene & Grazuleviciene, 2002; Sram et al., 2005; Dugandzic et al., 2006). Nitrogen dioxide (NO_2) is considered as a marker for air pollution from traffic associated with health effects (Belander et al., 2001; Rijnders et al.,

2001). In addition, a number of epidemiological studies have found various level relationships between exposures to traffic-related air pollution and birth outcomes, particularly for NO_2 and particulate matter, suggesting that exposure to these air pollutants may increase a woman's risk for preterm birth (Bobak, 2000; Maroziene & Grazuleviciene, 2002; Leem et al., 2006; Llop et al., 2010; Gehring et al., 2011).

The biological mechanisms by which air pollutants may interfere with the processes of prenatal development are still not clear. Several potential mechanisms have been hypothesised, including increased maternal susceptibility to inflammation, oxidative stress (Mohorovic, 2004; Becker et al., 2005; Risom et al., 2005), haematological factors (e.g., blood viscosity) (Pekkanen et al., 2000; Liao et al., 2005;) and the direct effect of specific pollutants on foetal development or on DNA and its transcription (Perera et al., 1999; Sram et al., 1999). Nitrogen dioxide is capable of oxidising tissue components (e.g., proteins, lipids) and of suppressing the antioxidant protective systems of organism (Sagai & Ichinose, 1991). Increased lipid peroxidation in the maternal and/or foetal compartment has been found in preterm birth (Moison et al., 1993). It was suggested that maternal exposure to NO_2 can increase the risk of pregnancy complications through stimulation of the formation of cell damaging lipid peroxides and from decrease in maternal antioxidant reserves (Tabacova et al., 1998). Recently a few potential biological mechanisms have been described through which air pollution could influence pregnancy outcomes, such as the induction inflammation of placenta, respiratory system and cardiovascular mechanisms of oxidative stress, coagulation, endothelial function, and hemodynamic responses (Kannan et al., 2006).

A crucial aspect of the study of prenatal exposure to air pollutants is the identification of vulnerable periods to the detrimental effects of the exposure during pregnancy and sensitive subjects (Hackley et al., 2007; Woodruff et al., 2009). Molecular epidemiological studies suggest possible biological mechanisms for the effect on preterm birth and intrauterine growth retardation (Shin, 2008). Population-based study data showed that 25% of the variation in preterm birth was explained by maternal genetic factors, fetal genetic factors only marginally influenced the variation in liability, while 70% of the variation in preterm birth was explained by the environmental effects (Svensson et al., 2009). More research is needed to clarify the role of traffic-related hazards on preterm birth, as well as their interactions with other environmental hazards and with specific genetic factors affecting maternal susceptibility.

In the present study, using individual cohort study data and adjusting for many important risk factors for preterm birth, we evaluated the effect of trimester-specific gestational THM internal dose and residential NO_2 exposure for preterm birth among genetically susceptible women. In our study individual exposure to THM was estimated as total internal dose based on monitoring of tap water THM levels and detailed water use behaviours. Controlling for influence of potential confounding variables, we seek to investigate whether the polymorphisms of metabolic genes GSTT1 and GSTM1 affect the association of maternal exposures to THMs and NO_2 with preterm birth risk.

2. Methods

We conducted a prospective cohort study of 4,161 pregnant women in Kaunas (Lithuania). We used tap water THM concentrations, geocoded maternal address at birth, individual information on drinking water ingestion, showering and bathing, and uptake factors of THMs in blood, to estimate an internal dose of THM. We estimated maternal residential

exposure to NO2 by Airviro dispersion models during entire pregnancy, and three pregnancy trimesters and used logistic regression to evaluate the relationship between internal THM dose and NO2 exposure and preterm birth controlling for potential confounding variables. To investigate whether the polymorphisms of metabolic genes GSTT1 and GSTM1 affect the association of maternal exposure to THMs and NO2 with preterm birth risk, a nested case-control study on preterm birth occurrence among 682 women with genotyping of GSTT1 and GSTM1 polymorphisms was conducted.

2.1 Participant recruitment and outcome assessment

We conducted a prospective cohort study of pregnant women in Kaunas city, Lithuania. On their first visit to a general practitioner, all pregnant women living in Kaunas city between 2007 and 2009 were invited to join the cohort. The women were enrolled in the study only if they consented to participate in the cohort. The study ethics complied with the Declaration of Helsinki (1996). The research protocol was approved by the Lithuanian Bioethics Committee and an oral informed consent was obtained from all subjects. In total 5,405 women were approached; 79% of them agreed to participate in the study.

The first interview was completed during the first pregnancy trimester. The median gestational age at interview was 8 weeks. The interview queried women regarding demographics, residence and job characteristics, chronic diseases (cardiovascular, hypertension, diabetes, renal), 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 preterm birth. Adjustment for these variables was made for studies of various subgroups. The women also were examined by ultrasound to determine the gestational age of the foetus.

A special water consumption and water use habits questionnaire was used to interview the 4,260 women who agreed to participate in the study; 76.4% of them were interviewed during the third pregnancy trimester before delivery at the hospital and 23.6% by telephone within the first month after delivery. Consumption was ascertained for three types of water: cold tape water or dinks made from cold tap water; boiled tap water (tea, coffee, and other); and bottled water, used at home, at work, other. In addition, number of showers, baths, swimming pools weekly, and their average length was asked of all subjects. The interviews were conducted by trained nurses who did not know the THM exposure status and birth outcome.

Pregnancy outcomes were abstracted from the medical records. Preterm birth was defined as infant's whose gestational age was less than 37 weeks. Gestational age of the foetus was estimated as the difference between the delivery date and the date of the last menstruation as reported by the women at the beginning of their pregnancies, and of an ultrasound examination. The reference group was defined as all term births (born at >37 weeks of gestation).

Women with multiple pregnancies (150), having inconsistent or invalid data for dating the pregnancy (5) or estimating THM exposure (mostly students moved out of the city during pregnancy, 839) or with newborn birth weight above 4,500 g (75) were excluded. We restricted our analyses to infants born with a birth weight below 4,500 g, leaving data for 3,341 women in the final analysis.

2.2 THM exposure assessment

The Kaunas city municipal drinking water is supplied by four water treatment plants system. The each treatment plant water supplied system is constituted of only one subsystem (i.e., one chlorination, and branchy water supplied to the users). Groundwater sources are used for the whole water supply system.

However, the four water treatment plants, which disinfected ground water with sodium hypochlorite (chlorine dose 0.26–0.91 mg/L, residual chlorine 0–0.22 mg/L), produced different concentrations of THMs in finished water. One treatment plant (Petrasiunai) supplied finished water with higher levels of THMs ("high level THM site," 54.9% subjects), 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 over a 3-year study period (2007–2009) in the morning in three locations: close to the treatment plant, at 5 km, and at 10 km or more from every treatment plant. A total of 85 water samples were collected from 12 monitoring sites in four water supply zones for THM analysis.

