Long-term exposure to trihalomethanes in drinking water and breast cancer in the Spanish multicase-control study on cancer (MCC-SPAIN)

Laia Font-Ribera[1,2,3,4], Esther Gràcia-Lavedan[1,3,4], Nuria Aragonés[4,5], Beatriz Pérez-Gómez[4,5], Marina Pollán[4,5,6], Pilar Amiano[4,7], Ana Jiménez-Zabala[7], Gemma Castaño-Vinyals[1,2,3,4], Aina Roca-Barceló[8], Eva Ardanaz[4,9], Rosana Burgui[4,9], Antonio José Molina[10], Tania Fernández-Villa[10], Inés Gómez-Acebo[11], Trinidad Dierssen-Sotos[11], Victor Moreno[12,13,4,14], Guillermo Fernandez-Tardon[4,15], Rosana Peiró[4,16], Manolis Kogevinas[1,2,3,4], Cristina M Villanueva[1,2,3,4*]

1. ISGlobal, Barcelona, Spain
2. IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain
3. Department of Experimental and Health Sciences, Universitat Pompeu Fabra (UPF), Barcelona, Spain
4. CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain
5. Cancer and Environmental Epidemiology Unit, National Centre for Epidemiology, Carlos III Institute of Health, Madrid, Spain
6. Cancer Epidemiology Research Group, Oncology and Hematology Area, IIS Puerta De Hierro, Madrid, Spain
7. Public Health Division of Gipuzkoa, Biodonostia Research Institute, San Sebastian, Spain

8. Epidemiology Unit and Girona Cancer Registry, Oncology Coordination Plan, Department of Health, Autonomous Government of Catalonia, Catalan Institute of Oncology, Girona, Spain.

9. Instituto de Salud Pública y Laboral de Navarra, Pamplona, Spain

10. Grupo de Investigación en Interacciones Gen-Ambiente y Salud. Universidad de León.

11. Department of Preventive Medicine and Public Health, University of Cantabria. Santander, Spain

12. Unit of Biomarkers and Susceptibility, Cancer Prevention and Control Program, Catalan Institute of Oncology (ICO). Hospitalet de Llobregat, Spain.

13. Colorectal Cancer Group, Bellvitge Biomedical Research Institute (IDIBELL). Hospitalet de Llobregat, Spain.

14. Department of Clinical Sciences, Faculty of Medicine, University of Barcelona, Barcelona, Spain

15. University of Oviedo, Asturias, Spain

16. Centre for Research in Public Health, Valencia, Spain

* Corresponding author.

Cristina M. Villanueva (cristina.villanueva@isglobal.org).

Barcelona Institute for Global Health (ISGlobal).

Doctor Aiguader, 88

08003 Barcelona, Spain

Tel. +34 93 214 73 44

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ABSTRACT

Background: Exposure to trihalomethanes (THMs) in drinking water has consistently been associated with an increased risk of bladder cancer, but evidence on other cancers including the breast is very limited.

Objectives: We assessed long-term exposure to THMs to evaluate the association with female breast cancer (BC) risk.

Methods: A multi case-control study was conducted in Spain from 2008 to 2013. We included 1,003 incident BC cases (women 20-85 years old) recruited from 14 hospitals and 1,458 population controls. Subjects were interviewed to ascertain residential histories and major recognized risk factors for BC. Mean residential levels of chloroform, brominated THMs (Br-THMs) and the sum of both as total THM (TTHMs) during the adult-lifetime were calculated.

Results: Mean adult-lifetime residential levels ranged from 0.8 to 145.7 µg/L for TTHM (median=30.8), from 0.2 to 62.4 µg/L for chloroform (median=19.7) and from 0.3 to 126.0 µg/L for Br-THMs (median=9.7). Adult-lifetime residential chloroform was associated with BC (adjusted OR=1.47; 95%CI=1.05, 2.06 for the highest (>24 µg/L) vs. lowest (<8 µg/L) quartile; p-trend=0.024). No association was detected for residential Br-THMs (OR=0.91; 95%CI=0.68, 1.23 for >31 µg/L vs. <6 µg/L) or TTHMs (OR=1.14; 95%CI=0.83, 1.57 for >48 µg/L vs. <22 µg/L).

Conclusions: At common levels in Europe, long-term residential total THMs were not related to female breast cancer. A moderate association with chloroform was suggested at the highest exposure category. This large epidemiological study with extensive exposure assessment overcomes several limitations of previous studies but further studies are needed to confirm these results.

1. INTRODUCTION

Breast cancer (BC) is the first cancer in incidence and mortality among women world-wide (Globocan 2012), with an increasing incidence during the last decades, also in Spain (Pollán et al. 2009). BC is more common in western countries and among favoured socioeconomic status (Brody and Rudel 2003). Main recognized risk factors affect endogenous estrogenic levels (Hankinson et al. 2004) and include sex, age, body mass index, age at menarche, at first delivery and at menopause, life-style factors such as alcohol consumption and low physical activity, and drugs with estrogenic action before or after menopause (Hankinson et al. 2004). Established risk factors explain approximately 50% of the variability in BC incidence, and other environmental factors may partly explain the remaining variation (Brody et al. 2007). Toxicological studies, and to a lesser extend epidemiological studies, have related some environmental exposures to BC (Macon et al. 2013), mainly through endocrine disruption (Brody and Rudel 2003). Drinking water disinfection by-products (DBP) are among the chemicals suggested by toxicologic research as potentially related to BC that have not been investigated enough in epidemiologic studies (Brody et al. 2007).

DBPs are a mixture of hundreds of chemicals formed in water during the disinfection process. This is a ubiquitous exposure through ingestion of tap water, inhalation and dermal exposure during showering, bathing or washing dishes (Villanueva et al. 2015). The most prevalent DBP in drinking water are trihalomethanes (THM), which are the only DBP group regulated in the EU with a maximum contaminant level of 100 μg/L. Several DBPs have been shown to be genotoxic in *in vitro* assays and carcinogenic in animal experiments (Richardson et al. 2007) and the WHO International Agency for Research on Cancer (IARC) classifies chloroform and other widespread DBP as possible humans carcinogens (Villanueva et al. 2015). Several epidemiological studies have related exposure to DBPs and cancer risk, being the most consistent evidence for bladder cancer and in a lower extent for colon and rectal cancer (Villanueva et al. 2015). Only sporadic epidemiological studies have assessed the impact of DBPs on other cancer sites including the breast (Villanueva et al. 2015).

Among the few epidemiological studies on DBP exposure and BC, some detected a positive association (Doyle et al. 1997; Gottlieb et al. 1982; Koivusalo et al. 1997; Wilkins and Comstock 1981), while others did not (Kanarek et al. 1982; Marcus et al. 1998; Vincetti et al. 2004; Young et al. 1981; Zierler et al. 1986). These are studies conducted 20 years ago (Doyle et al. 1997; Koivusalo et al. 1997; Marcus et al. 1998) or 30 (Gottlieb et al. 1982; Kanarek et al. 1982; Wilkins and Comstock 1981; Young et al. 1981; Zierler et al. 1986) with only one exception (Vincetti et al. 2004) and had important methodological limitations, including an ecological design (Marcus et al. 1998; Vincetti et al. 2004; Wilkins and Comstock 1981), a poor control for confounding and a very limited exposure assessment based on surrogates of DBP exposure (Doyle et al. 1997; Gottlieb et al. 1982; Kanarek et al. 1982; Koivusalo et al. 1997; Young et al. 1981). Furthermore, evidence that 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), a major mutagenic constituent of DBP, causes mammary tumours in rats (Komulainen et al. 1997) also suggests that the association between DBP exposure and BC should be further investigated in epidemiological studies overcoming the limitations of previous studies (Brody et al. 2007).

We aim to provide new epidemiological evidence on the association between lifetime exposure to DBPs and female BC risk in a large case-control study, including areas with contrasting THM concentrations in Spain and evaluating different routes of exposure and THM species.

2. METHODS

2.1. Study design and population

A multi case-control study was conducted from 2008 to 2013 in twelve provinces of Spain (MCC-Spain project) (Castano-Vinyals et al. 2015). Women 20-85 years old with histologically confirmed incident BC (International Classification of Diseases 10th Revision [ICD-10]: C50, D05.1, D05.7) without personal cancer history were recruited from oncologic and surgical services in fourteen hospitals from eight provinces. Controls were selected randomly from the rosters of General Practitioners at the Primary Health Centers participating in the study covering nearly all the population living in the corresponding area, allowing to identify population-based controls from the same study base as cases. Controls were frequency matched to cases by age in 5-year age groups and study area. They were contacted on behalf of their General Practitioner and invited to participate in the study. Subjects with serious barriers to communication were excluded. Average response rate was 71% for cases and 53% for controls. The study protocol was approved by the ethical review board from participating centers and all participants signed an informed consent before recruitment.

2.2. Individual information

A structured computerized questionnaire was administered by trained personnel in face-to-face interviews to collect data on residential history, water source in each residence (bottled, tap, other) and frequency and duration of bathing, showering and hand dish-washing. Several potential risk factors were also collected including age (continuous), educational level (less than primary school, primary school, secondary school and university), occupational status (working, not working, housewife, retired), race (white, others), weight and height to compute body mass index (BMI; <25, 25-29.9, =>30), family history of BC (yes/no), menopausal status (pre/post), menopause treatment (ever, never), oral contraceptive use (never, ever), nulliparity (yes, no), age at menarche (continuous, and categorized to: <=12, 13-14, >14 years), age at first birth (continuous, and categorized to: <25, 25-28, >28 years), breastfeeding (continuous, and categorized to: 0, >0-6, >6-12, >12 months), smoking (never, former or current), average leisure physical activity in the last 10 years (continuous frequency and duration converted to metabolic equivalents of task (METS)/hour/week). Diet habits and alcohol consumption was reported through a self-administered semi-quantitative food-frequency questionnaire and current energy intake (<1500, 1500-2000, >2000 kcal/day) and intake of alcohol in the past (0, 0-5.5, >5.5 grams/day) were calculated. Long-term waterborne ingested nitrate was also estimated (Espejo-Herrera et al. 2016) and levels were categorized in quartiles. Missing data in categorical variables were classified as a separate category.

2.3. Historical trihalomethane levels in the study area

We used trihalomethanes as a surrogate of DBPs. We collected historical information on water source, treatment and routine THM measurements in the study areas through water utilities, local authorities and health authorities. Historical THM levels back to 1940 were modeled at water zone level, the minimum geographic unit with homogeneous water source, treatment and THM levels (corresponding to municipality in most cases). Annual average THM levels were calculated using available measurements. For years when THM measurements were absent, available THM levels were averaged and imputed if water source and treatment were unchanged. Proportion of surface water and type of treatment were used as a weight to this average in the event of changes in water source and treatment. Before chlorination started, THM levels were assumed to be zero. Total THMs (TTHMs) was calculated summing up the concentrations of the four THMs (chloroforom, bromodichloromethane, dibromochloromethane, and bromoform). Brominated THMs (Br-THMs) were calculated as the TTHMs excluding chloroform. Correlation between residential levels of chloroform, Br-THMs and TTHMs was explored with Spearman correlation.

2.4. Individual exposure in the study population

THM levels and subjects' personal data were linked by year and water zone of residence to obtain an annual THM level in the residences where subjects lived from age 18 to 2 years before the interview. Average levels in all residences with THM estimates was then calculated and referred as adult-lifetime residential levels. Average residential THM levels in the last 10 years were also calculated as an alternative exposure window with more accurate exposure estimates. The type of water consumed in the residence and liters/day ingested were used to calculate ingested THM levels, by multiplying residential levels if tap water was consumed, and a zero THM level if water ingested was bottled (Font-Ribera et al. 2010). When water consumed was from private wells, levels assigned were 0.3, 0.3, 0.8, and 1.8 µg/L respectively for chloroforom, bromodichloromethane, dibromochloromethane and bromoform, according to unpublished records from wells in the study areas. Average ingested TTHMs and Br-THMs level in the residences was calculated for the years with available data and expressed as µg/day. Exposure through showering, bathing and hand dish-washing was estimated by multiplying minutes/week of each activity by the residential TTHMs or Br-THMs level and expressed as µg/L x min/week. When gloves were used "most of the time" for hand dish-washing (16.9% of subjects), half of the THM exposure was assigned.

