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Drinking water disinfection byproducts, genetic polymorphisms, and birth outcomes in a European mother-child cohort study

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Running Head: Water DBPs, genetic variation and birth outcomes

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Abstract

BACKGROUND. We examined the association between exposure during pregnancy to trihalomethanes, the most common water disinfection by-products, and birth outcomes in a European cohort study (HiWate). We took into account exposure through different water uses, measures of water toxicity, and genetic susceptibility.

METHODS. We enrolled 14,005 mothers (2002-2010) and their children from France, Greece, Lithuania, Spain, and the UK. Information on lifestyle- and water-related activities were recorded. We ascertained residential concentrations of trihalomethanes through regulatory records and *ad hoc* sampling campaigns and estimated route-specific trihalomethane uptake by trimester and for whole pregnancy. We examined single nucleotide polymorphisms and copy number variants in disinfection by-product metabolizing genes in nested case-control studies.

RESULTS. Average levels of trihalomethanes ranged from around 10µg/L to above the regulatory limits in the EU of 100 µg/L between centers. There was no association between birth weight and total trihalomethane exposure during pregnancy (beta= 2.2 g in birth weight per 10µg/L of THM, 95%CI -3.3, 7.6). Birthweight was not associated with exposure through different routes or with specific trihalomethane species. Exposure to trihalomethanes was not associated with low birth weight (OR per 10µg/L=1.02, 95%CI 0.95, 1.10), small-for-gestational age (OR=0.99, 0.94, 1.03) and preterm births (OR= 0.98, 0.9, 1.05). We found no gene-environment interactions for mother or child polymorphisms in relation to preterm birth or small-for-gestational age.

CONCLUSIONS. In this large European study we found no association between birth outcomes and trihalomethane exposures during pregnancy in the total population or in potentially genetically susceptible subgroups.

Introduction

Disinfection by-products are formed as a side reaction of water disinfection. Chlorinated water contains hundreds of disinfection by-products of which trihalomethanes and haloacetic acids are the most common compounds. Concern about the potential health risks of exposure to disinfection by-products have focused on cancer[1] and birth outcomes.[2,3] In animal studies high doses of chloroform and various haloacetic acids and haloacetonitriles have been associated with mental growth retardation.[4] At least 20 studies of different design and quality of information have examined the relation between fetal growth and disinfection by-products in humans. A recent meta-analysis found a 1% increased risk for small for gestational age but no evidence for associations between third trimester trihalomethane exposure and low birth weight (LBW), term LBW, preterm births.[5] Only two studies have examined whether genetic variation may affect risk of birth outcomes associated with exposure to trihalomethanes.[6,7]

Exposure assessment has been a major limitation of most studies that have used predominantly ecologic estimates of trihalomethane exposures in water supply zones. Some studies have combined individual information on water use with water zone estimates but fewer have examined different routes of exposure.[2,3,6,8-11] Exposure to trihalomethanes and other volatile disinfection by-products occurs predominantly through inhalation and absorption, during activities such as showering, bathing, and swimming.[12,13] For non-volatile disinfection by-products, such as haloacetic acids, ingestion is the main route of exposure.[14] Epidemiologic studies have used trihalomethanes as a proxy for total disinfection by-product exposure, which may underestimate exposure to disinfection by-products.[15,16]

Analyses of the association of trihalomethanes with birth outcomes for country-specific populations included in HiWate (Health Impacts of Long-Term Exposure to Disinfection By-Products in Drinking Water) have been published previously.[8,9,10,17,18] In this paper we report the results of the analysis of pregnancy outcomes in five pooled mother-child cohorts from France, Greece, Lithuania, Spain, and the UK. We examined exposure to trihalomethanes through drinking, bathing, and showering activities applying the same protocol for exposure assessment and also present associations in potentially genetically susceptible groups in nested case-control studies.

Methods

Study population

This European mother-child cohort study was conducted within the European HiWate project[19] and includes five cohorts (Table 1). We enrolled 14,005 mother-child pairs in 2002-2010 in obstetric units located in parts of five European countries: Greece (Rhea study, Heraklion [Crete]); Spain (INMA-Infancia y Medio Ambiente Project, Sabadell [Catalonia], Valencia, Asturias, Gipuzkoa [Basque Country]); United Kingdom (Born in Bradford study (BiB), Bradford); France (Pelagie study, Brittany); and Lithuania (Kaunas).

Detailed information regarding exposure and outcome of interest was collected from face-to-face interviews or self-reports during pregnancy together with birth records. Questionnaires were used in the five studies to collect information on sociodemographic, lifestyle, nutrition, occupation, medical, and reproductive history, family history, and environmental exposures. Information was available in all studies on the main *a priori* risk factors for birth outcomes, including maternal age and education, socioeconomic status, parity, smoking, and alcohol consumption.

Birth outcomes

Birth weight in grams, head circumference in cm, gestational age (weeks), gender, and mode of delivery were extracted from birth records, where available. Birth weight was analyzed as a continuous outcome, and was dichotomized as low birth weight (LBW), defined as weight less than 2500g, and term-LBW, defined as a birth weight below 2500g after at least 37 completed weeks of gestation.

Newborns small-for-gestational-age (SGA) were defined as those who weighed less than the 10th percentile of the cohort-specific reference of fetal growth, stratified by week of gestation and gender. In two studies (BiB and RHEA) we used customized (internal) models on fetal growth restriction.

Gestational age was based on last menstrual period and/or ultrasound-based estimated date of conception. Analyses related to gestational duration considered both the continuous outcome and dichotomized values of gestational duration (prematurity)

defined as gestation length <37 completed gestational weeks. Mode of delivery was grouped into vaginal deliveries and Caesarean sections.

Exposure assessment for water contaminants

All studies had information on water intake and sources of drinking water, information on showering, bathing and swimming pool use during pregnancy. Information from questionnaires was harmonized prior to the analysis. The evaluation of trihalomethane concentrations in drinking water in the study areas was done through the use of routinely collected trihalomethane data for regulatory purposes and was enhanced with information from disinfection by-product samples collected and measured within the HiWate project. [20] A description of trihalomethane levels available in each center is provided in eAppendix1.

Trihalomethane data were modeled based on available water quality parameters, treatment and water source for the study regions. A separate model was built in each country (region in the case of Spain) for total trihalomethanes, chloroform, and total brominated trihalomethanes following similar methods as discussed in study-specific published papers.[8-10,17, 18] Linear regression and generalized additive models were fitted using geographical and temporal variables (month, year). Among models retaining significant variables (p value<0.05), criteria to select the final model included the adjusted R-squared and the Akaike Information Criteria. General additive models were used to fit a smooth function of level by month that was used to predict levels for months without observations. Final models predicted a monthly level of trihalomethanes from conception until delivery in all study subjects.

Uptake of trihalomethanes (total dose log transformed to normalize) was estimated using a combination of modeled trihalomethanes, information on personal activities via ingestion, showering and bathing and uptake factors based on the literature[21-23] and in an earlier analysis in Spain.[9] Uptake factors used in the analysis for water ingestion were 0.0049 μg/μg/L for total trihalomethanes, 0.00490196 for chloroform and 0.00111848 for brominated trihalomethanes; for showers the corresponding values were 0.001321 μg/min/μg/L, 0.00153626 and 0.00135206; for baths the corresponding values were 0.001538 μg/min/μg/L, 0.00132075 and 0.00129571. Since a bromoform uptake factor was only available for showering, the average of bromodichloromethane and

dibromochloromethane uptake factors were used for the three brominated trihalomethanes. Swimming pool uptake factors and calculation of uptake from swimming was available only in the Spanish INMA cohort[9] and are not reported. A 90% reduction in ingestion was applied if a home filter was used; no information was available for factors that could affect efficiency of trihalomethane removal such as frequency at which filters were changed. Average trihalomethane uptake over the whole pregnancy was calculated, as well as in the first, second, and third trimesters separately in order to allow for evaluation of critical windows of exposure. Bathing and showering uptakes were added, and total household uptake was calculated by adding ingestion, showering, and bathing.

Genetic nested case-control study

We designed nested case-control studies on preterm births and a combination of small for gestational age (SGA) to evaluate the potential influence of genetic polymorphisms in connection to exposure to trihalomethanes. Controls were selected from the cohorts and were matched to cases on ethnic group or country of origin and sex, and were not preterm, not SGA and not large for gestational age. The same set of controls was used for the analysis of both phenotypes. A total of 2159 DNA samples were included initially in the study and of those 1908 were finally included in the analysis. There were 964 maternal samples (348 SGA, 251 preterm, 395 controls) and 944 child DNAs (349 SGA, 218 preterm and 400 controls); these numbers do not add because a small proportion of children were included in both the SGA and the preterm analysis. Maternal blood was not available in the Pelagie (France) cohort. Genotyping was not available for BiB at the time of this analysis. DNA extraction methods can be found elsewhere.[24] DNA was quantified using the PicoGreen dsDNA kit (Invitrogen) and normalized to 40-60 ng/μL.

