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## **Supplemental Material**

### **Colorectal Cancer and Long-Term Exposure to Trihalomethanes in Drinking Water: A Multicenter Case-Control Study in Spain and Italy**

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**Figure S3.** Spline of colorectal cancer risk (odds ratio, Y axis) associated with chloroform levels ( $\mu\text{g/l}$ , X axis) among men, from generalized additive models adjusted for age, sex, education, smoking, non-steroidal anti inflammatory drugs, smoking, physical activity and family history of colorectal cancer. P-value of gain from the linearity is statistically significant in Barcelona (p-value  $<0.001$ ), Leon (p-value 0.03), Madrid (p-value 0.04), and Navarra (p-value 0.01). Tick marks above the x-axes represent observations, and the dashed lines represent the 95% confidence intervals. Note that scale of x -axes differ by area.

**Figure S4.** Spline of colorectal cancer risk (odds ratio, Y axis) associated with total brominated THM levels ( $\mu\text{g/l}$ , X axis) among men, from generalized additive models adjusted for age, sex, education, smoking, non-steroidal anti inflammatory drugs, smoking, physical activity and family history of colorectal cancer. P-value of gain from the linearity is statistically significant in Barcelona (p-value  $<0.001$ ), Cantabria (p-value  $<0.01$ ), and Navarra (p-value  $<0.01$ ). Tick marks above the x-axes represent observations, and the dashed lines represent the 95% confidence intervals. Note that scale of x-axes differ by area.

**Figure S5.** Exposure-response relationship between ingested THM levels (X axis) and colorectal cancer (Y axis, expressed in odds ratios (OR) with 95% confidence intervals (95% CI)) among 2047 cases and 3684 controls. Adjusted for sex, age, area, education, smoking, physical activity, non-steroidal anti inflammatory drugs, and family history of cancer. Excludes unsatisfactory interviews and subjects with less than 70% THM estimated from the exposure window. P-value gain compared to linearity is  $<0.01$  for all models, except for chloroform in women (p-value=0.32). Tick marks above the x-axes represent observations, and the dashed lines represent the 95% confidence intervals. Note that scale of x-axes differ by area.

**Figure S6.** Exposure-response relationship between shower-bath THM levels (X axis) and colorectal cancer risk (Y axis, expressed in odds ratios (OR) with 95% confidence intervals (95% CI)) among 1702 cases and 3269 controls. Adjusted for (sex), age, geographical area, education, non steroidal anti inflammatory drugs consumption, smoking, physical activity and family history of colorectal cancer Tick marks above the x-axes represent observations, and the dashed lines represent the 95% confidence intervals. Note that scale of x-axes differ by area.

**Table S3.** Association between colorectal cancer and average THM concentrations in residential tap water by cancer site among 1273 colon cases, 537 rectal cases, and 3454 controls (27 cases had an unspecified localization).

**Table S1.** Previous studies evaluating colorectal cancer from individual-based studies (case-control or cohort designs) including incident cases with exposure assessment based on personal information from residential histories

Study design and reference	Country	Recruitment period	Study population	Colon cancer OR (95%CI) <sup>a</sup>	Rectal cancer OR (95%CI) <sup>a</sup>	Colorectal cancer OR (95%CI) <sup>a</sup>	Sex	Exposure index and levels	Exposure window
<b>Case-control</b>									
Bove et al. 2007	USA	1978-1986	128 rectal cancer cases 253 controls	-	<b>2.32 (1.22, 4.39)</b>	-	Men	Bromoform ingested 1.69-15.43 vs. 0.9-0.64 µg/day	Not specified
King et al. 2000	Canada	1992-1994	767 colon cancer cases 661 rectal cancer cases 1545 controls	<b>1.87 (1.15, 3.05)</b> 0.92 (0.49, 1.71)	0.98 (0.56, 1.72) 0.72 (0.34, 1.53)	Not shown	Men Women	Total trihalomethanes ≥75 vs. ≤24 µg/l	40 years before the study
Hildesheim et al. 1998	USA	1986-1989	560 colon cancer cases 537 rectal cancer cases, 1983 controls	1.06 (0.7, 1.6)	<b>1.66 (1.1, 2.6)</b>	Not shown	All	Total THM ≥46.4 vs. ≤0.7 µg/l	Lifetime
Young et al. 1987	USA	Not specified	347 colon cancer cases 611 population controls [and 639 cancer controls]	0.73 (0.44, 1.21) [0.93 (0.55, 1.57)]	-	-	All	>300 vs. <100 mg cumulative total THM exposure	Lifetime
Cragle et al. 1985	USA	1978-1980	200 colon cancer cases 407 controls	<b>3.36 (2.41, 4.61)</b>	-	-	All	Years living in households receiving chlorinated water, >15 vs. <15 years	1953-1978 (25 years)
<b>Cohort</b>				RR (95% CI) <sup>b</sup>	RR (95% CI) <sup>b</sup>	RR (95% CI) <sup>b</sup>			
Koivusalo et al. 1997	Finland	1970-1993	621431 subjects 1473 colon cancer cases 944 rectal cancer cases	0.83 (0.66, 1.04) 0.95 (0.78, 1.85) 0.90 (0.77, 1.04)	0.85 (0.66, 1.09) <b>1.38 (1.03, 1.85)</b> 1.04 (0.86, 1.26)	Not shown	Men Women All	Mutagenicity <sup>c</sup>	1971-1993 (22 years)
				HR (95% CI) <sup>a</sup>	HR (95% CI) <sup>a</sup>	HR (95% CI) <sup>a</sup>			
Doyle et al. 1997	USA	1985-1992	28237 subjects 178 colon cancer cases 78 rectal cancer cases	<b>1.68 (1.1, 2.53)<sup>a</sup></b>	1.07 (0.60, 1.93) <sup>a</sup>	Not shown	Women	Chloroform, 14-287 µg/l vs. <limit of detection	>10 years before the study

<sup>a</sup> Odds ratio (OR) or hazard ratio (HR) and 95% confidence intervals (CI) correspond to the highest versus lowest exposure categories reported.