2.5. Statistical analysis

The initial sample of BC cases and controls in the study areas with modeled THM was 3,322 (1,582 cases and 1,740 controls). Only subjects with known THM concentrations in the residential tap water for at least 70% of the years between age 18 to 2 years before the interview (87% of interviewed subjects) were included. In order to have a similar geographical distribution of cases and controls, only municipalities with at least one case and one control were included and 9 controls and 278 cases living in 117 small municipalities not accomplishing this criteria were excluded. One subject with unreliable interview was further excluded, as well as fourteen controls that had missing data in physical activity, a variable included in all final models. Analyzed sample included 2,461 subjects, 1,003 cases and 1,458 controls.

The main models estimated the association between BC and average adult-lifetime residential TTHMs, chloroform and Br-THMs levels. Generalized additive models (GAM) were used to evaluate the exposure-response relationships on continuous variables. Exposure variables were categorized into quartiles defined according to the exposure distribution among controls. We estimated odds ratios (OR) and 95% confidence intervals (CI) of BC using mixed models with recruitment area as random effect. Additional models explored the association with residential THM levels in the last 10 years, mutual adjustment between residential chloroform and residential Br-THMs as well as interaction by menopausal status. We estimated OR of BC for specific exposure routes: drinking water source in the longest residence, time showering, time washing dishes by hand, THM exposure through ingestion, through showering and through hand dish-washing.

All models were adjusted for age, area and education. Further adjustment included known risk factors for BC that were significant in the models (p-value <0.05) and those that changed the risk estimates (beta) >10%. Main models were adjusted for area, age, educational level, occupational status, family history of BC, BMI, energy intake, physical activity, oral contraceptive use and menopause treatment use. Multicollineality was explored using the variance inflation factor (VIF), having all variable categories a VIF<4 (except the highest quartile of TTHM and three study areas) with a mean of 2.66 in the model for life-time average residential TTHM. Statistical analyses were performed using STATA version 12.0 (Stata Corp, College Station, TX).

3. RESULTS

1003 cases and 1458 controls were included from ten study areas in Spain (Table 1). After adjusting by area, age and educational level, cases showed higher frequencies of family history of BC, overweight and obesity, occupational status, never use of menopause treatment, never use of oral contraceptives, being physically inactive and high energy intake. The OR of BC for these and other classical BC risk factors can be found in Table S1. Compared to women excluded for the final analysis, the included population had a higher proportion of controls, postmenopausal women, and a lower proportion of women of young age, working status and highest energy intake (Table S1). The geographical distribution of the residencies of cases and controls is shown in Figure S1.

Average adult-lifetime residential levels of TTHMs ranged from 0.8 to 145.7 µg/L among study participants (Figure 1), with a median level of 30.8 µg/L (interquartile range (IQR)=22.3, 51.6) for TTHMs, 19.7 µg/L (IQR=7.8, 24.5) for chloroform and 9.7 µg/L (IQR=5.3, 28.5) for Br-THMs. Exposure to residential chloroform ranged between 0.8 and 62.4 µg/L, while exposure to Br-THMs ranged from 1.9 to 126.0 µg/L. The variability of residential THMs within area was small for several areas, what precluded the estimation of overall effects through meta-analysis. The proportion of chloroform from TTHMs differed among areas, from 11% in Valencia to 88% in Madrid, and the Spearman correlation between chloroform and Br-THMs also differed between areas, being -0.26 overall (Figure S2).

Generalized additive models showed a positive linear relationship between BC and average adult-lifetime residential levels of TTHMs, chloroform and Br-THM (Figure 2). When exposure was categorized into quartiles, no significant association was seen between BC and TTHMs or Br-THMs (Table 2). The OR for the highest vs. lowest quartile of TTHM (>48.3 µg/L vs. <= 21.7µg/L) was 1.14 (95%CI=0.83, 1.57). Residential levels of chloroform were related to BC (OR=1.47 (95%CI=1.05, 2.06) for the highest vs. lowest quartile (>24.3 µg/L vs. <=7.6 µg/L)) and a p-trend value of 0.028. A positive association was also observed with residential chloroform as a continuous variable with an OR of 1.12 (0.98, 1.27) for a 10 µg/L increase. After further adjustment for residential levels of Br-THMs, the association between BC and residential chloroform remained very similar and there was no collinearity in the model (mean VIF for the two variables=1.89). No significant interaction was observed between residential THM levels and menopausal status on BC risk, although slightly higher associations among pre-menopausal women were found at the highest exposure category (Table S3). Likewise, no interaction was observed between residential TTHMs and chloroform levels and educational level on BC risk, while a significant interaction was detected for Br-THMs level. The OR for BC among those in the highest vs. lowest quartile of residential Br-THMs level was lower among those with primary or less education (OR=0.69; 95%CI=0.48, 1.01) than among those with secondary or more education (OR=1.36; 95%CI=0.83, 2.24) (Table S4). Further adjustment of the residential THM models by other socioeconomic status variables (partner educational level, social class by the largest occupation and parental socioeconomic status) gave similar results (Table S5).

In the Barcelona metropolitan area, with the highest levels of Br-THMs and TTHMs, exposure to residential Br-THMs and TTHMs were also related to BC (OR=1.76 (95%CI=0.80, 3.90) for the highest (>91.8 µg/L) vs. lowest (<48.8 µg/L) quartiles of Br-THMs and OR=1.72 (95%CI=0.79, 3.78) for the highest (>110.5 µg/L) vs. lowest (<71.1 µg/L) quartiles of TTHMs) (Table S6). Madrid, the largest city in Spain and the study area contributing with more subjects, had very low variability in THM levels and no association between those and BC was detected (Table S6).

Average residential THM levels during the last 10 years were highly correlated to average adult-life levels (spearman correlations of 0.80, 0.92 and 0.85 for TTHMs, Br-THMs and chloroform, respectively (all p-values <0.001). OR of BC for residential THM levels in the last 10 years were therefore very similar to those for adult-life time residential THM levels (Table S7).

Approximately 75% of the study population usually drank municipal water in their longest residence (28.4 years duration in average), while 21% drank bottled water, and type of water consumed was not related to BC (Table 3). The median weekly duration for showering and washing dishes by hand was 40 min (IQR=30, 70) and 140 min (IQR=35, 210), respectively, and they were not correlated (Spearman correlation = 0.004). Hand dish-washing was associated with BC with an OR of 1.39 (95%CI=1.05, 1.83) for the highest vs. lowest quartile (p-trend of 0.013), also after adjusting by residential TTHMs. When combining residential THM levels with water activities, the estimated ingested THM levels were not associated with BC (Table 4). Some intermediate category of exposure to TTHMs and Br-THMs through showering was protective for BC, while exposure through dish washing was positively related to BC (OR=1.93; 95%CI=1.47, 1.12 for the highest vs. lowest quartiles of TTHMs). Further adjustment by residential level of chloroform or Br-THMs did not affect the associations (results not shown).

4. DISCUSSION

For the first time, we estimated the association between life-time exposure to THMs in drinking water and female BC in a large case-control study, including areas with contrasting THM concentrations in Spain and evaluating different routes of exposure and THM species. At common levels in Europe, total THM exposure was not related to BC, but a positive association was suggested for exposure to chloroform.

This case-control study includes several areas in Spain with a large variability in Br-THMs and TTHM levels in drinking water. The proportion and correlation between chloroform and Br-THMs also varied considerably between areas, allowing detecting different associations with BC by THM species. When comparing the highest vs. lowest quartiles of exposure, residential chloroform in drinking water was associated with BC at >24 µg/L, while no association was detected for Br-THMs at >31 µg/L or for TTHM at >48 µg/L. However, the GAM models indicated a positive linear association with BC not only for chloroform but also for TTHM and Br-THMs, but a reduced number of subjects were exposed at the highest levels. A 7% of study participants had a median lifetime residential TTHM levels above the current maximum level in the EU of 100 µg/L. These subjects were from the Barcelona metropolitan area, where Br-THMs have been especially high (median of 64.4 µg/L and IQR=48.8, 91.8) due to a high bromide content in the raw water. In this area, an increased OR was also detected for life-time exposure to TTHMs and Br-THMs, although this was not statistically significant probably due to the reduced sample size.

These results are difficult to compare since previous studies on DBP exposure and BC did not report DBP levels (Gottlieb et al. 1982; Kanarek et al. 1982; Koivusalo et al. 1997; Vincetti et al. 2004; Young et al. 1981), but were based on surrogates of DBP exposure such as chlorinated vs. unchlorinated water (Kanarek et al. 1982, Koivusalo et al. 1997) or groundwater vs. surface water (Doyle et al. 1997). One exception is an ecological study in North Carolina published in 1998 (Marcus et al. 1998) that compared female BC rates by current levels of TTHMs and found a rate ratio of 1.1 (95%CI=0.9, 1.2) for >80 µg/L vs. <40 µg/L (Marcus et al. 1998). A large study using data from the cancer registry in Finland also found a small increased risk of BC (RR= 1.11 (95%CI=1.01, 1.22)) among women supplied by chlorinated surface water (Koivusalo et al. 1997). A part from a poor exposure assessment, previous epidemiological studies on BC and DBP exposure had other important methodological constrains, such as an ecological design (Marcus et al. 1998; Vincetti et al. 2004; Wilkins and Comstock 1981), the use of mortality instead of incidence data (Kanarek et al. 1982; Vincetti et al. 2004; Wilkins and Comstock 1981) or a poor assessment of relevant confounders. Therefore, the present study overcomes several limitations of previous studies and represents a step forward in the epidemiological evidence on DBP exposure and BC risk.

Another novelty of the present study is the evaluation of individual patterns of water use such as type of ingested water or the frequency and duration of showering and hand dish-washing. Different health effects could be expected by these water activities since they reflect different exposure routes (ingestion, dermal absorption or inhalation) and different THM uptakes (Gordon et al. 2006). The significant association detected between BC and exposure to chloroform was seen for residential levels but not for ingested chloroform. Similarly, residential TTHM levels has been the exposure indicator more related to bladder cancer (Costet et al. 2011; Villanueva et al. 2007) and ingested TTHM was also not related to this cancer in the largest international meta-analysis (Costet et al. 2011). On one hand, residential THM levels are considered an indicator of global exposure regardless of the route (Costet et al. 2011) and on the other hand, the lack of association between cancer and ingested THM levels could be attributed to limitations in the measurement of the indicator more than to a real lack of effect of ingested DBPs (Costet et al. 2011). This is the first study on THM exposure and cancer risk to consider exposure through hand dish-washing and it was positively related to BC. Although THM uptake is lower when washing dishes by hand than when showering (Gordon et al. 2006), the duration of exposure and variability was much higher for hand dish-washing than showering (median of 140 and 40 min/week, respectively) in this study population limited to women. However, the fact that some protective association was seen between BC and showering and that hand dish-washing was related to BC risk beyond THM levels, suggests potential confounding by other unmeasured factors.

The modelling of historical THM levels allowed us to estimate exposure for different temporal windows. However, very similar results were found between adult-lifetime exposure and exposure during the last 10 years, probably due to the high correlation between the levels at different exposure periods. Although the present study has done a huge improvement in exposure assessment compared to previous studies, some measurement error is probably still present due to the limited historical THM measurements. To minimize this, we exclude subjects having estimated THM levels for less than 70% of the exposure window. Inability to account for THM exposure outside the home may have introduced error in the estimation of ingested THM levels, although most of total water was consumed at home (74%). Selection bias might be another concern, since response rates were low especially among controls that were population-based and shared for different cancer sites within a larger multi-case control study, leading a slightly different age distribution than cases and higher educational level than general population. However, we assume that probability of participation is independent from the exposure, and we don't expect an impact on the results due to response rates. Finally, we cannot rule out uncontrolled confounding by other water contaminants beyond DBPs. However, long-term exposure assessment to nitrate was conducted in this study (Espejo-Herrera et al. 2016) and adjusting models by nitrate exposure did not modified the results. Furthermore, unpublished data on selected pesticides in drinking water in the study area (e.g., simazine, atrazine, terbuthylazine) showed levels below or around the quantification limit. Residual confounding by socioeconomic status could be another concern, although different sensitivity analysis indicate no major effects of socioeconomic status on the estimated risks.

