ANNEXES

Article 1

REVIEW

Meta-analysis of studies on individual consumption of chlorinated drinking water and bladder cancer

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Study objective: To evaluate whether consumption of chlorinated drinking water is associated with bladder cancer.
Design: A bibliographic search was conducted and the authors selected studies evaluating individual consumption of chlorinated drinking water and bladder cancer. The authors extracted from each study risk estimates for intermediate and long term (>40 years) consumption of chlorinated water, stratified by sex when possible, and performed meta-analysis for the two exposure levels. A meta-analysis was also performed of the dose-response regression slopes.
Setting: Populations in Europe and North America.
Participants: Those included in six case-control studies (6084 incident bladder cancer cases, 10 816 controls) and two cohort studies (124 incident bladder cancer cases) fulfilling the inclusion criteria.
Main results: Ever consumption of chlorinated drinking water was associated with an increased risk of bladder cancer in men (combined OR=1.4, 95%CI 1.1 to 1.9) and women (combined OR=1.2, 95%CI 0.7 to 1.8). The combined OR for mid-term exposure in both genders was 1.1 (95% CI 1.0 to 1.2) and for long term exposure was 1.4 (95%CI 1.2 to 1.7). The combined estimate of the slope for a linear increase in risk was 1.13 (95% CI 1.08 to 1.20) for 20 years and 1.27 (95% CI 1.15 to 1.43) for 40 years of exposure in both sexes.
Conclusions: This meta-analysis of the best available epidemiological evidence indicates that long term consumption of chlorinated drinking water is associated with bladder cancer, particularly in men. The observed relative risk is only moderately high, but the population attributable risk could be important as the vast majority of the population of industrialised countries is potentially exposed to chlorination byproducts for long time periods.

Chlorinated drinking water contains a complex mixture of chlorinated and brominated byproducts with mutagenic and carcinogenic properties. Several toxicological and epidemiological studies have found a positive association between chlorinated drinking water consumption and bladder cancer. An International Agency for Research on Cancer (IARC) working group evaluated the human carcinogenicity for chlorinated drinking water in 1991[1] concluding that there was inadequate evidence for its carcinogenicity to humans (Group 3). This evaluation was based mainly on ecological and death certificate studies. Several epidemiological studies on bladder cancer published after 1991 evaluated individual lifetime consumption to chlorinated drinking water overcoming partially the limitations of earlier studies. All of them found positive associations with bladder cancer. In 1999 the International Agency for Research on Cancer (IARC) re-evaluated individual chlorination byproducts such as chloroform and other trihalomethanes (THM) concluding that there was inadequate evidence for their carcinogenicity.[2] It was argued that although diverse studies had associated chlorinated drinking water intake with cancer, single compounds could not be evaluated as they always occur in mixtures. A more recent report on disinfectant byproducts by the WHO[3] considered that the evidence was insufficient to determine whether observed associations are causal and determine which specific byproducts or other contaminants play a part. Furthermore, it concludes that the health risks from disinfectant byproducts at the levels at which they occur in drinking water are extremely small in comparison with the risks associated to inadequate disinfection. Apart from bladder cancer, other health effects such as colorectal cancer and adverse pregnancy outcomes have also been associated with chlorinated drinking water.[4–6] We performed a meta-analysis of results from epidemiological studies on individual consumption of chlorinated drinking water and bladder cancer following established guidelines.[7] We provide a summary risk estimate of bladder cancer risk associated to chlorinated drinking water exposure, partially overcoming the criticisms raised in international or national evaluations of this risk.

METHODS

Literature search

A systematic bibliographic search was performed looking for studies on bladder cancer and chlorinated drinking water. We focused on those epidemiological studies with accurate exposure assessment—that is, with individual information on long term patterns of water consumption. The availability of residential history obtained from individual interviews linked with water source was defined as the inclusion criterion for the meta-analysis. This inclusion criterion was set because in previous evaluations on disinfection byproducts and cancer by the WHO,[3] the absence of individual information was determined as crucial for the evaluation of cancer risk in humans. According to this criterion, ecological and cancer mortality based studies were excluded. Firstly, we searched in Medline all articles published without using any publication date limit. Search terms included bladder cancer, chlorine, chlorination, trihalomethanes (thm), mx, disinfectant agent, and tap water. The search was performed by consecutively entering single or combination of search terms. The search strategy is summarised as: [(chlorine & bladder cancer) or ((bladder cancer) & (disinfectant agent or chlorination or mx or thm))]. Among the 46 articles identified, 14 were review articles, seven were ecological studies, three were mortality based studies, one discussion, one editorial, one in Russian

Table 1 Description of studies included in the meta-analysis

	Location	Study population	Exposure measurement selected for the meta-analysis	Exposure categories			Confounders considered in the statistical analysis or study designs
				Referent category	Mid-term exposure	Long term exposure	
Case-control studies			*Duration of exposure to:*				
Cantor *et al* 1998[9]	Iowa (USA)	732 cases 914 population controls	Chlorinated surface water	0 years	1–39 years	≥40 years	Age, sex, study period, high risk occupation, and cigarettes.
Koivusalo *et al* 1998[8]	Finland	1123 cases 1983 population controls	Substantially mutagenic drinking water	<15 years	15–44 years	≥45 years	Age, socio economic status and smoking. Results stratified by sex.
King *et al* 1996[10]	Ontario (Canada)	696 cases 1545 population controls	Chlorinated surface water	≤9 years	10–34 years	≥35 years	Age, gender, log pack years of smoking, current smoking, education, and calorie intake.
McGeehin *et al* 1993[11]	Colorado (USA)	327 cases 261 other cancer sites controls	Chlorinated water	0 years	1–30 years	>30 years	Coffee consumption, smoking, tap water intake, family history of bladder cancer, sex, and medical history of bladder infection or kidney stones.
Vena *et al* 1993[15]	New York state (USA)	351 cases 855 population controls	Tap water	0–49 years consuming 0–5 glasses/day	0–49 years consuming >10 glasses/day	≥50 years consuming >10 glasses/day	Age, education, cigarette smoking (pack years), sodium, carotene, and non-tap water. Only men.
Cantor *et al* 1987[13]	USA	2855 cases 5258 population controls	Chlorinated surface water	0 years	1–39 years	≥40 years	Age, sex, smoking habit, high risk occupation, population size of usual residence and reporting centre.
Cohort studies							
Wilkins and Comstock 1981[14]	Washington County (USA)	31000 study subjects, 81 bladder cancer cases	Drinking water source	Deep well users		Chlorinated surface water users	Age, marital status, education, smoking history, frequency of church attendance, adequacy of housing, and persons per room.
Doyle *et al* 1997[16a] Iowa USA		28237 study subjects, 43 bladder cancer cases	Drinking water source	100% ground water source	Mixed surface-ground water	100% surface water source	Age, education, smoking status, pack years of smoking, physical activity, fruit and vegetable intake, total energy intake, body mass index, and waist to hip ratio

with English abstract not available, 14 were not epidemiological studies on bladder cancer and chlorination (methodological, experimental, or clinical studies), and seven fulfilled our a priori inclusion criteria.[8–14] A second search was performed using bladder cancer and tap water as search terms. From the 25 articles found, only one fulfilled the inclusion criteria.[15] The searches were replicated in Cancerlit and Embase databases. All references retrieved from Cancerlit were included in Medline. One reference found in Embase was not included in Medline.[16] This was a review article in Chinese. The reference lists of the papers selected and the most recent review articles were checked for undetected published studies. A certain number of studies were identified, the studies by Doyle[16a] and Freedman[17] the only ones partially fulfilling the inclusion criteria.

Data

We finally included in the meta-analysis six case-control studies[8–11 13 15] and two cohort studies[14 16a] evaluating individual consumption of drinking water through personal interviews (table 1). The six case-control studies included 6084 incident bladder cancer cases and 10<thin<816 controls. The cohort studies included 124 incident bladder cancer cases (table 1).

The study by Lynch[12] was excluded from the analysis, because although it fulfilled the inclusion criteria, the population study was included in the study by Cantor 1987.[13] The study by Freedman[17] is a case-control study nested in the cohort of the study by Wilkins and Comstock.[14] The study by Freedman, however, evaluated water consumption patterns of the study population only for a limited time period, precisely at the time of the same private census used in the cohort study. We included in the main meta-analysis the cohort study.[14] Although the number of bladder cancer cases was smaller in the cohort study, the exposure assessment was more accurate and closer to the dates in which the study was conducted. The study by Freedman was considered in an alternative analysis. Death certificate based case-control studies,[18–20] although have been frequently quoted, were not included in this meta-analysis because exposure information was either ecological or based on interviews of proxies

Statistical analysis

For each study, odds ratios (OR) or relative risks (RR) and 95% confidence intervals (95% CI) by sex and exposure category were extracted. Two studies provided only gender specific risks[8 14] and overall risk estimates were calculated by us through a meta-analysis of male and female risks. One study included only men[15] and one only women.[16a] We used Wolf's method to combine risk estimates in all meta-analyses. This method is based on the study specific risk estimates and confidence intervals, applying the inverse of variance as the weighting factor.[21] The exposure indices analysed were duration of chlorinated drinking water consumption in the case-control studies and water source in the cohort study. Subjects were classified as whether they ever consumed or not chlorinated drinking water. When not presented in the original papers, combined risk estimates for ever-consumers were estimated through a meta-analysis of published risk estimates for exposed subcategories. Those consuming chlorinated drinking water were further grouped according to duration of consumption. Three a priori defined exposure categories were used: no/low exposure group (reference category) including subjects not drinking chlorinated drinking water or consuming chlorinated drinking water for short time periods; an intermediate exposure group, corresponding in most studies to a consumption of chlorinated drinking water from 1 to 40

Table 2 Odds ratios and 95% confidence intervals from the studies included in the meta-analysis according to duration of exposure to chlorinated drinking water

Case-control studies					
Cantor 98	Never exposed	1–19 years	20–39 years	40–59 years	≥60 years
Men	1.0	1.1 (0.8 to 1.3)	1.3 (0.9 to 1.8)	1.5 (0.95 to 2.3)	1.9 (1.1 to 3.6)
Women	1.0	0.9 (0.6 to 1.4)	0.7 (0.3 to 1.3)	0.7 (0.3 to 1.4)	0.7 (0.2 to 2.4)
Both sexes	1.0	1.0 (0.8 to 1.2)	1.1 (0.8 to 1.4)	1.2 (0.8 to 1.7)	1.5 (0.9 to 2.6)
Koivusalo 98	<15 years	15–29 years	30–44 years	≥45 years	
Men	1.0	1.07 (0.73 to 1.55)	1.67 (1.01 to 2.78)	2.32 (0.99 to 5.45)	
Women	1.0	0.92 (0.49 to 1.72)	1.19 (0.53 to 2.64)	1.88 (0.54 to 6.57)	
Both sexes*		1.03 (0.74 to 1.42)	1.52 (1.0 to 2.33)	2.2 (1.1 to 4.4)	
King 96	≤9 years	10–19 years	20–34 years	≥35 years	
Men	–	–	–	–	
Women	–	–	–	–	
Both sexes	1.0	1.04 (0.71 to 1.53)	1.15 (0.86 to 1.51)	1.41 (1.09 to 1.81)	
McGeehin 93	0 years	1–10 years	11–20 years	21–30 years	>30 years
Men	–	–	–	–	–
Women	–	–	–	–	–
Both sexes	1.0	0.7 (0.4 to 1.3)	1.4 (0.8 to 2.5)	1.5 (0.8 to 2.9)	1.8 (1.1 to 2.9)
Vena 93†	0–49 years	50–59 years	60–67 years	68–86 years	
Men	2.89 (1.47 to 5.67)	1.85 (0.96 to 3.57)	2.27 (1.14 to 4.50)	2.24 (1.05 to 4.74)	
Women	–	–	–	–	
Both sexes	–	–	–	–	
Cantor 87‡	0 years	1–19 years	20–39 years	40–59 years	≥60 years
Men	1.0	1.1 (0.7 to 1.6)	1.1 (0.7 to 1.5)	1.2 (0.8 to 1.7)	1.2 (0.7 to 2.1)
Women	1.0	1.8 (0.8 to 3.7)	1.5 (0.7 to 3.1)	2.2 (1.0 to 4.8)	3.2 (1.2 to 8.7)
Both sexes	1.0	1.2 (0.9 to 1.7)	1.1 (0.8 to 1.6)	1.3 (0.9 to 1.9)	1.4 (0.9 to 2.3)
Cohort studies					
Doyle 97	100% ground water	Mixed ground-surface water	100% surface water		
Women	1.0	2.27 (1.2 to 4.31)	0.62 (0.15 to 2.63)		
Wilkins and Comstock 81	Deep well users	Chlorinated surface water users			
Men	1.0	1.80 (0.8 to 4.75)			
Women	1.0	1.60 (0.54 to 6.32)			
Both sexes*		1.7 (0.8 to 3.5)			

*Risk for both sexes obtained from a meta-analysis of men and women risk. †OR for the quartile of ≥10 daily cups of tap water consumption. ‡OR for the stratum of water consumption above the population median (1·4 litres).

years; and a high exposure group corresponding in most studies to a consumption of chlorinated drinking water of more than 40 years. In the cohort studies information was provided only on water source and data from these studies are therefore not included in the analysis by duration. When the risk estimates of the intermediate and long term exposure categories we defined did not coincide with the published data, a meta-analysis of risk estimates collapsing exposure categories within study was performed. The cut off points used to define the exposure groups were study specific and did not coincide in all studies (table 1). The influence of the cut off points in determining results was examined in alternative analyses. Potential sources of heterogeneity were examined through graphical methods such as the Galbraith plot.[23] A heterogeneity test based on the Q statistic, following a χ^2 distribution, was performed in all meta-analysis. We considered that there was statistically significant heterogeneity when p value was below 0.10.[22] In cases with substantial heterogeneity random effects models were applied.[22]

The influence of each single study on the combined risk estimate was further examined by consecutively omitting each study from the meta-analysis.[24] Meta-regression was implemented to explain potential heterogeneity attributable to study design and year of publication,[25] fitting random effects models with two additive variance components (within and between studies).[22 26]

Under the assumptions of linear dose-response and independence of the dose specific OR, we estimated the dose-response regression slopes of each study for both sexes, using the OR, 95% confidence intervals and the midpoint of the exposure interval.[27] For open ended intervals a point 20% higher than the low end of the interval was used.[27] We then performed a meta-analysis of the slopes and their standard errors to get a combined dose-response slope for all the studies. The exponentiation of the slope gave the OR for a unit increase of the exposure index (one year of exposure). To overcome the problem of assuming independence of dose specific OR (which is incorrect as they have a common reference group), we adjusted the standard error of the within study slopes estimating the covariance. We applied the method previously described by Greenland and Longnecker[28] for the three studies reporting number of cases and controls by exposure category. We checked for publication bias through Egger's and Begg's graphical methods.[29 30] Analyses were done using Stata v6.0.

RESULTS

All selected studies reported excess risks of bladder cancer ranging from 1.4 to 2.2 for the study specific highest exposure category in both sexes combined (table 2) although only in four studies were results statistically significant. In all case-control studies OR tended to increase with duration of exposure.

Ever consumption of chlorinated drinking water was associated with bladder cancer with a combined risk estimate of 1.2, (95%CI 1.1 to 1.4) for both sexes, on the basis of six studies (fig 1). Sex specific combined risk estimates were 1.4 (95%CI 1.1 to 1.9) for men on the basis of five studies and 1.2 (95%CI 0.7 to 1.8) for women, on the basis of five studies (table 3).

Results from the meta-analysis show a statistically significant increased risk for bladder cancer, associated to long term exposure to chlorinated drinking water (table 3). The combined risk estimate for both sexes and the mid-term exposure was 1.1 (95% CI 1.0 to 1.2) on the basis of five studies. The combined risk estimate for the long term exposure was 1.4 (95%CI 1.2 to 1.7) on the basis of five studies. Combined risk estimates were slightly lower in women (combined OR=1.4) for the long term exposure category, compared with men (combined OR=1.6) (table 3). Inclusion of the

Table 3 Combined risk estimates from studies on bladder cancer and consumption of chlorinated drinking water by sex and exposure category

Exposure category	Meta-OR (95% CI)	Number of studies	Test for heterogeneity p value	Selected method
Both sexes				
Mid-term	1.1 (1.0 to 1.2)	5	0.84	Fixed effects
Long term	1.4 (1.2 to 1.7)	5	0.55	Fixed effects
Ever exposed	1.2 (1.1 to 1.4)	6*	0.61	Fixed effects
Men				
Mid-term	1.3 (1.0 to 1.7)	4	0.08	Random effects
Long term	1.6 (1.2 to 2.2)	4	0.11	Random effects
Ever exposed	1.4 (1.1 to 1.9)	5*	0.01	Random effects
Women				
Mid-term	1.0 (0.7 to 1.6)	3	0.09	Random effects
Long term	1.4 (0.6 to 3.6)	3	0.01	Random effects
Ever exposed	1.2 (0.7 to 1.8)	5*	0.01	Random effects

*Includes the cohort studies that do not provide risk estimates by duration of consumption.

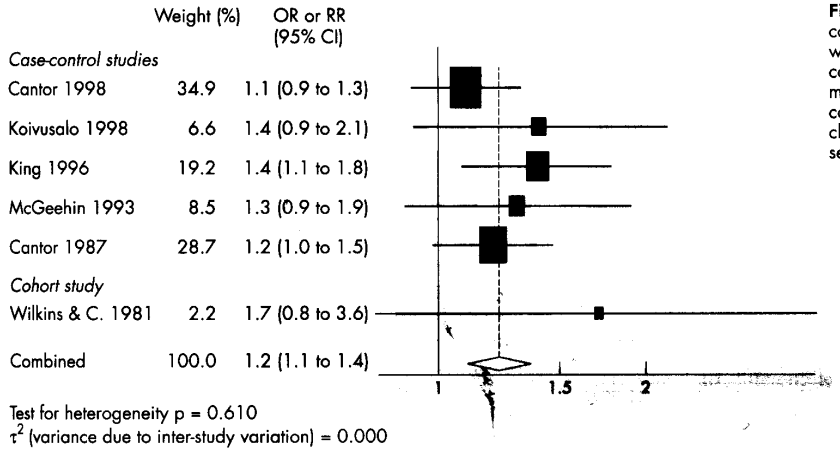

Figure 1 Odds ratios (OR), 95% confidence intervals (95% CI), study weight in the meta-analysis and combined risk estimate from meta-analysis of studies on bladder cancer and ever consumption of chlorinated drinking water. Both sexes.

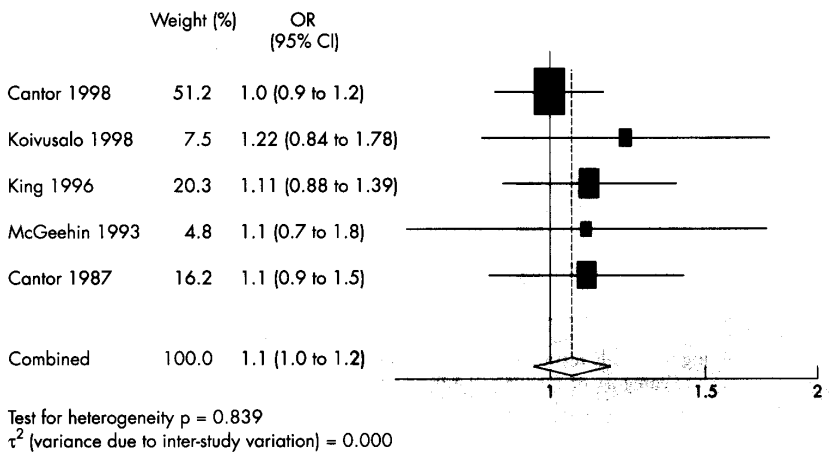

Figure 2 Odds ratios (OR), 95% confidence intervals (95% CI), study weight in the meta-analysis and combined risk estimates from meta-analysis of case control studies on bladder cancer and mid-term consumption of chlorinated drinking water. Both sexes.

cohort study or, alternatively, of the nested case-control study by Freedman *et al*, modified minimally the results. The combined OR for both sexes and long term exposure category was 1.5 (95% CI 1.3 to 1.7) when including the cohort study by Wilkins and Comstock, and the combined OR was 1.4 (95% CI 1.2 to 1.6) when including the nested case-control study.

