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

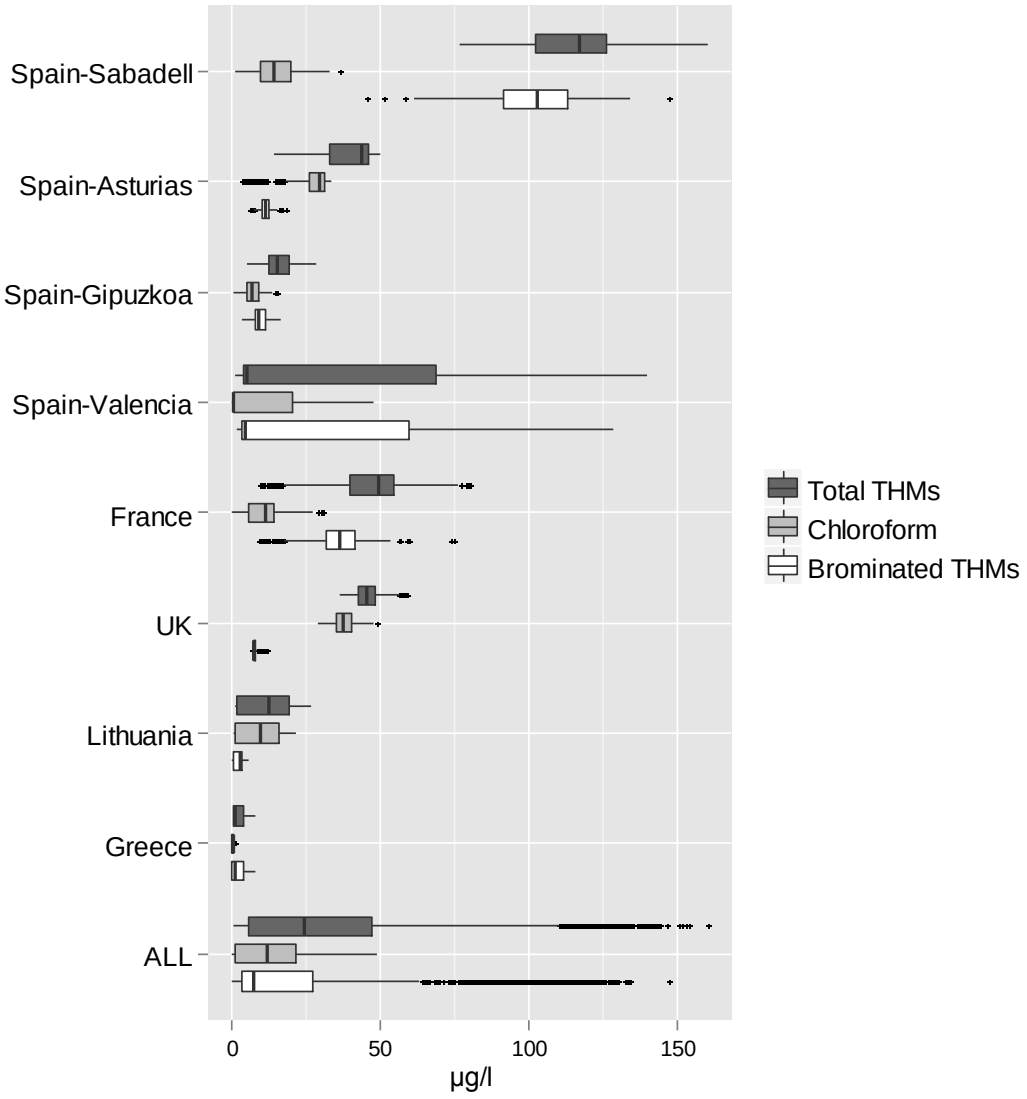
[b] Small for gestational age: mother's ethnicity, parity and cohort

[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.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Spain-Asturias
Spain-Gipuzkoa
Spain-Valencia
France
UK
Lithuania
Greece
ALL
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Total THMs
Chloroform
Brominated THMs