<sup>b</sup> Relative risk (RR) and 95% confidence interval (CI) based on continuous variable of exposure and calculated for the mutagenicity level of 3000 net rev/l.

<sup>c</sup> Mutagenicity estimated from an empirical equation relating Ames test results with raw water characteristics and treatment. The risk estimate represents the relative risk of an average exposure in a town using chlorinated surface compared with non chlorinated surface water)

**Table S2.** Single nucleotide polymorphisms (SNPs) of *CYP2E1* and *GSTZ1* examined in the Spanish population of the study, and nominal interaction p-value for total trihalomethanes (THM), chloroform (CHCl<sub>3</sub>), and brominated THMs (BrTHM) dichotomized at the percentile 75, that are respectively 60, 20 and 40 µg/l.

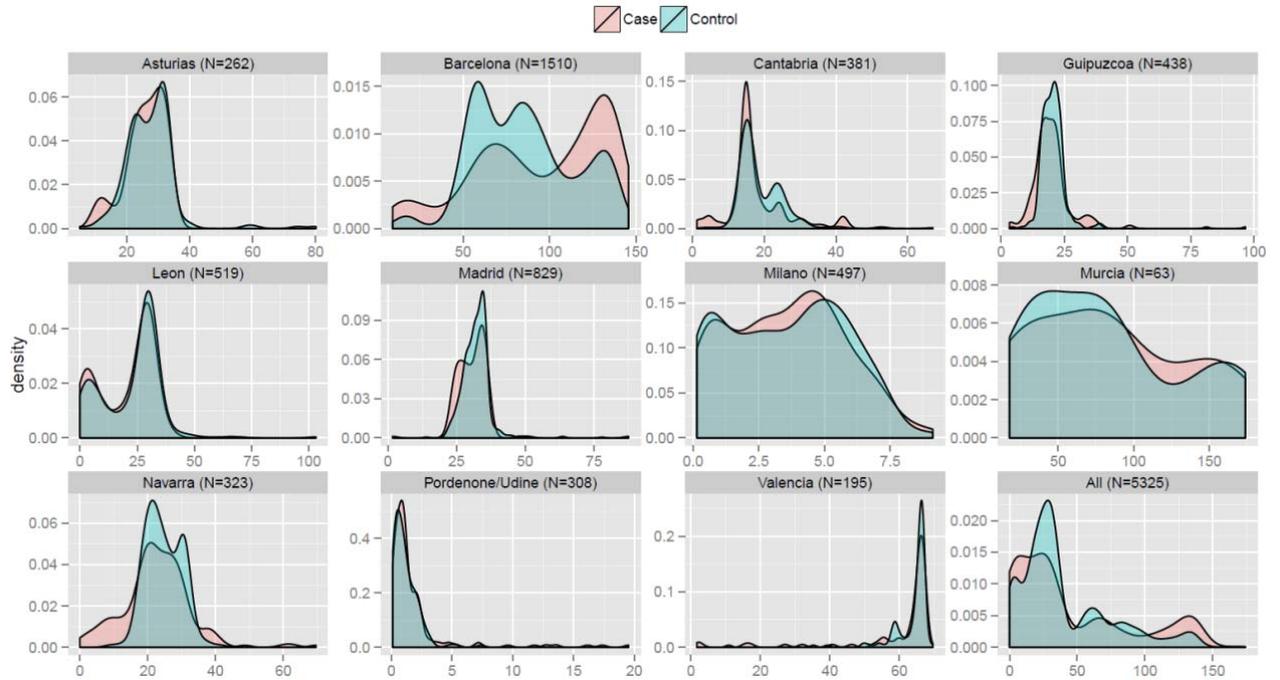
gene	SNP	chr	position	A1	A2	HWE	MAF	joint effect nominal p-values <sup>a</sup>		
								Total THM	CHCl <sub>3</sub>	BrTHM
<i>CYP2E1</i>	rs2070673	10	135340567	A	T	0.806	17.5	0.104	0.816	0.233
<i>CYP2E1</i>	rs6413420	10	135340829	T	G	0.748	5.9	0.709	0.613	0.788
<i>CYP2E1</i>	rs943975	10	135342260	C	T	0.911	8.7	0.715	0.969	0.658
<i>CYP2E1</i>	rs915906	10	135343738	C	T	0.359	16.1	0.346	0.221	0.403
<i>CYP2E1</i>	rs8192772	10	135344711	C	T	0.786	7.0	0.169	0.233	0.129
<i>CYP2E1</i>	rs6413421	10	135345811	C	T	1.000	6.0	0.684	0.586	0.730
<i>CYP2E1</i>	rs2070675	10	135346696	T	C	0.189	17.1	<b>0.029</b>	0.646	<b>0.009</b>
<i>CYP2E1</i>	rs915907	10	135346927	A	C	0.202	14.8	<b>0.031</b>	0.392	<b>0.009</b>
<i>CYP2E1</i>	rs915908	10	135346959	A	G	0.904	18.1	0.444	0.472	0.413
<i>CYP2E1</i>	rs8192775	10	135348026	A	G	0.365	7.4	0.103	0.383	<b>0.021</b>
<i>CYP2E1</i>	rs743535	10	135349367	A	G	0.733	8.5	0.056	0.063	<b>0.019</b>
<i>CYP2E1</i>	rs1329149	10	135349801	T	C	0.137	13.0	0.488	0.966	0.883
<i>CYP2E1</i>	rs2515642	10	135352013	C	T	0.916	21.4	0.137	0.436	0.183
<i>CYP2E1</i>	rs2480259	10	135352076	A	G	0.916	21.4	0.124	0.481	0.166
<i>CYP2E1</i>	rs2249694	10	135352153	A	G	0.916	21.4	0.140	0.471	0.186
<i>GSTZ1</i>	rs8177538	14	77787706	G	C	1.000	8.9	0.986	0.922	0.960
<i>GSTZ1</i>	rs3759733	14	77788838	A	G	0.093	41.3	0.837	0.740	0.839
<i>GSTZ1</i>	rs2363643	14	77788908	A	G	0.900	30.4	0.646	0.626	0.700
<i>GSTZ1</i>	rs2287395	14	77791519	G	A	0.119	26.1	0.953	0.868	0.977
<i>GSTZ1</i>	rs7975	14	77793207	A	G	0.294	30.2	0.772	0.632	0.774
<i>GSTZ1</i>	rs7972	14	77793237	A	G	0.423	8.4	0.781	0.750	0.753
<i>GSTZ1</i>	rs1046428	14	77794283	T	C	0.877	22.0	0.294	0.828	0.636