Samples were analysed at the University of the Aegean, Greece, by using gas chromatography with electron captures detection (Nikolaou et al., 2005). Measurements included specific values for the four regulated THMs (chloroform, bromoform, bromodichloromethane, and dibromochloromethane). We calculated the mean quarterly THM constituent concentrations for water zones and subsequently, depending on the TTHM levels within each zone, assigned "low level" and "high level" sites. We used tap water THM concentration, derived as the average of quarterly sample values over the time that the pregnancy occurred from all sampling sites located in the each distribution system, and geocoded maternal address at birth to assign the individual women residential exposure index. Estimates of exposure index to total and specific THMs from drinking water were tabulated first as an average level at the tap over the pregnancy period; this measure was then categorized at the tertiles of the distribution for birth outcomes. In addition, trimester-specific analyses were conducted. We combined every subject's residential exposure index and water-use questionnaire data to assess individual exposure through ingestion of THMs. Women were asked to indicate the cup or glass size and number of cups or glasses of tap water consumed per day, including hot and cold beverages made from tap water. With this information, we calculated daily amounts of hot and cold tap water ingested. Integration of the information on residential THM levels (μg/L), ingested amounts (L/day), and modifications by heating using an estimated uptake factor of 0.00490 to derive an integrated index of blood concentration, expressed in micrograms per day (μg/d) (Savitz et al., 2006; 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.

We assumed a null THM level for any bottled water consumption since in local bottled water production chlorination and ozonation is not used.

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 calculated each mother's trimester-specific and entire pregnancy average daily uptake of THM internal dose (μg/d). We derived indices of daily uptake by integrating THM concentrations, duration of bathing and showering reported in a questionnaire administered

to study participants, and estimated uptake factors of 0.001536 and 0.001321 of THMs in blood per minute per microgram from showering and bathing, respectively (Backer et al., 2000; Lynberg et al., 2001). 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
We then used average daily total uptakes in our analysis as continuous and categorized variables. We calculated tertiles of THM internal dose. This gave first (0.0025–0.0386 μg/d), second (0.0386–0.3496 μg/d), and third (0.3496–2.4040 μg/d) tertiles for average TTHM uptake. To reduce exposure misclassification errors in the subsequent analysis, we used a subset of women who through the entire pregnancy did not change their address.

2.3 NO_2 exposure assessment

In this study exposure to ambient NO_2 pollution estimates at each cohort number home address was assigned using GIS and AIRVIRO dispersion model, developed by the Swedish Meteorological and Hydrological (Airviro User Documentation 1997). The model integrates emissions inventories, meteorological data (wind direction and speed, temperature), background pollution measurements as input parameters and land use. Kaunas streets NO_2 emission data were used to create emission database within AIRVIRO Air Quality Management System. Gaussian plume dispersion simulations were run for a model domain encompassing the entire city area on a course grid resolution.
Geographic data for the Kaunas city streets, its type were measured by combination GIS and manual measurements. Total traffic counts and its composition (calculated as cars/day time's km street length) were measured based on the 2008 Municipal traffic-count data for Kaunas. If no counts were available for specific street, the numbers were estimated by a person with local information about the traffic conditions based on comparison with roads on which data were available. Traffic count data were available for 80% of the streets nearest to cohort addresses.
To attribute the NO_2 exposure to every study subject, the health data base and the environmental NO_2 pollution data base were joined. Every subject's full street address and residential NO_2 pollution level measurement data, and the current residence history data were combined to assess the individual NO_2 pollution exposure. A GIS assigning NO_2 pollution level was used for every woman by applying different GIS functions and possibilities. First, the study subjects data were converted to a database file structure for use in GIS software (ArcInfo version 9.3, ESRI). Geocoding was performed to obtain latitude and longitude coordinates for each patient's home address. Initially, 63 % records were matched and 37 % were left unmatched. All unmatched records were reviewed and corrected, leading to another 37 % matched addresses (total of 3287). Then, a spatial join was perform that allows the GIS user to append the attributes of one data layer (patient address points) to the attributes of another layer (nitrogen dioxide) assessed with AIRVIRO. We established the individual outdoor NO_2 exposure during three trimesters and entire pregnancy for every subject at the geocoded residential address.

2.4 Genotyping

Within the prospective cohort study, a nested case-control study on preterm birth occurrence among 682 women with genotyping of GSTT1 and GSTM1 polymorphisms was conducted. This study was assumed to identify gene–environment interaction that increases the risk of preterm delivery. We investigated the association between the risk of preterm delivery and each metabolic gene of glutathione *S*-transferases mu 1 (GSTM1) and theta 1 (GSTT1) along with exposure to THM and NO_2. The GSTM1-null and GSTT1-null genotypes were identified by the multiplex polymerase chain reaction (PCR) in peripheral blood DNA samples.

For genetic analysis 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 (SORPOclean Genomic DNA Extraction Kit, Vilnius, Lithuania). DNA concentrations were quantified with a spectrophotometer (Eppendorrf BioPhotometer, 61310488, Hamburg, Germany). The GSTM1- and GSTT1-null genotypes were identified by the multiplex polymerase chain reaction (PCR) in peripheral blood DNA samples. Multiplex PCR was performed as described by Arand et al. (1998) to determine the present (at least 1 allele present: AA or Aa) or absent (complete deletion of both alleles: aa) of GSTM1 and GSTT1 genes. PCR condition could be found elsewhere (Grazuleviciene et al., 2009).

2.5 Statistical analysis

The data analysis compared the preterm birth of low, medium and high exposed women to THMs. We used logistic regression to estimate adjusted odds ratios (ORs) and 95-percent confidence intervals (CIs) for preterm birth and the various exposure indices. We categorized TTHM internal dose in tertiles and evaluated the possible relationship between increases in preterm birth risk for an increase in estimated TTHM internal dose. We ran multivariate logistic regression models for the TTHMs, chloroform, dibromochloromethane, and bromodichloromethane for the total pregnancy and trimester specific periods. We also used multiple linear regressions for TTHM internal dose analysis as continuous variable to evaluate the relationship, if any between preterm birth and every 1 μg/d increase in TTHM internal dose.

In the logistic regression models for preterm birth outcome, using personal data of the cohort sample, we assessed a variety of potential confounders identified by univariate analysis. Further, we examined the association of THM exposure and preterm birth with a multivariable analysis controlling for effect of major covariates that changed the adjusted ORs for THM by 10% or more. The adjusted preterm birth outcome analyses included maternal smoking, education, family status, chronic diseases, previous preterm birth, stress and infant birth year.