Candidate genes that are known to participate in disinfection by-product detoxification were examined, including: *CYP1A locus* (*CYP1A1* and *CYP1A2*), *CYP2A6, CYP2D6, CYP3A locus* (*CYP3A4, CYP3A5, CYP3A7 and CYP3A43), CYP2E1, GSTZ1* and *GSTT locus* (*GSTT1, GSTT2* and *GSTT2B*). Genetic variants in detoxifying genes *GSTM* and *GSTA loci* were also explored. A detailed description of the selection of single nucleotide polymorphisms (SNPs) and copy number variants (CNVs), genotyping

procedures and quality control are presented in the online supplement on methods and in online eTable 1.

Statistical analysis

We evaluated the association between birth weight and average residential trihalomethane levels by linear regression, adjusting for gestational age and other potential confounders. Trihalomethane exposures were examined in three groups: total trihalomethanes, chloroform and brominated trihalomethanes. Adjustment for potential confounders was predefined based on prior knowledge on potential confounders with two levels of adjustment applied. A basic model included variables expected to be available in all subjects of all participating studies and that had been used in most previous studies: center (and in Spain, also area, Gipuzkoa [Basque Country], Sabadell, Asturias and Valencia), infant sex, gestational age linear and quadratic term, mother's ethnicity and parity. We included infant sex in the models to be compatible with previous studies although infant sex should not be considered a typical confounder; results changed minimally (second decimal) when not adjusting for sex (not shown). Further adjusted models included, in addition, maternal age, maternal height, maternal pre-pregnancy weight, maternal education, and maternal smoking during pregnancy; results for these models were similar to those of the basic adjustment models and are not shown. Logarithmic transformation of uptake of trihalomethanes was used in corresponding models. We used logistic regression was used to analyze dichotomous outcomes adjusting for potential confounders, and general additive models were used to evaluate the shape of the dose-response curve. A meta-analysis was performed to take into account the heterogeneity between the cohorts. A random effects model was used and a heterogeneity test based on the Q-statistic was performed considering a p-value below 0.10 as statistically significant.

We assessed gene-environment (G*E) interactions in the nested case-control study for total trihalomethanes only using unconditional logistic regression, adjusting for infant sex, ethnicity, parity, smoking during pregnancy, and cohort. Dominant genetic models were tested. In order to take into account the heterogeneous distribution of both DBPs and allele frequencies of selected SNPs in the cohorts, we included cohort-SNP and cohort-TTHM interaction terms in the models. Only those associations with consistent results between these statistical models were reported as significant results. To control

for multiple testing, we applied Bonferroni correction for the 43 independent associations examined (p-value 0.0011).

Ethics

The protocols of all studies were approved by local ethics committees. All subjects signed a consent form that includes the use of genetic data. Standard procedures for the protection of confidential individual information have been applied. Information that might identify a specific individual was never released or transferred between participating centers.

Results

Total trihalomethane levels in the water differed considerably between the regions of the European countries studied depending on water source and type of treatment (Figure). Differences were also observed within countries. The highest levels (average total trihalomethanes above the regulatory limit in the EU of 100μg/L) were observed in parts of Spain (Sabadell), levels around 50μg/L were observed in France and the UK, while the lowest levels (around 10μg/L) were observed in some parts of Spain and in Lithuania and Greece. The distribution of the individual trihalomethanes also differed between the parts of the countries studied with proportionally high levels of brominated compounds found in two of the regions in Spain studied (Sabadell, Valencia) and France compared to the UK or the Basque Country where the main exposure was to chloroform. Between the two low-level regions in the two other countries, Heraklion (Greece) had almost exclusive exposure to brominated compounds, whereas Kaunas (Lithuania) was dominated by chlorinated trihalomethanes.

The overall mean birth weight was 3333gr kg (SD 521) and differed between cohorts (Greece, 3179 (457); Spain, 3256 (478); France, 3391 (493); Lithuania 3447 (522); UK 3229 (567)). A high proportion of preterm births was observed in the Rhea cohort in Crete (11.6%). There were wide differences in the level of education and in smoking between cohorts in the regions studied (Table 1).

Water use and other water activities differed by region studied (Table 2). Tap water consumption was highest in Bradford (UK) (83%) and lowest in Heraklion (Greece) (18%). Baths were less frequent in the two regions studied in southern Europe.

Swimming pool use varied, with very low use in Heraklion (Greece) (2%) and higher use in Brittany (France) and Spain.

Main associations with trihalomethane exposure

There was no association between birth weight and total trihalomethane exposure during pregnancy (Table 3). The change in birth weight in grams per 10µg/L increase in total trihalomethanes was 2.2g (95%CI -3.3, 7.6) for the total cohort. The corresponding beta-coefficients and 95%CI for the individual cohorts were 2.7 (-9.9, 15.3) for Pelagie in Brittany (France), 73.8 (-17.9, 165) for Rhea in Heraklion (Greece), 8.3 (-4.9, 21.5) for Kaunas in Lithuania, -0.2 (-6.8, 6.4) for INMA in Spain, and -27.5 (-70.2, -15.2) for BiB in the UK. Differences in estimates between countries were examined through a random effects meta-analysis and were not statistically heterogeneous (chi-square 5.45, d.f. = 4, p-value = 0.244). We observed no association for exposure to chloroform or brominated trihalomethanes, nor any indication of a dose-response relationship evaluated through the use of quartiles of trihalomethane exposure (Table 3) or the use of splines (not shown). There was no indication of differences in risk by trimester of exposure for total trihalomethanes (Table 3) or for specific trihalomethanes (not shown).

There was no association of birth weight change with uptake of THMs. The change in birth weight in grams (and 95% confidence interval) for a 10% increase in total trihalomethanes, chloroform ($CHCl_3$) and total brominated concentrations through ingestion, shower/bath, or total uptake during the whole pregnancy adjusted for potential confounders are shown in Table 3. There were marked differences in uptake of trihalomethanes through ingestion, showers, and baths between cohorts, reflecting mostly the differences in trihalomethane concentrations in water in each region (country) studied. The total uptake is defined mostly (around 90%) by uptake from showers and baths.

We found no association with exposure to trihalomethanes per 10µg/L increase for term LBW (OR=1.04, 95%CI 0.96, 1.14), small-for-gestational age (OR= 0.99, 95%CI 0.94, 1.03) and preterm births (OR= 0.98, 95%CI 0.9, 1.05) (Table 4). Similarly, risks were close to null when examining separately exposure to chloroform or brominated compounds. No pattern was seen for a dose response when examining quartiles of exposure or using generalized additive models and these birth outcomes for total

trihalomethanes or for chloroform or brominated compounds (not shown). Uptake through any route or total uptake, were also not associated with any of these outcomes (not shown).

Nested case control studies on gene–environment interactions

We examined gene–environment interactions for maternal and child genotypes in relation to preterm births and SGA for total trihalomethanes (Table 5). Two genetic variants of the mother (rs743535 in *CYP2E1* and *GSTT1* CNV) modified the effect of trihalomethane levels on SGA with a p-value for the interaction of less than 0.05. In particular, among those mothers bearing *GSTT1* null genotype, an increased risk was observed for SGA for a 10 µg/L increase in total THMs (OR=1.4, 95% CI 0.9-2.1). None of these interactions persisted after Bonferroni correction for multiple comparisons. Detailed results both on the main genetic effects and on gene–environment interactions on birth outcomes are shown for preterm births in online eTable 2 and for SGA in eTable 3.

Discussion

In this large European study, exposure to trihalomethanes during pregnancy was not associated with birth weight, small for gestational age, low birth weight, or preterm birth despite the high concentrations of trihalomethanes and/or bromine-containing species in some areas. Results were consistent between European regions studied. We found little evidence of the potential modification of the effect of exposure to DBPs by genetic susceptibility.