SNP, single nucleotide polymorphism; chr (chromosome); A1, minor allele; A2, wild type allele; HWE, Hardy Weinberg Equilibrium test p-value; MAF, minor allele frequency.

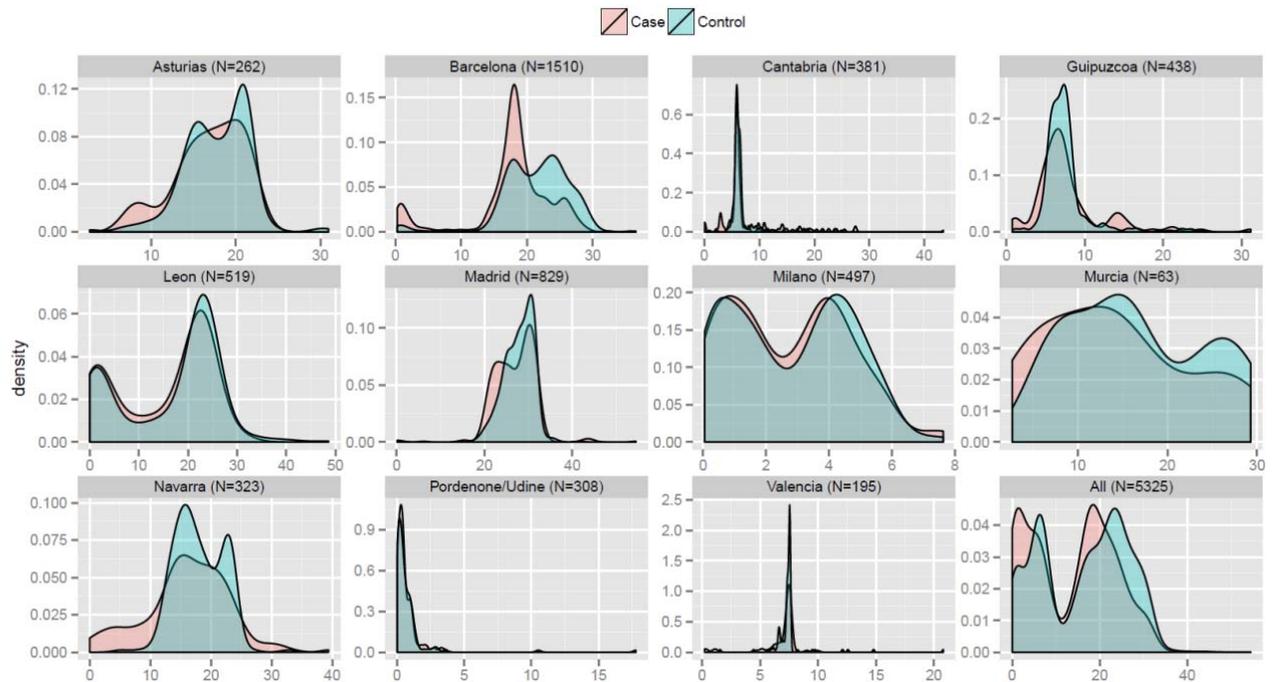
<sup>a</sup> p-value from a likelihood ratio test for the joint effect of the SNP and interaction term, using the dominant model (heterozygous and homozygous variant versus homozygous wild type).

**Figure S1.** Distribution of exposure density of average lifetime THM concentrations in residential tap water ( $\mu\text{g/l}$ ) among 1837 cases and 3488 controls with exposure estimates  $\geq 70\%$  of the exposure window. Note that scale of x- and y-axes differ by area.