The effect of ambient NO_2 exposure on preterm birth was estimated by logistic regression. We grouped the NO_2 concentrations into three categories (tertiles) and applied the exposure variable as both categorical and continuous parameters. We used exposure levels in the 1st tertile as the reference category (low exposure) and then also conducted an analysis of continuous exposure parameters on the basis of an increase of 10 $\mu g/m^3$ in NO_2 concentrations. We calculated crude and adjusted odds ratios (OR) and their 95 % confidence intervals (CIs) of preterm birth exposure categories. Statistical analyses were performed with SPSS software for Windows version 13.

To investigate whether the polymorphisms of metabolic genes GSTT1 and GSTM1 affect the association of maternal exposure to THMs and NO_2 with preterm birth risk, a nested case-control study data on preterm birth occurrence among 682 women with genotyping of

GSTT1 and GSTM1 polymorphisms was analysed. Logistic regression analyses were performed to explore the impact of each gene, THM and NO_2 exposure and their effect on the risk of preterm birth. 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, dibromochloromethane, and bromodichloromethane for total gestational and trimester-specific periods, while adjusting for potential confounders. Similarly we run analysis for the NO_2. We used logistic regression to estimate adjusted odds ratios (ORs) and 95-percent confidence intervals (CIs) for preterm birth, and the various exposure indices.
We estimated the exposure effect for GSTT1 (present, absent) and GSTM1 (present, absent) genotypes by a multivariable analysis controlling for influence of major covariates that changed the adjusted ORs for NO_2 by 10% or more. 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.

3. Results

3.1 Daily THM uptake

The mean tap water THM level in the low level site from three water treatment plants was 1.3 μg/L, and in the high level site (Petrasiunai) 21.9 μg/L (Table 1). The estimated individual total uptake of THMs ranged between 0.0025 and 2.40 μg/d. The total chloroform uptake ranged between 0.0013 and 2.13 μg/d. Mothers supplied with water who had a higher chloroform concentration generally also had a higher total internal dose. Daily uptake of bromodichloromethane ranged between 0.0001 and 0.34 μg/d and dibromochloromethane ranged between 0 and 0.064 μg/d. Bromoform was below the limit of detection.

Tap water sampling sites	TTHMs[c] Mean (SD[d])	$CHCl_3$ Mean (SD)	$CHBr_2Cl$ Mean (SD)	$CHBrCl_2$ Mean (SD)
All sites	9.8 (12.4)	7.8 (10.2)	0.3 (0.5)	1.7 (2.2)
Low THM level[a]	1.3 (1.2)	0.9 (1.0)	0.1 (0.2)	0.3 (0.5)
High THM level[b]	21.9 (10.9)	17.7 (9.0)	0.5 (0.6)	3.6 (2.1)

[a]Viciunai, Eiguliai, Kleboniskis. [b]Petrasiunai.
[c]TTHMs = total trihalomethanes: the sum of $CHCl_3$ (chloroform), $CHBr_2Cl$ dibromochloromethane), and $CHBrCl_2$ (bromodichloromethane).
[d]SD = standard deviation.

Table 1. Mean THM levels (μg/L) by sampling site and water supply zone

3.2 Preterm birth risk factors

The women recruited were predominantly Lithuanian in ethnic origin (97.4%) and did not smoke (93.1%) (Table 2). The mean age was 28.4 years, and the women tended to be highly educated (54.3% with a university degree). In general, mothers who were single, less educated, had previous preterm delivery, or reported a chronic stress delivered a higher proportion of preterm birth infants. We did not find a difference in preterm birth between water filter users and non-users.

Risk factors	Characteristics	All participants N(%)	Preterm birth N(%)
Maternal age	< 20 years	95 (2.8)	4 (2.1)
	20–29 years	1961 (58.7)	107 (55.7)
	≥ 30 years	1285 (38.5)	81 (42.2)
Marital status*	Married	2744 (82.1)	147 (76.6)
	Not married	597 (17.9)	45 (23.4)
Maternal education*	Primary school	166 (5.0)	16 (8.4)
	Secondary school	1361 (40.7)	88 (45.8)
	University degree	1814 (54.3)	88 (45.8)
Maternal smoking	Non-smoker	3110 (93.1)	176 (91.7)
	Smoker	231 (6.9)	16 (8.3)
Paternal smoking	Non-smoker	1748 (52.9)	88 (46.1)
	Smoker	1558 (47.1)	103 (53.9)
Alcohol consumption	No	3142 (94.0)	182 (94.8)
	Yes	199 (6.0)	10 (5.2)
Blood pressure (mm/Hg)	<140/80	2882 (86.3)	163 (84.9)
	≥140 or ≥ 90	459 (13.7)	29 (15.1)
Ethnic group	Lithuanian	3254 (97.4)	191 (99.5)
	Other	87 (2.6)	1 (0.5)
Parity	No child	1507 (45.1)	85 (44.3)
	≥ 1 child	1834 (54.9)	107 (55.7)
Infant gender	Male	1714 (51.3)	92 (47.9)
	Female	1627 (48.7)	100 (52.1)
Current residence	1–4 years	1401 (41.9)	84 (43.7)
	5–9 years	841 (25.2)	46 (24.0)
	≥ 10 years	1099 (32.9)	62 (32.3)
Work exposure	No	3048 (91.2)	177 (92.2)
	Yes	293 (8.8)	15 (7.8)
Chronic disease	No	2527 (75.6)	136 (70.8)
	Yes	814 (24.4)	56 (29.2)
Previous preterm delivery*	No	3279 (98.1)	180 (93.7)
	Yes	62 (1.9)	12 (6.3)
Socio economic status	Low income	1010 (30.2)	68 (35.4)
	Medium income	1824 (54.6)	95 (49.5)
	High income	507 (15.2)	29 (15.1)
Body mass index (kg/m^2)	<25 Normal	1974 (59.1)	118 (61.5)
	25–30 Overweight	947 (28.3)	49 (25.5)
	30 Obesity	420 (12.6)	25 (13.0)
Water filter	Yes	1015 (30.4)	64 (33.3)
	No	2326 (69.6)	128 (66.7)
Water supply area	Petrasiunai	1835 (54.9)	114 (59.3)
	Other	1506 (45.1	78 (40.7)
Birth year*	2007	681 (20.4)	48 (25.0)
	2008	1711 (51.2)	78 (40.6)
	2009	949 (28.4)	66 (34.4)
Maternal stress*	No	2432 (72.8)	128 (66,7)
	Yes	909 (27.2)	64 (33.3)

*$p<0.05$.