The study specific OR for both genders and the mid-term and long term exposure categories are shown in figures 2 and 3, respectively. OR for the long term exposure category are comparable and both the test for heterogeneity and the Galbraith plot, which is a more sensitive method than the χ^2 statistic, do not indicate substantial differences between studies (fig 4 and 5). Heterogeneity of results among studies is,

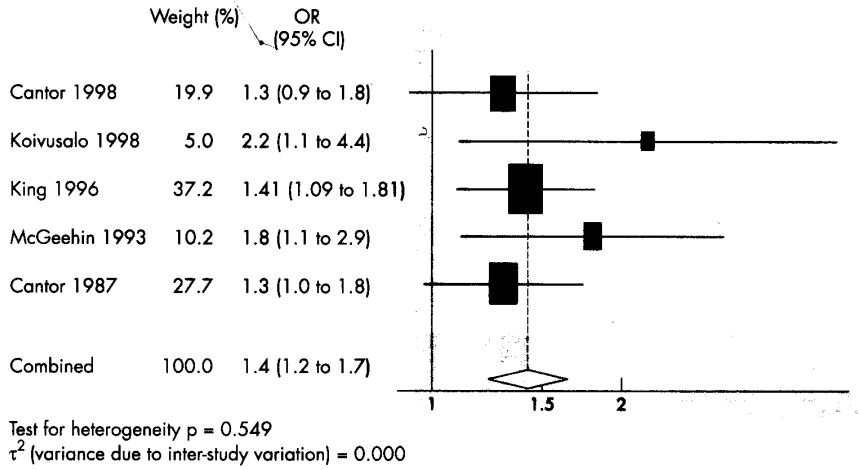

Figure 3 Odds ratios (OR), 95% confidence intervals (95% CI), study weight in the meta-analysis and combined risk estimate from meta-analysis of case-control studies on bladder cancer and long term consumption of chlorinated drinking water. Both sexes.

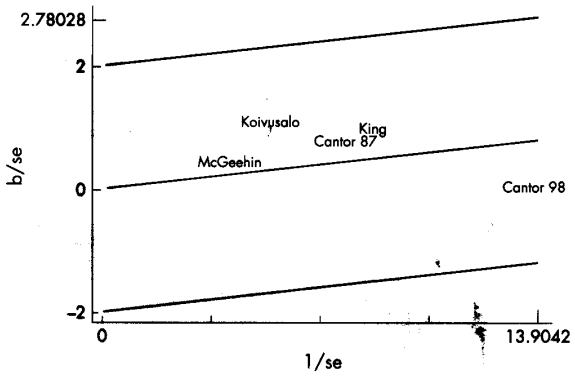

Figure 4 Galbraith plot for mid-term exposure, both sexes. The Galbraith plot provides a graphical display to get a visual impression of the amount of heterogeneity from a meta-analysis. For each study, the z statistic (β/s.e._β) is plotted against the reciprocal standard error 1/s.e._β. The (unweighted) regression line constrained through the origin, with its 95% confidence interval, has a slope equal to the overall log odds ratio in a fixed effects meta-analysis. The position of each study on the horizontal axis gives an indication of the weight allocated to it in a meta-analysis. The position on the vertical axis gives the contribution of each study to the Q statistic for heterogeneity. In the absence of heterogeneity we could expect all the points to lie within the confidence bounds (positioned two units over and below the regression line).

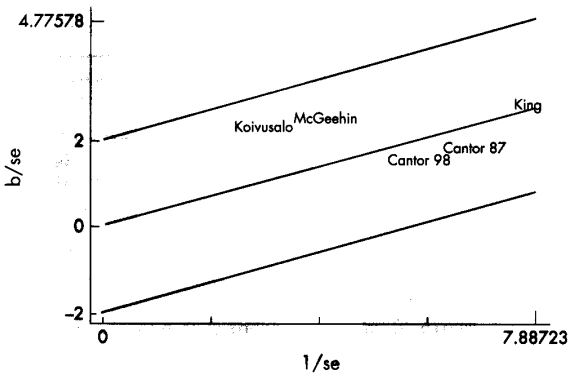

Figure 5 Galbraith plot for long term exposure, both sexes. (See legend to figure 4 for explanation of the Galbraith plot).

however, particularly evident for results in women that are based on three studies and on comparatively small numbers (table 3). In men, the main source of heterogeneity was attributable to the inclusion of the study by Vena,[15] particularly for mid-term exposure. Excluding this study resulted to an OR for men in the mid-term exposure category of 1.2 (95%CI 1.0 to 1.4) and p value for heterogeneity of 0.82. We explored through meta-regression whether year of publication was associated with the magnitude of the OR but found no statistically significant effect attributable to the year of publication for either of the two exposure categories.

We evaluated whether the cut off points selected could influence results, and calculated combined OR for two a priori defined "alternative" intermediate and high exposure categories. We selected as "alternative" intermediate exposure group the most comparable exposure category among studies, which corresponded to the strata including 25–26 years of consumption of chlorinated drinking water (table 2). The combined OR for this group was 1.2 (95% CI 1.0 to 1.4) with a p value for a test of heterogeneity of 0.36. The "alternative" high exposure group included the highest exposure strata in each study. The meta-OR for this group was 1.6 (95% CI 1.3 to 1.8) with a p value for a test of heterogeneity of 0.79. Similar to the overall combined OR, the gender specific combined OR calculated on the basis of these "alternative" exposure categories were slightly higher than those shown in table 3, and results for these categories were less heterogeneous.

The study by Cantor *et al*, 1987[13] reports OR by quantity of tap water consumption stratified below or above the median population level of daily water consumption (1.4 litres). Our model includes the OR of the most exposed stratum, tap water consumption above the median. We explored the effect on the combined risk estimate considering the OR from the stratum below the median. The risk estimate changed little for the overall combined risk estimate (OR=1.4, 95%CI 1.2 to 1.7); test for heterogeneity p value=0.528).

The study by Doyle reports results for three types of water source: subjects consuming 100% surface water (highest exposure), those consuming mixed surface and ground water, and those consuming 100% ground water (non-exposed). We included in our model the highest exposure category that included, however, only few subjects. We also checked for the effect on the results when using the intermediate exposure category that included the most exposed subjects. When using the intermediate category in the combined analysis, the OR increased (OR=1.4, 95%CI=0.9 to 2.2, test for heterogeneity=0.02).

Table 4 Dose-response regression slopes obtained from weighted least squares within study, and combined odds ratios (OR) with 95% confidence intervals (95% CI) obtained from the meta-analysis of the five slopes and their standard errors. Both sexes

Study	Slope	Standard Error	OR	(95% CI)
Cantor 1998	0.0039614	0.0021449		
Koivusalo 1998	0.0098449	0.003775		
King 1996	0.0072381	0.0025664		
McGeehin 1993	0.0159266	0.0057087		
Cantor 1987	0.0049595	0.0024032		
Combined				
unit increase	0.006	0.000128	1.006	1.004 to 1.009
20 years			1.13	1.08 to 1.20
40 years			1.27	1.17 to 1.43
60 years			1.43	1.27 to 1.72

Four studies applied elaborate exposure models and estimated long term level of exposure to trihalomethanes [9,10] or to water mutagenicity attributable to the presence of chlorination byproducts.[8] Risk estimates in the three studies examining both sexes were 1.4,[10] 1.5,[9] and 2.2[8] for long term exposure. The combined risk estimate for these three studies was 1.5 (95% CI 1.2 to 1.8) with a p value for the test of heterogeneity of 0.61.

The results of the dose-response analysis are shown in table 4. The combined OR for unit increase in duration of exposure is 1.006 (95%CI 1.004 to 1.009). For 20, 40, and 60 years of exposure, combined OR are respectively 1.13 (95%CI 1.08 to 1.20), 1.27 (95%CI 1.17 to 1.43), and 1.43 (95%CI 1.27 to 1.72). The comparison of "crude" and "adjusted" combined OR for the three studies that permitted the calculation of a covariance matrix, showed that adjusting for covariance led to a 20% lower combined estimate. For these three studies, the combined OR for unadjusted slopes was 1.005 per year of exposure (95%CI 1.003 to 1.008), standard error (SE)=0.00128. After adjusting for covariance, the combined OR was 1.004 (95%CI 1.001 to 1.007), SE=0.00153.

We found no evidence of publication bias. Egger's graph showed a slight negative slope indicating that the smaller and less precise studies tended to report higher risk estimates, while the bigger and more precise studies tended to report lower risk estimates. However, the evidence of this trend is not statistically significant. We performed also the Begg's and Egger's tests to check for publication bias in the models stratified by sex. Because of the small number of studies, the test is not reliable as the confidence intervals very wide.

DISCUSSION

Results of this meta-analysis indicate the presence of a moderate excess risk for bladder cancer attributable to consumption of chlorinated drinking water. A clear excess risk was observed among subjects consuming chlorinated drinking water for more than about 40 years. The risk estimate for the intermediate exposure category was only slightly increased, though it also was statistically significant. Overall, results for long term exposure to chlorinated drinking water were consistent between studies and fairly consistent exposure-response patterns were observed in all case-control studies. Previous meta-analyses or reviews[31] had reached the same conclusions but either did not provide a quantitative summary of the effect, or did not base the analyses and conclusions on those studies with individual information.

Exposure assessment has been identified as one of the main problems when evaluating results of epidemiological studies on chlorination by products[32] and recent studies have made considerable efforts in characterising lifetime exposure. All studies included in this meta-analysis recorded individual information on water consumption. Heterogeneity of the methods used in different studies and different background levels of chlorination byproducts remains, however, a main concern. Statistical heterogeneity of the results was present for the intermediate exposure category and particularly for results stratified by gender. Small numbers, especially in women, and differences in exposure assessment are probably the most important sources of heterogeneity of results between studies. The study by Vena *et al*[15] was the main source of statistical heterogeneity observed particularly in the mid-term category. In this study, OR were generally higher than in other studies, and is the least comparable to the other studies concerning various aspects of assessment of exposure including the exposure categories used.

The exposure categories used in the meta-analysis could correspond to different levels of exposure to chlorination byproducts between studies. The exposure strata used were not directly comparable between studies and also, levels of chlorination byproducts would be expected to differ between the geographical areas examined in these studies. Despite our effort to select the most comparable exposure categories identifying subjects with intermediate and long term consumption of chlorinated drinking water, exposure categories between studies differed. Even though the highly exposed group included in all studies subjects with long term consumption of chlorinated water the cut off points differed between studies. In one study the minimum was 30 years, in another 35 years, in two studies 40, in one study 45, and in two 50 years. More importantly, the groups considered as non-exposed in two studies had consumed chlorinated drinking water for a few years, which would have led to an underestimation of the risk. In an alternative analysis we examined the extent to which the definition of the cut off points could affect results. This analysis indicated that observed results are robust. Even though different estimates can be derived if the data are categorised in alternative ways, these differences are small and results tend to support a positive association between consumption of chlorination drinking water and bladder cancer.

Levels of trihalomethanes were measured and modelled only in three studies [9,10,16a] while a fourth study used a matrix of water mutagenicity that corresponds well with levels of chlorination byproducts.[8] In the two studies measuring trihalomethanes and evaluated both sexes, risks for specific contaminant levels were comparable. The extent to which this finding can be extrapolated to the other studies included in this analysis is unknown and, in principle, one should not expect that risks by duration of exposure should be directly comparable as levels of contaminants and type of contaminants differ between areas and time periods. It should be

noted that trihalomethanes have been traditionally used as markers of the whole mixture of chlorination byproducts because they are the most prevalent byproducts. Other chlorination byproducts such as haloacetic acids and MX, have also been shown to have mutagenic or carcinogenic properties.[33–35]

In one study the excess risk identified was present only among ever smokers.[9] All studies adjusted for important confounding factors like age, sex, and smoking and some studies also for occupation and socioeconomic status. Residual confounding attributable to smoking could still be present but, overall, confounding seems to be an unlikely explanation for the findings of individual studies and the results of the meta-analysis.

The alternative dose-response methods we used confirmed the existence of an excess risk, though they led to combined risk estimates of slightly different magnitude. According to the dose-response analysis described by Berlin[27] and Greenland,[28] we reached combined risk estimates slightly lower than according our intermediate term and long term exposure approach. The combined risk estimates obtained from both methods are comparable for the intermediate duration of exposure (about 20 years), and the difference seems to be larger for long term exposures. Both methodologies have their limitations. The dose-response slope approach is based on the assumption of a linear dose-response, which may be a simplification of the real dose-response trend. The mid-term, long term approach implies the combination of risk estimates from exposure categories that are not fully comparable among studies. These results obtained from different methodologies indicate the presence of an excess bladder cancer risk associated with exposure to disinfection byproducts, and also indicate that the limitations of each method are probably not producing a spurious association.

Publication bias is a concern for all meta-analyses. Our bibliographic search was limited to databases including published studies. There may exist other not published studies, for example, doctoral theses and congress communications. It is extremely difficult to identify such studies. Furthermore, their inclusion could be questioned as quality criteria are difficult to apply. A simple observation of the graphics presented (figs 1–3) indicates that there is no trend along the years. If publication bias did exist, reported risks would tend to be higher in the first published studies, and lower risks in more recent. We additionally examined with publication bias through statistical and graphical methods, showing no evidence of such bias.

To conclude whether disinfection byproducts (DBP) or chlorinated drinking water exposure is a risk factor for certain cancers, evaluations could be based on an evaluation of single compounds but should also be based on the effect of the total DBP mixture, as humans are exposed to complex mixtures of DBP and it is impossible to evaluate the effect of one single compound through epidemiological studies. Results of this meta-analysis of case-control studies of bladder cancer and chlorinated drinking water exposure provide an objective summary risk for one of the cancers most consistently associated to DBP exposure.

In industrialised countries disinfection and chemical protection of drinking waters should not be considered as antagonistic. The recommendations of a recent report by the WHO[3] similar to previous reports although applicable at a global level, do not correspond to the current situation of most industrialised societies where contamination of the water by microorganisms has been drastically, although not entirely,[36] reduced. Traditional drinking water treatment is highly chlorine and chemical based. There exist reasonable alternatives that keep the disinfection power and produce fewer byproducts. In the long term, the most efficient approach is the protection of source waters aimed at reducing the presence of natural organic matter.[3] Exposure to chlorination byproducts occurs through ingestion, inhalation, and dermal absorption.[37–39] Epidemiological studies have only evaluated ingestion. Changing drinking water practices, for example consuming bottled water, would reduce exposure to trihalomethanes by only about one third.[38]

In conclusion, on the basis of epidemiological evidence, chronic exposure to chlorinated drinking water is associated with a moderate increased risk for bladder cancer, particularly among men. The estimated relative risks are not high, but the population attributable risk could be important, as the vast majority of the population of industrialised countries is potentially exposed for long time periods.

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Article 2

Pooled analysis of case-control studies of bladder cancer and disinfection by-products

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Short title: Trihalomethanes and bladder cancer

Abstract

Background. Exposure to disinfection by-products in drinking water has been associated with an increased risk of bladder cancer. We pooled the primary data from six case-control studies of bladder cancer with individual-based exposure estimates using trihalomethanes as a marker of disinfection by-products.

Methods. Two studies were included from the USA, and one each from Canada, France, Italy and Finland. Inclusion criteria were availability of detailed data on trihalomethane exposure and individual water consumption. Primary data were combined using common definitions and coding. The analysis included 2806 cases and 5254 controls with known exposure for at least 70% of an exposure window of 40 years prior to interview. Cumulative exposure to trihalomethanes (mg) was estimated by combining individual year-by-year average trihalomethane level (μg /liter) and daily tap water consumption (liters/day).

Results. The adjusted odds ratio (OR) in men exposed to an average higher than 1 μg/l trihalomethanes, compared to those with lower or no exposure, was 1.24 (95% confidence interval (95% CI)=1.09-1.41). Relative risks increased with increasing exposure with an OR of 1.44 (95%CI 1.20-1.73) for exposure higher than 50 μg/l. Similar results were found for other trihalomethane exposure indices. Among women, trihalomethane exposure was not associated with bladder cancer risk.

Conclusions. These findings strengthen the hypothesis that long-term exposure to disinfection byproducts at levels currently observed in many industrialized countries is associated with an increased bladder cancer risk. Gender is an effect modifier, with a positive association observed only among men.

Keywords: bladder; neoplasms; disinfectants; water

INTRODUCTION

Chlorination is a widely used and highly cost-effective technique for disinfection of drinking water, and has conferred important public health benefits. Since the first identification of toxic by-products produced by the reaction of chlorine with organic matter in 1974 [1,2], a number of epidemiological studies evaluated the cancer risk associated with this exposure. The initial studies were ecological in design [3-7], and suggested bladder as one of the cancer sites associated with chlorinated water intake. Case-control studies based on death certificates [8-10], as well as a cohort study [11] strengthened these findings. When the International Agency for Research on Cancer [12] evaluated chlorinated drinking water as a potential human carcinogen in 1991, most of the available studies were ecological or death certificate-based. These studies typically used cross-sectional estimates of exposure (usually around the time of death), and were limited in their ability to adjust for other risk factors. These methodological limitations led the IARC to conclude that the evidence for the carcinogenicity of chlorinated drinking water in humans was limited (group 3). After this evaluation, several studies with improved exposure assessment at the individual level were published. Among them, the studies of bladder cancer reported positive associations with chlorination by-products exposure [13-18]. A recent meta-analysis of studies on bladder cancer with individual information on residence and water consumption reported an increased risk in subjects with long-term consumption of chlorinated drinking water. The meta-odds ratios were higher among men than women [19].

Recent evaluations by the IARC of single chlorination by-products, such as specific trihalomethanes and haloacetic acids [20,21], concluded that the evidence for their carcinogenicity in humans is inadequate or limited. It was argued that although some studies had associated chlorinated drinking water intake with cancer, single compounds could not be evaluated using these studies since they occur in mixtures. A recent report by the WHO [22] considered that the evidence

was insufficient to determine whether the observed associations are causal or to determine which specific by-product or other contaminants play a role.
We pooled the primary data from six case-control studies with individual-based exposure assessments conducted in five countries, using trihalomethanes (THM) as a marker for the total mixture of chlorination by-products. These studies evaluated the association between bladder cancer risk and exposure to chlorination by-products.

METHODS

Studies

We obtained the primary data from six studies (Table 1) that met the following inclusion criteria: (1) case-control studies of incident bladder cancer, (2) availability of detailed long-term exposure assessment to THM, and (3) accessibility to primary data. We identified the published studies through Medline searches. Unpublished studies were identified through personal contacts with research groups that had collaborated on another pooled analysis of bladder cancer [23-25]. The pooled database included two studies from the USA [18;26], and one each from Canada [15], Finland [17], France [27] and Italy (Porru, unpublished), conducted between 1978 and 2000. Data on THMs from the Finnish, French and Italian studies had not been previously published. Detailed THM information was available for only part of a large US study [13] and that part was incorporated in the pooled analysis [26]. Data from an additional study from the USA[14] were not accessible and were not included in this analysis. Overall results from this study are similar to those found in this pooled analysis with a twofold relative risk found for both men and women for consumption of chlorinated drinking water for more than 34 years. The principal investigators of the pooled project and of the individual studies met and discussed the protocol, operational decisions for the analysis, and the results of this analysis.

Data

We extracted from the original databases exposure information and covariates that might be potential confounders or effect modifiers: age, sex, smoking status (never smokers; ex-smokers, quitting two years before the interview; current smokers), duration of smoking, cigarettes smoked per day, ever worked in an *a priori* high-risk occupation [28], coffee consumption, total fluid consumption and socio-economic status (as years of education). Education was categorized in four groups: primary school completed or less, some secondary education, secondary education completed, higher education). We established common definitions and coding schemes for all variables. A separate occupational classification had to be used for the Canadian study and the high-risk occupations are therefore not identical to those used in the remaining five studies. We excluded subjects under 30 and over 80 years old (n=774) from the pooled database, as well as patients with more than two years between diagnosis and interview (n=166). The final pooled data set comprised 3419 cases and 6077 controls (Table 1). All cases included in the pooled analysis were histologically confirmed. Four studies enrolled population controls. The remaining two recruited hospital controls, one study using urological controls (Porru, unpublished) and the second patients from various wards diagnosed with osteoarticular, digestive and heart diseases [27]. Controls were individually or frequency matched to cases on age and geographic area.