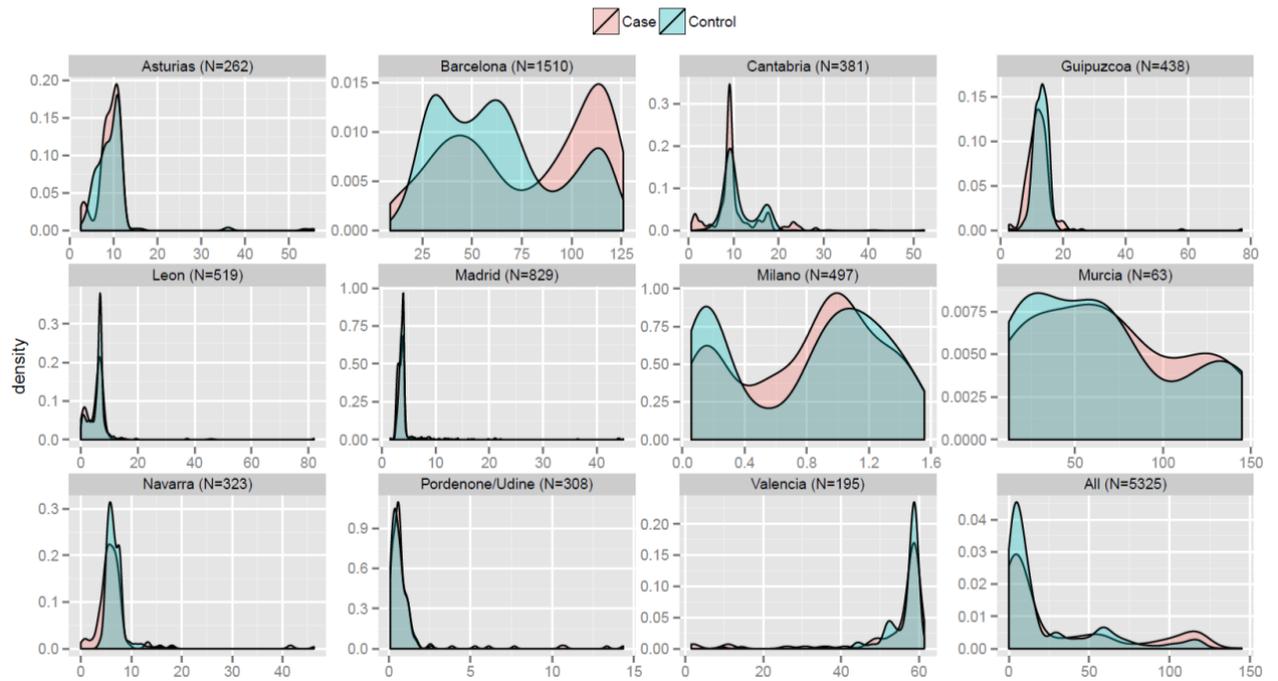
A) Total trihalomethanes



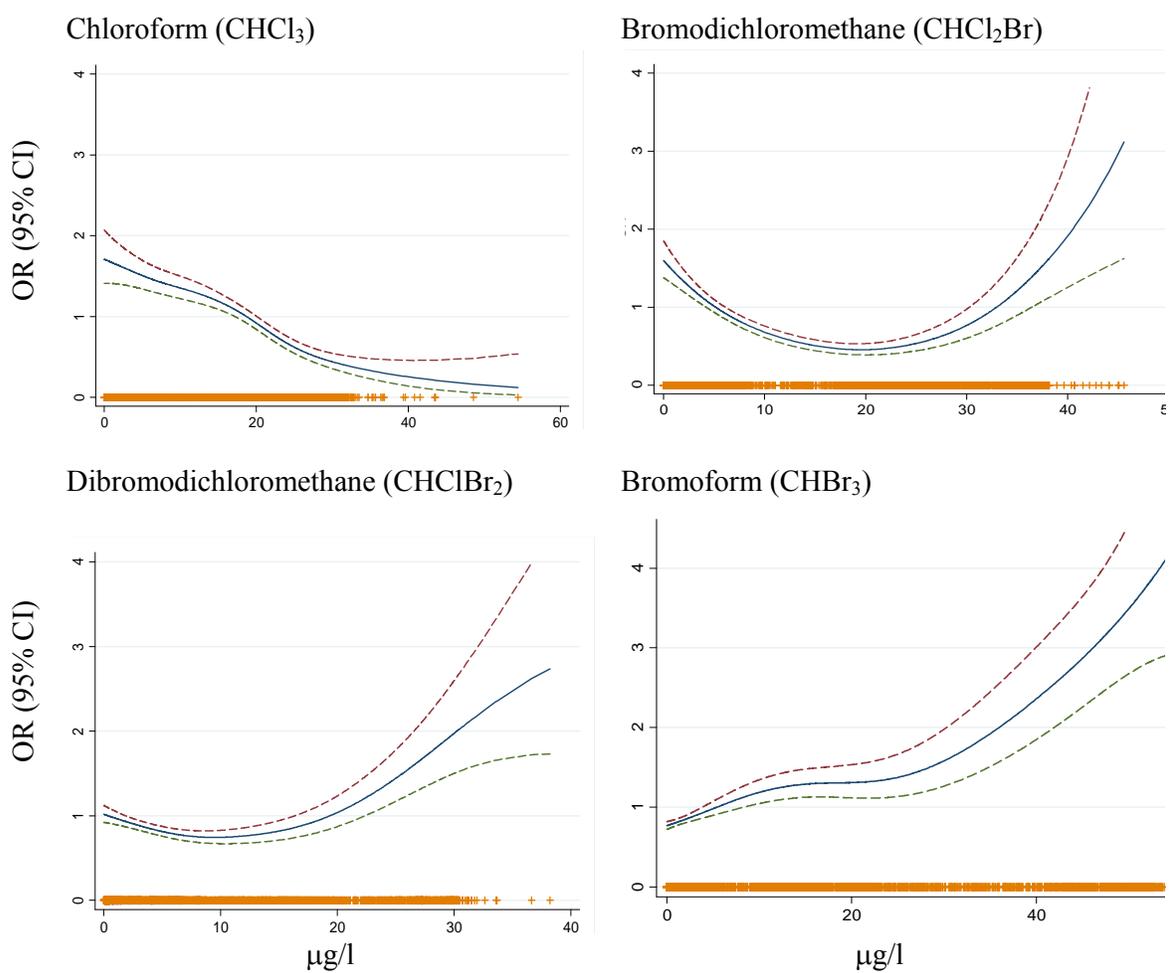
B) Chloroform



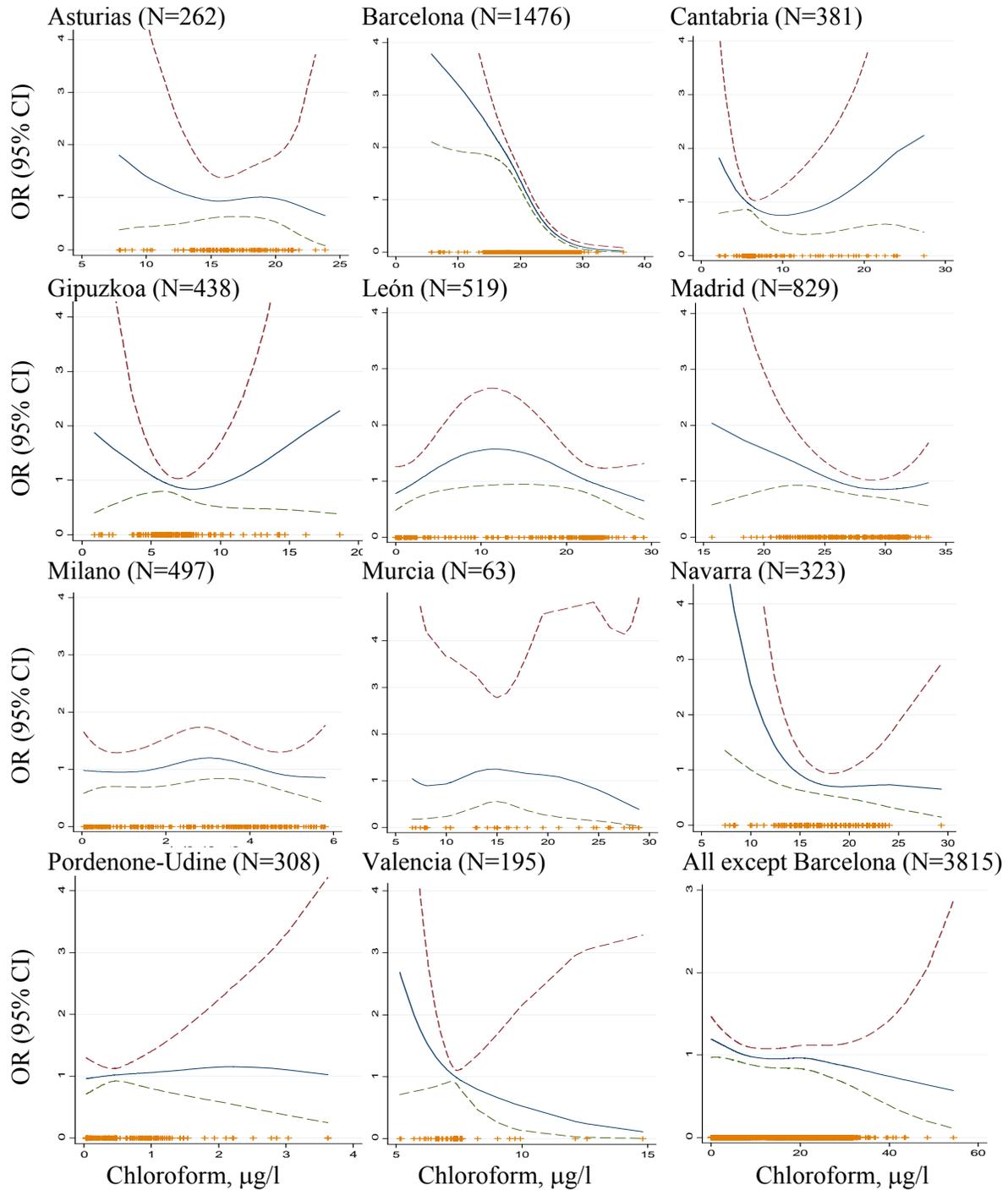
### C) Total brominated THMs



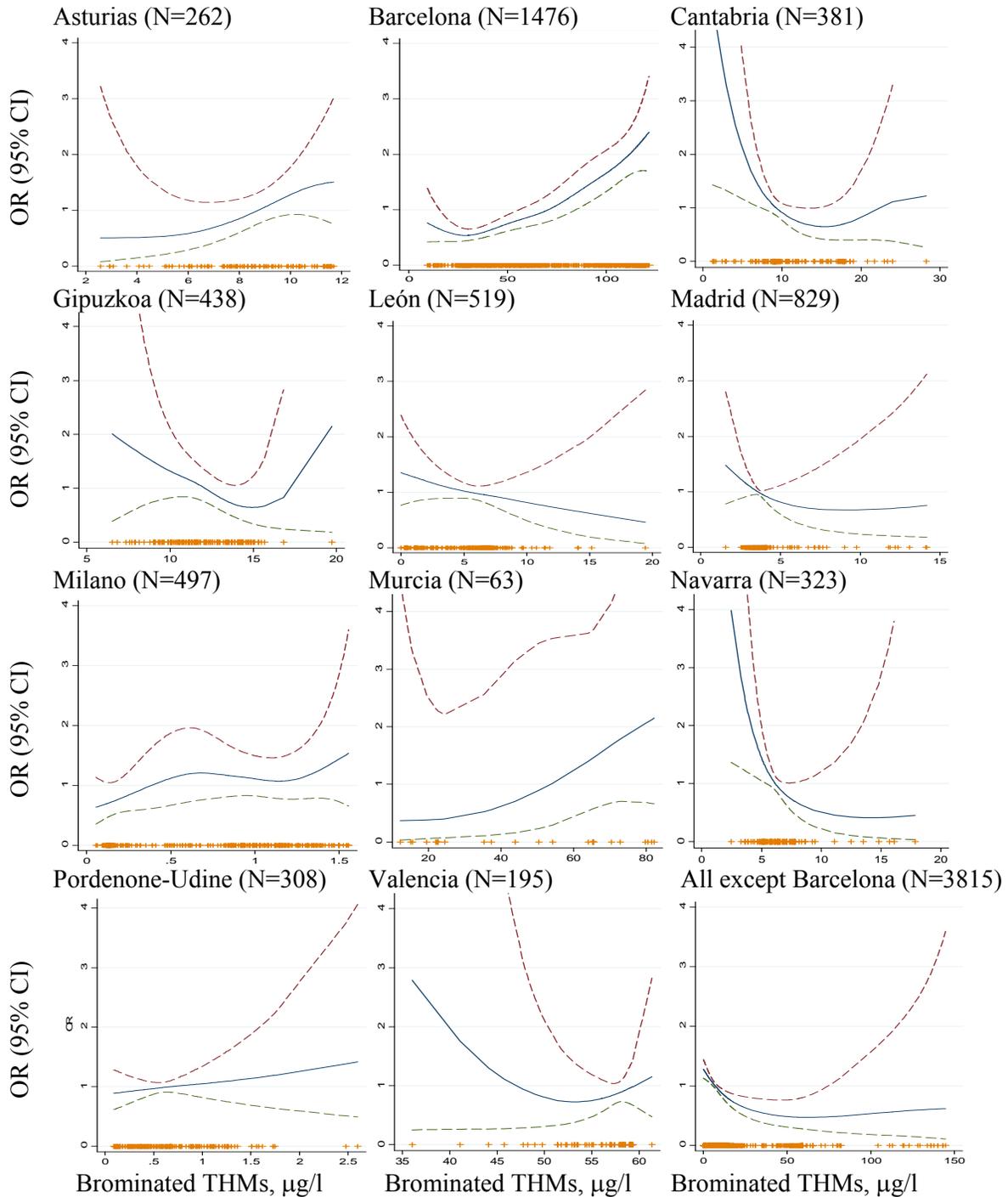
**Figure S2.** Exposure-response relationship between residential trihalomethane (THM) levels (X axis, in  $\mu\text{g/l}$ ) and colorectal cancer risk (Y axis, expressed in odds ratios) among 1837 cases and 3454 controls. Odds ratios (95 CI) are adjusted for sex, age, area, education, smoking, physical activity, non-steroidal anti inflammatory drugs and family history of colorectal cancer. Excludes unsatisfactory questionnaires and subjects with THM estimated less than 70% from the exposure window. Tick marks above the x-axes represent observations, and the dashed lines represent the 95% confidence intervals. Note that scale of x-axes differs by chemical.



