




Larry Stone



Understanding Preterm Birth

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Edited by Larry Stone



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Preface

An introductory account based on the topic of preterm birth has been provided in this profound book. The preterm parturition syndrome is presently one of the main obstetrical problems. Comprehending the mechanisms resulting in prematurity may help the clinicians to formulate the required treatment that is appropriate for the mechanisms leading to preterm parturition in a particular patient. The book provides up-to-date information regarding maternal stress, particular relation with periodontal disease, and activation of the hemostatic system and preterm childbirth. It also elucidates the effects of prematurity, both short term as well as long term, along with a long term aspect of late preterm parturition. It provides content for discussions between pediatricians, obstetricians and practitioners from other disciplines regarding the treatment, prevention and diagnosis of prematurity.

This book is the end result of constructive efforts and intensive research done by experts in this field. The aim of this book is to enlighten the readers with recent information in this area of research. The information provided in this profound book would serve as a valuable reference to students and researchers in this field.

At the end, I would like to thank all the authors for devoting their precious time and providing their valuable contribution to this book. I would also like to express my gratitude to my fellow colleagues who encouraged me throughout the process.

Editor

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Environmental Exposures, Genetic Susceptibility and Preterm Birth

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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; Maroziane & Grazuleviciene, 2002; Sram et al., 2005; Dugandzic et al., 2006). Nitrogen dioxide (NO₂) 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 NO₂ and particulate matter, suggesting that exposure to these air pollutants may increase a woman's risk for preterm birth (Bobak, 2000; Marozienne & 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 NO₂ 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 NO₂ 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 NO₂ 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 tap 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 (Petrašiūnai) 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 ($\mu\text{g/L}$), 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 ($\mu\text{g/d}$) (Savitz et al., 2006; Whitaker et al., 2003).

The actual algorithms of internal dose from ingestion were:

chloroform level ($\mu\text{g/L}$) \times water consumption (L/day) \times 0.00490196 $\mu\text{g}/\mu\text{g/L}$;

brominated THM level ($\mu\text{g/L}$) \times water consumption (L/day) \times 0.00111848 $\mu\text{g}/\mu\text{g/L}$.

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 ($\mu\text{g/L}$) 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 ($\mu\text{g/d}$). 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 $\times \mu\text{g/l}$ chloroform in water $\times 0.001536261 \mu\text{g/min}/\mu\text{g/l}$,

min/day showering $\times \mu\text{g/l}$ brominated THM in water $\times 0.001352065 \mu\text{g/min}/\mu\text{g/l}$,

min/day bathing $\times \mu\text{g/l}$ chloroform in water $\times 0.001320755 \mu\text{g/min}/\mu\text{g/l}$,

min/day bathing $\times \mu\text{g/l}$ brominated THM in water $\times 0.00129571 \mu\text{g/min}/\mu\text{g/l}$

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 $\mu\text{g/d}$), second (0.0386–0.3496 $\mu\text{g/d}$), and third (0.3496–2.4040 $\mu\text{g/d}$) 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 NO₂ exposure assessment

In this study exposure to ambient NO₂ 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 NO₂ 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 coarse 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 NO₂ exposure to every study subject, the health data base and the environmental NO₂ pollution data base were joined. Every subject's full street address and residential NO₂ pollution level measurement data, and the current residence history data were combined to assess the individual NO₂ pollution exposure. A GIS assigning NO₂ 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 performed 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 NO₂ 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 NO₂. 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 (Eppendorf 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 NO₂ exposure on preterm birth was estimated by logistic regression. We grouped the NO₂ 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 µg/m³ in NO₂ 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 NO₂ 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 NO₂ 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 NO₂. 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 NO₂ 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 ₂ Cl 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)

^aViciunai, Eiguliai, Kleboniskis. ^bPetrasiunai.
^cTTHMs = total trihalomethanes: the sum of CHCl₃ (chloroform), CHBr₂Cl dibromochloromethane), and CHBrCl₂ (bromodichloromethane).
^dSD = 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 Petrasinai 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 0.078–3.64) 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/m²)	<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