Table 2. Distribution of Kaunas cohort study subjects for various characteristic

The analysis by TTHM internal dose tertiles showed, that most characteristics of the exposure groups were similar (Table 3). There were no differences in social and demographic characteristics, health behaviour, pregnancy history, and maternal diseases. However, paternal smoking and alcohol consumption differed between exposure groups. All subjects of high THM exposure group were served by Petrasiunai water treatment plant while 99.8% subjects of low exposure group were served by other water treatment plants. Among 3,341 singleton infant, 192 (5.7%) were classified as preterm birth. The proportion of premature birth cases tended to be higher among women of medium and high THM exposure to compare to low THM exposure.

3.3 Association between THM internal dose and preterm birth risk

Using total gestational and trimester-specific daily uptakes tertiles of TTHM and individual THMs continuous variables, we examined the association between internal dose and preterm birth risk (Table 4). In THMs analysis by tertile, preterm birth risk tended to increase by increasing internal dose, however, data for TTHM and chloroform were not consistent. TTHM and chloroform analysed as continuous variables (increase of 0.1 μg/d) showed slightly elevated, but statistically non-significant increase in risk of preterm birth in all pregnancy trimesters. However, we found dose–response relationships for the first and second trimester's bromodichloromethane and dibromochloromethane internal dose and risk for preterm birth. The adjusted odds ratio for third tertile vs. first tertile dibromochloromethane internal dose of first trimester was 2.06, 95% CI 1.28–3.31; of second trimester the OR was 1.84, 95% CI 1.04–3.26; the OR per every 0.01 μg/d increase in dibromochloromethane internal dose was 1.28, 95% CI 1.04–1.57 and 1.21, 95% CI 1.01–1.45,respectively, for first and second trimester. The trend was not statistically significant when dibromochloromethane exposure were examined. The analyses were adjusted for the variables that have had effect on preterm birth risk: family status, maternal education, smoking, alcohol consumption, stress, previous preterm delivery, and infant birth year.

We used THM internal dose median level as a cut off (above median vs. below median) in a genetic polymorphism analyses. 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 bromodichloromethane: 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 dibromochloromethane exposures were analyzed. The OR for preterm birth among women exposed to dibromochloromethane 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 bromodichloromethane 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. Exposure to dibromochloromethane 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.

Risk factors	Characteristics	Low THM N (%)	Medium THM N (%)	High THM N (%)
Maternal age	< 20 years	19 (1.8)	17 (1.5)	23 (2.1)
	20–29 years	652 (60.1)	688 (59.7)	658 (59.6)
	≥ 30 years	414 (39.2)	447 (38.8)	423 (38.3)
Marital status	Married	876 (80.7)	958 (83.2)	910 (82.4)
	Not married	209 (19.3)	194 (16.8)	194 (17.6)
Maternal education	Primary school	59 (5.4)	50 (4.3)	57 (5.2)
	Secondary school	454 (41.8)	465 (40.4)	442 (40.0)
	University degree	572 (52.7)	637 (55.3)	605 (54.8)
Maternal smoking	Non-smoker	1003 (92.4)	1076 (93.4)	1031 (93.4)
	Smoker	82 (7.6)	76 (6.6)	73 (6.6)
Paternal smoking*	Non-smoker	574 (53.4)	629 (55.4)	545 (49.8)
	Smoker	501 (46.6)	507 (44.6)	550 (50.2)
Alcohol consumption*	No	1000 (92.2)	1094 (95.0)	1048 (94.9)
	Yes	85 (7.8)	58 (5.0)	56 (5.1)
Blood pressure (mm/Hg)	<140/80	969 (89.3)	1020 (88.5)	977 (88.5)
	≥140 or ≥ 90	116 (10.7)	132 (11.5)	127 (11.5)
Ethnic group	Lithuanian	1054 (97.1)	1117 (97.0)	1082 (98.1)
	Other	31 (2.9)	35 (3.0)	21 (1.9)
Parity	No child	492 (45.3)	499 (43.3)	516 (46.7)
	≥ 1 child	593 (54.7)	653 (56.7)	588 (53.3)
Infant gender	Male	559 (51.5)	611 (53.0)	544 (49.3)
	Female	526 (48.5)	541 (47.0)	560 (50.7)
Current residence	1–4 years	437 (40.3)	492 (42.7)	472 (42.8)
	5–9 years	257 (23.7)	288 (25.0)	296 (26.8)
	≥ 10 years	391 (36.0)	372(32.3)	336 (30.4)
Work exposure	No	996 (91.8)	1053 (91.4)	999 (90.5)
	Yes	89 (8.2)	99 (8.6)	105 (9.5)
Chronic disease	No	825 (76.0)	858 (74.5)	844 (76.4)
	Yes	260 (24.0)	294 (25.5)	260 (23.6)
Previous preterm	No	1069 (98.5)	1123 (97.5)	1087 (98.5)
	Yes	16 (1.5)	29 (2.5)	17 (1.5)
Socio economic status	Low income	335 (30.9)	337 (29.3)	338 (30.6)
	Medium income	582 (53.6)	642 (55.7)	600 (54.3)
	High income	168 (15.5)	173 (15.0)	166 (15.0)
Body mass index (kg/m^2)	<25 Normal	618 (57.0)	677 (58.8)	679 (61.5)
	25–30 Overweight	329 (30.3)	334 (29.0)	284 (25.7)
	30 Obesity	138 (12.7)	141 (12.2)	141 (12.8)
Water filter	Yes	341 (31.4)	336 (29.2)	338 (30.6)
	No	744 (68.6)	816 (70.8)	766 (69.4)
Water supply area*	Petrasiunai	2 (0.2)	728 (63.3)	1105 (100.0)
	Other	1084 (99.8)	422 (36.7)	0 (0.0)
Birth year*	2007	266 (24.5)	91 (7.9)	324 (29.3)
	2008	524 (48.3)	680 (59.1)	507 (45.9)
	2009	296 (27.3)	379 (33.0)	274 (24.8)
Maternal stress	No	794 (73.1)	848 (73.7)	790 (71.5)
	Yes	292 (26.9)	302 (26.3)	315 (28.5)
Preterm birth	No	1032 (95.0)	1079 (93.8)	1038 (93.9)
	Yes	54 (5.0)	71 (6.2)	67 (6.1)

*$p<0.05$.