Gastrointestinal and urinary tract, and not BC, are the cancer sites with higher biological plausibility to be affected by DBP exposure (Koivusalo 1997). However, biological mechanisms have been poorly investigated (Nieuwenhuijsen et al. 2009), being genotoxicity and carcinogenicity the most recognized ones (Richardson et al. 2007). Epigenetic changes in DNA methylation have also been suggested as another potential mechanism of DBP toxicity (Salas et al. 2015). DBPs are not considered important endocrine disruptors, but very little toxicological evidence is available (Klinefelter et al. 2004). A toxicological study described a delay in reproductive development in rats due to long-term exposure to brominated haloacetic acids (Klinefelter 2004) and MX was found to be a potent carcinogen in rats that increased mammary gland tumours in female rats (Komulainen et al. 1997). BC is a heterogeneous disease with potentially different aetiologies in pre- and postmenopausal women and in this study we found slightly higher associations between THM exposure and BC among pre-menopausal women at the highest exposure category.

DBPs constitute a complex mixture with around 600 identified chemicals with different toxicity (Richardson et al., 2007). THMs are usually the more prevalent DBPs in drinking water but have lower toxicity than other less prevalent DBPs, such as haloacetonitriles or MX (Richardson et al. 2007). During the recruitment period of the present study, several DBPs were analysed in drinking water of a representative sample across study areas (Villanueva et al. 2012). Haloacetic acids (HAAs) were in a very similar range than TTHMs (median of 26.4 μg/L). Haloacetonitries, haloketones, chloropicrin and chloralhydrate were in much lower levels and below the limit of detection in several samples. MX showed a median (range) concentration of 16.7 (0.8–54.1) ng/L. Chloroform concentration was positively correlated to chlorinated HAA and MX levels, while Br-THMs were positively correlated to brominated HAA (Villanueva et al. 2012).

Epidemiological studies on DBP exposure and cancer risk have mainly used TTHMs as an indicator of exposure to the total mixture of DBPs (Villanueva et al. 2015). This may result in the misclassification of exposure to the relevant chemicals for a given health outcome, since the correlation between DBP constituents is complex and varies across areas and over time (Villanueva et al. 2012). Measuring the exposure to chloroform and Br-THMs allows estimating separately the exposure to overall chlorinated DBPs and brominated DBPs, since these species are usually correlated between them (Villanueva et al. 2012). This may be especially relevant in epidemiological studies including areas with different chlorine-bromine speciation, like the present one. In this study, female

BC appeared to be associated with lifetime exposure to common levels of chloroform but not Br-THMs or TTHMs. Chlorinated DBPs are usually found in higher concentrations in drinking water than brominated DBPs, but toxicological evidence indicates that the brominated are more genotoxic and cytotoxic than their chlorinated analogues (Richardson et al. 2007). Furthermore, current concentrations of MX in the study area were positively correlated to current chloroform level and negatively correlated to Br-THMs (Villanueva et al. 2012).

5. CONCLUSIONS

At common levels in Europe, long-term residential total THMs were not related to female breast cancer. A moderate association with chloroform was suggested at the highest exposure category. These results should be confirmed in future large and well design epidemiological studies, since they would have a large public health impact due to the ubiquity of DBP exposure and the health burden of BC.

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Table 1. Description of the study population. N=2,461.

	Controls		Cases		
	N	%	N	%	p-value
Total	1458		1003		
Area					
Asturias	90	6.2	62	6.2	
Barcelona A	140	9.6	114	11.4	
Barcelona B	89	6.1	45	4.5	
Barcelona C	93	6.4	47	4.7	
Cantabria	149	10.2	86	8.6	
Guipuzcoa	239	16.4	119	11.9	
León	151	10.4	128	12.8	
Madrid	305	20.9	236	23.5	
Navarra	150	10.3	115	11.5	
Valencia	52	3.6	51	5.1	
Age, years					
Mean (SD)	59.4	(12.8)	57.1	(12.0)	
<=50	412	28.3	334	33.3	
51-60	346	23.7	278	27.7	
61-70	366	25.1	246	24.5	
>70	334	22.9	145	14.5	
Education					
< Primary school	243	16.7	144	14.4	0.417
Primary school	465	31.9	331	33.0	
Secondary school	448	30.7	325	32.4	
University	302	20.7	203	20.2	
Menopausal status					
Post	1,063	72.9	671	66.9	0.005
Pre	393	27.0	331	33.0	
DK/M	2	0.1	1	0.1	
Family history of breast cancer					
No	1,149	78.8	651	64.9	<0.001
Yes	252	17.3	321	32.0	
DK/M	57	3.9	31	3.1	
Body mass index, Kg/m^2					
<25	745	51.1	477	47.6	0.189
25-29.9	452	31.0	342	34.1	
30 or more	261	17.9	184	18.3	
Occupational status					
Working	556	38.1	483	48.2	<0.001
Not working	87	6.0	71	7.1	
Housewife	480	32.9	247	24.6	
Retired	335	23.0	202	20.1	
Oral contraceptive use					

Never	805	55.2	560	55.8	0.353
Ever	652	44.7	440	43.9	
DK/M	1	0.1	3	0.3	
Menopause treatment					
Ever	294	20.2	150	15.0	<0.001
Never	765	52.5	515	51.4	
Missing/Pre-menopause	399	27.4	338	33.7	
Physical activity [a], METs					
0	891	61.1	676	67.4	0.002
>0 to 8	272	18.7	162	16.2	
>10 to 16	121	8.3	84	8.4	
>16	174	11.9	81	8.1	
Energy intake, kcal/day					
<1500	452	31.0	251	25.0	0.007
1500-2000	485	33.3	352	35.1	
>2000	351	24.1	284	28.3	
DK/M	170	11.7	116	11.6	

a. physical activity: metabolic equivalents (MET) total h/week; annual median in the last 10 years.
DK/M: Don’t know or missing.

Figure 1. Average adult-lifetime residential levels of total trihalomethanes (TTHMs), chloroform and brominated trihalometanes (Br-THMs) among the study participants in the 10 study areas. N=2,461.

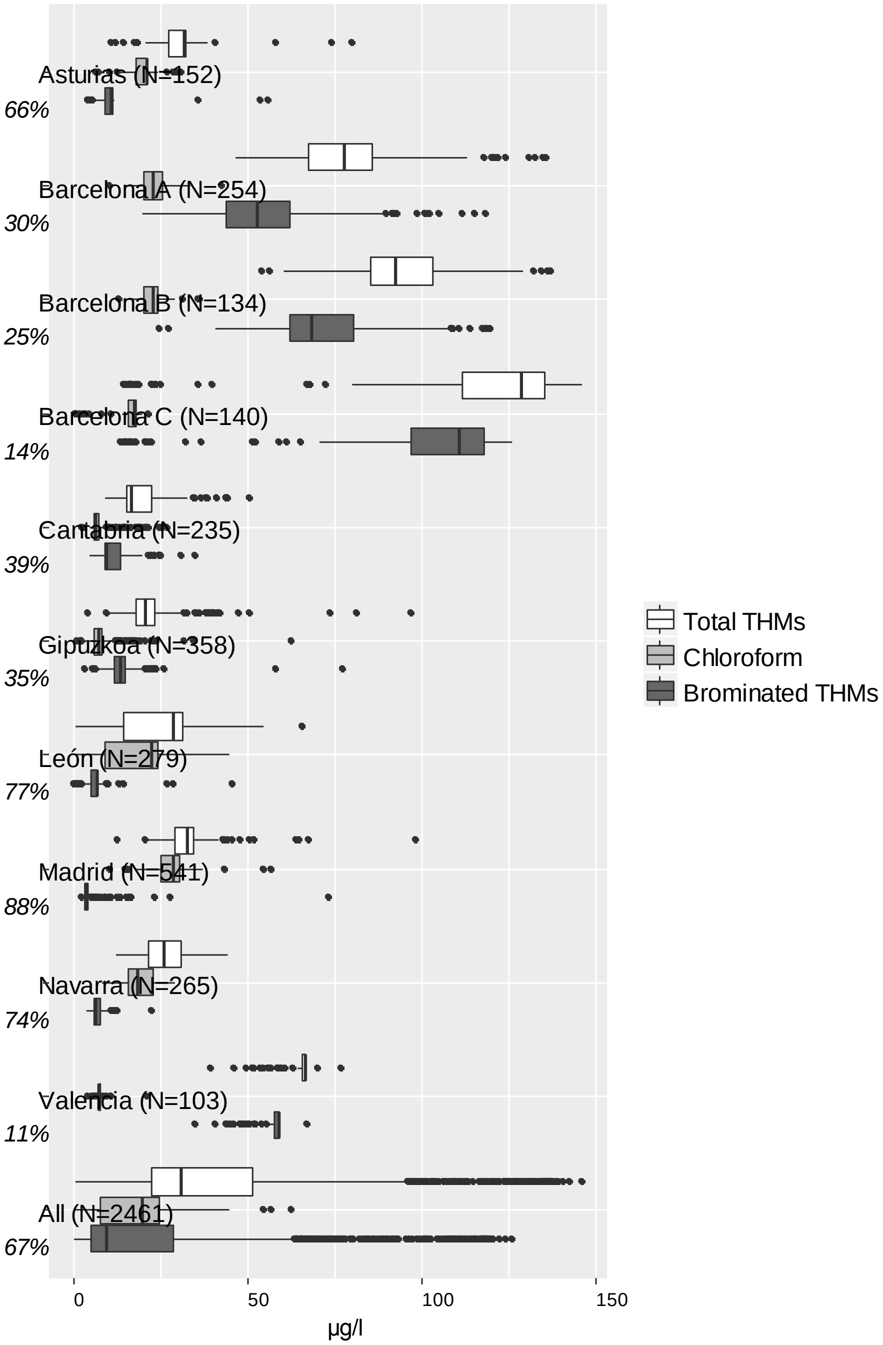

Figure legend. The percentage in italics indicates de proportion of TTHMs that is chloroform. The vertical line inside each box indicates the median value. The lower and upper hinges of the boxes indicate the 25th and 75th percentile.

Figure 2. Exposure-response curves between adult-lifetime residential THMs (μg/L) and breast cancer from generalized additive models. N=2,461 (1,003 cases and 1,458 controls).