The epidemiologic evidence evaluating associations between trihalomethane exposure during pregnancy and fetal growth is extensive. Different methodologies, particularly in exposure assessment, and different exposures and characteristics of the study populations hamper comparisons between studies. Experimental evidence suggests trihalomethanes have a harmful effect on fetal growth, but this has not been confirmed in epidemiologic studies. Our results support other recent studies that have applied robust exposure assessments of disinfection by-products. [2,3] Our results were consistent

across centers, particularly for residential trihalomethane uptake, providing robust combined risk estimates. There is no evidence for an association between preterm delivery and trihalomethane exposure during pregnancy, with most of the studies finding either a null association[25,26] or a protective effect. [2,27] The lack of any association in our study is thus consistent with the conclusions of a recent meta-analysis.[5]

There is limited evidence on the potential effect modification by genetic variants of key genes for disinfection by-product detoxification in relation to adverse reproductive outcomes. In this European study we have evaluated an extensive list of genes known to participate in disinfection by-product detoxification, exploring common polymorphisms by using tag SNPs and copy number variants. The two interactions reported here, which did not remain significant after multiple testing comparisons, account for maternal genetic variants in *CYP2E1* and in *GSTT1* genes, two of the most relevant genes for DBP detoxification. The lack of association between these same variants and SGA in the offspring might reflect time and tissue specificity expression of the detoxification genes. In contrast to what has been seen for bladder cancer,[28] we found that total trihalomethanes were associated with increased SGA risk among children whose mothers had no copies of a *GSTT1* copy number variant. Our results are in line with those reported by Kogevinas et al. (2010),[29] where exposure to total trihalomethanes had a mutagenic effect in lymphocytes (increased the number of micronuclei) among *GSTT1* null subjects, but not in subjects having at least one copy of the *GSTT1* gene. *GSTT1* gene lies in a complex genomic region rich in copy number variants and encompassing three highly similar genes: *GSTT1*, *GSTT2* and *GSTT2B*.[30]

A strength of our study is the prospective cohort design, with individual follow-up of pregnancies to determine reproductive outcomes. Most mothers in the European study self reported the last menstrual period during the first trimester of pregnancy when recall is more accurate, and we were able to correct with ultrasound dating. All of the cohorts had detailed individual-level information available on potential confounders/risk factors, which we were able to adjust for in the analyses. This is one of the few studies with detailed water and water-based fluids consumption and report of personal water-related activities during pregnancy. Similar to all the key recent studies, we were able to comprehensively evaluate uptake of trihalomethanes by routes of

exposure: ingestion, inhalation, and dermal exposure. The between-country comparisons are informative since cultural differences are related to differential use of water related activities. Assessment of exposure from showers and baths is particularly important since in several countries exposure to trihalomethanes through ingestion was very low due to the high consumption of bottled water by pregnant women, e.g., Spain and the regions in Greece and Lithuania studied.

We have based our exposure assessment on trihalomethanes, which are the most prevalent group of disinfection by-products, using thousands of measurements of these compounds in the study areas. Even though most measurements of trihalomethanes took place simultaneously with the development of the cohorts there is, undoubtedly, (nondifferential) misclassification regarding the evaluation of individual trihalomethane exposures. What is probably more important is misclassification regarding the evaluation of water toxicity when using trihalomethanes as the basis for the evaluation of the toxicity. Information on other major contaminants, particularly the haloacetic acids were not available for all centers. Similar trihalomethane concentrations in different regions/countries could potentially mask different mixtures of disinfection by-products with different toxicities. We analyzed chloroform separately from the brominated trihalomethanes that were more prevalent in some areas (e.g., two regions in Spain, and the regions studied in France and Greece) as compared to Lithuania and the UK sites and one of the Spanish regions. This is important, as bromine-containing disinfection by-products are of higher health concern. Moreover, the presence of high levels of bromine-containing trihalomethanes will mean there are high levels of other bromine-containing disinfection by-products,[31] some of which are much more toxic than the regulated trihalomethanes.[32, 33] New methods for evaluating water toxicity, requiring prospective collection of water samples will be needed in future epidemiologic studies to cover this complex pattern of water toxicity.

Overall, in this study of around 14,000 mother child pairs in eight regions in five European countries with a variation in average levels of total and specific trihalomethanes, we did not find substantial associations between exposure to trihalomethanes during pregnancy and reproductive outcomes. Although a large part of the population included subjects with low-level exposures, a considerable proportion was exposed to concentrations around 50 μ/L that have been associated with a 50%

increase in bladder cancer risk [1,34], while exposures measured by one center were above the US and EU regulatory limits (80 and 100 μ//L, respectively). The evaluation of genetically susceptible individuals indicated the possibility of gene-environment interactions for genes known to be associated with the metabolism of specific DBPs such as *GSTT1* or *GSTZ1*, but none of these interactions remained after correcting for multiple comparisons.

In conclusion, results from the HiWate study, together with those of a recent meta-analysis, indicate the lack of any important association of THMs with a number of adverse birth outcomes.

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Figure legend

Figure 1. Distribution of total trihalomethanes (THM), chloroform ($CHCl_3$) and total brominated trihalomethane concentrations at the residence during the whole pregnancy in the study population in the regions of the 5 countries studied, HiWate study.

Table 1. Subjects, birth outcomes, lifestyle and water related variables in the European study population and by cohort. Numbers and percentages or mean and standard deviation (SD), HiWate cohort (n= 14005) [a]

	ALL	**(Heraklion) Greece**	**(4 areas) Spain**	**(Britanny) France**	**(Kaunas) Lithuania**	**(Bradford) UK**
	14005	1359 (10%)	2473 (18%)	3322 (24%)	4158 (30%)	2693 (19%)
Sex of newborn						
Female	6835 (49%)	670 (49%)	1200 (49%)	1640 (49%)	2030 (49%)	1295 (48%)
Male	7164 (51%)	689 (51%)	1273 (52%)	1681 (51%)	2128 (51%)	1393 (52%)
Low birth weight	704 (5%)	73 (6%)	125 (5%)	108 (3%)	202 (5%)	196 (7%)
Term Low Birth Weight	295 (2%)	25 (2%)	68 (3%)	39 (1%)	72 (2%)	91 (4%)
Small for Gestational Age	1302 (10%)	102 (9%)	240 (10%)	332 (10%)	412 (10%)	216 (9%)
Gestational age (wks)	39.3 (1.7)	38.2 (1.6)	39.6 (1.7)	39.4 (1.5)	39.2 (1.7)	39.5 (1.9)
Preterm births	791 (6%)	154 (12%)	113 (5%)	126 (4%)	233 (6%)	165 (6%)
BMI mother (kg/m²)						
<20	2392 (18%)	192 (15%)	403 (16%)	854 (26%)	672 (16%)	271 (11%)
20-25	6664	664 (53%)	1421	1867	1749	963

	(49%)		(58%)	(57%)	(42%)	(38%)
	3057		456	413	1207	737
25-30	(22%)	244 (20%)	(18%)	(13%)	(29%)	(29%)
	1575		192			547
>30	(12%)	143 (12%)	(8%)	163 (5%)	530 (13%)	(22%)
Education mother						
	1614		605			477
Low	(12%)	265 (21%)	(25%)	23 (1%)	244 (6%)	(18%)
	6016		1018	1225	1719	1422
Medium	(43%)	632 (50%)	(41%)	(37%)	(41%)	(53%)
	6043		845	2066	2195	579
High	(44%)	358 (29%)	(34%)	(62%)	(53%)	(22%)
	184					184
Other	(1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	(7%)
Parity						
	6438		1390	1473	2032	1059
Nulliparous	(46%)	484 (38%)	(56%)	(45%)	(49%)	(40%)
	7425		1081	1837	2126	1583
1+	(54%)	798 (62%)	(44%)	(56%)	(51%)	(60%)
Smoking during pregnancy, mother						
	10150		1969	1217	3849	2234
Never	(84%)	881 (76%)	(82%)	(71%)	(93%)	(83%)
	1986		438	500		459
Ever	(16%)	280 (24%)	(18%)	(29%)	309 (7%)	(17%)

Second Hand Smoke, mother						
No	5822 (49%)	86 (7%)	900 (38%)	876 (61%)	2110 (51%)	1850 (69%)
Yes	6058 (51%)	1183 (93%)	1499 (63%)	553 (39%)	2000 (49%)	823 (31%)

[a] Totals may not add up because of missing values

Table 2. Tap water consumption and other water activities by region [a]