Exposure information

The approach followed in individual studies to estimate past exposures was fairly similar, although the detail of information available in each study and the specific models applied for the extrapolation differed. In the two US studies [18;26] the geometric means of contemporaneous THM levels by water source and treatment were estimated, and levels were then extrapolated to past periods taking into account water source and treatment. In the French study [27], information on water sources and treatments were collected retrospectively and THM mean levels were assigned to the different combinations of water sources and treatments as predicted by an experimental model using the same

parameters. In the Canadian study [15], retrospective THM data were estimated from a predictive model based on raw water parameters, treatment and current THM data. In the Italian study (Porru, unpublished), average THM levels from recent years were applied retrospectively to past years. A different approach was followed in the Finnish study [17]. Levels of mutagenicity in drinking water were estimated by an equation giving the level of mutagenicity on the basis of information on raw water quality (e.g., permanganate consumption, pH and color) and water treatment practices. A mutagenicity score was estimated for each person calculating an individual estimate of historical exposure to drinking water mutagenicity[29]. Individual THM levels were then derived applying a THM-mutagenicity correlation.

The exposure-related variables that we extracted from the six databases were amount of daily tap water consumption (liters/day, including water *per se*, coffee, tea and other beverages prepared with water from the tap) and yearly average THM level (μg/l). We created two exposure indices from these two variables: (1) average THM exposure (μg/l) that was calculated as the sum of the year-by-year annual mean THM level in each residence, divided by the number of years with non-missing THM data and (2) cumulative THM exposure (mg) that was calculated as the product of average THM exposure and total tap water consumption. (A subject reporting drinking of bottled water only, would have zero cumulative exposure irrespective of the average exposure). Average exposure reflects uptake through all exposure routes (ingestion, inhalation and skin absorption), while cumulative exposure is a better proxy for uptake through ingestion. It should be noted, however, that none of the studies included information on routes of exposure and that the two exposure indices are correlated (Pearson correlation coefficient=0.74). Average THM levels by study ranged between 10 and 30 (μg/L) with the exception of the smallest study (Porru, unpublished) where exposure levels were very low (Table 2). From the four studies that had available information on water source and chlorination status [15;18;26;27], we created variables for duration of exposure to

chlorinated surface water, chlorinated ground water and unchlorinated water. For all exposure indices, we defined a common exposure window of 40 years, extending from 45 years to 5 years prior to interview. A shorter time window was used for the French study since the earliest exposure data were available for 37 years prior to diagnosis.

Statistical analysis

We used unconditional logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (95% CI) for the different exposure indices. All ORs were adjusted by study, age (continuous), sex (when not stratified), socio-economic status (education), smoking status (never, ex-current smokers), ever worked in an *a priori* high-risk occupation [28], and heavy coffee consumption (>5 cups coffee/day). Models for average exposure were also adjusted for total fluid intake. Information on smoking status was available for all studies, but detailed comparable information on duration and intensity was available for five (15, 18, 26, 27 and Porru unpublished) out of six studies. Analyses of THM exposure in these five studies adjusting for smoking status and alternatively, for smoking status and pack-years of current smoking, indicated differences between the two sets of ORs only in the second decimal point. Exposure variables were initially treated as categorical variables. In initial analyses we estimated the risk for those ever exposed compared to those never exposed (average exposure equal to zero). The never exposed group was much smaller than those exposed and also it could be dissimilar to the remaining study population. Therefore, three alternative cut-off points were examined: 0.5, 1.0 and 1.5 μg/L average THM. The 1.0 μg/l average THM exposure cut-off corresponds approximately to the 15th percentile of the number of exposed subjects. A similar approach was followed for cumulative exposure with the cut-off of 15 mg corresponding to the 15th percentile. Using these cut-off points guaranteed that exposure of the reference group was sufficiently low, while allowing the inclusion of enough subjects from all studies in the reference group for the overall and sex-specific analyses. To examine exposure-response,

exposed subjects were grouped using quartiles as category boundaries. The pattern of the exposure-response relationship was also evaluated through a generalized additive model, using a natural spline (3 degrees of freedom) for the continuous average exposure variable adjusting by study, age, sex, education, smoking status, ever worked in high-risk occupations, heavy coffee consumption (>5 cups/day) and total fluid intake.

All analyses evaluating THM were limited to the 8060 subjects with known exposure for at least 70% of the exposure window (2806 cases and 5254 controls). Analyses were performed using the statistical packages STATA v.7.0., and S-Plus 2000. Adjusted ORs were calculated for the main effects within individual studies and the heterogeneity of effects among studies was evaluated through a meta-analysis [30].

RESULTS

Table 3 shows the distribution of covariates in the pooled study population. 80% of cases and 70% of controls were men, and the median age at interview was 67 years. After adjusting for study, sex and age, excess risks were found for ex- and current smokers, ever-worked in an *a priori* high-risk occupation, heavy coffee consumption (> 5 cups/day), and above-median intakes of total fluids, tap water and non-tap beverages. ORs for these covariates were similar between men and women except for occupation where ORs were higher among men.

Exposure to THM was associated with an excess relative risk among ever-exposed men (OR=1.32, 95% CI 1.10-1.59). Slightly lower ORs were obtained when the cut-off for defining ever exposed men were set at 0.5 μg/L (OR=1.23, 1.09-1.39), 1 μg/L 1.24 (1.09-1.41) or 1.5 μg/L (OR=1.15, 1.03-1.28). The relative risk tended to increase with increasing exposure (Table 4). The pattern of exposure response was further evaluated using natural splines (Figure 1) that

also showed increasing risk with increasing exposures. No association was found among women (OR for women with an average exposure higher than 1 μg/L =0.95, 95%=0.76-1.20). Sex was an effect modifier of the association between exposure to THMs and bladder cancer (p-value for interaction between exposure and gender =0.002).

Cumulative exposure to THM was associated with excess bladder cancer risk among men (Table 5). The OR in men ever exposed to THM as compared to those never exposed was 1.30 (95% CI=1.10-1.53). The risk was similar when including low-exposed men (0-15 mg THM) in the reference group (OR=1.30, 95% CI=1.14-1.50). The OR for women ever exposed to THM was 1.06 (95% CI=0.77-1.45). An exposure-response trend was observed among men with an OR of 1.50 for the highest quintile corresponding to more than 1000 mg THM during the 40-year exposure window examined. No exposure-response pattern was observed in women (Table 5). Similar to our findings with average exposure, sex was an effect modifier of the association between cumulative exposure and bladder cancer risk (p-value for interaction between exposure and gender <0.001).

The overall OR did not depend on any single study, as shown in Figure 2, where each study was excluded in turn, in the estimation of the pooled relative risk estimate. In the same Figure it can be seen that the ORs do not depend on whether the controls were hospital- or population-based. There was no observed heterogeneity of effects between studies for average THM exposure (Cochran's Q test for heterogeneity = 6.511, 4 d.f., p=0.164), and the meta-analysis of study-specific relative risk estimates gave the same OR as that obtained from the logistic regression adjusting for study (Table 4). This evaluation was also done separately by sex and did not indicate the presence of heterogeneity in men (Cochran's Q test for average THM exposure = 6.556, p-value= 0.256), or in women (Cochran's Q test = 5.113, p-value= 0.276). Similar results were obtained for cumulative exposure with a Cochran's Q test of 4.616,

p= 0.329 for both sexes, and no heterogeneity in men or in women (Figures 3A to 3C).

We evaluated whether bladder cancer risk was associated with specific time windows of exposure. We evaluated four ten-year periods, within the 40-year exposure period evaluated, specifically 5 to 14 years prior to interview, 15 to 24 years, 25 to 34 years and 35 to 45 years. All periods of exposure were associated with an increased risk (Table 6). Since exposure between periods could be correlated, the same analysis was repeated adjusting for exposure in all other periods. This analysis indicated that excess risks were associated with exposures that took place relatively early in life, prior to 25 years since interview.

Duration of exposure to chlorinated surface water was associated with an increase in bladder cancer risk among men (Table 7). In the group with the longest exposure to chlorinated surface water (30-40 years), the OR was 1.62 (95% CI=1.21-2.16) in relation to those never exposed. An increased risk was also found among subjects exposed to chlorinated ground water. Among women, no association was found for duration of exposure to chlorinated surface water (Table 7).

Adjusting for smoking status modified the OR of men ever exposed to an average of more than 1 μg/L THMs from 1.30, 95% CI=1.15-1.47 to 1.24, 95% CI=1.09-1.41. The OR among men never-smokers was 1.25 (95% CI=0.92-1.69) indicating that confounding or residual confounding by smoking could not have produced the observed excess risk. Neither was there effect modification by smoking of the risk associated with THM exposure, with an OR for current smokers being 1.23 (95% CI=0.99-1.52). Similarly in women no association with THM exposure was observed, regardless of smoking status (OR for never smokers=0.94, 95% CI= 0.69-1.27, OR for current smokers =0.99, 95% CI=0.63-

1.57). No differences were observed when subjects were stratified by occupation.

DISCUSSION

THM exposure was found to be associated with an increased risk of bladder cancer among men, while among women no association was observed with any of the exposure indices that we used. Pooling and jointly analyzing the data of studies with individual estimates of long-term exposure to chlorination by-products and potential confounding factors, although not devoid of methodological difficulties, has several advantages. The statistical power of the pooled data set to identify an excess risk overall and in dose-response analyses is considerably higher than that of the individual studies. The results from combined databases from many independent studies may have a stronger impact on public health policy than those of individual studies. Finally, the process of joint analysis fosters extensive communication among the contributing research groups, and can lead to improved methodologies.

A limitation of this pooled analysis is the use of THM as the common estimate of exposure from all studies, in the face of known and suspected differences among the studies. THM exposure has been used as a marker of exposure to chlorination by-products, which is a complex mixture of compounds with a variety of chemical and toxicological properties. THMs are the most prevalent chlorination by-products, but the proportion of THMs compared to other contaminants may vary, depending on factors such as raw water characteristics, temperature, and treatment practices. Consequently, the same level of THM did not necessarily represent the same mixture in all studies. Even within studies, the same THM level may have been a surrogate for mixtures that varied over space and time. Another source of heterogeneity in exposure assessment came from the different exposure models used. Three studies [18;26] and Porru, unpublished) estimated past THM levels by extrapolating contemporaneous or recent THM levels taking into account source and type of treatment. The

remaining three studies [15;17;27] applied models to predict past THM levels on the basis of historical data on raw water parameters, water source and treatment. The models applied in the French and the Canadian studies were similar, while the one of the Finnish study was based on totally different parameters. We found no cohort studies that estimated past THM levels.

The difference in relative risk by sex was observed in five out of the six studies, although differences between sexes were not pronounced. Among studies not included in the pooled data, some found a similar pattern [11;16], while one investigation reported higher relative risks in women [13] and another [14] reported no differences by gender. This latter study had been selected for inclusion in the pooled analysis, but data were not accessible. Given the large size of this pooled dataset and the trends observed, chance appears an unlikely explanation for our observation of a higher relative risk among men. Non-differential misclassification, attenuating risk only among women and not men also seems unlikely, although we had no means of testing this. Smoking and occupation are the most important risk factors for bladder cancer and did not substantially confound the association between THM exposure and bladder cancer. Knowledge of occupational risk factors is, however, more limited in women than in men[28] and residual confounding from occupation could occur, particularly among women. There are no published data on the importance of exposure to DBPs at work and it is therefore unknown to what extent differential mobility between sexes would affect results. Furthermore, the pooled analysis includes international data and the flow of populations to and from work may differ by country. Unpublished data from Iowa (Lynch personal communication) indicate that water intake at work is about a third of that at home and that subjects tended to be exposed to similar levels of THM at work and home. In addition, exposure to volatile compounds in the mixture occurs mostly at home, since a large part of this uptake is through inhalation and dermal absorption and occurs during bathing, showering, cleaning dishes etc.[31]. There is a sizable literature on bladder cancer and diesel exhaust and

other particulate matter air pollution but it refers to workers exposed to high exposure levels of these compounds[28]. If air pollution were a confounder, it is likely to be a weak one since any effect of air-pollution on bladder cancer can be expected to be small. Comparisons between subjects never exposed to DBPs with those exposed are, in part, also comparisons between subjects living in different geographical areas. The main analyses of this study, however, are not based on the never-ever comparisons but rather on the exposure–response and these are not purely comparisons between subjects of urban versus rural residences. "Urban" areas, that is areas that use water with elevated levels of THM and other chlorination byproducts, are frequently places as small as a few thousand inhabitants. Adjustment for education as a measure of socio-economic status did not make a difference in either gender, but this adjustment may not have adequately captured other socio-economic correlates of exposure. We did not have information in the pooled data set on other potential bladder cancer risk factors that probably have a differential gender distribution such as use of hair dyes, urinary infections, analgesics and diet [28]. However, their role as confounders of the association between THM and bladder cancer in either gender seems improbable.

Biological explanations for the observed gender difference should be considered, particularly since such differences in relation to a variety of outcomes have been observed in experimental animals [20]. Several authors have discussed the differences in bladder cancer risk between men and women and several mechanisms have been proposed [32]. These mechanisms could involve the role of sex hormones in the modulation of the enzymes that metabolize chlorination by-products into reactive metabolites, and possibly other factors such as voiding frequency and anatomical differences between genders, or the action of disinfection by-products as hormone disruptors [33;34]. Cytochrome P4502E1 (CYP2E1) is important in the metabolism of chloroform to active metabolites in humans [35;36] and, in laboratory animals, appears to be regulated by sex hormones [37]. Pharmacokinetic studies in humans show that the activity

of CYP2E1 may be higher in men than in women [38;39]. The metabolism of brominated THM is thought to involve a glutathione conjugation reaction leading either to formaldehyde or DNA-reactive intermediates via glutathione transferase-theta [40]. Several lines of evidence show that glutathione transferases are subject to regulation by thyroid and sex hormones [41-43]. In the absence of more complete experimental data the role of sex hormones in explaining gender differences in the effect of THM remains a hypothesis. There exist only few studies providing information on voiding frequency in population samples, most indicating that voiding frequency is higher in women than men[44-47], and one study showing equal voiding frequency among men and women[48]. These studies are not, however, comparable concerning design or the ages evaluated. Furthermore, although biologically plausible, the importance of voiding frequency in affecting bladder cancer risk has not been yet properly evaluated in epidemiological studies.

No epidemiological study has explicitly assessed routes of exposure other than ingestion of drinking water. Volatile compounds (trihalomethanes) enter the body not only through ingestion, but also through inhalation and dermal absorption in certain situations like showering, bathing or dishwashing. Recent estimates indicate that swimming, bathing and showering are the main routes of uptake of chloroform, which is the most prevalent by-product in most chlorinated water[31]. Non-volatile compounds (haloacetic acids, haloacetonitriles, etc.) incorporate mainly through ingestion of drinking water. We used two exposure indices, average and cumulative exposure as proxies for different exposure routes. Average exposure was defined, a priori, as the best proxy for exposure via all routes. Cumulative exposure was used as a proxy to exposure through ingestion since it was defined as the product of average exposure and amount of tap water consumed. The interpretation of the cumulative exposure index is more complicated since one of the two composite variables (fluid intake) defining this index could also be regarded as a

confounding variable[28;49;50] The two exposure indices were, however, highly correlated and results were very similar.

This pooled analysis of six epidemiological studies constitutes the most statistically robust analysis to-date on disinfection by-products and bladder cancer. We found an increased risk of bladder cancer and a clear dose-response pattern among men exposed to THM at levels currently observed in many industrialized countries. Since the potentially exposed population is large, the attributable risk could be considerably elevated. The observed difference in risk by gender is puzzling, although several plausible biological mechanisms could explain this difference. In view of growing evidence that exposure to disinfection by-products are associated with cancer risk and other health effects, consideration should be given to a more strict control of contaminant levels in chlorinated drinking water through water treatments that reduce the formation of such by-products without compromising the control of microbial contamination.

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Contributors

M Kogevinas and CM Villanueva planned and designed the study, wrote the report and analyzed the data; KP Cantor, S Cordier, JJK Jaakkola, WD King, CF Lynch and S Porru were primary responsible for the studies included in the pooled analysis, participated in the planning of the pooled study and in the interpretation and writing of results.

Conflict of interest statement

None declared.

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Table 1. Description of the studies included in the pooled analysis.

Study	Country	Cases		Controls		Source of controls	Period of enrolment
		N	%	N	%		
Porru S (unpublished)	Italy	123	3.6	150	2.5	Hospital	1997- 2000
King WD 1996	Canada	696	20.4	1545	25.4	Population	1992- 1994
Koivusalo M 1998	Finland	759	22.2	1292	21.3	Population	1991-1992
Cantor KP 1998	USA	959	28.0	1768	29.1	Population	1986-1992
Cordier S 1993	France	567	16.6	666	10.9	Hospital	1984-1987
Lynch CF 1989	USA	315	9.2	656	10.8	Population	1977- 1978
All studies		3419		6077			

Table 2. Estimated average THM levels (μg/L) and range for all the cases and controls (exposed and unexposed) by study. Levels are shown for subjects for whom exposure information is available for at least 70% of the time over the 40-year period evaluated.

Author (ref)	Average THM level (μg/l)					
	Cases			Controls		
	Mean (SD)	**Range**	**Subjects exposed to ≤ 1 μg/l**	**Mean (SD)**	**Range**	**Subjects exposed to ≤ 1 μg/l**
Cantor (18)	11.6 (19.7)	0.5-73.9	38%	10.0 (18.3)	0.5-73.9	42%
Cordier (27)	18.4 (21.9)	0-78.3	15%	17.2 (21.2)	0-78.3	16%
King (15)	32.2 (23.3)	0-124.7	13%	29.7 (23.0)	0-124.7	16%
Koivusalo (17)	23.5 (30.8)	0-130.0	49%	21.5 (29.4)	0-130.0	52%
Lynch (26)	14.8 (21.5)	0.5-73.9	40%	10.3 (17.7)	0.5-73.9	51%
Porru (*)	0.6 (0.8)	0-2.2	72%	0.4 (0.7)	0-2.2	84%

* unpublished

Table 3. Characteristics of cases and controls in the pooled study population*.

	Cases (%)	Controls (%)	OR (95%) †
	3419	6077	
Sex			
Men	2727 (79.8)	4227 (69.6)	
Women	692 (20.2)	1850 (30.4)	
Age			
Below the median (67 y)	1766 (51.6)	3415 (56.2)	
Above the median (>67 y)	1633 (48.4)	2662 (43.8)	
Smoking			
Never smoker	642 (18.9)	2379 (39.4)	1.00
Ex-smoker	1334 (39.3)	2140 (35.4)	2.13 (1.89-2.40)
Current smoker	1422 (41.8)	1526 (25.2)	3.55 (3.14-4.00)
Worked in high-risk occupations			
Never	2303 (67.4)	4276 (70.4)	1.00
Ever	653 (19.1)	851 (14.0)	1.30 (1.15-1.47)
Unclassifiable	463 (13.5)	950 (15.6)	1.45 (1.19-1.76)
Education			
≤ Primary school	699 (20.4)	1160 (19.1)	1.00
Some secondary	994 (29.1)	1405 (23.1)	1.15 (1.00-1.31)
Secondary completed	773 (22.6)	1546 (25.4)	0.95 (0.83-1.08)
> Secondary	657 (19.2)	1458 (24.0)	0.86 (0.75-1.00)
Other	296 (8.7)	508 (8.4)	0.85 (0.70-1.04)
Coffee			
0-5 cups/day	2832 (83.3)	5234 (86.8)	1.00
>5 cups/day	567 (16.7)	795 (13.2)	1.58 (1.39-1.79)
Total fluid consumption			
Below the median (2.4 l/day)	1650 (49.3)	3019 (50.5)	1.00
Above the median	1694 (50.7)	2960 (49.5)	1.21 (1.11-1.33)
Tap water consumption			
Below the median (1.4 l/day)	1756 (52.3)	3014 (50.3)	1.00
Above the median	1605 (47.7)	2983 (49.7)	1.20 (1.07-1.34)
Non-tap fluid consumption			
Below the median (0.9 l/day)	1547 (46.3)	2978 (49.8)	1.00
Above the median	1797 (53.7)	3001 (50.2)	1.17 (1.06-1.30)

* Numbers do not always add to a total of 9496 because of missing information.
† OR from logistic regression adjusted by study, sex and age.

Table 4. Odds ratios (OR) and 95% confidence intervals (95% CI) for bladder cancer by gender, and average THM exposure over a 40-year exposure window.