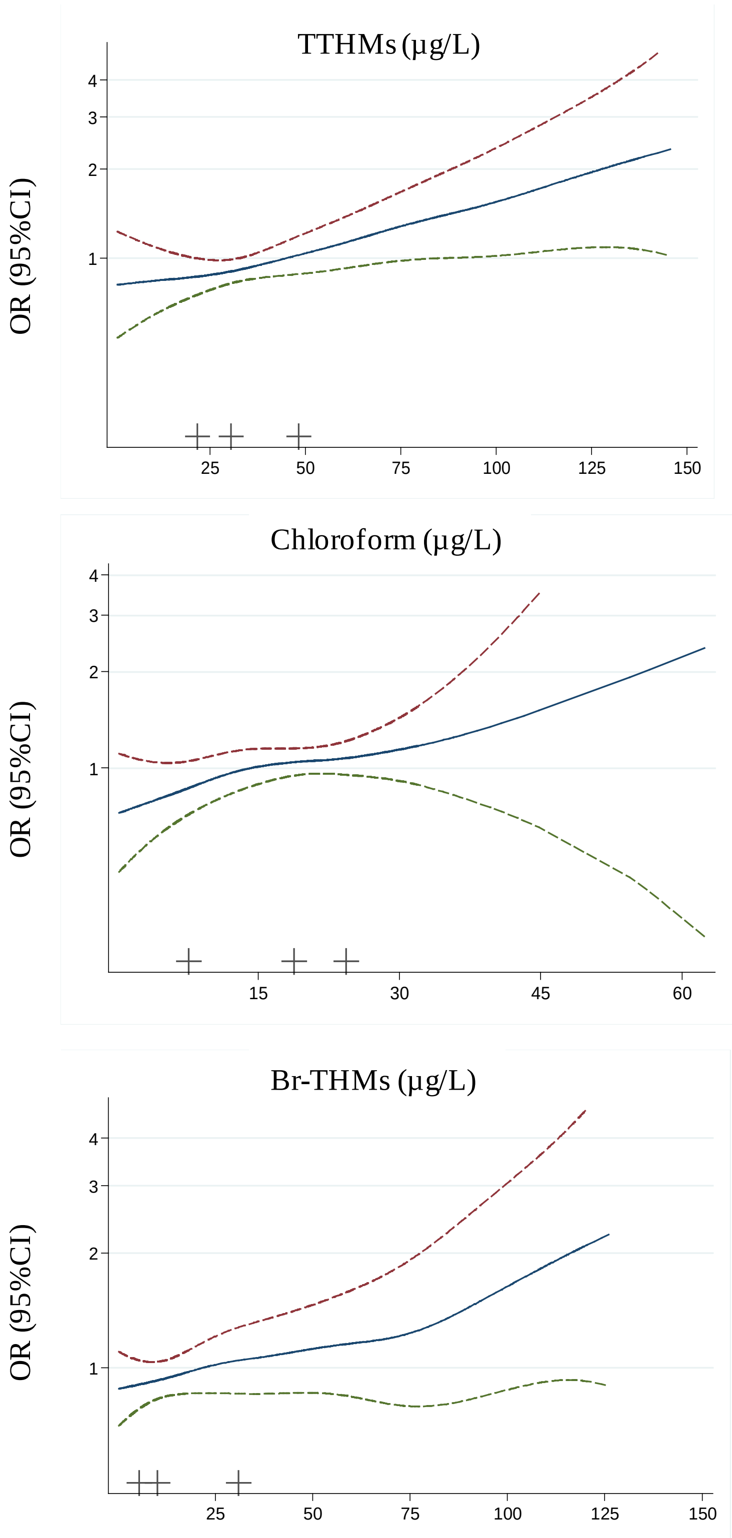

Figure legend. Tick marks above the x-axes represent quartiles of exposure. Models adjusted by area, age, educational level, occupational status, family history of BC, BMI, energy intake, physical activity, oral contraceptive use and menopause treatment use. P-values for nonlinearity: 0.616 for TTHMs, 0.473 for chloroform, 0.300 for Br-THMs.

Table 2. Odds ratio (OR) and 95% confidence interval (CI) of breast cancer associated with adult-lifetime residential levels of total trihalomethanes (TTHMs), chloroform and brominated trihalometanes (Br-THMs). N=2,461.

	Controls	**Cases**	**OR[a]**	**(95%CI)**	**OR[b]**	**95%CI**
TTHMs (μg/L)						
<=21.7	365	209	1			
>21.7-30.5	364	245	1.15	(0.88, 1.49)		
>30.5-48.3	365	290	1.09	(0.81, 1.46)		
>48.3	364	259	1.14	(0.83, 1.57)		
Total	1,458	1,003	*ptrend*	*0.503*		
Cont. (10 μg/L)			1.01	(0.97, 1.05)		
Chloroform (μg/L)						
<=7.6	365	198	1		1	
>7.6-18.8	364	233	1.25	(0.95, 1.65)	1.22	(0.92, 1.62)
>18.8-24.3	365	266	1.29	(0.96, 1.73)	1.25	(0.95, 1.65)
>24.3	364	306	**1.47**	**(1.05, 2.06)**	**1.40**	**(1.01, 1.95)**
Total	1,458	1,003	*ptrend*	***0.028***	*ptrend*	***0.038***
Cont. (10 μg/L)			1.12	(0.98, 1.27)	1.12	(0.98, 1.27)
Br-THMs (μg/L)						
<=5.5	365	276	1		1	1.00
>5.5-10.1	364	282	1.08	(0.82, 1.41)	1.16	(0.84, 1.59)
>10.1-31.0	365	197	0.79	(0.57, 1.10)	0.90	(0.64, 1.25)
>31.0	364	248	0.91	(0.68, 1.23)	0.95	(0.67, 1.35)
Total	1,458	1,003	*ptrend*	*0.275*	*ptrend*	*0.378*
Cont. (10 μg/L)			1.00	(0.96, 1.04)	0.99	(0.96, 1.03)

TTHMs: total trihalomethanes. Br-THMs: brominated trihalometanes. Mixed models with area as random effect. Exposure variables are categorized into quartiles. a. Models adjusted for age, educational level, occupational status, family history of BC, BMI, energy intake, physical activity, oral contraceptive use and menopause treatment use. b. Models mutually adjusted for chloroform and Br-THMs.

Table 3. Frequency of water-related activities in the study population and related odds ratio (OR) and 95% confidence interval (CI) of breast cancer.

	Controls		**Cases**		**OR**	**95%CI**
	N	%	N	%		
Drinking water source[a]						
Tap/municipal	1,101	75.6	749	75.0	1	
Bottled	304	20.9	207	20.7	0.99	(0.79, 1.23)
Wells/springs/other	51	3.5	42	4.2	1.07	(0.68, 1.68)
Total				2,454		
Showering (min/week)						
<=30	374	26.5	260	26.5	1	
>30-40	356	25.2	265	27.0	0.95	(0.75, 1.22)
>40-70	494	35.0	336	34.2	0.83	(0.66, 1.04)
>70	188	13.3	121	12.3	0.82	(0.61, 1.10)
				2,394	*ptrend*	*0.070*
Dish washing by hand (min/week)						
<=35	357	25.9	236	26.1	1	
35-140	439	31.9	271	29.9	0.95	(0.75, 1.20)
>140-210	279	20.2	182	20.1	1.10	(0.84, 1.45)
>210	303	22.0	216	23.9	**1.39**	**(1.05, 1.83)**
				2,283	*ptrend*	***0.013***

Mixed models with area as random effect. Exposure variables are categorized into quartiles. Models adjusted for age, educational level, occupational status, family history of BC, BMI, energy intake, physical activity, oral contraceptive use and menopause treatment use. a. Drinking water source in the longest residency.

Table 4. Odds ratio (OR) and 95% confidence interval (CI) of breast cancer associated with adult-lifetime exposure to total trihalomethanes (TTHMs), chloroform and brominated trihalomethanes (Br-THMs) through different exposure situations. N=2,461.

TTHMs	**Contr.**	**Cases**	**OR**	**95%CI**	**$CHCl_3$**	**Contr.**	**Cases**	**OR**	**95%CI**	**Br-THMs**	**Contr.**	**Cases**	**OR**	**95%CI**
Ingestion (µg /day)														
<=15.0	366	254	1		<=5.5	365	248	1		<=3.6	366	254	1	
>15.0-23.8	363	204	0.91	(0.71, 1.18)	>5.5-13.4	364	201	0.88	(0.68, 1.13)	>3.6-6.7	363	273	1.04	(0.81, 1.32)
>23.8-32.3	365	262	1.00	(0.77, 1.29)	>13.4-23.1	365	275	1.16	(0.90, 1.50)	>6.7-12.6	365	233	0.94	(0.72, 1.22)
>32.3	364	282	1.05	(0.82, 1.35)	>23.1	364	278	0.95	(0.71, 1.27)	>12.6	364	242	1.06	(0.81, 1.39)
Total	1458	1002	*ptrend*	*0.650*	Total	1458	1002	*ptrend*	*0.851*	Total	1458	1002	*ptrend*	*0.839*
Showering (µg/L*h/week)														
<=13.6	353	243	1		<=5.6	353	202	1		<=3.9	353	299	1	
13.6-23.1	353	270	0.99	(0.77, 1.26)	5.6-11.8	353	248	1.19	(0.92, 1.53)	3.9-7.6	353	236	**0.72**	**(0.56, 0.92)**
>23.1-40.0	353	202	**0.65**	**(0.50, 0.85)**	>11.8-21.0	353	285	1.16	(0.88, 1.52)	>7.6-18.9	353	192	**0.59**	**(0.45, 0.78)**
>40.0	353	267	0.91	(0.69, 1.20)	>21.0	353	247	0.93	(0.70, 1.24)	>18.9	353	255	0.76	(0.57, 1.01)
Total	1412	982	*ptrend*	*0.084*	Total	1412	982	*ptrend*	*0.497*	Total	1412	982	*ptrend*	***0.018***
Dishwashing (µg/L*h/week)														
<=13.6	345	222	1		<=4.8	345	211	1		<=3.8	345	245	1	
13.6-55.7	344	198	0.96	(0.74, 1.25)	4.8-26.5	344	196	1.05	(0.80, 1.36)	3.8-18.1	344	223	0.90	(0.70, 1.15)
>55.7-119.9	345	210	1.01	(0.78, 1.30)	>26.5-57.7	345	241	1.20	(0.93, 1.55)	>18.1-58.3	345	173	0.80	(0.61, 1.05)
>119.9	344	275	**1.47**	**(1.12, 1.93)**	>57.7	344	257	**1.34**	**(1.03, 1.73)**	>58.3	344	264	**1.41**	**(1.04, 1.92)**
Total	1378	905	*ptrend*	***0.009***	Total	1378	905	*ptrend*	***0.017***	Total	1378	905	*ptrend*	*0.154*

TTHMs: total trihalomethanes. Br-THMs: brominated trihalometanes. Mixed models with recruitment area as random effect. Exposure variables are categorized into quartiles. Models adjusted by age, educational level, occupational status, family history of BC, BMI, energy intake, physical activity, oral contraceptive use and menopause treatment use.

SUPPLEMENTAL MATERIAL

Long-term exposure to trihalomethanes in drinking water and breast cancer in the Spanish multicase-control study on cancer (MCC-SPAIN)

Laia Font-Ribera, Esther Gràcia-Lavedan, Nuria Aragonés, Beatriz Pérez-Gómez, Marina Pollán, Pilar Amiano, Ana Jiménez-Zabala, Gemma Castaño-Vinyals, Aina Roca-Barceló, Eva Ardanaz, Rosana Burgui, Antonio José Molina, Tania Fernández-Villa, Inés Gómez-Acebo, Trinidad Dierssen-Sotos, Victor Moreno, Guillermo Fernandez-Tardon, Rosana Peiró, Manolis Kogevinas, Cristina M Villanueva