	ALL	(Heraklion) Greece	(4 areas) Spain	(Brittany) France	(Kaunas) Lithuania	(Bradford) UK
Number of subjects	14005	1359	2473	3322	4158	2693
Tap water						
Yes	7732 (56%)	234 (18%)	1166 (47%)	1964 (60%)	2138 (51%)	2230 (83%)
No	6158 (44%)	1051 (82%)	1307 (53%)	1324 (40%)	2020 (49%)	456 (17%)
Average Liters/day (if yes)	0.83	1.16	0.96	0.29	0.79	1.23
Bottled water [b]						
Yes (main source)	7311 (69%)	972 (76%)	2172 (88%)	2904 (88%)	3249 (78%)	918 (34%)
No	3291 (31%)	313 (24%)	301 (12%)	286 (12%)	909 (22%)	1768 (66%)
Liters/day (if yes)	1.08	1.21	1.04	1.09	1.09	1.02
Showers						
Yes	10610 (76%)	1185 (87%)	2340 (95%)	1455 (99%)	3903 (94%)	1727 (64%)
Minutes/day	11.3	11.1	9.97	8.2	13.0	12
Baths						
Yes	4292 (31%)	71 (5%)	277 (11%)	564 (38%)	1660 (40%)	1720 (64%)
Minutes/day	10.9	12.2	8.95	5.8	8.0	15.7
Only bath						
Yes	981 (7%)	17 (1%)	47 (2%)	33 (1%)	166 (4%)	718 (27%)
Minutes/day	17.7	20.6	19.2	11.9	13.7	18.7
Swimming pools						
Yes	2223 (18%)	25 (2%)	1046 (43%)	603 (32%)	346 (8%)	203 (8%)
No	10136 (82%)	1228 (98%)	1364 (57%)	1258 (68%)	3814 (92%)	2472 (92%)

[a] Numbers may not add up because of missing values. Information on bottled water, showers and baths is available only for a subsample in the Pelagie cohort (France)

Table 3. Beta coefficients showing estimated change in birth weight in grams for a 10 μg/L increase in total trihalomethanes (THM), chloroform ($CHCl_3$), and total brominated trihalomethane levels in drinking water during whole pregnancy and by trimester, and for a 10% increase in uptake of the same compounds through different routes.[a]

	N	Beta coefficient (95% CI)
THMs in drinking water, whole pregnancy		
Total THMs	13098	2.17 (-3.3,7.6)
Chloroform	13098	0.97 (-9.5,11.4)
Brominated	13098	2.54 (-4.7,9.7)
Quartiles of total THM in drinking water, whole pregnancy,		
Quartile 1 (<5.2 μg/L)	3261	Reference
Quartile 2 (5.2-24.22 μg/L)	3301	22.04 (0.5,43.5)
Quartile 3 (24.24-47.4 μg/L)	3279	20.73 (-22.1,63.6)
Quartile 4 (>47.4 μg/L)	3257	16.94 (-24.8,58.6)
Total THMs in drinking water by pregnancy trimester		
First trimester	13098	1.05 (-3.7,5.8)
Second trimester	13098	1.49 (-3.3,6.3)
Third trimester	13090	2.75 (-2.2,7.7)
Uptake total THMs, whole pregnancy		
Ingestion	11036	0.04 (-0.26,0.35)
Shower, baths	11036	0.34 (-0.28,0.96)
Total uptake	11036	0.35 (-0.28,0.98)

Uptake Chloroform, whole pregnancy		
Ingestion	11036	0.02 (-0.26,0.3)
Shower, baths	11036	-0.03 (-0.55,0.49)
Total uptake	11036	-0.03 (-0.55,0.5)
Uptake Brominated THMs, whole pregnancy		
Ingestion	11036	0.06 (-0.17,0.29)
Shower, baths	11036	0.42 (-0.26,1.09)
Total uptake	11036	0.43 (-0.25,1.12)

[a] Beta coefficient (95% Confidence Interval) from linear regression, adjusted for infant sex, gestational age linear and quadratic term, mother ethnicity, parity and cohort

Table 4. Odds Ratio (95% Confidence Interval) of term low birth weight, small for gestational age and preterm births for a 10 μg/L increase of total trihalomethanes (THM), chloroform and total brominated THMs during whole pregnancy adjusted for potential confounders.

	N	OR (95%CI)
Term Low Birth Weight [a]		
Total THMs	12352	1.04 (0.96, 1.14)
Chloroform	12352	1.09 (0.92, 1.3)
Brominated THMs	12352	1.04 (0.94, 1.16)
Small for gestational age [b]		
Total THMs	12646	0.99 (0.94, 1.03)
Chloroform	12646	0.98 (0.89, 1.07)
Brominated THMs	12646	1 (0.94, 1.06)
Preterm births [c]		
Total THMs	13098	0.98 (0.9, 1.05)
Chloroform	13098	0.91 (0.79, 1.05)
Brominated THMs	13098	0.98 (0.89, 1.08)

[a] Term low birth weight: infant sex, gestational age linear and quadratic term, mother's ethnicity, parity and cohort

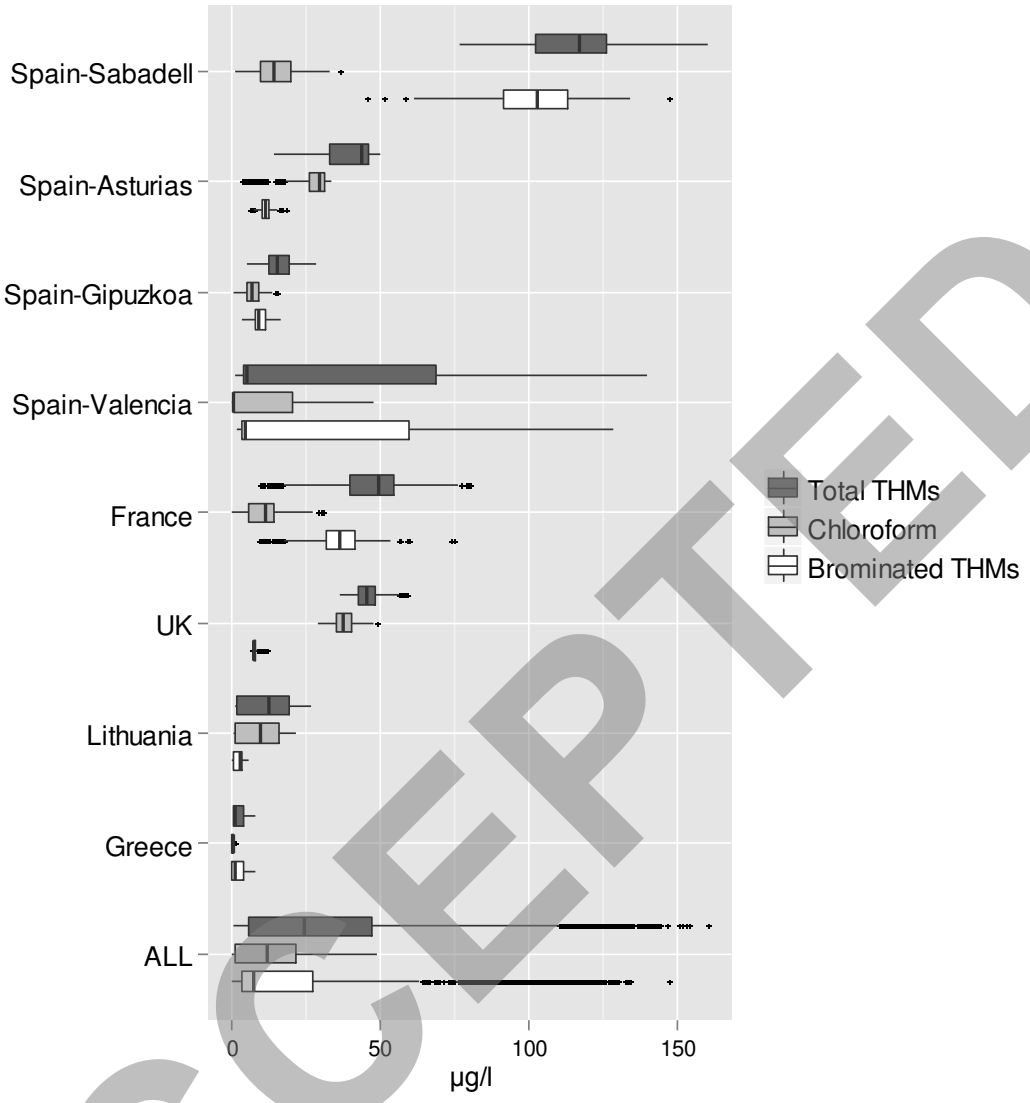
[b] Small for gestational age: mother's ethnicity, parity and cohort

[c] Preterm: birth weight, infant sex, mother's ethnicity, parity and cohort

Table 5. Effect of an increase in exposure by 10 µg/L total trihalomethanes (THM) on the association with small for gestational age (SGA), stratified by maternal genotype (genetic dominant model)

Gene	SNP/CNV	Genotype	N (%) controls	N (%) cases	OR (95%CI)	p value interaction
CYP2E1	rs743535	CC	342 (87.0)	286 (82.7)	1.1 (1.0,1.2)	0.028
		CT-TT	51 (13.0)	60 (17.3)	0.9 (0.6,1.1)	
GSTT1	GSTT1	-/-	69 (18.3)	68 (20.2)	1.4 (0.9,2.1)	0.037
	CNV	-/+, +/+	308 (81.7)	269 (79.8)	1.0 (0.9,1.1)	

Figure 1.



eAppendix1

Drinking water disinfection byproducts, genetic polymorphisms and adverse birth outcomes in a European mother-child prospective study (HiWate)

Authors

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Methods. Exposure assessment for THMs during pregnancy and exposure modeling

A summary for water DBP exposure assessment for each cohort is presented here below. A more detailed account can be found in specific article of each cohort [8,9,10,17, 18] .