Average THM	**Men**		**Women**		**Both sexes**
	OR (95% CI)*	Cases/controls†	OR (95% CI)*	Cases/controls†	OR (95% CI)*
Never (0 µg/l)	1.00	328/605	1.00	94/221	1.00
Ever (> 0 µg/l)	1.32 (1.10-1.59)	1798/2909	0.85 (0.60-1.19)	509/1415	1.18 (1.00-1.39)
0-1 µg/l	1.00	711/1365	1.00	189/506	1.00
> 1 µg/l	1.24 (1.09-1.41)	1415/2149	0.95 (0.76-1.20)	414/1130	1.18 (1.06-1.32)
0-1 µg/l	1.00	711/1365	1.00	189/506	1.00
>1-5 µg/l	1.10 (0.92-1.31)	366/574	0.99 (0.72-1.36)	96/231	1.08 (0.93-1.26)
>5-25 µg/l	1.26 (1.05-1.51)	314/499	0.86 (0.63-1.18)	97/309	1.15 (0.98-1.35)
>25-50 µg/l	1.25 (1.04-1.50)	399/647	1.04 (0.76-1.43)	128/356	1.22 (1.04-1.42)
>50 µg/l	1.44 (1.20-1.73)	336/429	0.93 (0.67-1.28)	93/234	1.31 (1.12-1.54)
p- linear trend	*<0.001*		*0.753*		*<0.001*

* OR from logistic regression adjusted by (sex), study, age, smoking status, ever worked in high-risk occupations, heavy coffee consumption (>5 cups/day), education and total fluid intake.

† Numbers do not always add to 8060 because of missing information.

Table 5. Odds ratios (OR) and 95% confidence intervals (95% CI) for bladder cancer by sex and level of 40-year cumulative ingestion exposure to THM

Cumulative ingestion THM	*Men*		*Women*		**Both sexes**
	OR (95% CI)*	Cases/controls†	OR (95% CI)*	Cases/controls†	OR (95% CI)*
Never (0 mg)	1.00	415/783	1.00	104/270	1.00
Ever (> 0 mg)	1.30 (1.10-1.53)	1720/2739	1.06 (0.77-1.45)	502/1371	1.24 (1.07-1.44)
0-15 mg	1.00	632/1233	1.00	159/406	1.00
> 15 mg	1.30 (1.14-1.50)	1503/2289	0.95 (0.74-1.23)	447/1235	1.22 (1.08-1.38)
0-15 mg	1.00	632/1233	1.00	159/406	1.00
>15-50 mg	1.22 (1.01-1.48)	333/532	0.92 (0.65-1.32)	87/243	1.14 (0.96-1.35)
>50-400 mg	1.28 (1.08-1.51)	500/744	0.94 (0.70-1.27)	147/386	1.21 (1.04-1.39)
>400-1000 mg	1.31 (1.09-1.58)	369/609	1.02 (0.74-1.41)	119/337	1.25 (1.07-1.47)
>1000 mg	1.50 (1.22-1.85)	301/404	0.92 (0.65-1.30)	94/269	1.34 (1.12-1.59)
p-linear trend	*<0.001*		*0.818*		*<0.001*

*OR from logistic regression adjusted by (sex), study, age, smoking status, ever worked in high-risk occupations, heavy coffee consumption (>5 cups/day) and education.
† Numbers do not always add to 8060 because of missing information.

Table 6. Odds Ratios (OR) for bladder cancer and average exposure to THMs higher than 1 μg/L versus no, or lower exposed subjects within specific time windows of exposures. ORs are for both sexes.

Time window prior to interview	OR (95% CI) *	OR (95% CI) * adjusting for the remaining time periods
5-14 years	1.12 (0.99-1.26)	0.99 (0.82-1.20)
15-24 years	1.15 (1.02-1.30)	0.92 (0.73-1.15)
25-34 years	1.25 (1.11-1.41)	1.20 (0.97-1.49)
35-45 years	1.28 (1.12-1.45)	1.19 (1.01-1.41)

* ORs are adjusted by sex, age, center, smoking status, education, ever worked in high-risk occupations, heavy coffee consumption (>5 cups/day), and total fluid intake. The analysis was conducted only among subjects with ≥70% exposure information.

Table 7. Odds ratios (OR) and 95% confidence intervals (95% CI) for bladder cancer by gender and duration of exposure to chlorinated drinking water.

	Men		Women		Both sexes
	OR (95% CI)*	Cases/controls	OR† (95% CI)*	Cases/controls	OR (95% CI) *
Years exposed to chlorinated surface water					
0 (and never to chlorinated ground water) †	1.00	252/537	1.00	58/163	1.00
>0-7	1.40 (1.02-1.94)	94/132	0.83 (0.47-1.47)	27/83	1.25 (0.95-1.64)
>7-15	1.01 (0.74-1.37)	96/173	1.24 (0.72-2.15)	36/68	1.09 (0.84-1.42)
>15-30	1.67 (1.22-2.29)	104/127	0.60 (0.32-1.12)	21/64	1.36 (1.03-1.79)
>30-40	1.62 (1.21-2.16)	146/158	1.08 (0.62-1.88)	32/67	1.50 (1.16-1.93)
p-linear trend	*<0.001*		*0.725*		*0.002*
Exposed only to chlorinated ground water	1.23 (1.01-1.50)	470/717	1.04 (0.71-1.53)	131/316	1.20 (1.00-1.43)

* OR from logistic regression adjusted by (sex), study, age, smoking status, ever worked in high-risk occupations, heavy coffee consumption (>5 cups/day) and education.

† This reference group includes subjects never exposed to chlorinated surface water and never exposed to chlorinated ground water.

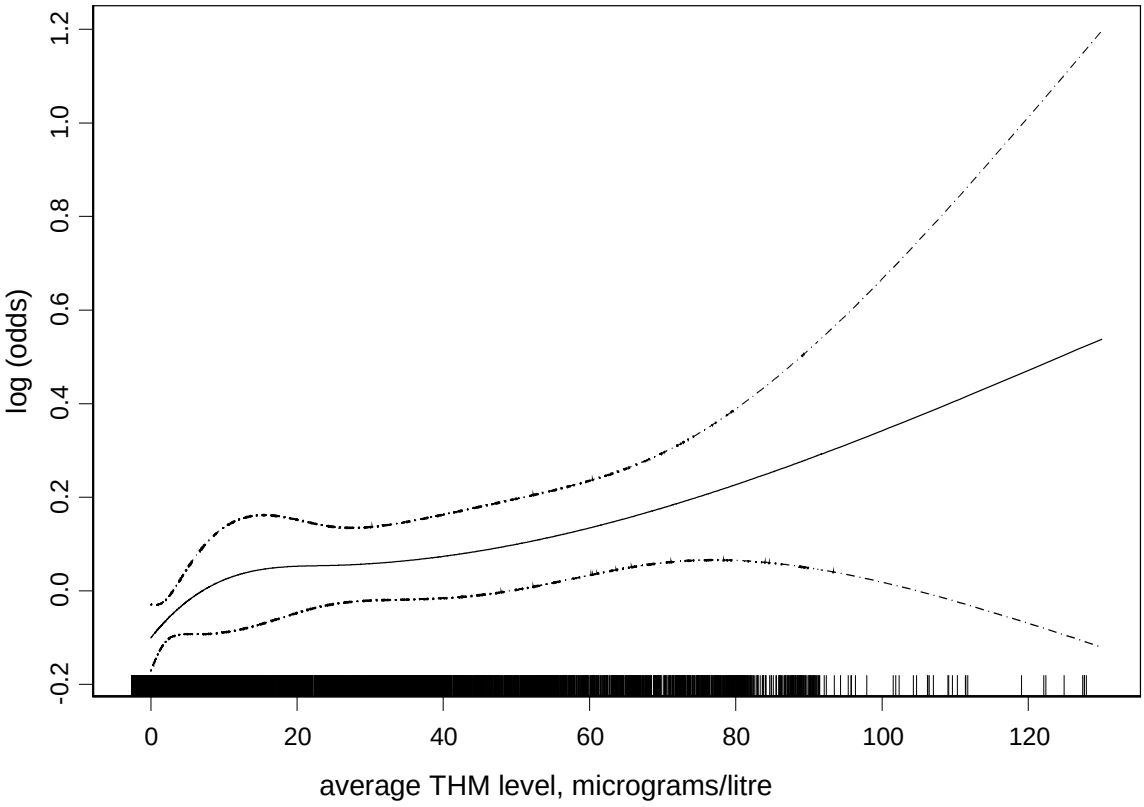
Figure Legends

Figure 1. Log odds ratio and 95% confidence interval for bladder cancer and average exposure to THMs (μg/L) using natural splines (3 degrees of freedom). Both sexes.

Footnote. The short vertical lines in the x-axis indicate the number of subjects by exposure level. ORs adjusted for age, sex, center, tobacco consumption, high-risk occupation, education, total fluid consumption, and high coffee consumption. Analysis limited to subjects with 70% or more lifetime exposure information.

Figure 2. Odds ratios and 95% CI for bladder cancer and average exposure to THMs for all studies (left) and subsequently ORs excluding each study in turn, both genders

Figure 3. Meta-analysis of study-specific adjusted odds ratios (OR) for those exposed to more than 1 μg/L THM in the 40-year exposure window. Figure 3A, both sexes; Figure 3B, men; Figure 3C, women.


log (odds)
-0.2
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
20
40
60
80
100
120
average THM level, micrograms/litre

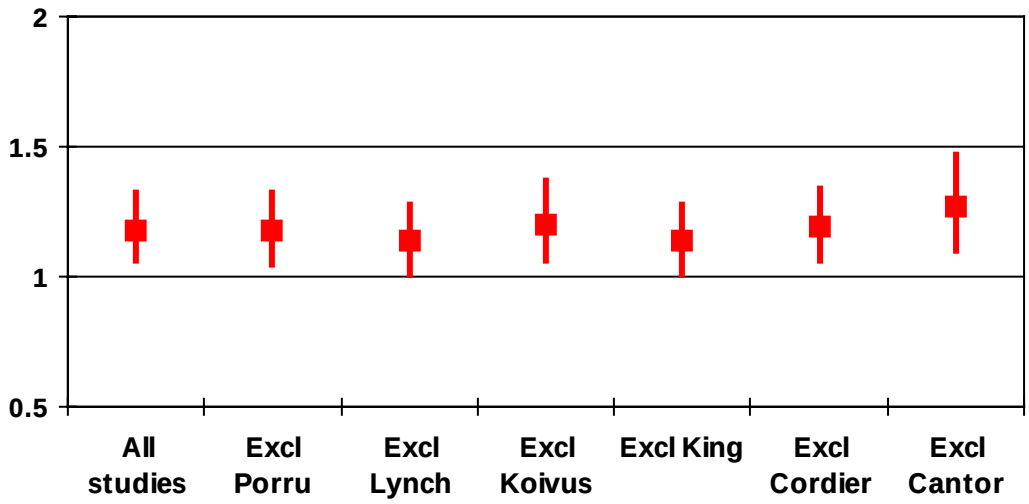
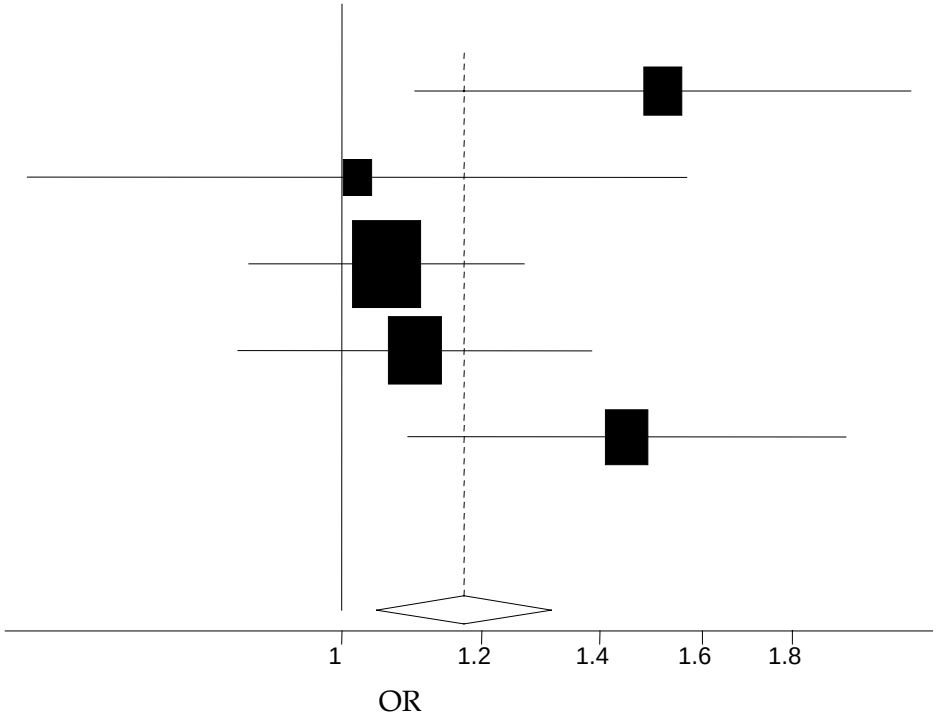

2
1.5
1
0.5
All studies
Excl Porru
Excl Lynch
Excl Koivus
Excl King
Excl Cordier
Excl Cantor

Figure 3A. Both sexes.

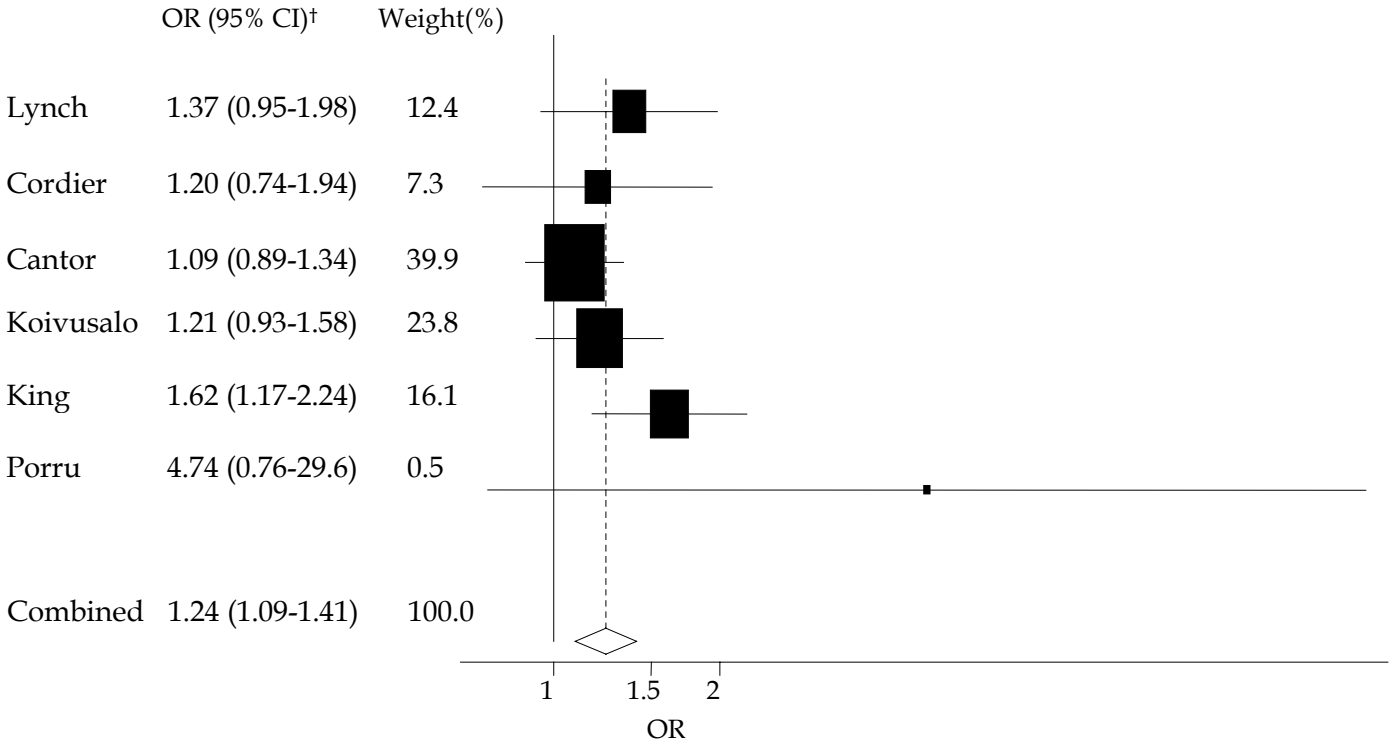
	OR (95% CI)†	Weight (%)‡
Lynch	1.52 (1.10-2.10)	12.4
Cordier	1.02 (0.66-1.57)	7.0
Cantor	1.06 (0.89-1.27)	40.3
Koivusalo	1.10 (0.87-1.39)	24.4
King	1.45 (1.09-1.93)	15.9
Combined	1.17 (1.05-1.31)	100



p value from Q test for heterogeneity=0.164
τ^2 (variance due to inter-study variation)=0.012

†Odds ratios (OR) and 95% confidence intervals (95% CI) from a logistic regression adjusting for sex, age, smoking status, ever worked in high-risk occupations, heavy coffee consumption (>5 cups/day), education and total fluid consumption.

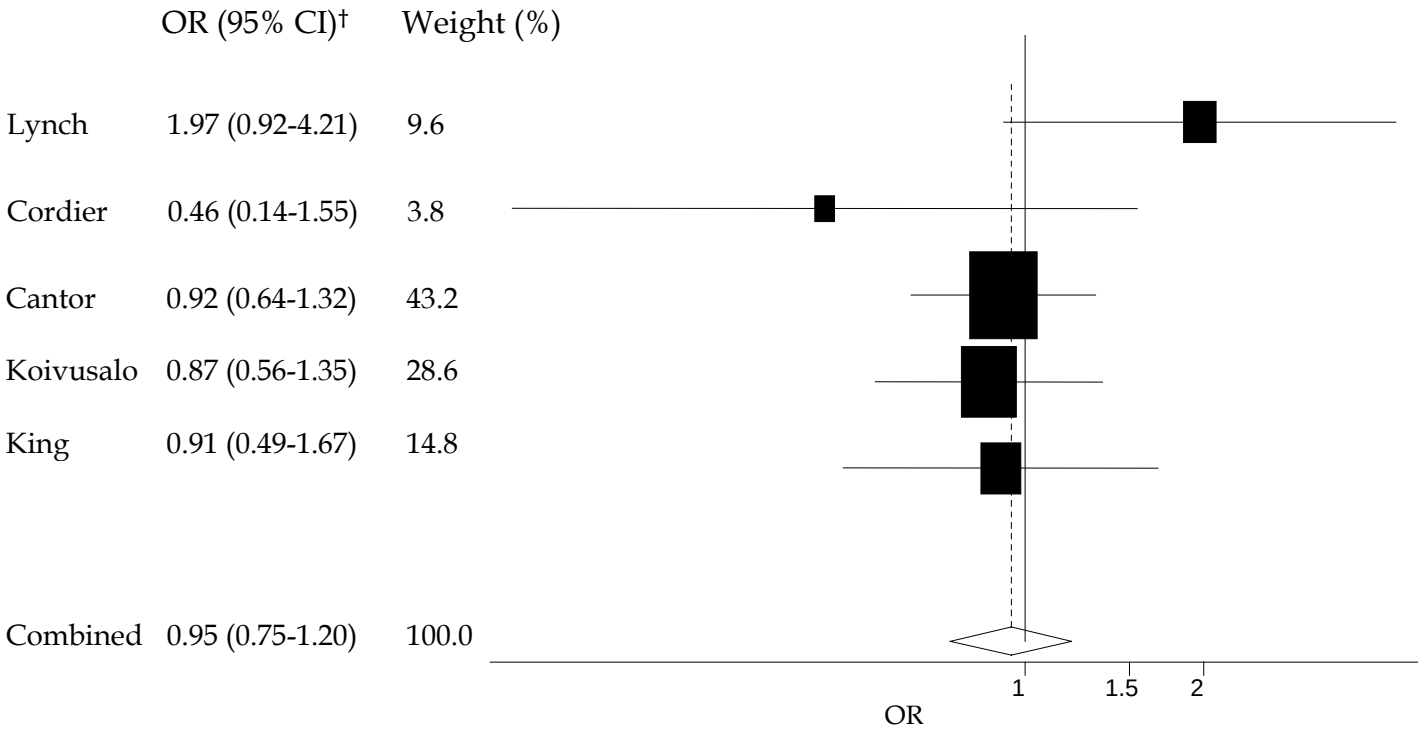
Figure 3B. Men.



p value from Q test for heterogeneity =0.256
τ^2 (variance due to inter-study variation)=0.009

† Odds ratio (OR) and 95% confidence intervals (95% CI) from a logistic regression adjusting by age, sex, smoking status, ever worked in high-risk occupations, heavy coffee consumption (>5 cups/day), education and total fluid consumption

Figure 3C. Women.



p value from Q test for heterogeneity =0.276
τ^2 (variance due to inter-study variation)=0.023

† Odds ratio (OR) and 95% confidence intervals (95% CI) from a logistic regression adjusting by age, sex, smoking status, ever worked in high-risk occupations, heavy coffee consumption (>5 cups/day), education and total fluid consumption

Article 3

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Research note

Haloacetic acids and trihalomethanes in finished drinking waters from heterogeneous sources

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Abstract

Trihalomethanes (THM) and haloacetic acids (HAA) are the most frequent chlorination by-products (CBP) in finished drinking waters. Traditionally, THM have been used as surrogates for CBP although the quantitative association between THM and other CBP is not well established. This problem is addressed in the present study from the analysis of THM and HAA in drinking water samples from four Spanish regions, representing areas with very different CBP composition, e.g. between 86 and 8.0 μg/l of THM and 50–3.0 μg/l of HAA.