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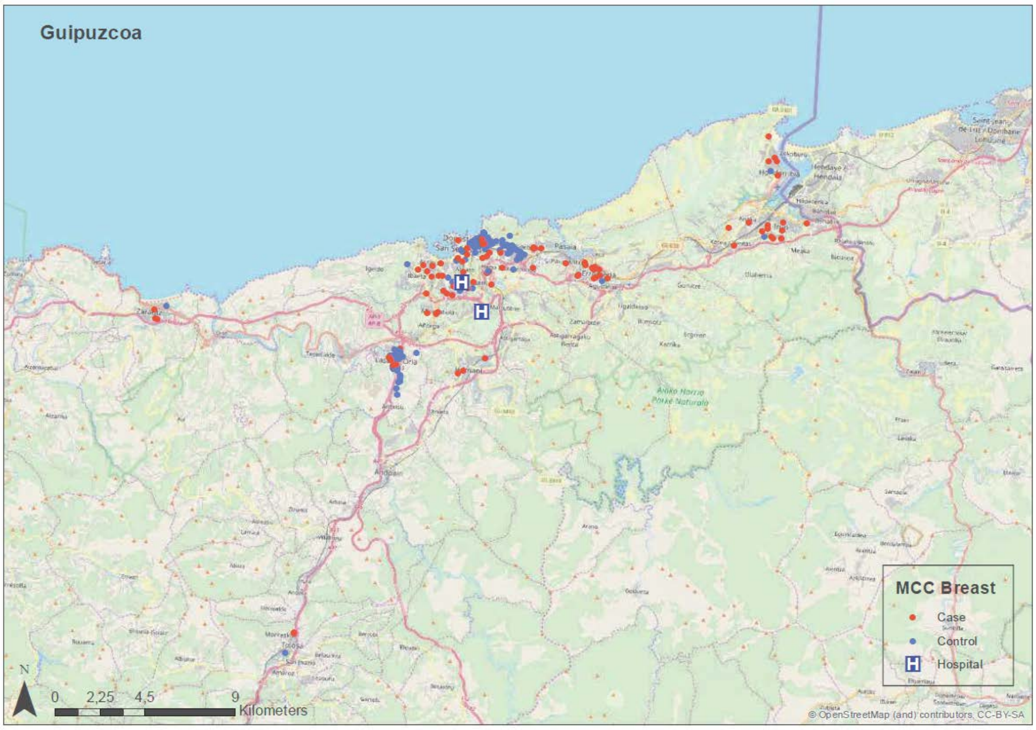
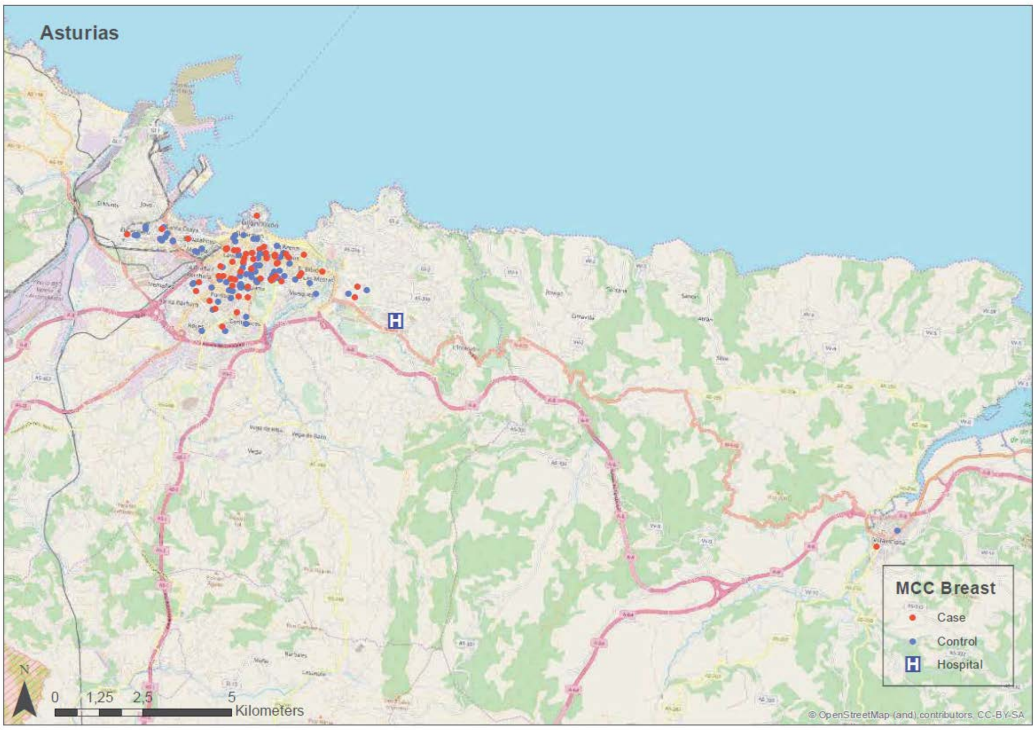
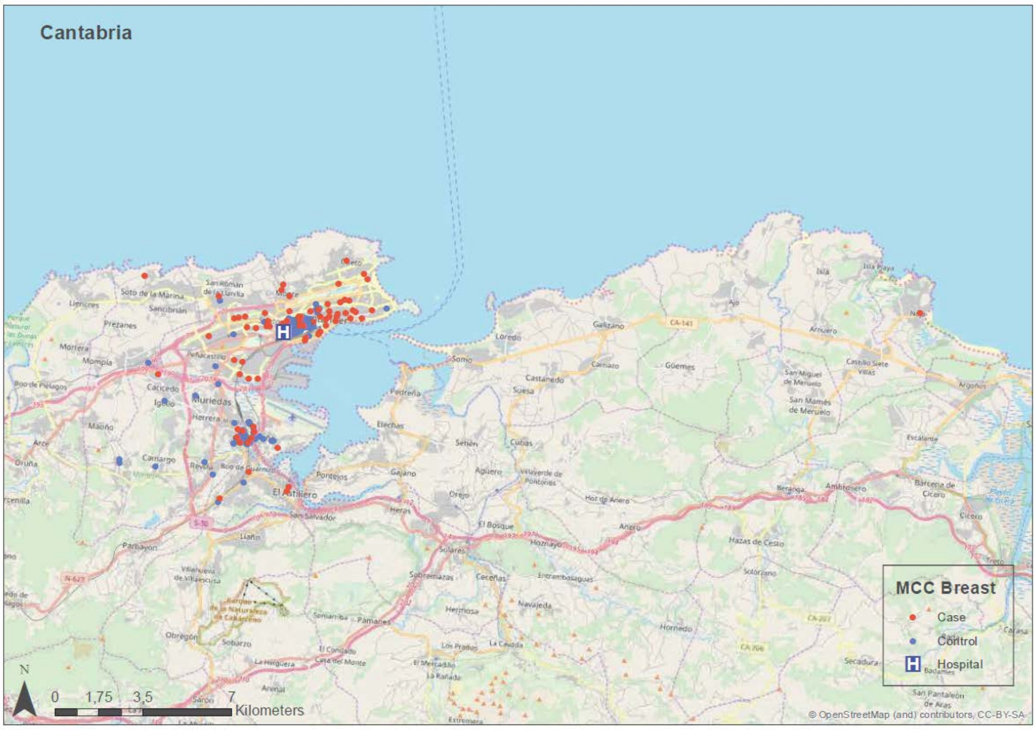
chloroform, and brominated trihalomethanes (Br-THMs) in the last 10 years. N=2,428.

Figure S1. Geographical distribution of the residences of cases and controls of study subjects in the 10 study areas.