Table 3. Distribution of Kaunas cohort study subjects for various characteristic by THM exposure

THM dose tertile limits (µg/d)	Cases	Controls	Entire pregnancy OR (95% CI)	I trimester OR (95% CI)	II trimester OR (95% CI)	III trimester OR (95% CI)
THM						
0.0025-0.0386	54	1032	1	1	1	1
0.0386-0.3496	71	1079	1.35 (0.93-1.96)	1.51 (1.04-2.18)	1.34 (0.92-1.95)	1.47 (1.01-2.14)
0.3496-2.4040	67	1038	1.24 (0.85-1.80)	1.09 (0.74-1.61)	1.18 (0.81-1.72)	1.10 (0.76-1.59)
Continuous (0.1µg/d)			1.02 (0.98-1.07)	1.01 (0.97-1.06)	1.03 (0.98-1.07)	1.02 (0.98-1.07)
Chloroform						
0.0013-0.0249	55	1035	1	1	1	1
0.0249-0.2868	73	1072	1.38 (0.95-2.02)	1.65 (1.13-2.42)	1.38 (0.94-2.02)	1.67 (1.13-2.47)
0.2868-2.1328	64	1042	1.14 (0.78-1.66)	1.15 (0.78-1.69)	1.16 (0.79-1.68)	1.20 (0.82-1.76)
Continuous (0.1µg/d)			1.03 (0.97-1.08)	1.01 (0.96-1.07)	1.03 (0.98-1.08)	1.03 (0.98-1.08)
BDCM						
0.0001-0.0124	62	1029	1	1	1	1
0.0124-0.0501	61	1085	1.02 (0.70-1.49)	1.26 (0.87-1.83)	1.21 (0.83-1.76)	1.02 (0.70-1.50)
0.0501-0.3359	69	1035	1.17 (0.82-1.68)	1.27 (0.88-1.85)	1.32 (0.91-1.90)	1.11 (0.78-1.60)
Continuous (0.01µg/d)			1.02 (0.99-1.05)	1.02 (0.98-1.06)	1.02 (0.98-1.06)	1.02 (0.99-1.05)
DBCM						
0.0000-0.0000	64	1051	1	1	1	1
0.0000-0.0039	59	1065	1.40 (0.82-2.39)	1.51 (0.91-2.51)	1.79 (1.03-3.13)	0.83 (0.51-1.36)
0.0039-0.0644	69	1033	1.67 (0.97-2.85)	2.06 (1.28-3.31)	1.84 (1.04-3.26)	1.11 (0.70-1.77)
Continuous (0.01µg/d)			1.20 (0.99-1.46)	1.28 (1.04-1.57)	1.21 (1.01-1.45)	1.12 (0.94-1.32)

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

Table 4. Preterm birth adjusted OR and 95% confidence intervals for entire pregnancy and trimester–specific internal THM dose

THM exposure*	GSTM1-1 GS (95% PI)	GSTM1-0 GS (95% CI)	GSTT1-1 GS (95% CI)	GSTT1-0 GS (95% CI)
TTHM				
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)
Chloroform				
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**				
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**				
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)

*Referent group bellow median.
Adjusted for: family status, smoking, education, stress, previous preterm birth, and infant birth year.

Table 5. 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

3.4 Distribution preterm birth risk factors by NO_2 exposure levels

Distribution of pregnancy outcomes and NO_2 pollution levels are presented in Figure 1. The mean levels of NO_2 to which the women were exposed outside their homes throughout their pregnancies ranged from 5.3 to 36.0 μg/m^3. Table 6 shows the prevalence of distribution of Kaunas cohort study subjects for various characteristic by nitrogen dioxide exposure. There were no differences in social and demographic characteristics, health behaviour, pregnancy history, maternal diseases and health behaviour between the three NO_2 exposure groups.

Risk factors	All participants N(%)	Low NO_2 N (%)	Medium NO_2 N (%)	High NO_2 N (%)
Maternal age				
< 20 years	95 (2.8)	34 (3.0)	31 (2.9)	28 (2.6)
20–29 years	1935 (58.8)	655 (58.3)	663 (61.2)	617 (56.8)
≥ 30 years	1264 (38.4)	434 (38.6)	389 (35.9)	441 (40.6)
Marital status				
Married	2707 (82.2)	920 (81.9)	888 (82.0)	899 (82.8)
Not married	585 (17.8)	203 (18.1)	195 (18.0)	187 (17.2)
Maternal education				
Primary school	162 (4.9)	52 (4.6)	55 (5.1)	55 (5.1)
Secondary school	1340 (40.7)	483 (43.0)	412 (38.0)	445 (41.0)
University degree	1790 (54.4)	588 (52.4)	616 (56.9)	586 (54.0)
Maternal smoking				
Non-smoker	3066 (93.1)	1049 (93.4)	1009 (93.2)	1008 (92.8)
Smoker	226 (6.9)	74 (6.6)	74 (6.8)	78 (7.2)
Paternal smoking				
Non-smoker	1721 (52.8)	589 (52.6)	593 (55.4)	539 (50.5)
Smoker	1536 (47.2)	530 (47.4)	477 (44.6)	529 (49.5)
Alcohol consumption				
No	3095 (94.0)	1054 (93.9)	1016 (93.8)	1025 (94.4)
Yes	197 (6.0)	69 (6.1)	67 (6.2)	61 (5.6)
Blood pressure				
<140/80 mm/Hg	2841 (86.3)	989 (88.1)	925 (85.4)	927 (85.4)
≥140 or ≥ 90 mm/Hg	451 (13.7)	134 (11.9)	158 (14.6)	159 (14.6)
Ethnic group				
Lithuanian	3205 (97.4)	1094 (97.4)	1054 (97.3)	1057 (97.3)
Other	87 (2.6)	29 (2.6)	29 (2.7)	29 (2.7)
Parity				
No child	1487 (45.2)	493 (43.9)	500 (46.2)	494 (45.5)
≥ 1 child	1805 (54.8)	630 (56.1)	583 (53.8)	592 (54.5)
Infant gender *				
Male	1690 (51.3)	615 (54.8)	543 (50.1)	532 (49.0)
Female	1602 (48.7)	508 (45.2)	540 (49.9)	554 (51.0)
Current residence				
1–4 years	1381 (42.0)	480 (42.7)	468 (43.2)	433 (39.9)
5–9 years	831 (25.2)	266 (23.7)	256 (23.6)	309 (28.5)
≥ 10 years	1080 (32.8)	377 (33.6)	359 (33.1)	344 (31.7)