The resulting dataset exhibit a statistically significant correlation between total THM and HAA (Pearson's correlation coefficient, $r_p = 0.815$, $p < 0.0005$). Furthermore, specific HAA are highly correlated with specific THM or their combinations. Accordingly, multivariate linear regression analysis of the concentrations observed show that the levels in total and specific HAA can be predicted from the THM content. These results are relevant for epidemiological studies on health effects from CBP exposure since they usually involve comparison of populations consuming waters of very distinct quality.

Keywords: Drinking water; Chlorination by-products (CBP); Trihalomethanes (THM); Haloacetic acids (HAA); Bromide; Speciation

1. Introduction

Since the first chlorination by-products (CBP) were detected in drinking waters early in the 1970s, several studies have evaluated their chemical properties, toxicology and human health effects [1]. Although epidemiological studies suggest that probable health effects of these compounds in humans may be related to cancer [2] and birth defects [3], causal association is still undemonstrated [4].

Traditionally, trihalomethanes (THM) have been the most studied CBP and have been usually used as surrogates of these products. However, the association to other compounds such as haloacetic acids (HAA) has not been previously addressed in environmental epidemiology studies. Chlorinated drinking water contains a complex mixture of CBP with different chemical and toxicological properties that may eventually enter the human body by ingestion, inhalation and dermal absorption [5]. In this context, speciation of CBP deserves increasing attention, e.g. toxicological differences between brominated and chlorinated CBP (International Programme on Chemical Safety) [6].

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PII: S0043-1354(02)00411-6

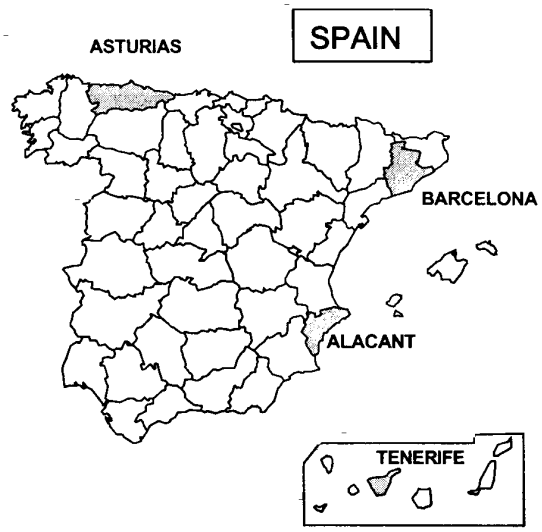
Chlorine is the drinking water disinfectant mostly used in Spain, a country with different climates and heterogeneous quality of water sources. Accordingly, four waters of diverse origins have been chosen to investigate the quantitative relationships between these compounds. These diverse compositions give rise to different levels and CBP composition in chlorinated drinking waters providing a good case for the study of the changes in THM and HAA levels in finished drinking waters from heterogeneous sources. The results are aimed to model HAA levels on the basis of THM levels, those more systematically determined in drinking waters, in order to provide more ground for the interpretation in epidemiology.

2. Materials and methods

2.1. Areas of study

Four provinces of Spain with different drinking water sources were selected for study: Barcelona, Alacant, Asturias and Tenerife (Fig. 1). Drinking water supplied in Barcelona, Alacant and Asturias mainly comes from surface sources. Drinking water supplied in the island of Tenerife (Canary Islands) originates from ground sources.

Eighty-eight drinking water samples were taken for the analysis of THM, 34 in Asturias, 25 in Barcelona, 19 in Alacant and 10 in Tenerife. HAA were analysed in a subset of 18 samples: five each in Barcelona, Asturias and Alacant and three in Tenerife.



ig. Situation of the four sampling areas.

2.2. Sampling

For the analysis of THM, tap water samples were collected avoiding bubble formation and stored by duplicate in 40 ml glass vials. At sampling, 3 mg of sodium thiosulphate were added to each vial for quenching additional CBP formation. The vials were sealed with Teflon-faced rubber septums and open-top screw plugs. No air camera was left between the water and the screw plug.

For the analysis of HAA, water samples were taken in 250 ml glass bottles that were also sealed with Teflon-faced rubber screw plugs.

All samples were kept at 4°C until analysis, which was performed no later than 14 days after sampling.

2.3. Experimental analysis

Four THM (chloroform, bromodichloromethane, dibromochloromethane and bromoform) were analysed following a previously optimised procedure [7]. Five millilitres of water was introduced into a purge and trap device and was bubbled with helium at a flow-rate of 40 ml/min for 11 min. The volatile compounds were retained in a tube packed with Tenax TA. These compounds were then analysed with a Perkin–Elmer Automatic Thermal Desorption Model 400 coupled to a Perkin–Elmer Autosystem gas chromatograph with an electron capture detector.

Nine HAA (monochloroacetic, dichloroacetic, trichloroacetic, monobromoacetic, dibromoacetic, tribromoacetic, bromochloroacetic, dibromochloroacetic and dichlorobromoacetic acids) were analysed. The analytical procedure has been described in detail elsewhere [8]. Briefly, 30 ml water samples were treated with 2-bromopropinoic acid, concentrated sulphuric acid, anhydrous sodium sulphate, copper (II) sulphate pentahydrate and methyl *tert*-butyl ether (MtBE). One micro litre of the MtBE extract was finally injected into a gas chromatograph equipped with electron capture detection.

2.4. Statistical analysis

Correlation between total THM and HAA was examined through simple linear regression analysis using an SPSS 9.0 package, introducing total THM level as the independent variable, and total HAA level as the dependent variable.

Multivariate linear regression analysis was used to elucidate which specific THM were the best predictors for the HAA compounds. For each single HAA the four THM were correlated as independent variables and those lacking statistical significance ($p > 0.05$) were excluded. Consecutive multiple linear regression models were performed and further THM were excluded if they were not significantly correlated to the HAA. The

procedure was repeated until the model only included statistically significant THM species.

3. Results and discussion

3.1. Raw water characteristics

Chloride content and organic carbon (measured as chemical oxygen demand) in the four sampling sites are reported in Table 1. As expected, the surface waters from the Mediterranean regions, Barcelona and Alacant are those exhibiting higher chloride and organic carbon, since they are reused several times for agricultural irrigation and they also receive urban and industrial effluent discharges. Ground waters in Tenerife exhibit higher chloride concentration than those in Asturias, which probably witness the intrusion of some proportion of saline water in the aquifer. The waters from this island are those with lower organic matter content, which is in agreement with the low pollution load expected for ground waters. The waters from Asturias exhibit the lowest chloride concentration of the series, which is consistent with the high amounts of rain collected by the river system in this region.

3.2. THM in finished drinking water

Total and specific THM levels are shown in Table 2. The Mediterranean area (Alacant and Barcelona) exhibits the highest levels (85.9 and 63.6 μg/l, respectively) while Tenerife exhibits the lowest (8 μg/l). Thus, total THM levels increase from low levels in ground waters (Tenerife) to low-intermediate levels in good-quality surface waters (Asturias) and high levels in poor-quality surface waters (Barcelona and Alacant).

THM speciation varies significantly among areas. The northern province (Asturias) presents the highest proportion of chloroform (60%) whereas both Mediterranean regions (Barcelona and Alacant) show a higher proportion of brominated and chloro-brominated species (70–80%). The island of Tenerife exhibits the highest proportion of brominated and chloro-brominated THM (> 90%). The levels of CBP are related to the amount of organic matter in the water samples [9]. Thus, the waters exhibiting higher CBP content (Table 2) are those with higher organic matter load (Table 1).

These results are consistent with those from a European survey conducted recently [10] that showed average THM levels of 78 μg/l (standard deviation, s.d., 100, $n = 19$) and 7.6 μg/l (s.d. 6.7, $n = 3$) for surface and ground waters, respectively.

THM speciation varies greatly according to water source.

Table 1
Concentration of chloride and organic matter (measured as chemical oxygen demand, COD) in the raw waters from the areas selected for study

	Chloride (mg/l)	COD (mg O_2/l)
Alacant		17 (18)
Barcelona		5.1 (0.62)
Asturias		4.2 (4.0)
Tenerife		0.60 (0.11)

Table 2
Concentrations of trihalomethanes (μg/l) in the different areas of study

	Alacant	Barcelona	Asturias	Tenerife
Chloroform ($CHCl_3$)				
Average (s.d.)	13.60 (5.33)	20.00 (10.56)	14.57 (7.14)	0.39 (0.21)
Min.	4.93	8.26	2.56	<0.1
Max.	24	35.25	29.54	0.68
Median	17.63	19.21	12.91	0.42
Bromodichloromethane ($CHCl_2Br$)				
Average (s.d.)	24.73 (5.07)	22.68 (5.46)	4.96 (2.59)	0.85 (0.67)
Min.	11.2	7.00	1.1	<0.5
Max.	30.8	31.46	12.43	1.95
Median	21.07	22.20	5.13	0.69
Dibromochloromethane ($CHBr_2Cl$)				
Average (s.d.)	25.67 (11.36)	10.81 (8.32)	2.23 (1.61)	1.18 (0.76)
Min.	6.55	2.86	0.07	0.5
Max.	40.99	36.10	6.7	3.2
Median	30.37	8.12	1.66	0.93
Bromoform ($CHBr_3$)				
Average (s.d.)	21.93 (12.96)	10.19 (12.24)	0.56 (0.52)	5.55 (3.23)
Min.	4.5	0.02	0.05	2.8
Max.	44.28	40.10	1.84	11.8
Median	23.4	2.28	0.48	3.96
Total trihalomethanes				
Average (s.d.)	85.93 (30.62)	63.64 (20.57)	22.25 (5.54)	8.00 (3.44)
Min.	35.2	34.56	6.36	5.08
Max.	125.34	121.7	44.54	16.28
Median	69.23	55.59	22.34	7.07
	19	25	34	10

3.3. HAA in finished drinking water

Total and specific HAA levels in the areas of study are shown in Table 3. Similarly to THM, the Mediterranean

Table 3
Concentrations of haloacetic acids (μg/l) in the areas of study

	Alacant	Barcelona	Asturias	Tenerife
Chloroacetic acid ($ClCH_2CO_2H$)				
Average (s.d.)	<0.3 (0)	0.87 (1.61)	<0.3 (0)	<0.3 (0)
Min.	<0.3	<0.3	<0.3	<0.3
Max.	<0.3	3.75	<0.3	<0.3
Median	<0.3	0.15	<0.3	<0.3
Dichloroacetic acid (Cl_2CHCO_2H)				
Average (s.d.)	12.03 (5.79)	7.10 (6.84)	4.29 (1.94)	0.25 (0.02)
Min.	6.75	0.62	1.74	0.24
Max.	21.45	15.44	6.24	0.27
Median	11.85	5.30	4.93	0.24
Trichloroacetic acid (Cl_3CCO_2H)				
Average (s.d.)	10.61 (8.39)	5.90 (4.82)	6.65 (1.64)	0.13 (0.03)
Min.	5.70	0.42	4.87	0.10
Max.	25.49	10.22	8.60	0.16
Median	7.25	8.52	7.26	0.13
Bromoacetic acid ($BrCH_2CO_2H$)				
Average (s.d.)	0.44 (0.56)	1.53 (1.31)	0.69 (0.36)	0.62 (0.14)
Min.	<0.1	0.51	<0.1	0.48
Max.	1.27	3.51	0.93	0.75
Median	0.05	0.82	0.83	0.62
Dibromoacetic acid (Br_2CHCO_2H)				
Average (s.d.)	5.16 (1.86)	6.49 (6.88)	0.40 (0.22)	31 (1.11)
Min.	2.40	0.39	<0.1	0.14
Max.	7.38	17.00	0.59	2.36
Median	5.72	5.22	0.50	1.42
Tribromoacetic acid (Br_3CCO_2H)				
Average (s.d.)	0.62 (0.70)	1.67 (1.93)	<0.1 (0)	0.35 (0.27)
Min.	<0.1	<0.1	<0.1	<0.1
Max.	1.74	3.812	<0.1	0.58
Median	0.45	0.72	<0.1	0.50
Bromochloroacetic acid ($BrClCHCO_2H$)				
Average (s.d.)	9.26 (5.33)	5.08 (3.80)	1.08 (0.41)	0.20 (0.04)
Min.	2.84	1.35	0.70	0.15
Max.	17.22	11.09	1.72	0.22
Median	9.38	5.12	1.03	0.22
Dibromochloroacetic acid (Br_2ClCCO_2H)				
Average (s.d.)	4.10 (1.61)	2.57 (2.04)	0.24 (0.27)	<0.1 (0)
Min.	2.01	0.90	<0.1	<0.1
Max.	6.00	5.77	0.54	<0.1
Median	4.47	1.72	0.05	<0.1
Bromodichloroacetic acid ($BrCl_2CCO_2H$)				
Average (s.d.)	8.04 (4.00)	4.60 (3.65)	1.74 (0.16)	0.09 (0.04)
Min.	3.06	0.90	1.52	<0.1
Max.	14.06	9.45	1.94	0.12
Median	8.32	5.09	1.78	0.10
Total haloacetic acids				
Average (s.d.)	50.41 (22.35)	35.81 (19.41)	15.30 (1.32)	3.14 (1.41)
Min.	31.15	12.81	13.81	1.72
Max.	87.00	65.42	16.92	4.54
Median	46.03	34.61	14.92	3.71
	5		5	3

coastal waters (Alacant and Barcelona) exhibit the highest HAA levels (50.4 and 35.8 μg/l, respectively) and Tenerife the lowest (3.1 μg/l). Mean HAA levels in European surface and ground waters are 9.25 (s.d. 7.7) and 0.83 μg/l (s.d. 0.76), respectively [10]. Cancho et al. [8] described HAA levels in water from Barcelona in the order of 21.6 μg/l.

The highest proportion of chlorinated HAA is found in the Asturian waters (monochloroacetic, dichloroacetic and trichloroacetic acids > 60%) whereas both Mediterranean regions (Barcelona and Alacant) show a higher proportion of brominated and chloro-brominated species (50–60%). The waters from Tenerife exhibit the highest proportion of brominated and chloro-brominated HAA (> 80%).

Dichloroacetic acid is the most abundant compounds in the Mediterranean areas. Trichloroacetic and dibromoacetic acids are the second most abundant compounds in Alacant and Barcelona, respectively. The waters from Asturias contain trichloroacetic acid in highest abundance, dichloroacetic acid being the second. In Tenerife waters the most abundant compounds are brominated species, their composition being dominated by dibromoacetic and bromoacetic acids which rank as first and second main compounds, respectively.

Chloroacetic acid is the least common HAA in all areas, which is in agreement with analyses from other sites [11]. Tribromoacetic acid is the second less common.

3.4. THM–HAA correlations

The correlation between THM and HAA is based on the dataset in which HAA concentrations were available. The representativeness of this sub-sample for the whole dataset was checked by comparison of the average THM levels in both groups and no significant differences ($p<0.05$) were found between them.

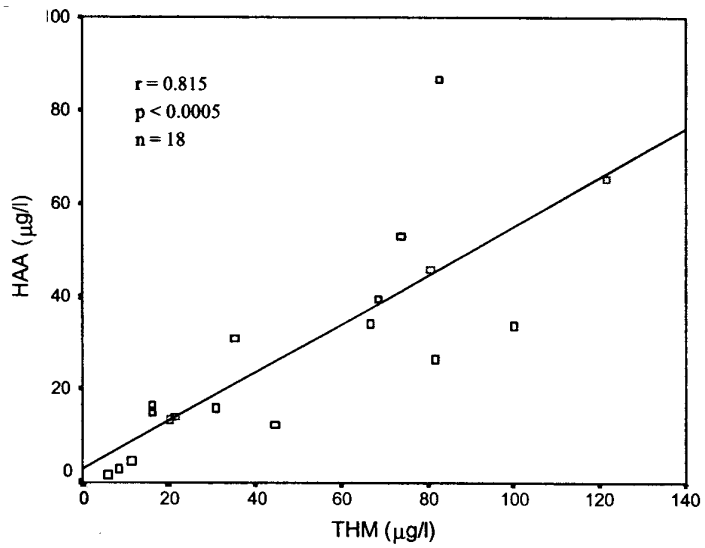


Fig. 2. Scatter plot showing the correlation between total THM and HAA levels.

Table 4
Models predicting the concentrations of specific HAA from those of THM

Haloacetic acids (Y_i)	Best predictor (X_i)[a]	Model[a]	Regression coefficient	Model statistical significance
Dibromochloroacetic	$CHClBr_2$	$Y_1 = -0.119 + 0.159X_3$	$r = 0.967$	$p<0.0005$
Dichloroacetic	$CHCl_2Br$ $CHBr_3$	$Y_2 = 1.534 + 0.566X_2 - 0.258X_4$	$r = 0.939$	$p<0.0005$
Tribromoacetic	$CHBr_3$	$Y_3 = -0.13 + 0.0898X_4$	$r = 0.922$	$p<0.0005$
Bromodichloroacetic	$CHCl_3$ $CHClBr_2$ $CHBr_3$	$Y_4 = -0.154 + 0.131X_1 + 0.277X_3 - 0.125X_4$	$r = 0.911$	$p<0.0005$
Dibromoacetic	$CHClBr_2$ $CHBr_3$	$Y_5 = -0.046 + 0.143X_3 + 0.192X_4$	$r = 0.876$	$p<0.0005$
Bromochloroacetic	$CHClBr_2$ $CHBr_3$	$Y_6 = 1.045 + 0.409X_3 - 0.212X_4$	$r = 0.844$	$p<0.0005$
Monobromoacetic	$CHBr_3$	$Y_7 = 0.442 + 0.0459X_4$	$r = 0.680$	$p = 0.003$
Trichloroacetic	$CHCl_3$	$Y_8 = 1.269 + 0.375X_1$	$r = 0.658$	$p = 0.003$
Monochloracetic	$CHBr_3$	$Y_9 = -0.028 + 0.0399X_4$	$r = 0.573$	$p = 0.016$

$X_1 = [CHCl_3]$, $X_2 = [CHCl_2Br]$, $X_3 = [CHClBr_2]$ $X_4 = [CHBr_3]$.

Linear regression analysis reveals a high and statistically significant correlation between total THM and total HAA, $Y = 2.643 + 0.526X$, Pearson's correlation coefficient $(r) = 0.851$, $p<0.0005$; Y = total HAA (µg/l), X = total THM (µg/l) (Fig. 2).

Multivariate linear regression analysis shows that some HAA are highly correlated to specific THM. The concentrations of dibromochloroacetic, dichloroacetic, tribromoacetic, and bromodichloroacetic acids are highly correlated with those of some THM ($r>0.9$, $p<0.0005$, Table 4). Thus variation of some THM may explain > 81% of the variability of these four HAA. Dibromoacetic and chlorobromoacetic acids are also correlated with THM but with a lower correlation

coefficient ($r > 0.8$, $p < 0.0005$). Chloroacetic, trichloroacetic and bromoacetic acids are less correlated with THM ($r = 0.573, 0.658$ and 0.680 respectively).

The more brominated THM (bromoform and dibromochloromethane) are those with highest predictive capacity, fully or partially, explaining the concentrations of eight of the nine HAA examined. Chloroform is only correlated to trichloroacetic acid and bromodichloromethane to dichloroacetic acid (in part). Surprisingly, the only THM that correlates significantly with monochloracetic acid is bromoform.

4. Conclusions

- Total THM and HAA vary considerably between different drinking waters according to their water sources. Low CBP levels are observed in ground waters, low-intermediate levels in good-quality surface waters and high levels in poor-quality surface waters. THM and HAA speciation also varies substantially among different water sources.
- Despite these differences, total and specific HAA species show a high correlation with total and specific THM compounds. Thus, HAA levels could be predicted from THM concentrations.
- The linear regression equations calculated from these correlations may be useful for the estimation of HAA concentrations from THM. This approach may find applicability in environmental and toxicological studies for assessment of human health risk of chlorinated by-products in drinking water.