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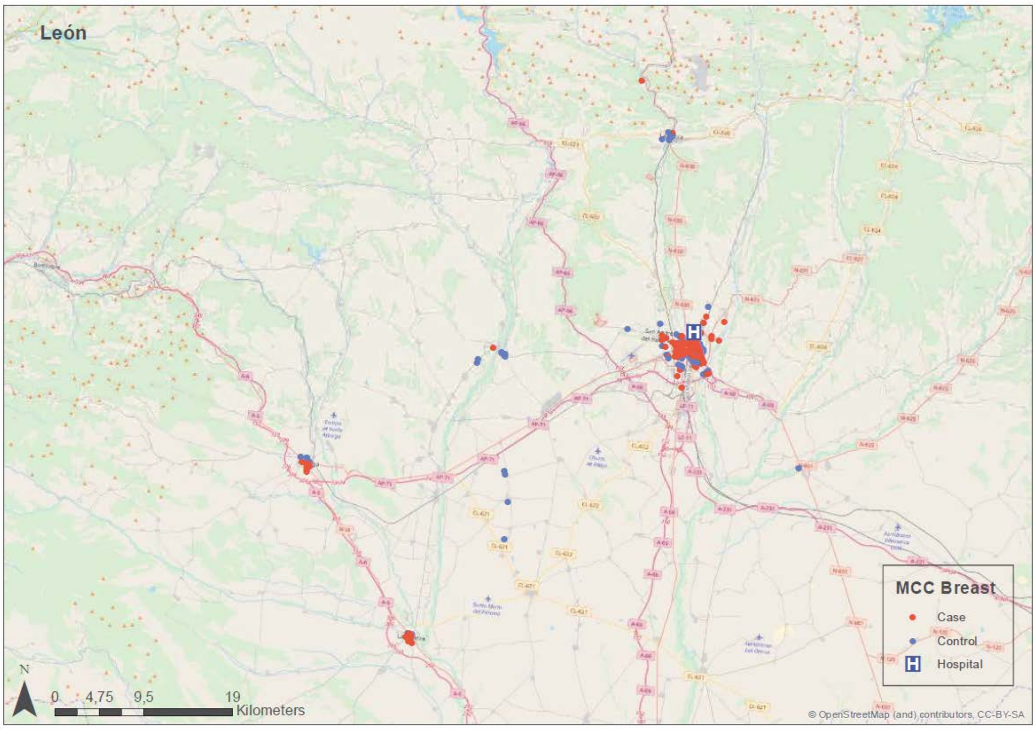
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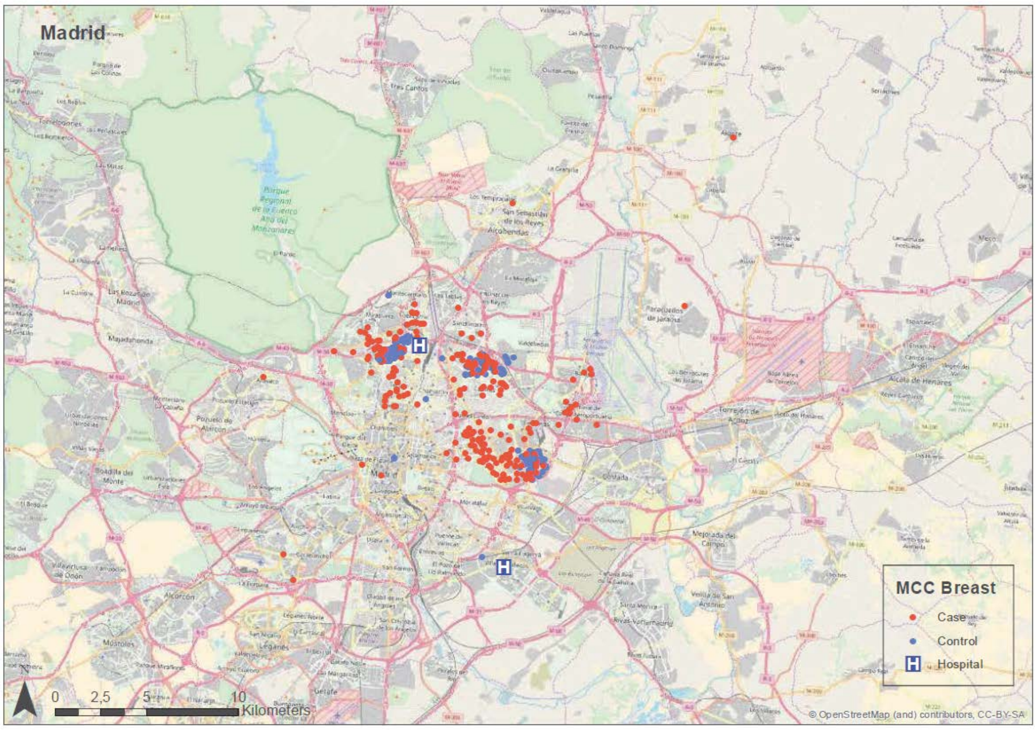
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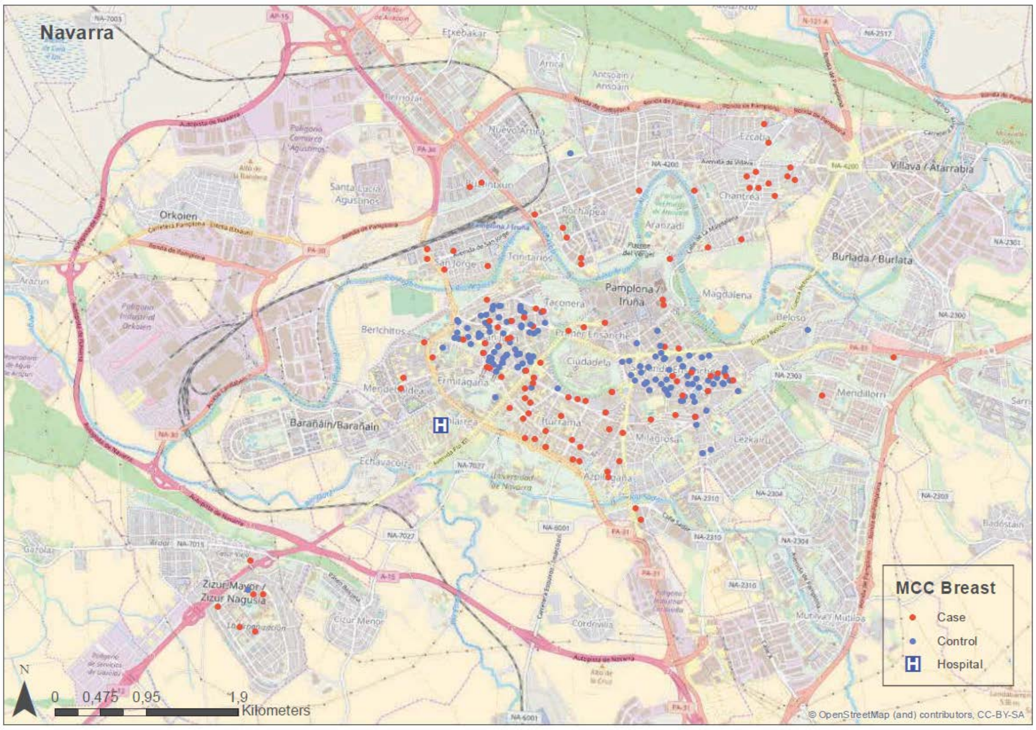
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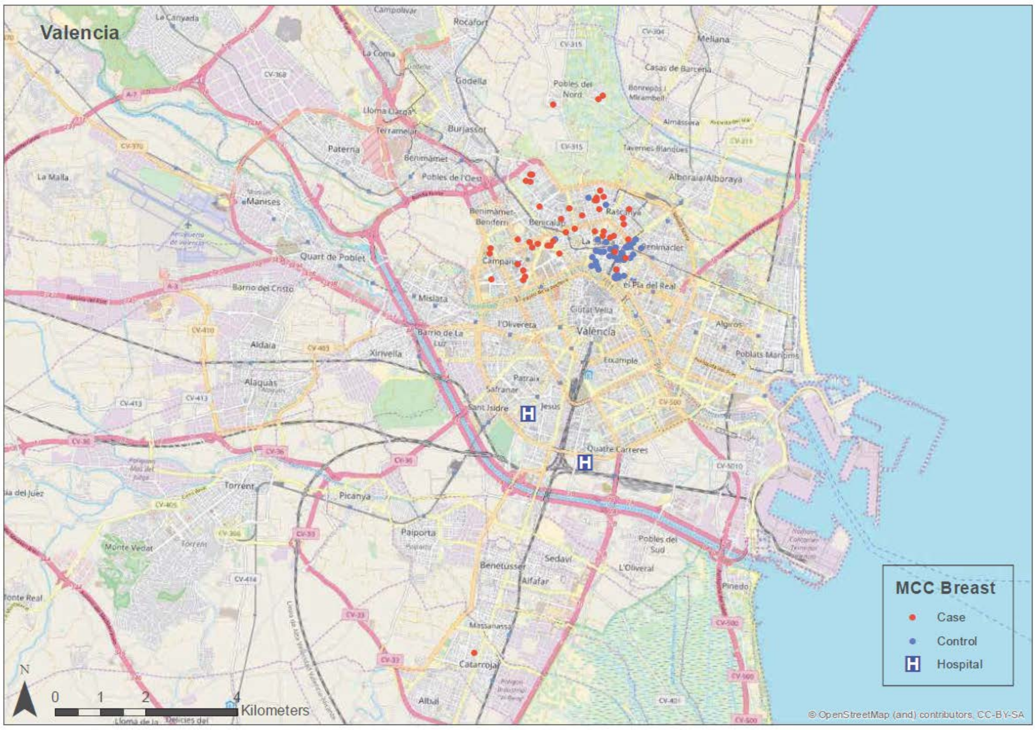
1 
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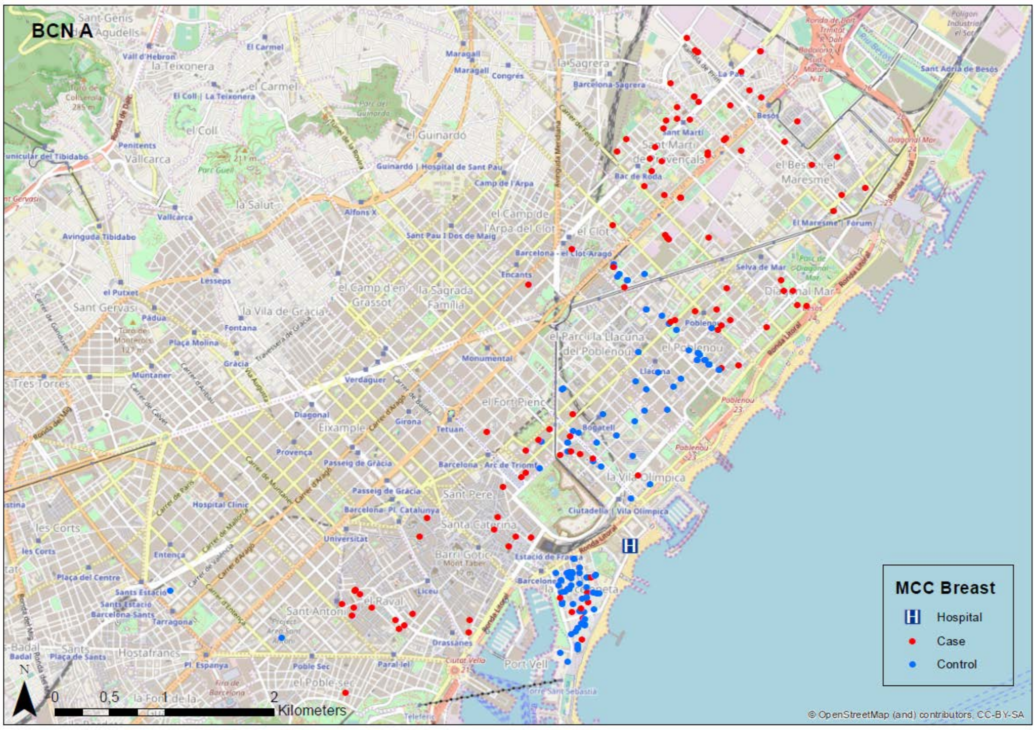
2 
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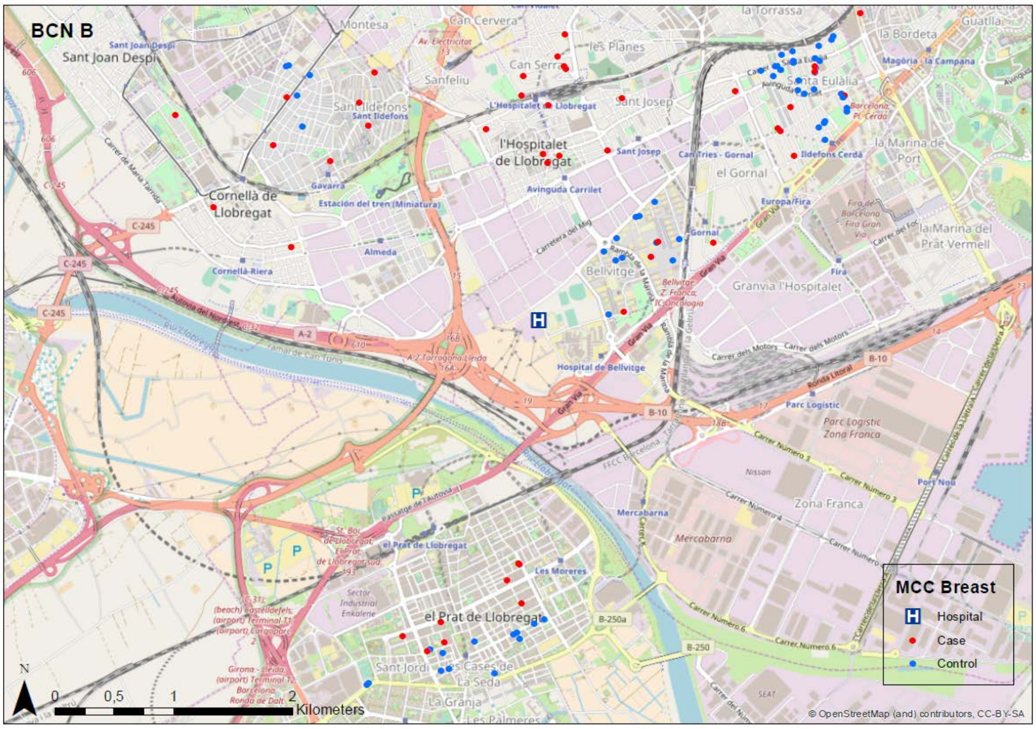
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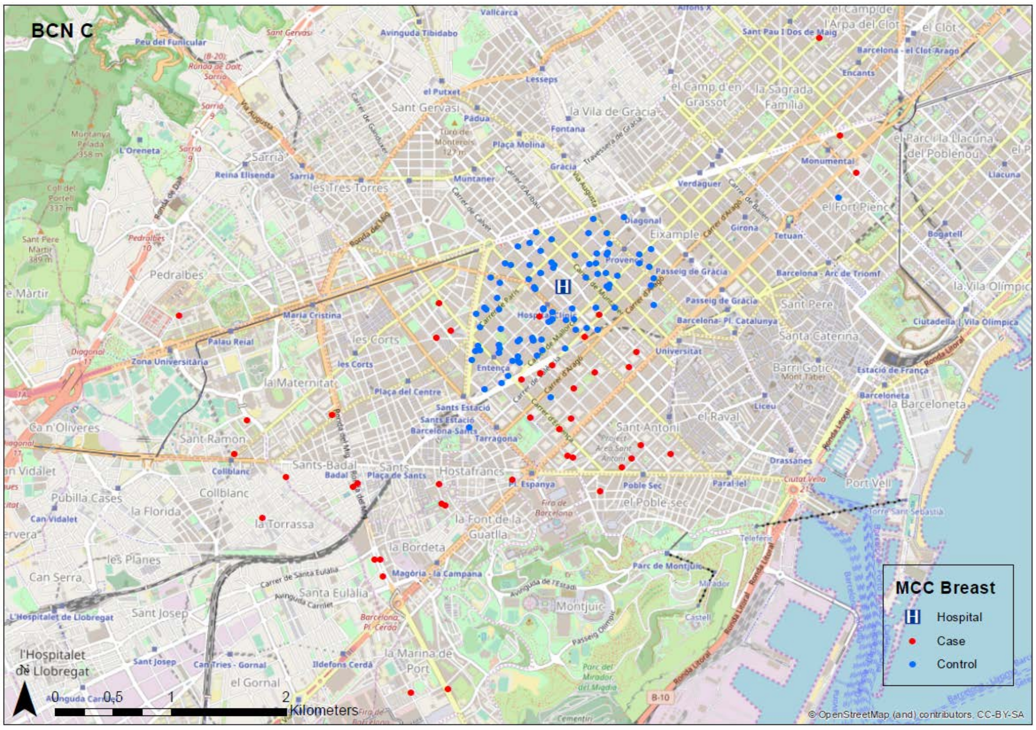
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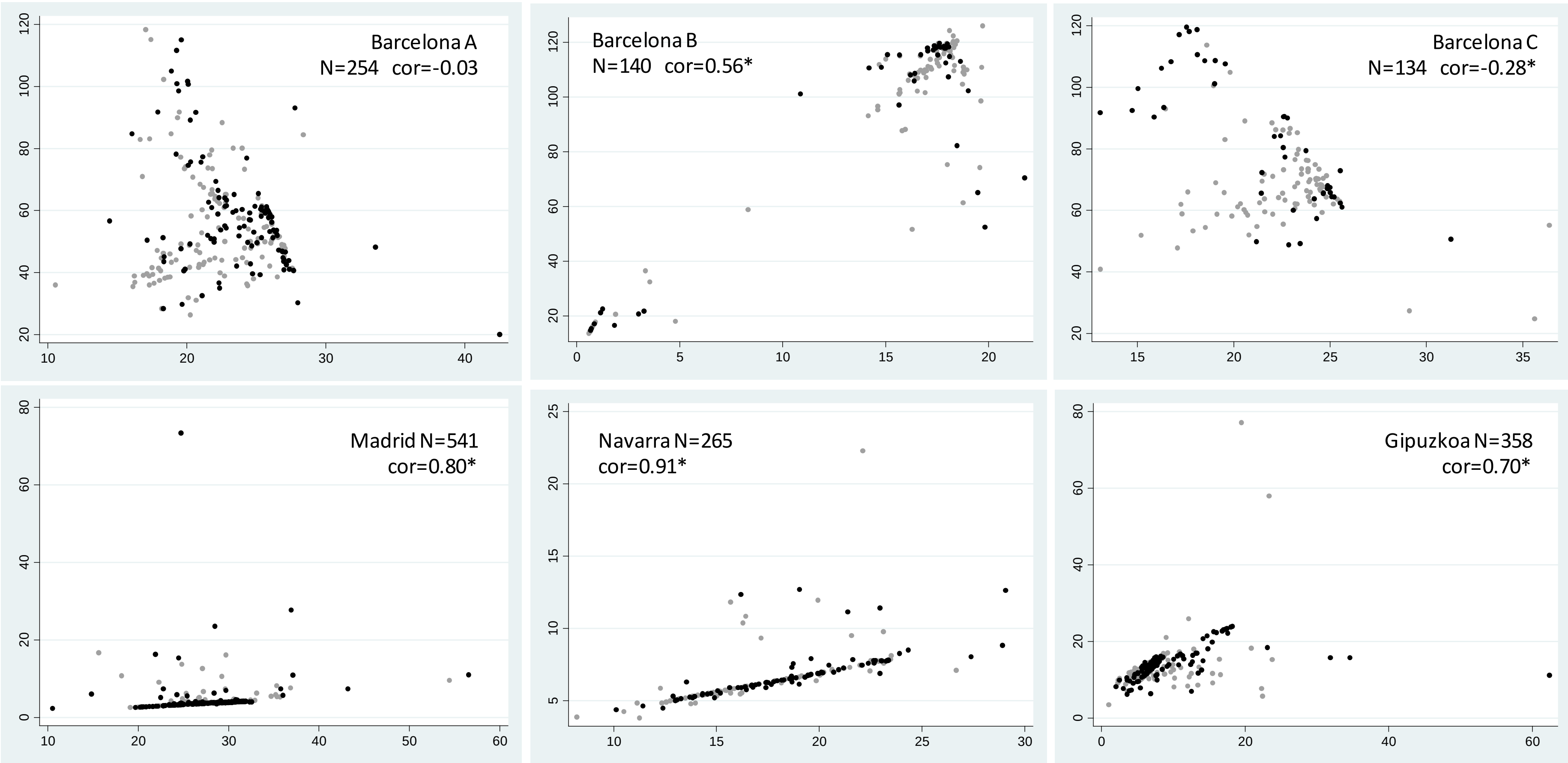
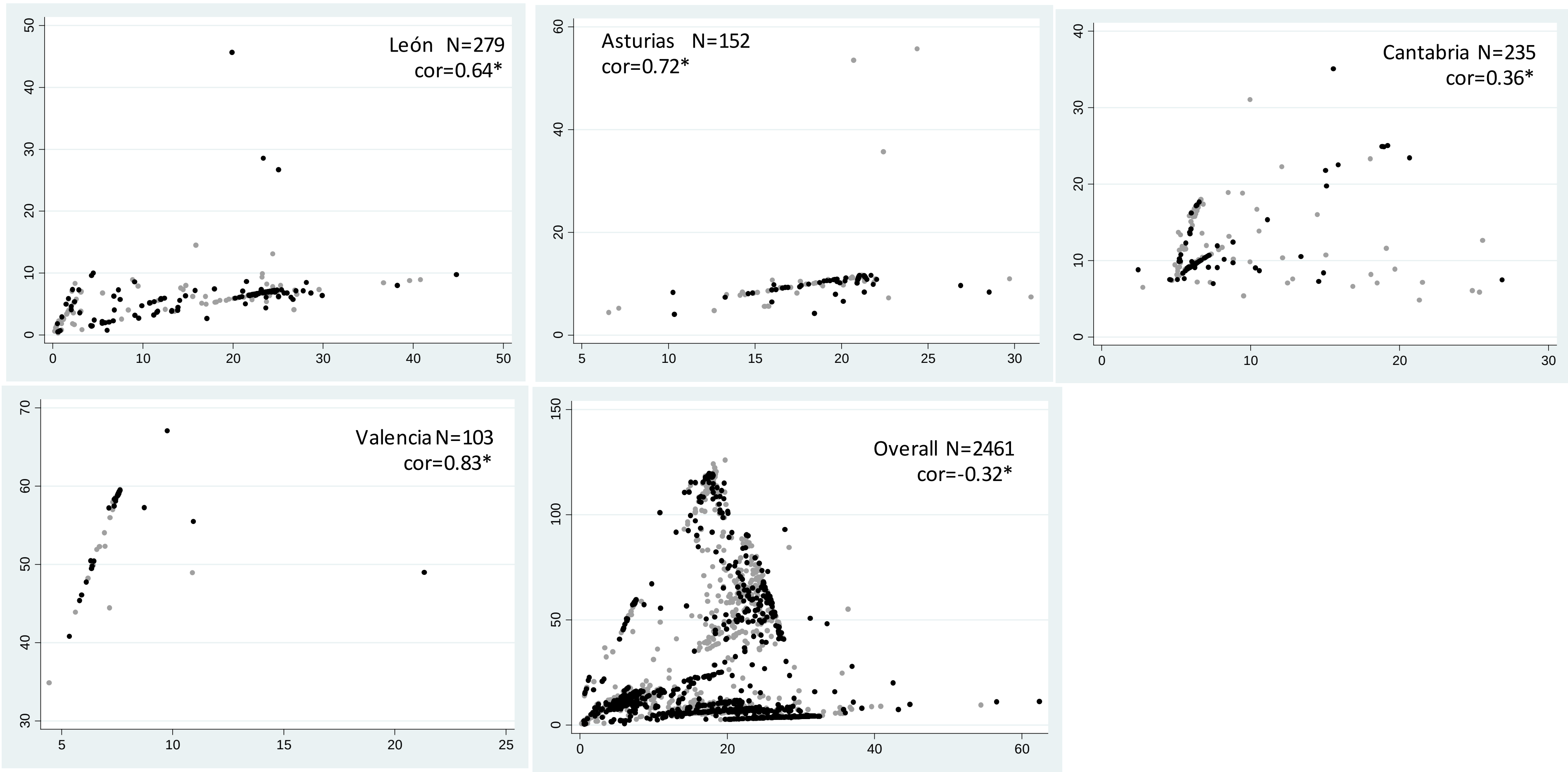
Figure S2. Scatter plot and Spearman correlations between adult-lifetime residential chloroform (X axes) and adult-lifetime residential Br-THMs (Y axes) in the 10 study areas and overall.