INMA cohort, several locations, Spain. Interviews with pregnant women at week 32 of gestation included questions on water use during pregnancy specifically source of drinking water at home (municipal, bottled, private well, other), use of a home water filter, changes in water ingestion since getting pregnant, and frequency and duration of showering, bathing, and swimming pool attendance. Information on ingestion of tap water and beverages made with tap water was requested at weeks 12 and 32 using a food frequency questionnaire. Information on levels of THMs was based on sampling campaigns and regulatory data from local authorities and water companies. Sampling locations were defined to be geographically representative of the study areas, and water samples were collected from taps. THMs were determined in 1092 samples of which 828 were from own sampling. Separate models were conducted for each area of the study to predict levels for each THM (chloroform, bromodichloromethane, dibromochloromethane, bromoform), and for total THM and to assign a concentration to the distribution system of the municipality where women resided. Final models predicted average monthly THMs levels from conception until delivery in each participant's residential water supply.

RHEA cohort, area around Heraklion, Crete, Greece. The main water source in Crete is ground water, while chlorination is the main method of disinfecting drinking water. Information on water-related habits was requested at the first interview at around the third month of pregnancy including drinking water source (municipal, bottled, private well, spring water) at home and other places, average frequency and duration for showering and bathing, use of filter both for drinking and cooking water, type of water used to cook, usual method of dishwashing, use of gloves for dishwashing by hand, frequency and duration of dishwashing per day and swimming pool attendance. Information on water consumption including water and water-based fluids (coffee, tea, and other herbs) was requested through a food frequency questionnaire in two times during pregnancy. Water supply zones for each of the areas of the study were provided by the water supply company. 12 sampling points in urban areas and six in rural areas were selected covering geographically all the water zones of the residences of pregnant women. Women were visited four times at home to collect tap water samples between 2007 and 2009 (72 tap water samples in total). Residential THM and brominated THM levels were calculated for each trimester of pregnancy.

PELAGIE, area around Rennes, France. THM levels in water networks of the maternal residence study areas were estimated from a Ministry of Health database in France which includes regulatory measurements of contaminant levels in water networks including four THMs (chloroform, bromoform, dibromochloromethane and bromodichloromethane) since 2004. Frequency of monitoring depends on the size of the population served by the network. Only women living within water networks with THM measurements were included (88%).Of those 41% had at least 2 THM measurements per year while 19 had monthly measurements. Mixed hierarchical linear models separately for each type of water source were used to impute missing monthly levels of THMs and average environmental THM levels were estimated by trimester of pregnancy. THM levels were estimated for each constituent THM and for the sum of all 4 THMs (total THMs). Information about maternal daily water intake and the percentage of bottled water was collected in early pregnancy while data on shower and bath habits and swimming pool use were collected at the 2-year follow-up after birth and at that time women were asked retrospectively for water habits during pregnancy.

Kaunas, Lithuania. The Kaunas city municipal drinking water is supplied by four water treatment plants systems using groundwater sources and using sodium hypochlorite. Levels in different areas differed considerably with one plant supplying water with higher THM levels than other. 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. Levels of the four regulated THMs (chloroform, bromoform, bromodichloromethane, and dibromochloromethane) were determined. Tap water THM concentration for geocoded maternal address at birth, were calculated as the average of quarterly sample values over the time of pregnancy. Information on water consumption per day including tap water and hot and cold beverages made from tap water was retrieved from questionnaires delivered during pregnancy. Dermal absorption and inhalation was estimated requesting information on showering and bathing.

Born in Bradford, Bradford, UK. The questionnaire delivered during around the 28th week of pregnancy ascertained typical daily consumption of tap water, bottled water, and water based beverages e.g. tea, coffee, squash, at home or elsewhere, use of water filters and frequency and duration of showering, bathing and swimming. The local water treatment supply company provided routine monitoring data for the eight water supply zones covering the study area from 2006 to 2011. On average, each water zone was sampled 9 times per year, with a total of 374 data points. For places for which data were sparse, predictive modeling was applied to estimate DBP concentrations. THM samples below the limit of detection were assigned a value equal to half the LOD. Time-weighted average THM concentrations were calculated for each woman using modeled THM concentrations (µg/l) for the water supply zone of her residence postcode at the time of recruitment for each trimester.

Methods: Selection of genes and SNPs, genotyping and QC

Metabolism of DBPs has been reported to be mediated by enzymes from the GST and CYP families, but little is known about the importance of these genes in an evaluation of the effect of a complex mixture of DBPs. *CYP2E1*, *CYP1A2*, *CYP3A4,* and *CYP2A6* genes have been involved in the metabolism of chloroform and bromodichloromethane (Allis and Zhao 2002; Gemma et al. 2003; Leavens et al. 2007; Zhao and Allis 2002), and *CYP2E1* is believed to be the major THM detoxifier. *CYP2D6* variants have been found to modify THM blood levels after showering (Backer et al. 2008). Among *GST* genes, *GSTZ1* catalyzes the oxygenation of dichloroacetic acid (DCA) to glyoxylic acid

and plays a critical role in the tyrosine degradation pathway and in alpha-haloacid metabolism (Board and Anders 2005). Expression of the human *GSTT1* gene in a strain of *Salmonella* was seen to activate brominated THMs to mutagens, but not chloroform (DeMarini et al. 1997; Pegram et al. 1997). An evaluation of a CNV including *GSTT1* gene indicated significantly stronger associations between total THM exposure and bladder cancer among subjects with the presence of at least one copy of the *GSTT1* gene than among subjects with deletions in both alleles (Cantor et al. 2010).

Tag Single Nucleotide Polymorphisms (SNPs) as well as putative functional variants were selected. Tag SNPs were obtained using a pair-tagging strategy (r^2>0.8) from CEU HapMap data (Rel24, phase II Nov08, on NCBI B36 assembly, dbSNP b126) after filtering for a MAF>0.05, HWE p value>0.05, 1 Mendelian error and <20% missing genotypes. Five kb upstream and downstream of each gene were included in the design. When possible, putative functional variants were forced to be included as tags. Functional variants were defined as variants that modify enzymatic activity, that produce an amino acid change or that change expression and were obtained from publicly available libraries and bibliography (http://www.cypalleles.ki.se/, http://www.pharmgkb.org/search/annotatedGene/index.jsp, http://eqtl.uchicago.edu/). A minimum MAF of 0.01 was defined for functional variants. Some of the DBP detoxification genes are located in Copy Number Variant (CNV) regions and 4 common CNVs (encompassing *GSTT1, GSTT2B* or *GSTM1* genes; and near *GSTM4 gene*) were selected. Finally a SNP in chrY was included to detect sex inconsistencies (Supplementary Table 2).

A total of 75 SNPs were genotyped using Illumina Golden Gate technology at the Barcelona Node of the Spanish Genotyping Center (CEGEN-Barcelona). Genotype calling was performed with the GenomeStudio software (Illumina). Each 96-plate contained 1 HapMap trio as a positive control giving consistent results among replicates and in comparison with genotypes listed in the HapMap database. Additionally, 69 HiWATE samples were replicated giving consistent results.

A total of 2159 DNA samples (maternal or child) were included initially in the study. Thirty-six samples failed genotyping, 14 mother-child pairs accumulated Mendelian errors (>3), 24 mother-child pairs had sex errors, and they were excluded from the analysis. After merging genetic data with exposure data and covariates, the following number of subjects were available: 395 mother controls, 348 mother SGA, 251 mother preterm, 400 child controls, 349 child SGA, 218 child preterm (N total 1908). These numbers do not add because a small proportion of children were included in both the SGA and the preterm analysis. After SNP quality control (Hardy Weinberg equilibrium p value<0.05, call rate, MAF), 39 SNPs were included in subsequent analysis (Supplementary Table S2).