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Article 4

Retrospective exposure assessment to disinfection by-products in a multicentre case-control study of bladder cancer

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ABSTRACT

Background

Accurate retrospective exposure assessment is of paramount importance in assessing health effects related to environmental exposures, and it becomes especially complex when studying chronic effects like cancer. Limitations in exposure assessment have been a major issue in studies linking exposure to disinfection by-products (DBP) with an increased cancer risk. We describe the methodology we followed to assess the retrospective DBP exposure of the study population through ingestion, inhalation and dermal absorption, in a multicentre case-control study of bladder cancer conducted in Spain.

Methods

Total trihalomethanes (THM), chloroform, bromodichloromethane, dibromochloromethane and bromoform levels were used as DBP surrogates. Historical THM levels in the study areas were ascertained through questionnaires to water companies and local authorities. Current THM levels were measured in drinking water samples from the study areas. Individual information on water-related habits was obtained from personal interviews to cases and controls. Residential history from birth, occupational history from age 6, and water source (municipal/bottled/private well/other) in each residence and job was asked, as well as average daily water consumption (litres/day) and water-related habits associated with inhalation and dermal absorption (showering/bathing/swimming pool attendance). Retrospective missing THM levels were attributed to past years at municipal level, using all the available data on THM levels, water source and year chlorination started. The assumption was that THM levels in a municipality remained constant over time if source was the same. Proportion of surface water was used as a weight when water source changed along the years. Average THM levels from current years were applied to past years. Municipal THM database was then merged with individual database by municipality and year. Individual THM exposure indices were then created.

Results

Water source and chlorination status (year of start chlorination) of 129 municipalities were collected, covering 79% of the total person-years from the study population (1226 cases and 1271 controls). THM levels from the main study cities were obtained. On average and for the last residence, 61% of the population reported drinking municipal water, 2% drank water from a private well, 24% drank bottled water and 13% drank water from other sources. After merging the municipal THM data with the individual data, and for the selected exposure window defined from age 15, 51% of the study population had 100% known THM exposure, 59% of the study population had ≥90% known THM exposure, 66% had ≥80 known THM exposure, and 71% of the population had ≥70% known THM exposure from the exposure window.

*Conclusion*s

Due to the lack of a valid biomarker of chronic exposure to DBP, THM or other DBP levels in the drinking water appear as valid markers of exposure in epidemiological studies of chronic effects.

INTRODUCTION

Accurate retrospective exposure assessment is of paramount importance in assessing health effects related to environmental exposures. Exposure assessment becomes more complex for assessing chronic effects like cancer. Limitations in exposure assessment have been the main criticism to the epidemiological studies linking disinfection by-products (DBP) exposure with an increased cancer risk. And it has been the argument for the International Agency for Research on Cancer (IARC) to conclude that human evidence linking chlorinated drinking water consumption with cancer was not conclusive[1], even though many epidemiological studies reported a positive association between DBP exposure and increased risk of cancer[2-5]. First studies were ecological in design[6-10], then mortality based-case control studies were conducted[4;11;12], followed by incidence case-control or cohort studies[2;3;5;13;14]. Those studies used surrogates of the exposure, such as water source (surface *vs*. ground)[2], duration of residence with municipal water source[14], disinfectant used[4;10], etc. Quantitative and individual assessment of the exposure or surrogates of the exposure suspected to be risk factor (total DBP mixture or specific compounds), like trihalomethane (THM) level or mutagenicity, was only performed in the 1990s, after the IARC evaluation[15;16-18]. Bladder is one of the cancer sites more consistently associated to DBP exposure, and many epidemiological studies with an improved exposure assessment have been published after the IARC evaluation, reporting positive associations[14;15;17-19]. However, some concerns about the exposure assessment still remain. DBP are a complex mixture of compounds with heterogeneous chemical, mutagenic and carcinogenic properties. THM levels have been traditionally used as markers of the total mixture since they are the most prevalent ones, although not necessarily the risk factors of cancer. New DBP are being detected as analytical techniques are developed[20;21]. THM are volatile compounds and apart from ingestion, THM can enter the body through inhalation and dermal absorption while showering, bathing, swimming pool attendance, dishwashing, and other water-related habits[22-24]. Other DBPs, as haloketones are also permeable to the skin and can enter the body through dermal absorption, while the haloacetic acids (HAA) have shown a low skin permeability[25]. Inhalation is also a significant route of exposure for haloketones during the shower, but insignificant for HAA[26]. DBP speciation has become important, as studies have shown that mutagenic and carcinogenic potential varies among specific DBB, especially with the bromine content within

a group of DBP. Brominated DBP have been shown to be more mutagenic and carcinogenic than chlorinated ones[27]. Since the same THM level can be associated to a different composition of DBP, the use of total THM level as a surrogate of DBP exposure is a limited marker. Specific THM levels may provide a better estimate of the real exposure, giving an idea of the proportion of brominated and chlorinated DBP species.
We conducted a multicentre case-control study of bladder cancer, and assessed the retrospective exposure of the study population to THM (total and four specific compounds: chloroform, bromodichloromethane, dibromochloromethane and bromoform) as markers of DBP exposure. Also, for the first time in an epidemiological study, we addressed the issue of alternative exposure pathways to DBP: inhalation and dermal absorption. Here we describe the methodology followed to assess the retrospective exposure to THM of the study population.

METHODS

STUDY DESIGN AND POPULATION

A multicentre case-control study of bladder cancer was conducted from June 1998 until October 2001 in Spain. Cases and controls were identified in 18 participating hospitals from 4 Spanish provinces (figure 1). The definition of case was a patient with a histollogically confirmed diagnosis of primary bladder cancer, living in the catchment area of the participating hospital. Controls were individually matched to cases by gender, age (5-years period) and residence area. Additionally, disease of controls should not be related with the known risk factors of the bladder cancer.

RESPONSE RATES

Trained interviewers administered the study subjects a comprehensive computer assisted personal interview (CAPI) while their stay in the hospital. Those subjects reluctant to answer the CAPI were administered a reduced interview of critical items. Response rates of the study population was 84% for the cases and 88% among the controls. Among respondents, 20% answered the interview of critical

items (21% of cases and 19% of controls). 1226 cases and 1271 controls were finally included in the study.

THM LEVELS IN THE STUDY AREAS

Levels of THM in the drinking water from the study areas was ascertained through questionnaires to water companies and local authorities, as well as our THM measurements in drinking water samples from the study areas.

Historical THM levels

Two structured questionnaires were developed. One was specifically aimed to water companies and the other was aimed to local authorities. Water companies were asked for general information (name of the company, contact information, date of answer and covered municipalities); source of the water (proportion of ground/surface currently and in the past, from 1920); treatment process (type of disinfectants used and other treatments); year-by-year annual mean levels of THM in finished water plant effluent from 1970, and yearly average levels of organic matter, chlorine demand, pH, temperature and other raw water parameters from 1950; year when chlorination started was also asked. The questionnaire to local authorities was a reduced version of the water company questionnaire. The collected information was: general information (name of municipality and contact information); history of water source (proportion of ground and surface water along the years, from 1920); year of start chlorination; water treatment; and year-by-year annual mean THM levels.

A procedure was followed to select water companies and municipalities to be sent the questionnaires. Firstly, the water companies from the main cities in each study region were identified and were sent the questionnaire. After posting the questionnaire, telephone calls of reinforcement were done to ensure the water companies answered the questionnaire. This way we gathered information from big water companies covering large amounts of people. Secondly, when the collection of individual data was in an advanced phase, residential histories from the personal interviews (CAPI) were analysed to ascertain in which municipalities the study subjects had lived for longer. Total years lived by the population in each municipality were calculated (person-years by municipality). Municipalities were sorted in decreasing order of person-years. The position of each municipality in the list provided an objective criterion of preference order to

gather information about the municipality. Questionnaire to local authorities were sent by regular mail following this priority order. Telephone calls were done to ensure the authorities filled out the questionnaire. When the questionnaires were received back filled out, the answers were checked. If an answer was unclear or doubtful, the contact person that filled out the questionnaire was telephoned to clarify the responses.

Current THM levels

From September until December 1999, 105 drinking water samples distributed among the study areas were collected from the tap to measure THM levels. Distribution of water samples among the areas was approximately proportional to the population coming from each area. 34 samples were taken in Asturias, 42 in Barcelona (24 in Barcelona city, 9 in Manresa and 9 in Sabadell), 19 in Alacant and 10 in Tenerife. Haloacetic acids were determined in a subset of 18 samples, equally distributed in the four study areas. Sampling and experimental analysis is detailed elsewhere[28].

Estimation of retrospective missing THM levels

When historical THM levels were not available, those were estimated according to established criteria. Estimation was performed at local (municipality) level, only in those municipalities with available information on water source history along the years (% ground/surface) and year of start chlorination.

Municipalities were grouped into 6 categories according to the type of available information. *Municipalities A* were defined as those with only available source history and year of start chlorination. *Municipalities B* had the same information as A, plus available THM data from one single year. *Municipalities C* had the same information as A, plus average THM data from 2 years or more. *Municipalities D* had the same information as C plus data on water treatment and other raw water parameters in past years. *Municipalities E* had THM level available from some years, but water source history and year of start chlorination were not available. *Municipalities F* had only available information on water source history but year of start chlorination was missing.

To ensure a systematic estimation of historical THM levels some rules were established by type of municipality. For the municipalities A, B, C and D, the THM estimation was done using all available information on source, year of start chlorination and THM levels (data obtained from both the questionnaires and the

own determinations). For the years before the chlorination started, the THM level attributed was zero. For the years in which the water was chlorinated but THM levels were missing, average THM levels of recent years with the same source were attributed. This assumed that within a municipality, for the same water source THM levels remained constant along the years. If source changed from surface to 100% ground, average THM levels of geographically close municipalities with 100% ground source were applied. For other changes in water source, proportion of surface water was used as weight to calculate THM levels. Few municipalities changed treatment along the years, others didn't change, and for the rest we didn't have this information available. When we had information on water treatment and it changed along the time, to avoid a different THM estimation between municipalities with and without treatment information, we assumed that treatment didn't affect THM levels. Municipalities E were omitted and no estimation of retrospective THM levels was performed on those. Only the years with available THM level were used for the analysis. For municipalities F, estimation of past THM levels was only performed if any geographically close municipality with information (type A, B, C or D) and the same water source was available.

The city of Barcelona was treated separately, since within the same municipality two water sources of different quality and chemical characteristics supplied water, giving rise to different levels of total and specific THM within the city. Although both were surface sources (rivers), the southwest was supplied with a river quite polluted and very rich in bromine (Llobregat river), providing a drinking water after treatment with relatively high levels of total THM and a high proportion of brominated and chloro-brominated species. In contrast, the northeast was supplied from a less polluted river with lower levels of bromine (Ter river). It provides a finished drinking water with lower total THM levels and a high proportion of chloroform. In Barcelona, estimation of past THM levels was performed at the zip code level. The Barcelona Water Company provided us with the proportion of each water source by zip code along the years since early the XXth century. This proportion was used as a weight to calculate historical THM levels in Barcelona. Zip codes without information on proportion of each source available, or the source was different within zip code were excluded and estimation of retrospective THM levels was not performed.

According to these procedures, we obtained a database containing year-by-year annual average THM level (total and specific ones), chlorination status and water source by municipality from 1920.

INDIVIDUAL DATA

Individual information on water-related habits was ascertained through a structured personal interview to cases and controls. Interviews included questions about socio-demographics, smoking habits, coffee consumption, occupational history, residential history, medical history, familiar history of cancer, quality of life and personal information. A diet questionnaire was also administered, as well as a diary of urinary pH and frequency. Relevant information for assessment of DBP exposure was:

- Residential history: street, city, province, autonomy and country of all the residences from birth, including year start and year end living in all the residences during one year or more
- Occupational history: city, province, autonomy and country of all the occupations from 16 years old, including year start and year end working in each job during at least 6 consecutive months
- Water source (municipal/bottled/private well/other) at each residence
- Water source (municipal/bottled/private well/other) at each job
- Daily water consumption (litres/day) was asked both in the self-administered diet questionnaire and in the computer assisted personal interview.
- Water-related habits: frequency of bathing/showering/swimming pool attendance; duration of bath/shower/swimming in the pool; water temperature of shower or bath (hot/cold/both); indoor/outdoor swimming pool.

MERGING INDIVIDUAL INFORMATION WITH MUNICIPAL THM LEVELS

The individual database with residential history and water source in each residence contained as many registers by subject as residences. This database was expanded to obtain year-by-year information by subject. This expanded individual database was merged with the database containing year-by-year average THM levels by municipality. Merging variables were municipality and year. Accordingly, we obtained a database with individual year-by-year annual

average THM level, as well as water source and chlorination status of the residential drinking water.

To get a THM exposure variable by subject we added all the year-by-year annual THM levels. The number obtained was then divided by the number of years with non-missing THM levels, obtaining an average THM exposure. The proportion of years with missing THM data in the exposure window (due to missing water source or missing municipal THM data) was also calculated.

The same procedure was followed to calculate lifetime average $CHCl_3$, $CHCl_2Br$, $CHCl_2Br$ and $CHBr_3$ exposure.

By adding the number of years with unchlorinated water source, chlorinated ground and chlorinated surface, the following three variables were created: duration of unchlorinated, chlorinated ground and chlorinated surface water in the residence. The proportion of years with missing water source and chlorination status in the exposure window (due to missing water source in the residential history or missing municipal water source or chlorination status) was also calculated.

The same procedure was followed with the occupational history data than for the residential history data to obtain individual exposure indexes.

To select the exposure window to analyse, different databases were created with different exposure windows: lifetime; from age 10; from age 15; from birth and 5 years lagged, from birth and 10 years lagged (lifetime excluding 5 and 10 years prior interview, respectively); 5 years lagged from age 10; 5 years lagged from age 15 and 10 years lagged from age 10. We explored the distribution of % known THM in the population in each exposure window and selected the exposure window that maximised the % of known exposure.

In the multivariate statistical analysis relating bladder cancer risk with THM exposure, certain subjects with a certain degree of exposure misclassification will have to be excluded. If we include all the study population, we will be including subjects with exposure misclassification. If we include only those subjects with 100% of exposure information, we will have a reduced population. Thus, the

selection of the cut point will be a compromise between the number of subjects excluded and the quality of information of the subjects included. The cut point selected for the logistic analysis was 70%, so only subjects with at least 70% of exposure information were included in the analysis.

INDIVIDUAL EXPOSURE INDICES

We created two individual exposure indices for THM exposure.
For the *ingestion THM exposure*, municipal THM levels were attributed when the subject reported drinking municipal water. If the subject reported drinking water from a private well, bottle or other sources, a null THM level was attributed. We obtained an average THM exposure level by adding the year-by-year THM level and then dividing by the number of non-missing THM levels. We multiplied this level by the daily amount of tap water consumption (litres/day, including water *per se*, plus coffee and tea). Consequently, we obtained an average THM exposure through ingestion, in μg/day.

For the *overall or global THM exposure index*, municipal THM levels were attributed to the subjects independently on the reported drinking water source. THM exposure can occur through inhalation and dermal absorption. We calculated the addition of year-by-year THM levels and then divided by the number of years with non-missing THM level. Thus we obtained a variable of average THM exposure with μg/litre units. This overall exposure index relies in the fact that a person reporting drinking bottled water won't be exposed through ingestion but indeed will be exposed to the municipal THM levels through inhalation and dermal absorption while showering, bathing, dishwashing, or other water-related activities. Additionally, if the subject works in the same municipality, this overall exposure index also implies the exposure in the job

Showering and bathing variables were analysed by duration of these activities, since all the population is exposed. Total minutes per month showering and bathing separately were calculated by combining frequency and usual duration of each shower/bath.

Swimming in the pool was analysed by never/ever attendance, duration, and type of swimming pool (outdoor/indoor). Lifetime minutes swimming in the pool

was calculated by combining frequency of attendance and minutes spent in the water each time.

Lifetime hours showering, bathing and swimming in the pool were combined to generate an overall non-ingestion exposure index. The weighting factors used to combine the three activities were based on the bibliography[29]. The equation used was: [2/3(lifetime minutes showering)+2/3(lifetime minutes bathing)+minutes swimming in the pool].

RESULTS

Water source and chlorination status (year start chlorination) of 129 municipalities, covering 79% of the total person-years from the study were collected (table 1). Municipalities with best data (type D municipality) covered about 41% of total person-years. Information from three municipalities was lacking on water source history and year of start chlorination, and thus they were excluded from the past THM estimation. However, these three municipalities were equivalent to only 0.4% of the study population.

Barcelona was the first province where THM were analysed in the drinking water (starting regularly in 1979), and also the study area with highest past THM levels. Mean levels obtained from all the annual average levels provided by the water companies supplying Barcelona was 164.4 μg/l. In Alacant THM were sporadically analysed in the drinking water in 1983, although regular analyses started in the 1990's. Average levels from the water companies were 77.6 μg/l. The other regions began analysing THM after 1990 (table 2). Quenching status of the water samples is unknown.

Table 3 shows the available year-by-year annual average THM data from the water companies in the main municipalities of the study, indicating that from all cities, 1999 data from our own THM determinations are available.

The results of our THM determinations are shown in the table 4. Following a similar pattern as the data from the water companies, the cities located in the Mediterranean coast (Alacant, Sabadell and Barcelona) show the highest THM levels: 85.9, 82.5 and 63.6 μg/l respectively. Maximum levels of these places are

above 100 μg/l, which is the maximum admissible level in any individual sample established by the European and Spanish legislation. The drinking water from Tenerife show the lowest THM levels (8.0 μg/l). Asturias show intermediate-low levels (22.3 μg/l), and Manresa (close to Sabadell, but taking water from a different source) has intermediate levels (56.1 μg/l). HAA levels distribution in the study areas is similar to that of THM levels. HAA concentrations are also higher in the Mediterranean areas: Alacant and Barcelona show the highest levels among the study areas with 50.4 and 35.8 μg/l respectively. Tenerife exhibit the lowest total HAA levels with 3.1 μg/l, and Asturias show intermediate levels: 15.3 μg/l. More detailed results on current exposure (THM and HAA species) in the study areas are published elsewhere[28].

Drinking water source in the last residence (mean duration=28.6 years) by area is shown in table 5. Sabadell and Alacant are the areas where municipal water consumption is lower (around 40% in relation to all types of water), while in Barcelona, Manresa and Asturias more than 60% of people drunk municipal water in the last residence, which was above the median population (60%). Around 2% of the population drunk water from a private well, although 12% people from Alacant drunk water from a private well. Tenerife and Barcelona are the areas with lowest private well water consumption (0.2 and 0.8% respectively). Mean proportion of bottled water consumption is around 24%. In Sabadell, Alacant and Barcelona the population drunk bottled water above the median (50, 46 and 29% respectively). Asturias, Manresa and Tenerife drunk bottled water below the median consumption (12, 22 and 24% respectively). Water from other sources was around 12% for all the population. Asturias, Tenerife and Manresa are the areas with higher consumption of these alternative water sources.

Table 6 shows the distribution of the cumulative percentage of known THM ingestion exposure by cumulative population for different exposure windows. For any given cumulative percentage of known exposure, the exposure window that maximised cumulative population was that defined from age 15. Consequently, this was the exposure window selected for the main analysis. The rest of exposure windows will be used for alternative analyses.

The figure 2 shows the distribution of the cumulative percentage of known THM ingestion exposure *versus* cumulative population for the exposure window

defined from age 15. Since the cut point selected for the analysis was 70%, only subjects with 70% or more THM exposure information from the exposure window are included in the analysis. That represents 71% of the study population.

For statistical analysis of showering and bathing, THM levels in the residence are used as adjusting variables, and are not incorporated into the exposure index. THM levels in the swimming pool are *a priori* independent on the THM levels in the residences. Consequently, the statistical analysis for swimming pool attendance is performed independently on the municipal THM levels.

DISCUSSION

We used chloroform, bromodichloromethane, dibromochloromethane, bromoform and total THM levels to characterize the exposure to disinfection by-products of the study population in a multicentre case-control study of bladder cancer. The retrospective assessment of exposure to the four single THM with comprehensive collection of exposure data in the study areas is one of the strengths of the study. Additionally, the thorough personal interview of cases and controls providing a lifetime profile of the type of water drunk in home and in the job, daily average water consumption, as well as information on water-related activities where inhalation and dermal absorption to DBP takes place, represents an improvement in the exposure assessment to DBP in comparison to the previous epidemiological studies. However, although THM level correlates well with total halide content and is probably the best marker of DBP so far, is still a surrogate and have certain limitations. THM level does not necessarily correlate with other DBP, and consequently does not necessarily correlate with the real and unknown risk factor/s. Additionally, for being a multicentre study, the same THM level can be associated to a different mixture of DBP in different areas, implying certain misclassification. This bias, however, can be controlled in the statistical analysis if adjusting by area.