Work h/week during 1st trimester				
Nonemployed				
<10 h.	537 (16.0)	172 (15.3)	161 (14.9)	194 (17.9)
10-20 h.	305 (9.3)	102 (9.1)	104 (9.6)	99 (9.1)
20-40 h.	138 (4.2)	45 (4.0)	40 (3.7)	53 (4.9)
> 40 h.	1785 (54.2)	597 (53.2)	612 (56.5)	576 (53.0)
	537 (16.3)	207 (18.4)	166 (15.3)	164 (15.1)
Chronicle disease				
No	2487 (75.5)	847 (75.4)	839 (77.5)	801 (73.8)
Yes	805 (24.5)	276 (24.6)	244 (22.5)	285 (26.2)
Previous preterm				
No	3231 (98.1)	1102 (98.1)	1061 (98.0)	1068 (98.3)
Yes	61 (1.9)	21 (1.9)	22 (2.0)	18 (1.7)
Socio economic status				
Low income	994 (30.2)	344 (30.6)	300 (27.7)	350 (32.2)
Medium income	1799 (54.6)	605 (53.9)	623 (57.5)	571 (52.6)
High income	499 (15.2)	174 (15.5)	160 (14.8)	165 (15.2)
Body mass index				
<25 Normal	1951 (59.3)	663 (59.0)	642 (59.3)	646 (59.5)
25–30 Overweight	926 (28.1)	320 (28.5)	309 (28.5)	297 (27.3)
30 Obesity	415 (12.6)	140 (12.5)	132 (12.2)	143 (13.2)
Birth year*				
2007	671 (20.4)	205 (18.3)	209 (19.3)	257 (23.7)
2008	1688 (51.3)	572 (50.9)	551 (50.9)	565 (52.0)
2009	933 (28.3)	346 (30.8)	323 (29.8)	264 (24.3)
Maternal stress				
No	2397 (72.8)	830 (73.9)	782 (72.2)	785 (72.3)
Yes	891 (27.2)	293 (26.1)	301 (27.8)	301 (27.7)
Preterm birth				
No	3105 (94.3)	1061 (94.5)	1026 (94.7)	1018 (93.7)
Yes	187 (5.7)	62 (5.5)	57 (5.3)	68 (6.3)

*p<0.05

Table 6. Distribution of Kaunas cohort study subjects for various characteristic by nitrogen dioxide exposure

3.5 Association between NO_2 exposure and preterm birth risk

In crude analyses, we found consistently higher, statistically non-significant, ORs for preterm birth before 37 weeks associated with higher NO_2 levels during the entire pregnancy and during the three trimesters of pregnancy (Table 7). Fully adjusted models by trimesters revealed exposure–response relationships for the entire pregnancy and trimester-specific NO_2 tertile and risk for preterm birth, however, none of these associations reached statistical significance. After adjustment for confounding variables, strongest relation between preterm birth and NO_2 levels was in the first and in the second trimesters of pregnancy. The OR for preterm birth among women exposed to third tertile NO_2 during the first trimester was 1.28 (95% CI 0.87–1.90) and 1.42 (95% CI 0.96–2.11) for the second trimester, respectively, to compare to the first NO_2 exposure tertile. During the third pregnancy trimester third NO_2 exposure tertile was associated with OR 1.12 (95% CI 0.76–1.67), compared with the lowest NO_2 exposure. Using a continuous measure, we found that the risk of preterm birth for entire pregnancy tended to increase by 22 % (adjusted OR = 1.22, 95% CI 0.94–1.56) per every 10 $\mu g/m^3$ increase in NO_2 concentrations. There was no statistically significant association between preterm birth and NO_2 exposure.

NO_2 exposure tertiles	Preterm birth (<37 weeks)		Control (>37 weeks)		Crude odds ratio OR (95% CI)	Adjusted* odds ratio OR (95% CI)
	N	%	N	%		
Entire pregnancy						
1st tertile (6.4-18.7 μg/m³)	50	5.0	958	95.0	1	1
2nd tertile (18.7-23.7 μg/m³)	50	4.9	966	95.1	0.99 (0.66-1.48)	0.99 (0.66-1.49)
3rd tertile (23.7-44.3 μg/m³)	67	6.8	922	93.2	1.39 (0.96-2.03)	1.44 (0.98-2.11)
Continuous variable (per 10 μg/m³ increase in concentration)					1.19 (0.93-1.53)	1.22 (0.94-1.56)
First trimester						
1st tertile (5.3-16.7 μg/m³)	55	5.2	994	94.8	1	1
2nd tertile (16.7-24.0 μg/m³)	51	5.2	921	94.8	1.00 (0.68-1.48)	1.04 (0.70-1.55)
3rd tertile (24.0-53.2 μg/m³)	61	6.1	931	93.9	1.18 (0.81-1.72)	1.28 (0.87-1.90)
Continuous variable (per 10 μg/m³ increase in concentration)					1.07 (0.88-1.28)	1.11 (0.91-1.35)
Second trimester						
1st tertile (5.3-16.7 μg/m³)	47	4.8	936	95.2	1	1
2nd tertile (16.7-24.5 μg/m³)	56	5.4	989	94.6	1.13 (0.76-1.68)	1.15 (0.77-1.73)
3rd tertile (24.5-53.2 μg/m³)	64	6.5	921	93.5	1.38 (0.94-2.04)	1.42 (0.96-2.11)
Continuous variable (per 10 μg/m³ increase in concentration)					1.14 (0.95-1.37)	1.15 (0.96-1.39)
Third trimester						
1st tertile (5.3-16.7 μg/m³)	53	5.1	977	94.2	1	1
2nd tertile (16.7-24.2 μg/m³)	58	5.7	958	94.2	1.12 (0.76-1.64)	1.12 (0.76-1.66)
3rd tertile (24.2-51.9 μg/m³)	56	5.8	911	94.6	1.13 (0.77-1.67)	1.12 (0.76-1.67)
Continuous variable (per 10 μg/m³ increase in concentration)					1.10 (0.92-1.32)	1.09 (0.90-1.31)

Adjusted for: maternal smoking, education, family status, chronic diseases, previous preterm birth, stress, gender, and birth year. Parity below 4 children.

Table 7. Crude and adjusted odds ratios (OR) and their 95% confidence intervals (CI) for preterm birth by trimester–specific and entire pregnancy NO_2 exposure

Genotype	NO2 tertiles limits (μg/m³)	Total N	Preterm birth (%)	Crude OR 95% CI	Adjusted* OR 95% CI
GSTT1-1	I tertile (5.3-16.7)	171	16 (9.4)	1	1
	II tertile (16.7-24.5)	146	14 (9.6)	1.03 (0.48-2.18)	1.05 (0.47-2.36)
	III tertile (24.5-53,2)	187	25 (13.4)	1.50 (0.77-2.90)	1.64 (0.80-3.38)
GSTT1-0	I tertile (5.3-16.7)	46	6 (13.0)	1	1
	II tertile (16.7-24.5)	30	5 (16.7)	1.33 (0.37-4.83)	1.21 (0.27-5.44)
	III tertile (24.5-53,2)	26	9 (34.6)	3.53 (1.09-11.5)	5.44 (1.29-22.9)
GSTM1-1	I tertile (5.3-16.7)	107	12 (11.2)	1	1
	II tertile (16.7-24.5)	96	6 (6.3)	0.53 (0.19-1.47)	0.50 (0.17-1.44)
	III tertile (24.5-53,2)	111	17 (15.3)	1.43 (0.65-3.16)	1.42 (0.62-3.26)
GSTM1-0	I tertile (5.3-16.7)	110	10 (9.1)	1	1
	II tertile (16.7-24.5)	80	13 (16.3)	1.94 (0.80-4.68)	1.90 (0.68-5.25)
	III tertile (24.5-53,2)	102	17 (16.7)	2.00 (0.87-4.60)	2.65 (1.03-6.83)

* Adjusted for: maternal smoking, education, family status, chronic diseases, previous preterm birth, stress, and birth year.