Cases are shown in black and controls in light grey. *p-value<0.001.

Table S1. Odds ratio (OR) and 95% confidence interval (CI) of breast cancer associated with classical BC risk factors.

	Controls	Cases	OR	95%CI
Education				
< Primary school	243	144	1	
Primary school	465	331	1.03	(0.79, 1.34)
Secondary school	448	325	0.93	(0.70, 1.23)
University	302	203	0.81	(0.59, 1.11)
Menopausal status				
Post	1,063	671	1	
Pre	393	331	1.00	(0.77, 1.29)
DK/M	2	1	0.60	(0.05, 6.74)
Family history of breast cancer				
No	1,149	651	1	
Yes	252	321	**2.22**	**(1.83, 2.70)**
DK/M	57	31	0.98	(0.62, 1.55)
Body mass index, Kg/m^2				
<25	745	477	1	
25-29.9	452	342	**1.35**	**(1.12, 1.64)**
30 or more	261	184	**1.27**	**(1.00, 1.60)**
Occupational status				
Working	556	483	1	
Not working	87	71	0.90	(0.64, 1.27)
Housewife	480	247	**0.69**	**(0.47, 0.77)**
Retired	335	202	**0.74**	**(0.55, 0.98)**
Oral contraceptive use				
Never	805	560	1	
Ever	652	440	**0.82**	**(0.69, 0.98)**
DK/M	1	3	4.33	(0.44, 42.86)
Menopause treatment				
Ever	294	150	1	
Never	765	515	**1.30**	**(1.04, 1.64)**
Missing/Pre-menopause	399	338	1.26	(0.93, 1.71)
Physical activity [a], METs				
0	891	676	1	
>0 to 8	272	162	**0.75**	**(0.60, 0.93)**
>10 to 16	121	84	0.89	(0.66, 1.20)
>16	174	81	**0.60**	**(0.45, 0.79)**
Energy intake, kcal/day				
<1500	452	251	1	
1500-2000	485	352	**1.29**	**(1.05, 1.59)**
>2000	351	284	**1.39**	**(1.11, 1.74)**
DK/M	170	116	1.22	(0.91, 1.62)
Age at first birth				
<25	378	243	1	

25-28	422	277	1.07	(0.86, 1.34)
>28	389	262	1.07	(0.84, 1.35)
DK/M	269	221	1.26	(0.98, 1.63)
Nulliparity				
No	1197	213	1	
Yes	260	213	1.20	(0.97, 1.48)
Age at menarche (years)				
<=12	600	433	1	
13-14	668	453	0.96	(0.81, 1.15)
>14	178	104	0.87	(0.66, 1.14)
DK/M	12	13	1.77	(0.79, 3.95)
Breastfeeding (months)				
0	464	349	1	
>0-6	418	317	1.00	(0.81, 1.22)
>6-12	235	169	0.97	(0.76, 1.24)
>12	341	168	**0.70**	**(0.55, 0.89)**

Mixed models with recruitment area as random effect and adjusted by age and educational level.

1Table S2. Differences between the included and the excluded population.

		Excluded		Included		
		N	%	N	%	p-value
Total		861	25.9	2,461	74.1	
Case/control	Controls	282	32.75	1,458	59.24	<0.001
	Cases	579	67.25	1,003	40.76	
Area	Asturias	21	2.44	152	6.18	<0.001
	Barcelona A	39	4.53	254	10.32	
	Barcelona B	27	3.14	134	5.44	
	Barcelona C	76	8.83	140	5.69	
	Cantabria	94	10.92	235	9.55	
	Guipuzcoa	123	14.29	358	14.55	
	León	149	17.31	279	11.34	
	Madrid	165	19.16	541	21.98	
	Navarra	142	16.49	265	10.77	
	Valencia	25	2.9	103	4.19	
Age, years	<=50	356	41.35	746	30.31	<0.001
	51-60	202	23.46	624	25.36	
	61-70	130	15.1	612	24.87	
	>70	173	20.09	479	19.46	
Education	<Primary school	150	17.42	387	15.73	0.33
	Primary school	253	29.38	796	32.34	
	Secondary school	284	32.98	773	31.41	
	University	174	20.21	505	20.52	
Menopausal status	postmenopausal	510	59.23	1,734	70.46	<0.001
	premenopausal	350	40.65	724	29.42	
	DK/M	1	0.12	3	0.12	
Family history of breast cancer	No	606	70.38	1,800	73.14	0.283
	Yes	223	25.9	573	23.28	
	missing	32	3.72	88	3.58	
Body mass index, Kg/m^2	<25.00	410	48.46	1,222	49.65	0.634
	25.00-29.99	288	34.04	794	32.26	
	30.00 or more	148	17.49	445	18.08	
Menopause treatment	Ever	99	11.5	444	18.04	<0.001
	Never	410	47.62	1,280	52.01	

	DK/M	352	40.88	737	29.95	
Occupational status	Working	426	49.48	1,039	42.22	<0.001
	Not working	66	7.67	158	6.42	
	Housewife	202	23.46	727	29.54	
	Retired	167	19.4	537	21.82	
Oral contraceptive use	Never	476	55.28	1,365	55.47	0.915
	Ever	383	44.48	1,092	44.37	
	DK/M	2	0.23	4	0.16	
Physical activity[a], METs	0	562	66.51	1,567	63.67	0.102
	>0 to 8	118	13.96	434	17.64	
	>10 to 16	76	8.99	205	8.33	
	>16	89	10.53	255	10.36	
Energy intake, **kcal/day**	<1500	208	24.16	703	28.57	<0.001
	1500-2000	269	31.24	837	34.01	
	>2000	225	26.13	635	25.8	
	missing	159	18.47	286	11.62	

a. physical activity: metabolic equivalents (METs) total h/week; annual median in the last 10 years. DK/M: Don't know or missing.
p-values for the chi^2 test.

Table S3. Odds ratio (OR) and 95% confidence interval (CI) of breast cancer associated with adult-lifetime residential levels of total trihalomethanes (TTHMs), chloroform and brominated trihalometanes (Br-THMs), stratified by menopausal status.