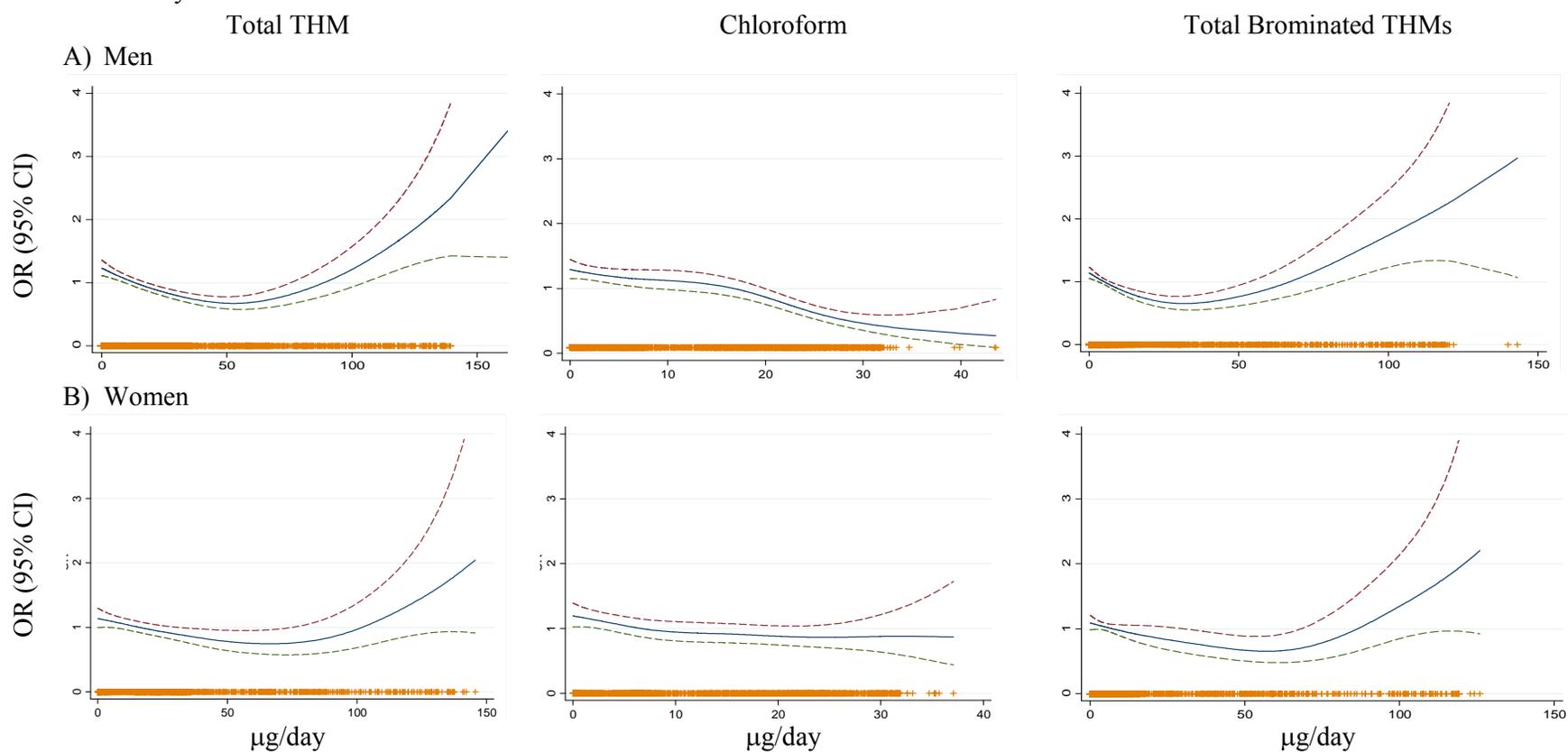
**Figure S3.** Spline of colorectal cancer risk (odds ratio, Y axis) associated with chloroform levels ( $\mu\text{g/l}$ , X axis) among men, from generalized additive models adjusted for age, sex, education, smoking, non-steroidal anti inflammatory drugs, smoking, physical activity and family history of colorectal cancer. P-value of gain from the linearity is statistically significant in Barcelona (p-value <0.001), Leon (p-value 0.03), Madrid (p-value 0.04), and Navarra (p-value 0.01). Tick marks above the x-axes represent observations, and the dashed lines represent the 95% confidence intervals. Note that scale of x -axes differs by area.



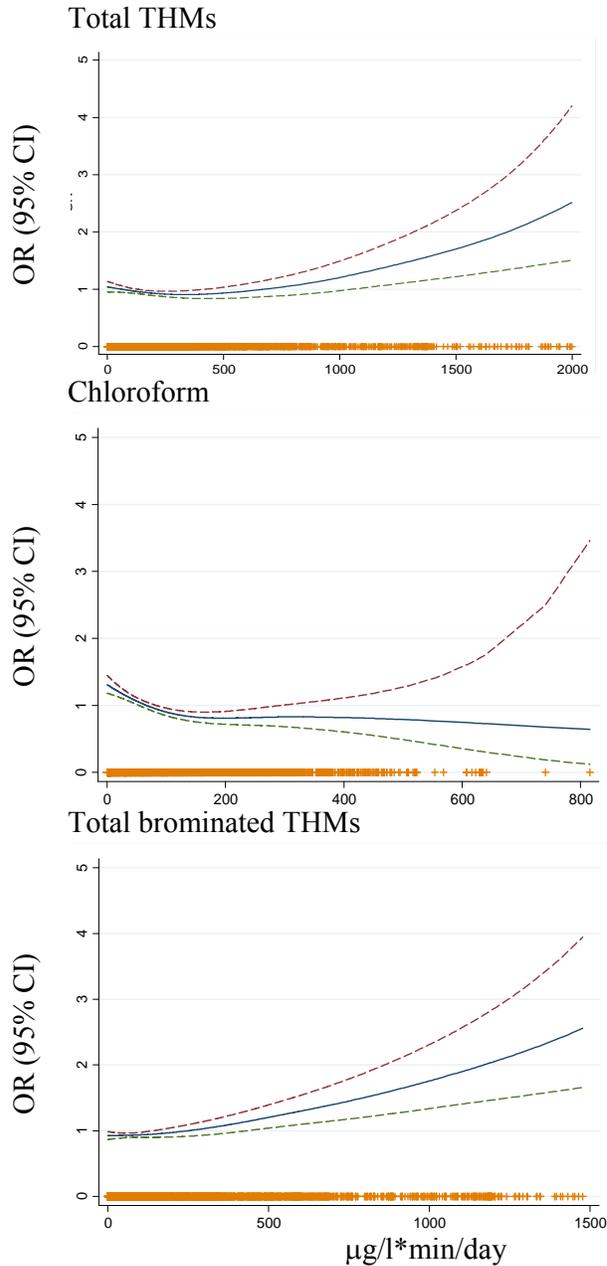
**Figure S4.** Spline of colorectal cancer risk (odds ratio, Y axis) associated with total brominated THM levels ( $\mu\text{g/l}$ , X axis) among men, from generalized additive models adjusted for age, sex, education, smoking, non-steroidal anti inflammatory drugs, smoking, physical activity and family history of colorectal cancer. P-value of gain from the linearity is statistically significant in Barcelona (p-value <0.001), Cantabria (p-value <0.01), and Navarra (p-value <0.01). Tick marks above the x-axes represent observations, and the dashed lines represent the 95% confidence intervals. Note that scale of x-axes differs by area.