Four common CNVs (GSTM1, GSTM4, GSTT1 and GSTT2B) were genotyped using the Multiplex Ligation-dependent Probe Amplification (MLPA) method (MRC-Holland) following the manufacturer's instructions with minimal modifications. A detailed protocol and probe sequences can be found elsewhere (Bustamante et al. 2012). A HapMap trio, with known genotypes for the four common CNVs was included in each 96-plate. All HapMap genotypes for the common CNVs were consistent among plates and comparing with genotypes generated by PCR in the lab or in the literature (Zhao et al. 2009) or by aCGH in the literature (http://www.sanger.ac.uk/humgen/cnv/). We also included 62 HiWATE replicates and error rate was <1%. CNV genotypes were extracted from MLPA data using the CNV assoc package (Subirana et al. 2011) and all of them had high classification scores. A 90% certainty was used to determine CNV

genotype as 0, 1 or 2 copies. CNV genotypes were available for 873 child and 929 mother samples (Supplementary Genetics Table S2).

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eTables

eTable 1. Descriptive of the SNPs genotyped in mothers and children

						Maternal subset			Child subset		
Gene	SNP	Selection	Chr	hg18	alleles	call rate	MAF	HWE *P* value	call rate	MAF	HWE *P* value
CYP3A4-A5-A7-A43	rs4646450	tag	7	99104253	C/T	99.8	15.5	0.377	100.0	16.8	0.861
CYP3A4-A5-A7-A43	rs776746	functional, eQTL, tag	7	99108474	G/A	99.9	7.0	0.213	99.9	6.9	0.030
CYP3A4-A5-A7-A43	rs2257401	functional, tag, T409R	7	99144620	G/C	99.9	8.7	0.745	100.0	8.7	0.016
CYP3A4-A5-A7-A43	rs2014764	tag	7	99185441							
CYP3A4-A5-A7-A43	rs12333983	tag	7	99192049	T/A	99.9	9.4	0.192	99.9	9.9	0.067
CYP3A4-A5-A7-A43	rs2242480	tag	7	99199401	C/T	99.6	9.1	0.340	99.6	9.2	0.034
CYP3A4-A5-A7-A43	rs28371759	functional	7	99199561	T	98.4	0.0	-	98.2	0.0	-
CYP3A4-A5-A7-A43	rs4646437	tag	7	99203018	C/T	100.0	9.5	0.514	99.9	10.0	0.141
CYP3A4-A5-A7-A43	rs2246709	tag	7	99203654	G	98.4	0.0	-	98.2	0.0	-
CYP3A4-A5-A7-A43	rs2740574	functional	7	99220031	A/G	99.7	3.4	0.437	99.7	3.3	0.498
CYP3A4-A5-A7-A43	rs651430	tag	7	99267778	T/C	100.0	49.6	0.314	100.0	48.6	0.318
CYP3A4-A5-A7-A43	rs678040	tag	7	99284981	T/C	99.9	8.1	1.000	99.7	6.7	0.130
CYP3A4-A5-A7-A43	rs680055	tag, A340P	7	99295540							
CYP3A4-A5-A7-A43	rs472660	tag	7	99298042	C/T	100.0	12.4	0.817	100.0	11.4	0.051
CYP3A4-A5-A7-A43	rs474229	tag	7	99304493	A/C	100.0	41.7	0.473	100.0	41.6	0.836
CYP3A4-A5-A7-A43	rs559239	tag	7	99304933	T/C	99.4	5.0	1.000	99.9	5.0	0.255
CYP2E1	rs10857735	tag	10	135186219	C/A	100.0	9.0	0.502	100.0	9.3	0.036
CYP2E1	rs2070673	tag	10	135190556							
CYP2E1	rs6413420	tag	10	135190818	G/T	99.9	5.5	1.000	99.6	4.6	0.618
CYP2E1	rs915906	tag	10	135193727	T/C	98.4	14.4	0.300	98.9	14.8	0.852
CYP2E1	rs2070674	tag	10	135195329	C/T	99.9	2.6		99.6	2.5	0.036
CYP2E1	rs2070675	tag	10	135196685	C/T	98.5	17.3	1.000	98.8	16.8	0.862
CYP2E1	rs915908	tag	10	135196948	G/A	98.8	14.9	0.137	98.8	15.2	0.329

						Maternal subset			Child subset		
Gene	SNP	Selection	Chr	hg18	alleles	call rate	MAF	HWE *P* value	call rate	MAF	HWE *P* value
CYP2E1	rs743535	tag	10	135199356	C/T	99.0	7.6	0.413	98.9	8.5	0.732
CYP2E1	rs2070676	tag	10	135201126							
CYP2E1	rs2249695	tag	10	135202157	C/T	94.2	18.8	1.000	93.3	19.4	0.873
CYP2E1	rs4512750	eQTL	10	135208653	C/T	94.2	18.8	0.737	93.2	19.3	0.874
GSTZ1	rs4899651	tag	14	76854214	G/A	100.0	24.8	0.574	100.0	24.0	0.780
GSTZ1	rs8177539	tag	14	76857511	G/A	100.0	4.5	1.000	100.0	5.6	0.687
GSTZ1	rs2363643	tag	14	76858660	G/A	99.9	32.9	0.575	99.9	31.1	0.255
GSTZ1	rs2270422	tag	14	76862576	G/C	99.9	40.4	1.000	99.7	40.4	0.400
GSTZ1	rs7972	tag, R42G	14	76862989							
GSTZ1	rs2287396	tag	14	76863944	C/T	99.7	17.2	0.125	99.3	16.9	0.021
GSTZ1	rs1046428	tag, T82M	14	76864035	C/T	99.9	18.2	0.210	100.0	19.7	0.871
GSTZ1	rs11624726	tag	14	76870567	C/T	99.9	30.0	0.406	99.9	30.6	0.421
GSTZ1	rs7975	K32E	14	77793207							
CYP1A1-A2	rs1048943	I462V	15	72800037	A/G	99.7	3.6	0.100	100.0	3.5	0.000
CYP1A1-A2	rs4646421	tag	15	72803244	C/T	89.5	9.2	0.055	97.5	9.3	0.036
CYP1A1-A2	rs2470893	tag	15	72806501	G/A	99.9	22.8	0.790	100.0	24.7	0.894
CYP1A1-A2	rs4886605	tag	15	72813040	C/T	100.0	15.8	0.675	99.8	16.0	0.463
CYP1A1-A2	rs2472297	tag	15	72814932	C/T	99.8	16.1	0.725	99.9	17.9	0.244
CYP1A1-A2	rs2069514	functional	15	72825272							
CYP1A1-A2	rs762551	tag, functional	15	72828969	A/C	98.1	34.8	0.580	99.2	36.0	1.000
CYP1A1-A2	rs11854147	tag	15	72839823	C/T	99.3	38.0	0.665	99.8	38.7	0.914
CYP2A6	rs7246742	tag	19	46037234	T/G	99.4	18.0	0.407	99.7	16.2	0.267
CYP2A6	rs1801272	functional	19	46046372	T/A	99.6	2.6	0.370	98.9	2.6	0.000
CYP2A6	rs28399433	functional	19	46048218							
CYP2A6	rs4105144	tag	19	46050463							
CYP2A6	rs12973598	tag	19	46077673							
GSTT1-2-2B	rs4820571	eQTL	22	22572972	G/A	100.0	40.4	0.674	99.9	40.6	0.098
GSTT1-2-2B	rs6003959	eQTL	22	22594394	C/T	100.0	23.8	1.000	99.9	24.1	0.166
GSTT1-2-2B	rs1892715	tag	22	22599429	T/C	99.9	19.0	0.302	100.0	18.5	1.000
GSTT1-2-2B	rs1006771	tag GSTT2B	22	22644005	T/G	96.4	34.5	0.724	99.2	36.0	0.740