Table 8. Crude and adjusted associations as odds ratios (OR) of NO_2 exposure during entire pregnancy with preterm birth by maternal genotypes

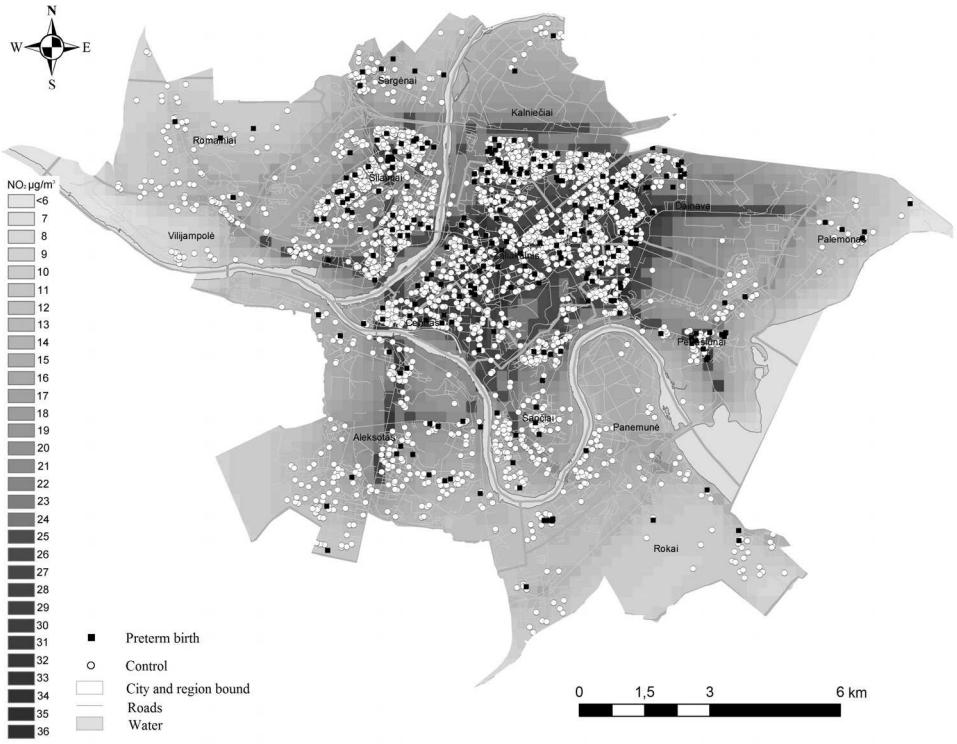

Fig. 1. Modelled NO_2 concentration and geocoded study subjects addresses

4. Discussion

4.1 Trihalomethanes effects

We conducted a prospective cohort study to examine the effects of internal dose of THM during the entire pregnancy and during three trimesters on preterm births before 37 weeks. TTHM and chloroform analysed as continuous variables (increase of 0.1 µg/d) showed slightly elevated, but statistically non-significant increase in risk of preterm birth in all pregnancy trimesters. However, we found dose–response relationships for the first and second trimester's bromodichloromethane and dibromochloromethane internal dose and risk for preterm birth. The adjusted ORs for third tertile vs. first tertile dibromochloromethane internal dose of first trimester was 2.06, 95% CI 1.28–3.31; of second trimester the OR was 1.84, 95% CI 1.04–3.26. The probability of delivering a preterm birth infant was elevated by 28% per every 0.01 µg/d increase in dibromochloromethane internal dose (OR 1.28, 95% CI 1.04–1.57) and by 21% (OR 1.21, 95% CI 1.01–1.45), respectively, for first and second trimester. The lack of statistically significant effects for other TTHM constituents may be due to low exposure because of low levels, and lack of power in our study sample.

Consistent with other recent studies (Källén & Robert; 2000; Lewis et al., 2007; Nieuwenhuijsen et al., 2009) our findings suggest that THM exposures might increase the risk of preterm birth. The epidemiological evidence for an association between exposure to THM and preterm birth is relatively inconsistent. A number of prior investigations have evaluated crude exposure during the pregnancy. An epidemiological study reported a statistically significant increased risk of delivering a preterm birth infant among women users of chlorinated with sodium hypochlorite water vs. no chlorinated water, with OR of 1.09 (CI 1.01–1.17) (Källén and Robert; 2000). Some authors found no association between preterm birth and THM (Aggazzotti et al., 2004; Hinckley et al., 2005). Some authors find small increases in gestation age with increased TTHM exposure and with both chloroform and BDCM (Wright et al., 2003; 2004). A negative association during the second trimester were reported, however, women who depended on a governmental source of payment for prenatal care were at increased risk when exposed at high levels TTHM late in gestation (OR 1.39; 95% CI, 1.06–1.81) (Lewis et al., 2007).

Lack of a consistent effect of the epidemiologic studies may be result of a study design, be a result of exposure misclassification or inadequate control for confounding variables, or a lack of power in studies sample, or actual lack of an effect of DBP on reproductive effects. This study offered advancement in individual internal dose assessment based on residential THM levels, detailed water use behaviours and exposure during pregnancy. Every subject's exposure indices were estimated as daily internal dose of the THM constituents (mg/d) and birth outcome effects were assessed by using indices categorical variable and also as a continuous variable. An additional strength of our study is that pregnant women were prospectively followed, and did not move during pregnancy. This allowed collection of self reported data on potential confounding covariates and decreased of exposure misclassification errors. However, there is a possibility of confounding in our study, because of lack information on maternal nutrition, infection diseases, and occupational physical exposures (Magann et al., 2005). Furthermore, lack of information regarding the validity of the internal dose assessment models that we used is one of the limitations of this study, but again validity studies are difficult to conduct and are expensive.

4.2 Maternal GSTT and GSTM genotypes effects

Our study adds to the findings of researchers who studied genetic polymorphism along with to 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. 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 bromodichloromethane 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 and GSTM1 null genotypes tended to be more susceptible to THM exposure.

Our findings raises the possibility that the effect of TTHM exposure on preterm birth may be associated with one or two variant alleles for the GSTT and GSTM genes involved in the

metabolism of low doses of THMs. Further tests with larger sample sizes are needed to verify these observations.