**Figure S5.** Exposure-response relationship between ingested THM levels (X axis) and colorectal cancer (Y axis, expressed in odds ratios (OR) with 95% confidence intervals (95% CI)) among 2047 cases and 3684 controls. Adjusted for sex, age, area, education, smoking, physical activity, non-steroidal anti inflammatory drugs, and family history of cancer. Excludes unsatisfactory interviews and subjects with less than 70% THM estimated from the exposure window. P-value gain compared to linearity is <math><0.01</math> for all models, except for chloroform in women (p-value=0.32). Tick marks above the x-axes represent observations, and the dashed lines represent the 95% confidence intervals. Note that scale of x-axes differs by area.



**Figure S6.** Exposure-response relationship between shower-bath THM levels (X axis) and colorectal cancer risk (Y axis, expressed in odds ratios (OR) with 95% confidence intervals (95% CI) among 1702 cases and 3269 controls. Adjusted for (sex), age, geographical area, education, non steroidal anti inflammatory drugs consumption, smoking, physical activity and family history of colorectal cancer Tick marks above the x-axes represent observations, and the dashed lines represent the 95% confidence intervals. Note that scale of x-axes differs by area.



**Table S3.** Association between colorectal cancer and average THM concentrations in residential tap water by cancer site among 1273 colon cases, 537 rectal cases, and 3454 controls (27 cases had an unspecified localization).

Exposure	cases <sup>a</sup>	contr. <sup>a</sup>	OR <sup>b</sup> (95% CI)	cases <sup>a</sup>	contr. <sup>a</sup>	OR <sup>b</sup> (95% CI)
	Colon			Rectum		
Chloroform (µg/l), men						
<6	277	446	1	104	445	1
6-17.4	173	404	0.61 (0.44, 0.86)	94	401	0.82 (0.52, 1.27)
17.4-23.4	222	384	0.68 (0.48, 0.98)	108	380	0.75 (0.47, 1.19)
>23.4	120	567	0.24 (0.16, 0.36)	60	567	0.24 (0.14, 0.41)
<i>p-trend<sup>c</sup></i>			<0.001			<0.001
Chloroform (µg/l), women						
<6	147	298	1	46	298	1
6-17.4	120	491	0.63 (0.42, 0.95)	46	491	1.08 (0.52, 2.26)
17.4-23.4	127	414	0.52 (0.34, 0.81)	53	413	1.21 (0.56, 2.62)
>23.4	85	450	0.40 (0.24, 0.67)	26	450	0.64 (0.26, 1.56)
<i>p-trend<sup>c</sup></i>			0.001			0.336
Total brominated THM (µg/l), men						
<3.7	247	483	1	93	481	1
3.7-9	154	419	0.73 (0.51, 1.05)	81	416	0.74 (0.45, 1.21)
9-41.8	173	479	0.67 (0.39, 1.14)	65	478	0.34 (0.16, 0.71)
>41.8	218	420	1.42 (0.76, 2.65)	127	418	1.44 (0.60, 3.46)
<i>p-trend<sup>c</sup></i>			0.093			0.037
Total brominated THM (µg/l), women						
<3.7	133	309	1	51	309	1
3.7-9	119	524	0.79 (0.51, 1.25)	30	524	0.68 (0.33, 1.40)
9-41.8	89	484	0.35 (0.17, 0.72)	37	484	1.12 (0.39, 3.27)
>41.8	138	336	0.31 (0.13, 0.72)	53	335	1.21 (0.35, 4.25)
<i>p-trend<sup>c</sup></i>			0.017			0.894

<sup>a</sup> Numbers do not always add 1273 colon cases, 537 rectal cases, and 3454 controls due to observations dropped from the analyses because of insufficient observations in some covariable categories.

<sup>b</sup> Odds ratio (OR) and 95% confidence interval (CI) adjusted for sex, age, area, education, smoking, physical activity, non-steroidal anti inflammatory drugs, family history of colorectal cancer. Excludes unsatisfactory questionnaires and subjects with THM estimated less than 70% from the exposure window.

<sup>c</sup> Linear trend p-value, derived from a likelihood ratio test comparing a model with the categorical nitrate variable as an ordinal variable (0, 1, 2), with a model that excluded the variable.

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