Chlorine has been the most widely used drinking water disinfectant, but gradually it's getting substituted in many western countries by alternative disinfectants as chlorine dioxide, ozone, chloramines, etc. These alternative disinfectants produce less THM and generate different DBP from chlorination by-products. Consequently, as chlorine is substituted by other disinfectants, non-

THM DBP become more prevalent, becoming THM levels worse surrogates of DBP. In Spain, chlorine has been the most used disinfectant for drinking water, and it is still in the present. As a consequence, THM have probably been the most prevalent DBP in the study areas and are a valid indicator of the whole DBP mixture in our study.

One potential source of error in the estimation of historical THM levels could be due to the fact that THM levels vary from the treatment plant effluent to the drinking point (the tap). According to the literature, concentration of DBP formed in the distribution system increase from those detected in the plant effluent[30], and this increase depend on the distance (contact time), the amount of residual chlorine and the residual organic matter. We didn't model the effect of the distance and remaining time in the water system from the plant until the tap for the data from the water companies. THM levels from the water company will probably be underestimating the real exposure. Additionally, we combined indistinctly THM data provided from the water companies (measured in water from the plant effluent) and our THM determinations (measured in water from the tap). Certain misclassification of the exposure have probably been produced, with the effect of attenuating the magnitude of the risk estimate.

The assumption that THM level along the years remained constant in a municipality as long as water source was the same, could also represent a source of error in the estimation of historical THM levels. We ignored the effect over the THM levels due to changes in the water treatment, since this information was missing from most of the municipalities. These are simplifications that don't reflect reality. However, assumptions are necessary to perform the retrospective exposure assessment, since THM data from past years doesn't exist (retrospective assessment goes back even before THM were detected).

A third potential source of error in the historical THM estimation could arise from the combination of our THM measurements (determined in a centralized laboratory), and THM levels provided from water companies (and thus determined in different laboratories). Technical procedures to analyse THM levels have probably been different, as well as sampling procedures, storing conditions, lag between sampling and analysis and use of quenching compound. Due to these differences, the THM determinations obtained from water companies and

local authorities are less comparable. However, we still find a comparable and consistent pattern between historical THM levels and the results from our measurements (that followed a standard procedure from sampling until analytical determination).

No valid biological markers of chronic exposure to DBP have been found. THMs are rapidly eliminated through the exhaled breath. Chloroform level in breath air or blood can be a good marker of recent exposure (after swimming in the pool, bathing or showering)[31], but it is not useful as a marker of cumulative exposure during decades, since half live in the body of the volatile compounds is too short[23;32-34]. HAA levels in human tissue have been investigated as biological markers of exposure[35-37]. Trichloroacetic acid (TCAA) levels in urine was correlated with the levels in the water[35], so TCAA levels in urine appears as a potentially valid marker of chronic exposure, since half live in the body is longer than successive intervals of exposure. However, TCAA is the metabolite of other pollutants like trichloroethylene and tetrachloroethylene[38], and those can be present in the drinking water or in the general environment. In conclusion, to date, environmental levels of DBP appear as the best markers of exposure, and become better if measured in the point closest to the consumption (the tap).

Figure 1. Location of the study areas.

note: Barcelona area includes three sub-areas with different characteristics: *Barcelona city, Sabadell and Manresa*

Table 1. Frequency of municipalities and person-years by the type of exposure information available

Type of information	Number of municipalities (%)	Persons-years (%)
A (source history+year start chlorination)	75 (58%)	28 654 (25.6%)
B (A+ THM level from 1 year)	9 (7%)	6 522 (5.8%)
C (A+average THM level from ≥2 years)	14 (11%)	30 169 (27.0%)
D (A+C+water treatment+raw water parameters)	25 (20%)	45 614 (40.7%)
E (some THM levels, no A)	3 (2%)	432 (0.4%)
F (water source history, no year start chlorinat.)	3 (2%)	558 (0.5%)
Total municipalities with available information	129 (17%)	111 949 (79.4%)
Total study	757 (100.0%)	141 036 (100%)

Table 2. Average trihalomethane level (µg/l) in the study areas obtained from water companies, and year they start analysing THM

	Mean¦ (Stand.Dev.)	median	minimum	maximum	n*	Year start THM determinations
Barcelona†	164,4 (73.5)	157,3	53,2	380,1	28	1979
Sabadell	77.6 (10.2)	82.5	65.9	84.5	16	1996
Alacant¥	72.3 (18.9)	76.8	28.4	94.1	16	1983
Manresa	57.6 (15.7)	61.0	32.3	73.6	17	1980
Astúries‡	34.1 (15.2)	30.0	14.3	63.9	16	1995
Tenerife§	11.1 (15.8)	5.3	2.0	58.6	13	1992

¦ Mean levels obtained from all the yearly average levels provided by the water companies supplying each area

* This mean has been calculated averaging all the average annual THM levels provided by different water companies in each area.

† Includes data from the three treatment plants supplying the city of Barcelona

¥ Inclues data from Crevillente, Santa Pola and Orihuela

‡ Includes data from the three main cities: Gijón, Oviedo and Avilés

§ Includes data from Santa Cruz de Tenerife, La Laguna, Arona and Santa Cruz de la Palma

Table 3. Available THM data from the questionnaire to water companies in the main cities of the study.

	Barcelona†	Sabadell†	Manresa	Gijón	Oviedo	Avilés	Elche	Sta. Cruz Tenerife
1979	X							
1980	X		X					
1981	X							
1982	X							
1983								
1984	X							
1985	X							
1986	X							
1987	X							
1988	X							
1989	X							
1990	X							
1991	X							
1992	X							
1993	X							
1994	X							
1995	X				X	X		
1996	X	X	X		X	X		
1997		X		X	X	X		X
1998				X	X	X		
1999			X	X	X	X		
2000			X	X	X	X		
own 1999‡	X	X	X	X	X	X	X	X

† Only total THM level available.
‡ Data from our THM determinations in 1999.

Table 4. Current trihalomethane and haloacetic acid levels (µg/l) in the study areas, obtained from our determinations

	Average (SD)	Median	min.-max.	# samples
Total trihalomethane levels (µg/l)				
Alacant	85.9 (30.6)	69.2	35.2-125.3	19
Sabadell	82.5 (16.5)	86.6	64.1-100.9	9
Barcelona	63.6 (20.6)	55.6	34.6-121.7	24
Manresa	56.1 (22.2)	59.4	28.3-101.8	9
Asturias	22.3 (5.5)	22.3	6.4-44.5	34
Tenerife	8.0 (3.4)	7.1	5.1-16.3	10
Total haloacetic acid levels (µg/l)				
Alacant	50.4 (22.4)	46.0	31.2-87.0	5
Barcelona	35.8 (19.4)	34.6	12.8-65.4	5
Asturias	15.3 (1.3)	14.9	13.8-16.9	5
Tenerife	3.1 (1.4)	3.7	1.7-4.5	3

Table 5. Drinking water source in the last residence by study area (n, % from all sources).

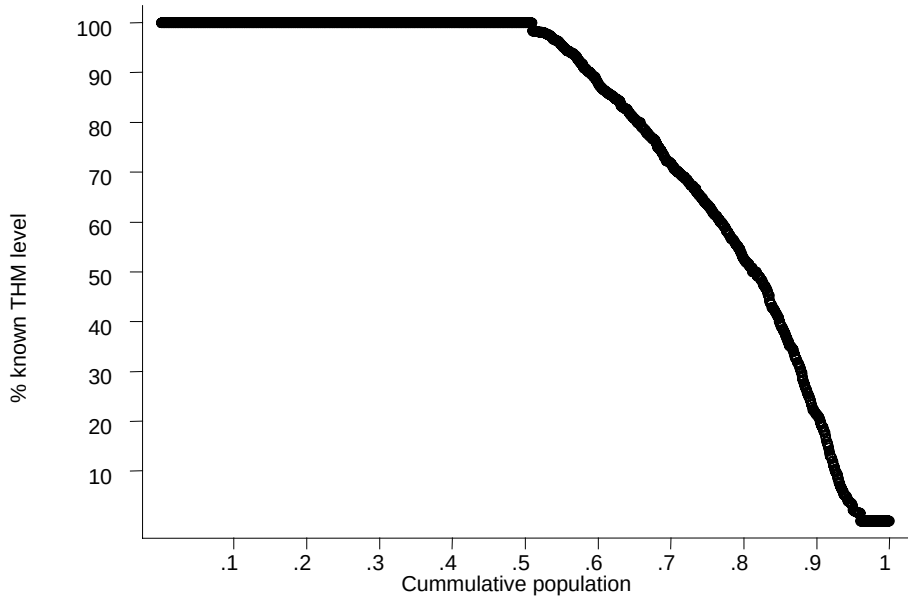
Current residence	**municipal**	**Private well**	**bottled**	**other**	**Missing/ DK† / refused**	**Didn't drink water**	**total**
Barcelona	317 (67.6)	4 (0.8)	136 (29.0)	12 (2.6)	7	0	476 (100.0)
Sabadell	94 (41.4)	5 (2.2)	113 (49.8)	15 (6.6)	3	0	230 (100.0)
Manresa	92 (62.2)	2 (1.4)	33 (22.3)	21 (14.2)	0	0	148 (100.0)
Alacant	68 (39.3)	21 (12.1)	79 (45.7)	5 (2.9)	0	0	173 (100.0)
Tenerife	262 (60.2)	1 (0.2)	104 (23.9)	68 (15.6)	10	1	446 (100.0)
Asturias	670 (67.2)	14 (1.4)	124 (12.4)	189 (19.0)	26	1	1024 (100.0)
total	1503 (61.4)	47 (1.9)	589 (24.0)	310(12.7)	46	2	2497

† don't know

Table 6. % of population with at least 60, 70, 80, 90 or 100% of known THM ingestion exposure by different exposure windows.

% exposure data	EXPOSURE WINDOWS							
	Lifetime exposure	5-years lagged	10-years lagged	5-years lagged from age 10	5-years lagged from age 15	10-years lagged from age 10	From age 10	From age 15
≥60%	71.0	69.7	67.9	72.9	74.8	70.1	74.6	77.0
≥70%	64.5	63.5	62.8	67.8	69.3	66.2	69.2	71.1
≥80%	59.3	58.9	58.6	62.0	64.6	61.0	62.9	65.8
≥90%	53.1	53.2	52.8	56.1	58.3	55.7	56.7	59.0
100%	44.2	44.9	45.2	49.4	51.3	49.5	48.9	51.0

Figure 1. Distribution of cummulative percentage of known THM ingestion exposure versus cumulative population, for the exposure window defined from 15 years old



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Article 5

Disinfection by-products and bladder cancer risk: a multicentre case-control study conducted in Spain

cmv, kpc, md, jog, ..., mk

INTRODUCTION

Drinking water disinfectants are highly reactive compounds that generate undesired by-products due to the reaction with organic matter. Chlorine has been the more widespread disinfectant used for drinking water treatment, and long-term exposure to chlorination by-products has been associated with an increased of cancer risk in a series of studies[1-4], reinforced by experimental evidence of carcinogenic activity [5-8]. Bladder is one of the cancer sites most consistently associated with chlorination by-products exposure[9-12]. However, methodological limitations and the low increased risk reported require the replication of the studies in different settings. We conducted a multicentre case-control study of bladder cancer in Spain, and evaluated the association between DBP exposure and bladder cancer risk. We used THM level (the most prevalent chlorination by-product) as marker of DBP exposure, and evaluated the role of inhalation and dermal absorption of DBP associated with the bladder cancer risk.

METHODS

STUDY DESIGN AND POPULATION

A multicentre case-control study of bladder cancer was conducted from June 1998 until October 2001 in Spain. Cases and controls were identified in 18 participating hospitals from 6 Spanish areas (Barcelona, Sabadell, Manresa, Alicante, Tenerife and Asturias). The definition of case was a patient with a histologically confirmed diagnosis of primary bladder cancer, living in the catchment area of the participating hospital. Controls were individually matched to cases by gender, age (5-years period) and residence area. Additionally, disease of controls should not be related with the known risk factors of the bladder cancer.

RESPONSE RATES

After obtaining a written consent by the patient, trained interviewers admistered the study subjects a comprehensive computer assisted personal interview (CAPI) while their stay in the hospital. Those subjects reluctant to answer the CAPI were administered a reduced interview of critical items. Response rates of the study population were 84% for the cases and 88% among the controls. Among respondents, 20% answered the interview of critical items (21% of cases and 19% of controls). 1226 cases and 1271 controls were finally included in the study.

INDIVIDUAL DATA COLLECTION

Following a structured questionnaire, subjects were asked about socio-demographics, smoking habits, coffee consumption, occupational history, residential history, medical history, familiar history of cancer, quality of life and personal information. A diet questionnaire was also administered, as well as a diary of urinary pH and frequency. Collected information on water-related habits included: drinking water source in each residence and job (municipal/bottled/private well/other), average daily water consumption (litres/day), shower and bath frequency, time spent showering and bathing

and usual temperature of the water (cold/warm/hot), swimming pool attendance, time spent swimming in the pool, and type of swimming pool (indoor/outdoor).

THM DATA COLLECTION

We developed a questionnaire aimed to water companies, and a second questionnaire aimed to local authorities, obtaining historical annual average THM level, water source (from 1920) and year of start chlorination from 129 study municipalities. These municipalities covered 79% of the total study person-years.

THM (chloroform, bromodichloromethane, dibromochloromethane and bromoform) were measured in 105 water samples from the study areas taken from the tap, from September to December 1999. Samples were distributed in the study areas following a similar distribution to the population each area provided to the study.

When historical THM levels were missing, we estimated those by municipality and year-by-year according to established criteria. Retrospective exposure assessment is detailed elsewhere (ref. Methodological article). Briefly, before chlorination started, attributed THM level to those years was zero. After chlorination started, average THM levels from recent years in the same municipality were attributed. Proportion of surface water was used as a weight when water source changed. If water source changed to totally ground, THM levels of a geographically close municipality with ground source were applied.

INDIVIDUAL EXPOSURE INDICES

Individual database was merged with municipal THM level database by year and municipality, obtaining an individual year-by-year annual THM level, water source and chlorination status.

We created two individual exposure indices for THM exposure.

For the ingestion THM exposure, municipal THM levels were attributed when the subject reported drinking municipal water. If the subject reported drinking water from a private well, bottle or other sources, a null THM level was attributed. We obtained an average THM exposure level by adding the year-by-year THM level and then dividing by the number of non-missing THM levels. We multiplied this average level by the daily amount of tap water consumption (litres/day, including water per se, plus coffee and tea). Consequently, we obtained an average THM exposure through ingestion, with the unit μg/day.

We had tap water consumption from the main questionnaire and from the diet questionnaire. We decided to include in the analysis the variable with less missing values, and that was the tap water variable from the diet questionnaire (1/3 more subjects than for the main interview variable).

For the global THM exposure index, municipal THM levels were attributed to the subjects independently on the reported drinking water source. We calculated the addition of year-by-year THM levels and then divided by the number of years with non-missing THM level. Thus we obtained a variable of average THM exposure with μg/litre units. This overall exposure index relies in the fact that a person reporting drinking non-municipal water won't be exposed through ingestion but indeed will be exposed to the municipal THM levels through inhalation and dermal absorption while showering, bathing, dishwashing, or other water-related activities. Additionally, if the subject works in the same municipality, this global exposure index also implies the exposure in the job

Showering and bathing variables were analysed by duration of these activities, since all the population is exposed. Total minutes per month showering and bathing separately were calculated by combining frequency and usual duration of each shower/bath.

Swimming in the pool was analysed by never/ever attendance, duration, and type of swimming pool (outdoor/indoor). Lifetime minutes swimming in the pool was calculated by combining frequency of attendance and minutes spent in the water each time.

Lifetime hours showering, bathing and swimming in the pool were combined to generate an overall non-ingestion exposure index. The weighting factors used to combine the three activities were based on the bibliography[13]. The equation used was: [2/3(lifetime minutes showering)+2/3(lifetime minutes bathing)+minutes swimming in the pool].

STATISTICAL ANALYSIS

Different exposure windows were explored, and we selected the one that maximised the cumulative population with known exposure. This exposure window was defined from age 15 until the time of interview.

We used unconditional logistic regression to calculate odds ratios (OR) and 95% confidence intervals (95% CI) for the different exposure indexes. All OR were adjusted by area, age (continuous), sex (when not stratified), smoking status (never/ex/current), urbanicity of longest residence, fruit and vegetable intake, overall quality of interview and education. The analysis was restricted to those subjects with exposure information of at least 70% of the exposure window. Subjects with unsatisfactory or questionable overall quality of interview were excluded from the analysis.

RESULTS

Table 1 shows the characteristics of the study population. 87% were men, and the median age at interview was 67 years. 41% of the study area came from Asturias, 19% from Barcelona, 18% from Tenerife, 9% from Sabadell, 7% from Alicante, and 6% from Manresa. After adjusting by age, gender and area, statistically significant excess risks were found for former and current smokers. Those high consumers of fruits and vegetables were protected with statistically significance referred to those with low fruit and vegetable consumption (last quartile referred to the first quartile of consumption). Subjects who had their longest residence in a village were protected referred to those whose longest residence was in a metropolitan area. Cases and controls had a similar educational level. Overall quality of interview had a different distribution among cases and controls. More controls than cases had an interview with reliable overall quality, and this difference was statistically significant.

Subjects exposed to an average global THM level above 49 μg/l in the residence have an odds ratio (OR)=1.99, 95% confidence interval (95% CI)=1.03-3.86, in relation to those subjects exposed to an average THM level below or equal 8 μg/l (Table 2). By gender and the same categories of exposure, OR is 2.41, 95% CI=1.16-4.99 among men and 1.48 (95% CI=0.24-9.28) among women. A dose-response trend is observed among men (p value for linear trend=0.006), but not among women (p value for linear trend=0.693).

For average ingestion exposure, men exposed to an average THM level above 35 μg/day show an OR=1.55 (95% CI=1.02-2.35) in relation to those unexposed (Table 2). A statistically significant dose-response trend is also observed among men (p value for linear trend=0.030). Among women and for the same exposure categories, OR is 0.63 (95% CI=0.19-2.04), and no dose-response trend is observed (p value for linear trend=0.403).

Water source and chlorination status is associated with an increased bladder cancer risk among men. If the predominant water source in the residence (>=70% of the lifetime) has been surface chlorinated, men show an increased bladder cancer risk in relation to those subjects with predominant water source in the residence unchlorinated or ground chlorinated, with an OR=2.13 (95%CI=1.19-3.83). Among women and for the same exposure categories, OR was 0.86 (0.13-5.57).

Table 3 shows the distribution of cases and controls in the study areas according to the exposure categories used for the global average exposure. Barcelona, Sabadell, Manresa and Alicante concentrate the subjects in the high-exposure categories, and there are few subjects with low exposure. In contrast, Tenerife concentrate subjects in the low exposure categories. Population coming from Asturias are distributed in the low and intermediate exposure categories.

Showering and bathing shows no association with bladder cancer risk. Subjects in the highest category of exposure to shower and bath (>6 hours/month) in relation to the lowest category of exposure (<= 2 hours/month) have an OR=1.28 (95%CI=0.90-1.82), and no dose-response trend is observed (p value for linear trend=0.064). Among men and for the same exposure categories, OR is 1.30 (95%CI=0.89-1.91) and p value for linear trend=0.081. Among women OR is 1.00 (0.35-2.87), and p-value for linear trend is 0.833.

Swimming in the pool is associated with an increased bladder cancer risk among men. OR for the highest category of exposure (>630 hours swimming in the lifetime) in relation to those who had never swam in the swimming pool had an OR=2.08 (95% CI=1.06-4.09), with a dose-response trend (p-value for linear trend=0.007). Among women and for the same exposure categories, OR is 0.68 (95% CI=0.09-5.22), without showing a dose-response trend (p-value for linear trend=0.895).

For the overall inhalation and dermal absorption index, exposure is not associated with an increased bladder cancer risk. Men exposed more than 1000 hours in their lifetime to swimming, bathing or showering have an OR for bladder cancer of 1.30 (95% CI=0.92-1.82) in relation to those exposed <=297.3 hours, with no dose-response trend (p-value for linear trend=0.127). Among women and for the same exposure categories, OR is 1.73 (95% CI=0.71-4.22) and p-value for linear trend=0.155.