						Maternal subset			Child subset		
Gene	SNP	Selection	Chr	hg18	alleles	call rate	MAF	HWE *P* value	call rate	MAF	HWE *P* value
GSTT1-2-2B	rs5996646	functional	22	22653226							
GSTT1-2-2B	rs140199	tag GSTT2B	22	22656782							
GSTT1-2-2B	rs5760147	tag	22	22664947							
GSTT1-2-2B	rs140289	tag	22	22666326	C/T	98.8	26.0	0.419	99.8	25.4	0.680
GSTT1-2-2B	rs2266637	I169V	22	22706844	G	79.1	0.0	-	79.0	0.0	-
GSTT1-2-2B	rs8138555	tag	22	22730359	A/C	99.9	17.4	0.268	100.0	18.3	0.483
GSTT1-2-2B	rs5760176	tag GSTT1	22	22732320	G/A	99.8	48.0	0.420	99.9	49.5	0.045
GSTT1-2-2B	rs738809	tag, eQTL	22	22735491	A/G	99.8	29.4	0.629	99.9	28.4	0.168
GSTT1-2-2B	rs422674	tag	22	22736777	C/A	99.7	33.4	0.909	99.9	34.3	0.740
GSTT1-2-2B	rs11090305	tag	22	22737482	T/C	97.9	21.8	0.398	99.5	21.0	0.888
GSTT1-2-2B	rs62623405	R196X	22	24325648							
CYP2D6	rs1062753	eQTL	22	40722756	G/A	99.7	27.4	0.200	99.9	27.9	0.802
CYP2D6	rs8138080	eQTL	22	40726316	G/A	99.9	27.4	0.201	100.0	27.9	0.802
CYP2D6	rs5751222	tag	22	40847865	T/A	98.0	18.9	0.462	99.9	20.4	0.078
CYP2D6	rs6002626	tag	22	40847932	G/C	99.9	43.6	0.613	99.8	42.6	0.919
CYP2D6	rs764481	tag	22	40848369	G/A	100.0	37.1	0.594	100.0	36.7	0.667
CYP2D6	rs1135840	functional, S486T	22	40852556	C/G	97.0	43.2	0.177	97.5	42.2	0.029
CYP2D6	rs28371725	functional	22	40853748							
CYP2D6	rs16947	functional, R296C	22	40853886	G	98.4	0.0	-	98.1	0.0	-
CYP2D6	rs1065852	functional, P34S	22	40856637							
CYP2D6	rs769258	functional, M11V	22	40856706							
CYP2D6	rs17478227	eQTL	22	40984270	C/G	99.9	17.3	0.061	99.9	18.6	0.305
GSTM-45	GSTM-45	CNV	22	-	-/+	100.0	37.8	0.330	99.9	38.4	0.830
GSTM1	GSTM1	CNV	22	-	-/+	98.6	28.5	0.224	98.6	30.6	0.643
GSTT2B	GSTT2B	CNV	22	-	-/+	100.0	43.2	0.750	99.7	42.6	0.748
GSTT1	GSTT1	CNV	22	-	-/+	99.9	43.7	0.833	99.5	42.5	0.011

HWE= Hardy Weinberg equilibrium
eQTL=expression quantitative trait locus
functional=produce a change in enzyme activity or inducibility in vitro

eTable 2. Main genetic effects and G*E interaction between SNPs and CNVs and tTHMs (per 10µg/L) in relation to preterm*.

Maternal and child genotypes (genetic dominant model)

		Maternal DNA			Child DNA		
		Genetic main effects		G*E	Genetic main effects		G*E
Gene	SNP	OR (95% IC)	*P* value	*P* value	OR (95% IC)	*P* value	*P* value
CYP3A4-A5-A7-A43	rs4646450	0.79 (0.55,1.14)	0.210	0.741	0.94 (0.65,1.36)	0.763	0.114
CYP3A4-A5-A7-A43	rs12333983	1.17 (0.76,1.80)	0.465	0.131	0.99 (0.63,1.54)	0.963	0.702
CYP3A4-A5-A7-A43	rs4646437	1.16 (0.75,1.78)	0.502	0.118	1.02 (0.65,1.58)	0.928	0.632
CYP3A4-A5-A7-A43	rs651430	1.32 (0.90,1.96)	0.154	0.466	1.08 (0.73,1.61)	0.701	0.455
CYP3A4-A5-A7-A43	rs678040	1.04 (0.67,1.60)	0.866	0.582	1.06 (0.65,1.70)	0.815	0.759
CYP3A4-A5-A7-A43	rs472660	1.34 (0.91,1.96)	0.134	0.983	1.08 (0.71,1.62)	0.708	0.983
CYP3A4-A5-A7-A43	rs474229	0.86 (0.61,1.22)	0.401	0.570	0.96 (0.67,1.38)	0.824	0.929
CYP2E1	rs915906	0.90 (0.62,1.31)	0.587	0.539	0.60 (0.39,0.90)	0.015	0.455
CYP2E1	rs2070675	1.18 (0.82,1.69)	0.376	0.983	0.89 (0.60,1.30)	0.548	0.520
CYP2E1	rs915908	0.84 (0.57,1.23)	0.374	0.062	1.14 (0.77,1.68)	0.516	0.340
CYP2E1	rs743535	1.30 (0.79,2.12)	0.298	0.574	1.09 (0.67,1.77)	0.720	0.172
CYP2E1	rs2249695	0.94 (0.65,1.34)	0.719	0.505	0.75 (0.51,1.09)	0.134	0.173
CYP2E1	rs4512750	0.96 (0.67,1.38)	0.831	0.559	0.72 (0.49,1.05)	0.091	0.147
GSTZ1	rs4899651	1.20 (0.86,1.67)	0.283	0.045	1.10 (0.77,1.56)	0.608	0.074

		Maternal DNA			Child DNA		
		Genetic main effects		G*E	Genetic main effects		G*E
Gene	SNP	OR (95% IC)	*P* value	*P* value	OR (95% IC)	*P* value	*P* value
GSTZ1	rs2363643	0.87 (0.62,1.21)	0.406	0.253	0.79 (0.56,1.12)	0.190	0.194
GSTZ1	rs2270422	1.00 (0.71,1.41)	0.997	0.679	1.07 (0.74,1.53)	0.731	0.854
GSTZ1	rs1046428	1.21 (0.84,1.73)	0.307	0.926	1.25 (0.87,1.80)	0.228	0.301
GSTZ1	rs11624726	1.02 (0.73,1.42)	0.928	0.653	0.78 (0.55,1.11)	0.171	0.055
CYP1A1-A2	rs2470893	0.62 (0.43,0.88)	0.008	0.651	0.76 (0.54,1.09)	0.136	0.095
CYP1A1-A2	rs4886605	1.35 (0.93,1.98)	0.118	0.217	1.19 (0.81,1.73)	0.375	0.335
CYP1A1-A2	rs2472297	0.68 (0.46,1.01)	0.056	0.654	0.73 (0.49,1.07)	0.105	0.078
CYP1A1-A2	rs762551	0.93 (0.66,1.30)	0.662	0.334	1.17 (0.82,1.67)	0.377	0.595
CYP1A1-A2	rs11854147	1.19 (0.84,1.68)	0.326	0.638	1.23 (0.86,1.76)	0.255	0.589
CYP2A6	rs7246742	0.95 (0.67,1.35)	0.790	0.909	0.94 (0.64,1.36)	0.734	0.682
GSTT1-2-2B	rs4820571	1.22 (0.86,1.73)	0.265	0.930	1.13 (0.79,1.63)	0.490	0.837
GSTT1-2-2B	rs6003959	1.26 (0.90,1.76)	0.173	0.934	1.07 (0.75,1.51)	0.705	0.668
GSTT1-2-2B	rs1892715	1.13 (0.80,1.59)	0.498	0.609	1.14 (0.79,1.64)	0.476	0.960
GSTT1-2-2B	rs1006771	1.33 (0.94,1.88)	0.103	0.589	1.22 (0.86,1.74)	0.269	0.702
GSTT1-2-2B	rs140289	1.22 (0.87,1.70)	0.251	0.080	1.17 (0.82,1.65)	0.382	0.238
GSTT1-2-2B	rs8138555	1.11 (0.78,1.57)	0.576	0.316	1.27 (0.89,1.82)	0.186	0.186