	ALL N=2,461		Pre-menopause N= 723		Post-menopause N=1,734	
	OR	(95%CI)	OR	95%CI	OR	95%CI
TTHMs (μg/L)						
<=21.7	1		1		1	
>21.7-30.5	1.15	(0.88, 1.49)	1.08	(0.66, 1.76)	1.15	(0.85, 1.58)
>30.5-48.3	1.09	(0.81, 1.46)	1.21	(0.80, 1.81)	0.91	(0.62, 1.34)
>48.3	1.14	(0.83, 1.57)	1.45	(0.90, 2.35)	1.12	(0.72, 1.74)
Chloroform (μg/L)						
<=7.6	1		1		1	
>7.6-18.8	1.22	(0.92, 1.62)	1.33	(0.79, 2.22)	1.31	(0.93, 1.84)
>18.8-24.3	1.25	(0.95, 1.65)	1.20	(0.77, 1.89)	1.38	(0.95, 2.01)
>24.3	1.40	(1.01, 1.95)	1.50	(1.00, 2.26)	1.39	(0.89, 2.16)
Br-THMs (μg/L)						
<=5.5	1		1		1	
>5.5-10.1	1.16	(0.84, 1.59)	1.18	(0.77, 1.79)	1.00	(0.69, 1.43)
>10.1-31.0	0.90	(0.64, 1.25)	0.88	(0.57, 1.35)	0.70	(0.45, 1.08)
>31.0	0.95	(0.67, 1.35)	1.26	(0.78, 2.03)	0.78	(0.51, 1.21)

TTHMs: total trihalomethanes. Br-THMs: brominated trihalometanes. Mixed models with area as random effect. Exposure variables are categorized into quartiles. Models adjusted for age, educational level, occupational status, family history of BC, BMI, energy intake, physical activity, oral contraceptive use and menopause treatment use. Chloroform and Br-THMs models mutually adjusted for chloroform and Br-THMs. p-values for interaction between THMs and menopausal status on BC > 0.05.

Table S4. Odds ratio (OR) and 95% confidence interval (CI) of breast cancer associated with adult-lifetime residential levels of total trihalomethanes (TTHMs), chloroform and brominated trihalometanes (Br-THMs), stratified by educational level in two categories.

	ALL N=2,461		Primary or less N=1183		Secondary & university N=1278	
	OR	(95%CI)	OR	95%CI	OR	95%CI
TTHMs (μg/L)						
<=21.7	1		1		1	
>21.7-30.5	1.15	(0.88, 1.49)	1.21	(0.83, 1.75)	1.10	(0.74, 1.62)
>30.5-48.3	1.09	(0.81, 1.46)	0.94	(0.59, 1.50)	1.15	(0.76, 1.73)
>48.3	1.14	(0.83, 1.57)	1.12	(0.73, 1.72)	1.17	(0.71, 1.93)
Chloroform (μg/L)						
<=7.6	1		1		1	
>7.6-18.8	1.22	(0.92, 1.62)	1.23	(0.86, 1.76)	1.21	(0.82, 1.80)
>18.8-24.3	1.25	(0.95, 1.65)	1.41	(0.98, 2.01)	1.05	(0.69, 1.58)
>24.3	1.40	(1.01, 1.95)	1.16	(0.75, 1.77)	1.62	(1.03, 2.54)
Br-THMs (μg/L)						
<=5.5	1		1		1	
>5.5-10.1	1.16	(0.84, 1.59)	0.67	(0.45, 1.00)	1.93	(1.22, 3.05)
>10.1-31.0	0.90	(0.64, 1.25)	0.74	(0.49, 1.11)	1.19	(0.72, 1.99)
>31.0	0.95	(0.67, 1.35)	0.69	(0.48, 1.01)	1.36	(0.83, 2.24)

TTHMs: total trihalomethanes. Br-THMs: brominated trihalometanes. Mixed models with area as random effect. Exposure variables are categorized into quartiles. Models adjusted for age, educational level, occupational status, family history of BC, BMI, energy intake, physical activity, oral contraceptive use and menopause treatment use. Chloroform and Br-THMs models mutually adjusted for chloroform and Br-THMs.

Table S5. Odds ratio (OR) and 95% confidence interval (CI) of breast cancer associated with adult-lifetime residential levels of total trihalomethanes (TTHMs), chloroform and brominated trihalometanes (Br-THMs), adjusted by further socioeconomic variables.

	Main models N=2,461		Models further adjusted by social class based on largest occupation N=2,123		Models further adjusted by parental socioeconomic status N=2,398		Models further adjusted by partner educational level N=2,446	
	OR	(95%CI)	OR	95%CI	OR	95%CI	OR	95%CI
TTHMs (µg/L)								
<=21.7	1		1		1		1	
>21.7-30.5	1.15	(0.88, 1.49)	1.10	(0.83, 1.47)	1.19	(0.91, 1.54)	1.16	(0.89, 1.51)
>30.5-48.3	1.09	(0.81, 1.46)	1.10	(0.81, 1.50)	1.15	(0.86, 1.53)	1.09	(0.81, 1.46)
>48.3	1.14	(0.83, 1.57)	1.35	(0.97, 1.86)	**1.38**	**(1.03, 1.84)**	1.17	(0.84, 1.62)
Chloroform (µg/L)								
<=7.6	1		1		1		1	
>7.6-18.8	1.22	(0.92, 1.62)	1.29	(0.98, 1.70)	**1.37**	**(1.06, 1.77)**	1.23	(0.92, 1.64)
>18.8-24.3	1.25	(0.95, 1.65)	1.17	(0.89, 1.53)	1.24	(0.96, 1.60)	1.25	(0.93, 1.68)
>24.3	**1.40**	**(1.01, 1.95)**	1.26	(0.92, 1.72)	**1.38**	**(1.03, 1.85)**	**1.43**	**(1.01, 2.02)**
Br-THMs (µg/L)								
<=5.5	1		1		1		1	
>5.5-10.1	1.16	(0.84, 1.59)	1.26	(0.94, 1.70)	1.17	(0.89, 1.54)	1.16	(0.84, 1.59)
>10.1-31.0	0.90	(0.64, 1.25)	0.87	(0.63, 1.21)	0.87	(0.65, 1.18)	0.91	(0.64, 1.28)
>31.0	0.95	(0.67, 1.35)	1.19	(0.90, 1.58)	1.16	(0.89, 1.51)	0.96	(0.67, 1.38)

TTHMs: total trihalomethanes. Br-THMs: brominated trihalometanes. Mixed models with area as random effect. Exposure variables are categorized into quartiles. Models adjusted for age, educational level, occupational status, family history of BC, BMI, energy intake, physical activity, oral contraceptive use and menopause treatment use. Chloroform and Br-THMs models mutually adjusted for chloroform and Br-THMs.

Table S6. Odds ratio (OR) and 95% confidence interval (CI) of breast cancer associated with residential levels of total trihalomethanes (TTHMs), chloroform and brominated trihalomethanes (Br-THMs) in the two largest metropolitan areas in Spain.

			Controls	Cases	OR[a]	95%CI	OR[b]	95%CI
Barcelona (N=528)	TTHMs (µg/L)	<=71.1	82	36	1			
		>71.1-87.3	80	74	1.77	(0.96, 3.26)		
		>87.3-110.5	80	37	0.80	(0.39, 1.65)		
		>110.5	80	59	1.72	(0.79, 3.78)		
		Total	322	206	*ptrend*	*0.441*		
	Chloroform (µg/L)	<=17.8	81	42	1		1	
		>17.8-20.6	81	45	1.13	(0.62, 2.07)	1.08	(0.56, 2.06)
		>20.6-23.9	80	44	1.16	(0.54, 2.50)	1.31	(0.54, 3.19)
		>23.9	80	75	1.57	(0.72, 3.40)	1.62	(0.67, 3.91)
		Total	322	206	*ptrend*	*0.219*		*0.223*
	Br-THMs (µg/L)	<=48.8	82	40	1		1	
		>48.8-64.4	80	70	1.56	(0.87, 2.78)	1.46	(0.80, 2.67)
		>64.4-91.8	80	39	0.77	(0.38, 1.54)	0.76	(0.37, 1.57)
		>91.8	80	57	1.76	(0.80, 3.90)	1.87	(0.78, 4.50)
		Total	322	206	*ptrend*	*0.478*		*0.478*
Madrid (N=541)	TTHMs (µg/L)	<=29.4	78	67	1			
		>29.4-32.4	76	49	0.67	(0.40, 1.12)		
		>32.4-34.4	75	57	0.89	(0.54, 1.48)		
		>34.4	76	63	1.15	(0.70, 1.91)		
		Total	305	236	*ptrend*	*0.595*		
	Chloroform (µg/L)	>=25.6	77	69	1		1	
		>25.6-28.5	76	45	0.65	(0.39, 1.09)	0.64	(0.33, 1.25)
		>28.5-30.3	78	62	0.92	(0.56, 1.53)	0.79	(0.36, 1.75)
		>30.3	74	60	1.19	(0.71, 1.98)	1.16	(0.51, 2.59)
		Total	305	236	*ptrend*	*0.674*		*0.572*
	Br-THMs (µg/L)	<3.5	77	64	1		1	
		>3.5-3.8	76	50	0.72	(0.43, 1.21)	0.91	(0.44, 1.88)
		>3.8-4.0	76	69	1.04	(0.62, 1.73)	1.24	(0.53, 2.92)
		>4.0	76	53	0.78	(0.46, 1.32)	0.74	(0.33, 1.67)
		Total	305	236	*ptrend*	*0.962*		*0.703*

TTHMs: total trihalomethanes. Br-THMs: brominated trihalometanes. Models in the Barcelona metropolitan area are mixed models with recruitment area as random effect. Models in Madrid are logistic regressions. Exposure variables are categorized into quartiles. a. Models adjusted by age, educational level, occupational status, family history of BC, BMI, energy intake, physical activity, oral contraceptive use and menopause treatment use. b. Models mutually adjusted for chloroform and Br-THMs.

Table S7. Odds ratio (OR) and 95% confidence interval (CI) of breast cancer associated with average residential levels of total trihalomethanes (TTHMs), chloroform, and brominated trihalomethanes (Br-THMs) in the last 10 years. N=2,428.

	Controls	**Cases**	**OR[a]**	**95%CI**	**OR[b]**	**95%CI**
TTHM (µg/L)						
<=21.2	391	225	1			
21.2-30.3	361	242	1.03	(0.71, 1.51)		
>30.3-40.7	329	262	1.21	(0.84, 1.72)		
>40.7	360	258	1.16	(0.84, 1.60)		
Total	1441	987	*ptrend*	*0.215*		
Cont. (10 µg/L)			0.99	(0.95, 1.03)		
Chloroform (µg/L)						
<=7.6	386	203	1		1	
7.6-21.7	352	271	**1.61**	**(1.13, 2.29)**	**1.79**	**(1.20, 2.67)**
>21.7-24.8	358	220	1.11	(0.77, 1.60)	1.31	(0.84, 2.03)
>24.8	345	293	**1.53**	**(1.08, 2.18)**	**1.84**	**(1.20, 2.82)**
Total	1441	987	*ptrend*	***0.038***	*ptrend*	***0.022***
Cont. (10 µg/L)			**1.12**	**(1.00, 1.26)**	1.11	(0.99, 1.25)
BrTHM (µg/L)						
<=7.0	368	296	1		1	
7.0-8.1	377	205	0.71	(0.49, 1.03)	0.73	(0.48, 1.11)
>8.1-37.9	338	237	1.05	(0.71, 1.54)	1.25	(0.80, 1.96)
>37.9	358	249	0.90	(0.59, 1.36)	0.90	(0.51, 1.57)
Total	1441	987	*ptrend*	*0.815*	*ptrend*	*0.371*
Cont. (10 µg/L)			0.98	(0.95, 1.03)	0.99	(0.96, 1.03)

TTHMs: total trihalomethanes. Br-THMs: brominated trihalometanes. Mixed models with recruitment area as random effect. a. Models adjusted by age, educational level, occupational status, family history of BC, BMI, energy intake, physical activity, oral contraceptive use and menopause treatment use. b. Models mutually adjusted for chloroform and Br-THMs.