Although the effects of unmeasured risk factors could not be excluded with certainty, our findings suggest that genetically determined differences in maternal detoxification may contribute to the risk of preterm birth. In particular, women carriers of GSTT1 or GSTM1 null genotypes and exposed to high levels of NO_2 during the pregnancy were at a higher risk for preterm birth to compare to the GSTT1-1 and GSTM1-1 genotypes carriers. We observed a statistically significant association between maternal GSTT1 null genotype (OR 5.44, 95% CI 1.29–22.9) and GSTM1 null genotype (OR 2.65, 95% CI 1.03–6.83) and the risk for preterm birth infants in the presence of maternal NO_2 exposure of third tertile during pregnancy. This result provided evidence for a gene–environment interaction regarding a risk for preterm birth.

To the best of our knowledge, this is the first study to explore the synergistic effect of the GSTT1 and GSTM1 polymorphism and exposure to NO_2 on preterm birth. Limitations in interpreting these results include: there are other external exposures which we did not measure; the presence of internal uncontrolled exposures; GSTT1 and GSTM1 genes are linked to other genes responsible for preterm birth.

4.3 NO_2 exposure effects

Our findings provide little support to the hypothesis of an adverse effect of maternal exposure to NO_2 during pregnancy on preterm birth. NO_2 exposure during entire pregnancy and during the three trimesters of pregnancy tended to be associated with increase in risk of preterm birth after adjustment for the main possible confounders: maternal smoking, education, family status, chronic diseases, previous preterm birth, stress, infant gender, and birth year. In this study we also were able to estimate residential NO_2 exposure to every cohort study subject during pregnancy trimesters and to control for effect of change residence during pregnancy. Adjusted odds ratios for preterm birth for entire pregnancy exposed to third tertile NO_2 was found to be 1.44, 95% CI 0.98–2.11. The risk of preterm birth tended to increase by 22% (adjusted OR = 1.22, 95% CI 0.94–1.56) per 10 $\mu g/m^3$ increase in NO_2 concentrations. A limited statistical power of the study may be associated with a low prevalence of preterm birth in our cohort (5.7 %) and also be a consequence of low NO_2 exposure level, since only a low percentage of pregnant women were exposed to the levels exceeding established limit value of the annual mean NO_2 concentration (40 $\mu g/m^3$).

The results of the study confirm to a data of epidemiological studies performed in other countries. The reported NO_2 effect on preterm birth was small with odds ratios in the range 1.1–1.2 per 10 $\mu g/m^3$ increase in NO_2 levels or effect was not found (Gehring et al. 2011; Liu et al. 2003; Hansen et al. 2006; Ritz et al. 2007). Association of NO_2 exposure and increased risk of preterm birth was reported during different trimesters (Bobak 2000; Lee et al. 2003). A study in Valencia, Spain, find that highest association between NO_2 levels during pregnancy and preterm birth was found in second trimester (1.11, 95 % CI 1.03- 1.21) (Llop et al. 2010). Consistent with the findings of a cohort study in Vancouver, Canada where association between preterm birth and NO_2 concentrations was found during different periods of pregnancy (Liu et al. 2003), our findings suggest that NO_2 exposure might increase the risk of preterm birth.

In our previous study we found a moderately increased preterm birth risk for NO_2 exposures estimated at the entire residential district level (Maroziene and Grazuleviciene 2002). Adjusted ORs of preterm birth for the medium and high NO_2 tertile exposures were OR = 1.14 (95% CI 0.77–1.68) and OR = 1.68 (95% CI 1.15–2.46), respectively. Using a continuous measure, the risk of preterm birth increased by 25% (adjusted OR = 1.25, 95% CI 1.07–1.46) per 10 μg/m^3 increase in NO_2 concentrations.

Potential mechanisms by which air pollution might increase preterm birth include inflammation, endotelian dysfunction, endocrine disruption and genetic polymorphisms (Slama et al., 2010; Suh et al., 2008; Yorifuji et al., 2011).

Consistent with our finding, some previous studies found that exposure to maternal smoking or high levels of particulate matter in the presence of the GSTM1 null genotype is associated with the adverse birth outcomes (Sram et al., 2006; Suh et al., 2008; Grazuleviciene et al., 2009).

To make progress, this research field needs input from molecular epidemiology, toxicology, exposure assessment, and epidemiology, especially to aid in the identification and exposure assessment of reproductive-toxic agents in water and ambient air, and in the development of early markers of adverse reproductive outcomes. Additional research that clearly defines the mechanisms by which risk factors are related to preterm birth is crucial. Improved understanding of these mechanisms should allow clinicians to design appropriate interventions so that the incidence of preterm birth and related fetal and neonatal morbidity and mortality will be reduced. Improved methods of characterizing patients based on etiology that allow for the evaluation of multiple candidate genes simultaneously are most likely to be successful in identifying genetic susceptibility that increase the risk for preterm birth. Futher research should focus on the use of individual environmental exposures and genetic susceptibility in the studies of environmental toxicants effects on birth outcomes.

5. Conclusion

This study presented epidemiological evidence for a dose-response relationship between dibromochloromethane internal dose and the risk for preterm birth. The analyses were adjusted for the confounding variables that have had effect on preterm birth risk: single women, low maternal education, smoking, alcohol consumption, stress, previous preterm delivery, and infant birth year. In addition, increased THM internal dose tended to increase preterm birth risk and maternal GSTM1 and GSTT1 genotypes modified the THM exposure effects on preterm birth. Furthermore, we found an association between exposure to high levels of NO_2 during pregnancy and the presence of the GSTM1 null and GSTT1 null genotypes for the risk of preterm birth, providing evidence that both genetic and environmental factors determine complex traits such as preterm delivery. Genes involved in metabolic detoxification processes such as GSTM1 and GSTT1 may be treated as candidate risk factors for preterm birth.

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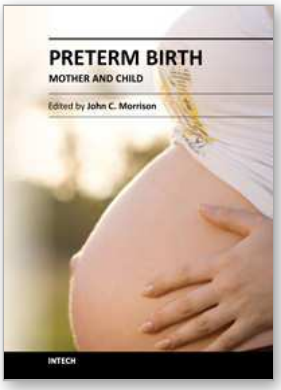

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While there are many studies and books regarding preterm birth, both the obstetric and in the neonatal/pediatric literature, what is missing is the integration of data from obstetrics through neonatal course and into pediatrics as the neonate transverses childhood. A continued dialogue between specialties is essential in the battle against preterm birth in an attempt to relieve the effects or after-effects of preterm birth. For all of our medical advances to date, preterm birth is still all too common, and its ramifications are significant for hospitals, families and society in general.

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University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821