		Maternal DNA			Child DNA		
		Genetic main effects		G*E	Genetic main effects		G*E
Gene	SNP	OR (95% IC)	*P* value	*P* value	OR (95% IC)	*P* value	*P* value
GSTT1-2-2B	rs738809	0.96 (0.69,1.34)	0.827	0.945	1.12 (0.79,1.58)	0.525	0.729
GSTT1-2-2B	rs422674	0.85 (0.61,1.19)	0.338	0.427	0.89 (0.63,1.27)	0.525	0.706
GSTT1-2-2B	rs11090305	0.86 (0.61,1.22)	0.397	0.243	0.77 (0.54,1.10)	0.156	0.221
CYP2D6	rs1062753	0.94 (0.68,1.31)	0.735	0.560	0.98 (0.70,1.39)	0.927	0.138
CYP2D6	rs8138080	0.95 (0.68,1.32)	0.762	0.573	0.98 (0.69,1.37)	0.889	0.262
CYP2D6	rs5751222	1.29 (0.90,1.83)	0.167	0.862	1.32 (0.93,1.89)	0.122	0.685
CYP2D6	rs6002626	0.73 (0.52,1.04)	0.083	0.885	0.76 (0.53,1.09)	0.137	0.357
CYP2D6	rs764481	0.95 (0.68,1.33)	0.776	0.729	0.89 (0.63,1.26)	0.520	0.562
CYP2D6	rs17478227	1.18 (0.83,1.67)	0.366	0.459	1.15 (0.80,1.65)	0.455	0.428
GSTM-45	GSTM-45	0.98 (0.69,1.38)	0.898	0.894	0.88 (0.61,1.28)	0.516	0.773
GSTM1	GSTM1	0.80 (0.57,1.13)	0.209	0.240	0.66 (0.46,0.95)	0.027	0.066
GSTT2B	GSTT2B	1.21 (0.85,1.75)	0.297	0.475	1.42 (0.97,2.11)	0.078	0.683
GSTT1	GSTT1	0.83 (0.54,1.27)	0.386	0.151	1.15 (0.72,1.86)	0.564	0.928

*Adjustment variables: race, cohort, parity, child sex, smoking during first trimester of pregnancy.

eTable 3. Main genetic effects and G*E interaction between SNPs and CNVs and tTHMs (per 10µg/L) in relation to SGA*. Maternal and child genotypes (genetic dominant model)

		Maternal DNA			Child DNA		
		Genetic main effects		G*E	Genetic main effects		G*E
Gene	SNP	OR (95% IC)	*P* value	*P* value	OR (95% IC)	*P* value	*P* value
CYP3A4-A5-A7-A43	rs4646450	0.77 (0.55,1.07)	0.115	0.987	0.95 (0.69,1.31)	0.770	0.746
CYP3A4-A5-A7-A43	rs12333983	1.13 (0.76,1.68)	0.558	0.475	1.32 (0.91,1.93)	0.149	0.274
CYP3A4-A5-A7-A43	rs4646437	1.10 (0.74,1.63)	0.645	0.920	1.31 (0.90,1.90)	0.165	0.405
CYP3A4-A5-A7-A43	rs651430	1.25 (0.90,1.76)	0.191	0.256	0.88 (0.63,1.21)	0.430	0.835
CYP3A4-A5-A7-A43	rs678040	0.81 (0.53,1.23)	0.327	0.497	0.85 (0.54,1.34)	0.489	0.433
CYP3A4-A5-A7-A43	rs472660	0.85 (0.59,1.21)	0.373	0.487	0.95 (0.66,1.36)	0.771	0.659
CYP3A4-A5-A7-A43	rs474229	0.84 (0.61,1.14)	0.258	0.360	1.24 (0.91,1.70)	0.175	0.641
CYP2E1	rs915906	1.02 (0.73,1.43)	0.888	0.716	0.95 (0.69,1.31)	0.761	0.593
CYP2E1	rs2070675	1.01 (0.73,1.38)	0.968	0.237	1.00 (0.73,1.37)	0.982	0.845
CYP2E1	rs915908	0.79 (0.56,1.09)	0.155	0.841	0.98 (0.70,1.36)	0.896	0.658
CYP2E1	rs743535	1.40 (0.93,2.12)	0.111	0.028	1.25 (0.84,1.85)	0.269	0.858
CYP2E1	rs2249695	1.03 (0.75,1.42)	0.838	0.405	1.02 (0.75,1.40)	0.900	0.721
CYP2E1	rs4512750	1.10 (0.80,1.51)	0.564	0.432	1.00 (0.73,1.36)	0.986	0.624
GSTZ1	rs4899651	1.09 (0.81,1.46)	0.584	0.124	1.08 (0.80,1.46)	0.608	0.385
GSTZ1	rs2363643	0.92	0.602	0.562	0.80	0.141	0.442

		Maternal DNA			Child DNA		
		Genetic main effects		G*E	Genetic main effects		G*E
Gene	SNP	OR (95% IC)	*P* value	*P* value	OR (95% IC)	*P* value	*P* value
		(0.69,1.24)			(0.60,1.08)		
		0.95			1.08		
GSTZ1	rs2270422	(0.70,1.29)	0.735	0.541	(0.80,1.47)	0.613	0.881
		1.17			1.04		
GSTZ1	rs1046428	(0.85,1.61)	0.324	0.870	(0.77,1.42)	0.786	0.444
		0.89			0.80		
GSTZ1	rs11624726	(0.66,1.20)	0.438	0.892	(0.59,1.07)	0.132	0.663
		0.84			1.13		
CYP1A1-A2	rs2470893	(0.62,1.13)	0.247	0.867	(0.84,1.51)	0.432	0.243
		1.38			0.92		
CYP1A1-A2	rs4886605	(0.99,1.92)	0.060	0.361	(0.66,1.27)	0.606	0.969
		0.88			1.09		
CYP1A1-A2	rs2472297	(0.63,1.22)	0.441	0.617	(0.80,1.50)	0.580	0.053
		0.88			1.13		
CYP1A1-A2	rs762551	(0.65,1.18)	0.385	0.972	(0.84,1.52)	0.423	0.955
		0.91			1.12		
CYP1A1-A2	rs11854147	(0.68,1.23)	0.557	0.622	(0.83,1.51)	0.469	0.499
		0.93			0.97		
CYP2A6	rs7246742	(0.68,1.27)	0.633	0.061	(0.70,1.33)	0.835	0.867
		1.11			0.99		
GSTT1-2-2B	rs4820571	(0.81,1.51)	0.515	0.788	(0.73,1.34)	0.946	0.407
		1.07			0.91		
GSTT1-2-2B	rs6003959	(0.80,1.45)	0.643	0.079	(0.68,1.22)	0.532	0.624
		1.06			1.40		
GSTT1-2-2B	rs1892715	(0.77,1.44)	0.730	0.310	(1.03,1.91)	0.032	0.383
		1.17			1.00		
GSTT1-2-2B	rs1006771	(0.86,1.58)	0.321	0.997	(0.74,1.35)	0.992	0.120
		1.14			1.24		
GSTT1-2-2B	rs140289	(0.84,1.54)	0.393	0.479	(0.92,1.66)	0.154	0.281
		1.07			1.15		
GSTT1-2-2B	rs8138555	(0.78,1.47)	0.689	0.299	(0.84,1.58)	0.371	0.523
GSTT1-2-2B	rs738809	1.07	0.673	0.721	1.07	0.640	0.909

		Maternal DNA			Child DNA		
		Genetic main effects		G*E	Genetic main effects		G*E
Gene	SNP	OR (95% IC)	*P* value	*P* value	OR (95% IC)	*P* value	*P* value
		(0.79,1.43)			(0.80,1.44)		
		1.01			0.91		
GSTT1-2-2B	rs422674	(0.75,1.37)	0.926	0.468	(0.68,1.23)	0.545	0.426
		0.76			0.75		
GSTT1-2-2B	rs11090305	(0.55,1.03)	0.079	0.075	(0.55,1.02)	0.070	0.688
		1.05			1.11		
CYP2D6	rs1062753	(0.78,1.41)	0.761	0.078	(0.82,1.48)	0.502	0.295
		1.06			1.10		
CYP2D6	rs8138080	(0.79,1.42)	0.706	0.085	(0.82,1.47)	0.524	0.284
		1.34			1.11		
CYP2D6	rs5751222	(0.98,1.84)	0.065	0.313	(0.82,1.51)	0.502	0.911
		0.86			0.85		
CYP2D6	rs6002626	(0.63,1.19)	0.361	0.846	(0.62,1.16)	0.314	0.060
		0.89			0.98		
CYP2D6	rs764481	(0.66,1.20)	0.457	0.639	(0.73,1.32)	0.890	0.394
		1.09			1.12		
CYP2D6	rs17478227	(0.80,1.50)	0.576	0.126	(0.82,1.54)	0.468	0.784
		1.00			0.83		
GSTM-45	GSTM-45	(0.73,1.36)	0.990	0.353	(0.61,1.13)	0.242	0.082
		0.77			0.84		
GSTM1	GSTM1	(0.56,1.04)	0.092	0.987	(0.62,1.14)	0.258	0.647
		1.19			1.35		
GSTT2B	GSTT2B	(0.87,1.65)	0.280	0.288	(0.98,1.88)	0.072	0.067
		0.90			1.09		
GSTT1	GSTT1	(0.62,1.32)	0.600	0.037	(0.75,1.59)	0.662	0.612

*Adjustment variables: race, cohort, parity, child sex, smoking during first trimester of pregnancy.