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Table 1. Description of the study population.

	Cases (%)	Controls (%)	OR (95% CI)†
	1226	1271	
Gender			
Men	1072 (87.4)	1105 (86.9)	
Women	154 (12.6)	166 (13.1)	
Age			
mean (SD)	66 (9.9)	65 (9.9)	
min-max	22-81	19-88	
percentiles 25, 50, 75	61, 68, 74	59, 67, 72	
Area			
Barcelona	229 (18.7)	247 (19.4)	
Sabadell	119 (9.7)	111 (8.7)	
Manresa	69 (5.6)	79 (6.2)	
Alicante	89 (7.3)	84 (6.6)	
Tenerife	220 (17.9)	226 (17.8)	
Asturias	500 (40.8)	524 (41.3)	
Smoking status			
Never	221 (18.0)	464 (36.5)	1.00
Former	502 (40.9)	510 (40.1)	2.99 (2.33-3.85)
Current	496 (40.5)	286 (22.5)	6.27 (4.79-8.20)
DK/missing	7 (0.6)	11 (0.9)	
Education			
< Primary school	565 (46.1)	592 (46.6)	1.00
< High school	474 (38.7)	491 (38.6)	1.08 (0.91-1.29)
≥ High school	167 (13.6)	164 (12.9)	1.17 (0.91-1.51)
Other	15 (1.2)	15 (1.2)	1.15 (0.55-2.39)
Refused/DK/missing	5 (0.4)	9 (0.7)	
Overall quality of interview			
Unsatifactory	18 (1.5)	8 (0.6)	1.00
Questionable	92 (7.5)	85 (6.7)	0.49 (0.20-1.19)
Reliable	857 (69.9)	970 (76.3)	0.42 (0.18-0.98)
High	213 (17.4)	179 (14.1)	0.61 (0.26-1.44)
Refused/DK/missing	46 (3.7)	29 (2.3)	
Fruit and vegetable consumption (grams, in quartiles)			
0-421.8	258 (21.0)	216 (17.0)	1.00
>421.8-671	229 (18.7)	216 (17.0)	0.87 (0.67-1.13)
>671-1000.6	236 (19.3)	216 (17.0)	0.88 (0.67-1.14)
>1000.6	194 (15.8)	215 (16.9)	0.72 (0.55-0.94)
Missing	309 (25.2)	408 (32.1)	
Size of longest residence			
Metro/city	389 (31.7)	366 (28.8)	1.00
Small city	157 (12.8)	156 (12.3)	0.93 (0.71-1.21)
Village	455 (37.1)	543 (42.7)	0.77 (0.63-0.93)
Farm	4 (0.3)	2 (0.2)	1.99 (0.36-10.93)
Refused/DK/missing	221 (18.0)	204 (16.0)	

† OR and 95% CI calculated by logistic regression adjusting by age, gender and area

Table 2. Odds ratios (OR) and 95% confidence intervals (95% CI) for bladder cancer by gender for different exposure indices to THM and water source.

	MEN		WOMEN		ALL
	OR (95% CI)†	cases/controls	OR (95% CI)†	cases/controls	OR (95% CI)†
Global THM exposure. Average THM in the exposure window (μg/l)					
≤8.0	1.00	137/171	1.00	24/25	1.00
>8.0, ≤26.0	1.45 (0.89-2.36)	141/158	0.39 (0.12-1.28)	19/33	1.18 (0.76-1.85)
>26.0, ≤49.0	2.16 (1.25-3.74)	184/160	1.04 (0.28-3.88)	23/22	1.84 (1.12-3.02)
>49.0	2.41 (1.16-4.99)	158/180	1.48 (0.24-9.28)	25/22	1.99 (1.03-3.86)
p-trend‡	0.006		0.693		0.012
Ingestion THM exposure. Average THM in the exposure window (μg/day)					
0	1.00	119/140	1.00	28/19	1.00
>0, ≤10	0.99 (0.67-1.48)	119/124	0.58 (0.17-2.03)	17/18	0.92 (0.63-1.33)
>10, ≤35	1.28 (0.85-1.92)	132/119	0.61 (0.18-2.03)	14/16	1.15 (0.78-1.67)
>35	1.55 (1.02-2.35)	131/114	0.63 (0.19-2.04)	17/18	1.35 (0.92-1.98)
p-trend‡	0.030		0.403		0.110
Predominant water source and chlorination status in the residence					
≥70% unchlorinated or ground chlorinated	1.00	151/200	1.00	25/27	1.00
≥70% surface chlorinated	2.13 (1.19-3.83)	153/182	0.86 (0.13-5.57)	20/18	1.78 (1.04-3.04)

† OR and 95% CI obtained from logistic regression adjusting by smoking status, age, gender, education, urbanicity of longest residence, fruit and vegetable intake, overall quality of interview, and area. Analysis excluding subjects with overall quality of interview unsatisfactory and questionable, and limited to subjects with percentage known exposure ≥70% from the exposure window

‡ p value for linear trend

Table 3. Distribution of cases and controls in the exposure categories by area

	Barcelona	Sabadell	Manresa	Alicante	Tenerife	Asturias
Average THM in the exposure window (μg/l)						
≤8.0	0/1	-	3/4	-	125/137	33/54
>8.0, ≤26.0	1/0	0/1	1/0	0/1	9/14	149/175
>26.0, ≤49.0	30/38	44/34	10/8	1/1	-	122/101
>49.0	91/100	18/20	13/16	61/66	-	-

Table 4. Odds ratio (OR) and 95% confidence intervals (95% CI) for bladder cancer by gender and duration of showering or bathing per month.

Shower+bath, hours/month	**Men**		**Women**		**All**
	OR (95% CI)†	cases/controls	OR (95% CI)†	cases/controls	OR (95% CI)†
≤2 hours/month	1.00	129/171	1.00	28/32	1.00
>2, ≤3.5 h/month	0.78 (0.52-1.16)	84/120	0.25 (0.06-1.02)	4/17	0.71 (0.49-1.03)
>3.5, ≤6 h/month	1.11 (0.77-1.59)	131/156	0.87 (0.33-2.28)	17/18	1.09 (0.78-1.52)
>6 h/month	1.30 (0.89-1.91)	133/121	1.00 (0.35-2.87)	19/19	1.28 (0.90-1.82)
p-trend‡	*0.081*		*0.833*		*0.064*

† OR and 95% CI obtained from logistic regression adjusting by smoking status, age, gender, education, urbanicity of longest residence, fruit and vegetable intake, overall quality of interview, THM level in the residence, and area. Analysis excluding subjects with overall quality of interview unsatisfactory and questionable, and limited to subjects with percentage known exposure ≥70% from the exposure window
‡ p value for linear trend

Table 5. Odds ratio (OR) and 95% confidence intervals (95% CI) for bladder cancer by gender and duration of swimming in the pool in the lifetime.

Swimming in the pool. Lifetime hours	**Men**		**Women**		**All**
	OR (95% CI)†	cases/controls	OR (95% CI)†	cases/controls	OR (95% CI)†
Never swam	1.00	539/679	1.00	96/128	1.00
>0, ≤36 hours	1.43 (0.77-2.65)	24/25	0.41 (0.04-4.63)	1/3	1.30 (0.72-2.35)
>36, ≤165 hours	1.86 (0.99-3.49)	29/21	1.61 (0.20-12.94)	2/2	1.77 (0.97-3.21)
>165, ≤630 hours	1.29 (0.70-2.39)	25/27	2.04 (0.27-15.50)	3/2	1.30 (0.73-2.33)
>630 hours	2.08 (1.06-4.09)	26/18	0.68 (0.09-5.22)	2/3	1.84 (0.98-3.44)
p-trend‡	*0.007*		*0.895*		*0.012*

† OR and 95% CI obtained from logistic regression adjusting by smoking status, age, gender, education, urbanicity of longest residence, fruit and vegetable intake, overall quality of interview, and area. Analysis excluding subjects with overall quality of interview unsatisfactory and questionable.
‡ p value for linear trend

Table 6. Odds ratio (OR) and 95% confidence intervals (95% CI) for bladder cancer by gender and duration of time equivalent in the shower, bath and swimming in the pool (total inhalation and dermal absorption).

Inhalation and dermal absorbtion (lifetime hours)	**Men**		**Women**		**All**
	OR (95% CI)†	cases/controls	OR (95% CI)†	cases/controls	OR (95% CI)†
≤297.3 hours	1.00	132/188	1.00	28/36	1.00
>297.3, ≤573.3 h.	1.06 (0.76-1.48)	153/195	0.68 (0.30-1.52)	19/39	0.96 (0.71-1.30)
>573.3, ≤1000 h.	1.15 (0.82-1.61)	155/188	1.39 (0.59-3.28)	24/22	1.17 (0.86-1.59)
>1000 hours	1.29 (0.91-1.82)	171/165	1.73 (0.71-4.22)	25/24	1.30 (0.94-1.78)
p-trend‡	*0.127*		*0.129*		*0.056*

† OR and 95% CI obtained from logistic regression adjusting by smoking status, age, gender, education, urbanicity of longest residence, fruit and vegetable intake, overall quality of interview, and area. Analysis excluding subjects with overall quality of interview unsatisfactory and questionable.
‡ p value for linear trend

Article 6

Cloración del agua potable y efectos sobre la salud: revisión de estudios epidemiológicos

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La disponibilidad de agua potable es una de las prioridades en todas las sociedades humanas. Además de la cantidad suficiente para cubrir las necesidades básicas, el agua debe tener una calidad que garantice su inocuidad para la salud. La desinfección es una etapa esencial en el proceso de potabilización del agua para eliminar microorganismos patógenos y evitar infecciones de origen hídrico. El cloro es el desinfectante del agua potable más extendido en España. La introducción de la cloración a principios del siglo xx supuso un importante avance en salud pública gracias a la reducción de las enfermedades infecciosas trasmitidas por el agua. Brotes epidémicos recientes de legionelosis indican que el proceso de desinfección del agua en España no se cumple de manera adecuada. Además, es posible que en España, como en otros países, existan microepidemias que nunca sean identificadas[1]. A pesar del beneficio de la desinfección del agua mediante la cloración, el cloro se caracteriza por ser altamente reactivo y producir subproductos indeseados al reaccionar con la materia orgánica natural del agua. Los subproductos de la cloración tienen propiedades mutágenas y cancerígenas, por lo que han sido extensamente estudiados desde que se detectaron por primera vez, en 1974. La exposición humana a dichos compuestos se ha asociado en estudios epidemiológicos, principalmente a cáncer de diversos tipos y a efectos adversos en neonatos de madres expuestas.

El objetivo de este trabajo consiste en realizar una revisión sistemática de los estudios epidemiológicos que han evaluado los efectos adversos asociados a la exposición a subproductos de la cloración, y exponer el contexto en que se encuentra España con relación a esta exposición ambiental.

Los subproductos de la cloración

El origen del agua y el tipo de desinfectante utilizado determinan la concentración de subproductos de la cloración. Las aguas subterráneas, al tener una menor cantidad de precursores orgánicos y requerir una dosis inferior de cloro, darán lugar a concentraciones más reducidas de subproductos de la cloración que las aguas superficiales. Desinfectantes alternativos al cloro como, por ejemplo, el dióxido de cloro, las cloraminas o el ozono, con similar o mayor poder desinfectante[2], producen menor cantidad de subproductos clorados. La presencia de dichos compuestos en el agua embotellada es mínima o inexistente.

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Los subproductos de la cloración son una mezcla compleja de diferentes sustancias con diversas propiedades fisicoquímicas y cancerígenas, a las que la población puede estar expuesta a través del agua potable. A continuación se mencionan las características químicas, las evidencias de potencial carcinógeno en animales y la información disponible sobre el metabolismo para los diferentes subproductos de la cloración.

Características químicas

Subproductos mayoritarios

Trihalometanos. Los trihalometanos (THM) son los subproductos de la cloración que se forman en mayor concentración y han sido utilizados tradicionalmente como indicadores de la concentración total de subproductos de la cloración. El grupo de los THM está formado por el cloroformo, bromodiclorometano, dibromoclorometano y bromoformo. Los valores de THM en el agua clorada pueden variar de manera notable en función de las características del origen del agua: puede ir desde menos de 10 µg/l en aguas cloradas de origen subterráneo a más de 200 µg/l en aguas cloradas de origen superficial. Los cuatro THM, igual que otros subproductos de la cloración, se forman en diferente proporción según las características del origen del agua. La principal propiedad química de los THM es su elevada volatilidad, por lo que la inhalación y la absorción dérmica[3] son vías de exposición importantes en situaciones como la ducha[4,5], el baño[6] o las piscinas[7-11]. Para actividades cotidianas típicas de ducha e ingestión de agua, las tres vías de exposición serían equivalentes[6].

Ácidos acéticos halogenados. Los ácidos acéticos halogenados (HAA) forman un conjunto de 9 compuestos con diferente contenido de cloro y bromo: ácidos cloroacético, dicloroacético, tricloroacético, bromoacético, dibromoacético, tribromoacético, bromocloroacético, dibromocloroacético y bromodicloroacético. Son los segundos subproductos de la cloración más abundantes después de los THM. La concentración total de HAA en el agua clorada suele ser la mitad que la de THM[12]. Los HAA más abundantes son los que contienen dos cloros y/o bromos.

Subproductos minoritarios

MX (mutágeno X). Con este nombre se conoce la 3-cloro-4-diclorometil-5-hidroxi-2(5H)-furanona. Después de los THM es uno de los subproductos de la cloración más investigados. Se detectó por primera vez en 1984 en emanaciones de fábricas de pulpa de papel blanqueada con cloro en Finlandia. Dos años después se detectó en aguas potables. Generalmente se encuentra en concentraciones muy bajas en las aguas de consumo, del orden de pocos µg/l[13], pero tiene una elevada actividad mutágena en el test de Ames[14]. Se ha

estimado que representa un tercio del potencial mutágeno total en las aguas de Finlandia[15].

Acetonitrilos halogenados, hidrato de cloral, haloacetonas, cloropicrina, cloruro y bromuro de cianógeno. Son subproductos de la cloración formados en concentraciones de pocos µg/l[16].

Efectos en animales de experimentación y metabolismo

Subproductos mayoritarios

Trihalometanos. Se ha observado que los THM pueden inducir tumores en animales de laboratorio[17] y todos, excepto el cloroformo, son mutágenos[18,19]. Experimentos de laboratorio con roedores han puesto de manifiesto que el bromodiclorometano tiene una actividad espermatotóxica[20]. El mecanismo de acción es diferente para cada THM, pero la vía común pasa por la acción genotóxica de los metabolitos. Los trihalometanos son absorbidos de manera extensa en el tracto gastrointestinal y el cloroformo también se absorbe extensamente por los pulmones[21]. La excreción de los compuestos no metabolizados tiene lugar principalmente a través del aire exhalado, y una pequeña cantidad se excreta por la orina[21]. La vida media de los compuestos volátiles en el cuerpo es corta, desde media hora[22] hasta 5 u 8 h[23] según el compuesto. Por su elevada lipofilidad, la acumulación de los trihalometanos es mayor en tejidos de alto contenido lipídico como la grasa corporal, el hígado y los riñones[21].

Ácidos acéticos halogenados. Los ácidos dicloroacético, tricloroacético y los ácidos acéticos bromados son inductores de tumores en roedores de laboratorio[24], pero no se ha podido demostrar la capacidad del ácido cloroacético para inducir tumores en animales de experimentación[25]. Diversos estudios han evaluado la capacidad genotóxica de estos compuestos utilizando metodologías diferentes, pero no se ha podido concluir inequívocamente el potencial mutágeno de estos compuestos[26,27]. Se ha observado actividad espermatotóxica de los ácidos dicloroacético y dibromoacético[28]. El ácido tricloroacético ha demostrado ser teratógeno en experimentos con animales[29]. El metabolismo es diferente para cada HAA. El ácido dicloroacético se absorbe con rapidez en el intestino y es metabolizado de manera inmediata[30]. En cambio, el ácido tricloroacético se metaboliza en una pequeña proporción, y la mayoría del compuesto no reaccionado se excreta por la orina[31,32]. Un 50% de la dosis de ácido cloroacético se excreta por la orina[33]. La concentración de ácido tricloroacético en la orina se correlaciona con las concentraciones en el agua, a diferencia de otros ácidos acéticos halogenados[34,35].

Subproductos minoritarios

MX. El MX induce tumores en animales de laboratorio expuestos a dosis bajas que no producen toxicidad general[36], pero los datos de potencial carcinógeno no son concluyentes[37]. Resultados de estudios *in vitro* sugieren que el MX puede ser un teratógeno de acción directa[38]. El MX es extensamente destoxificado en experimentos *in vivo* y es improbable que provoque daño genético en los tejidos excepto a dosis relativamente elevadas en que las vías de destoxificación se saturan[39]. Estudios de farmacocinética de MX marcado radiactivamente demuestran que éste se absorbe en el tracto intestinal en un grado considerable y se excreta por la orina con rapidez. La vida media de eliminación de la radiactividad en sangre es de 3,8 horas y la vía principal de eliminación es la orina[40].

Acetonitrilos halogenados, hidrato de cloral, haloacetonas, cloropicrina, cloruro y bromuro de cianógeno. La mayor parte de estos compuestos tiene propiedades mutágenas y potencial cancerígeno en experimentos con animales[26,41-44]. El hidrato de cloral ha demostrado poseer una actividad espermatotóxica en roedores de laboratorio[20]. Se absorbe con rapidez y se metaboliza a ácido tricloroacético o tricloroetanol. Los metabolitos se excretan principalmente por la orina[26]. El dicloroacetonitrilo se absorbe en el tracto intestinal y la mayor parte se excreta por la orina[26].

Metodología

Se ha utilizado la base de datos bibliográfica MEDLINE, las monografías de la IARC/OMS y los archivos personales de los autores; así mismo, se ha examinado la bibliografía de los estudios identificados. En la literatura científica se ha encontrado que los dos principales grupos de efectos evaluados con relación a esta exposición ambiental son cáncer de diversos órganos y defectos en neonatos de madres expuestas. El cáncer de vejiga es el efecto del que existe una bibliografía más extensa. Para la revisión de artículos sobre cáncer de vejiga se seleccionaron solamente aquellos publicados de estudios con información individual sobre consumo de agua que consideraban la exposición a subproductos de la cloración o agua clorada. Se identificaron 8 estudios de casos y controles[45-52] y dos estudios de cohortes[53,54] sobre cáncer de vejiga y cloración de agua, con información individual extraída de entrevistas personales. Se identificaron 3 estudios con información individual extraída de censos y registros poblacionales[55-57]. Debido a la disponibilidad de suficientes estudios con información individual sobre consumo de agua, se excluyeron los artículos con información de tipo ecológico. Dichos estudios corroboran de manera clara los hallazgos de los estudios con información individual[58-62].

Para la revisión de estudios sobre cáncer de colon y recto se seleccionaron trabajos con información individual de la población de estudio. Se identificaron 8 estudios: cinco se basan en información individual extraída de entrevistas personales[63-67]; dos estudios de diseño de casos y controles sobre mortalidad por cáncer basados en información individual extraída de registros de mortalidad y compañías de agua[57,56], y un estudio de cohortes que asociaba actividad mutágena del agua con incidencia de diversos tipos de cáncer[53]. Se excluyeron los estudios de diseño ecológico, dado que existían suficientes resultados provenientes de estudios con información individual. Los estudios ecológicos[60,68] corroboran los resultados de los estudios de casos controles y de cohortes.

Para la revisión de los artículos sobre cáncer de otros órganos y de los defectos reproductivos en neonatos de madres expuestas se seleccionaron todos los estudios que evaluaban la exposición a agua clorada o a subproductos de la cloración con relación a estos efectos, incluyendo estudios ecológicos. La inclusión de dichos estudios se hizo porque existen menos estudios epidemiológicos sobre estos efectos en comparación con los que investigan el cáncer de vejiga, colon y recto. De forma similar que para los estudios de cáncer de vejiga, colon y recto, los estudios ecológicos corroboran los hallazgos (o la falta de hallazgos) de estudios con información individual.

Se identificaron 7 estudios de diseños heterogéneos que evaluaban el riesgo de diversos tipos de cáncer por exposición a subproductos de la cloración[54,56,57,62,68-70]. También se identificaron artículos que estudiaban un tipo de cáncer específico: uno sobre cáncer de esófago[71], dos sobre cáncer de páncreas[72,73] y uno sobre cáncer de cerebro[74].

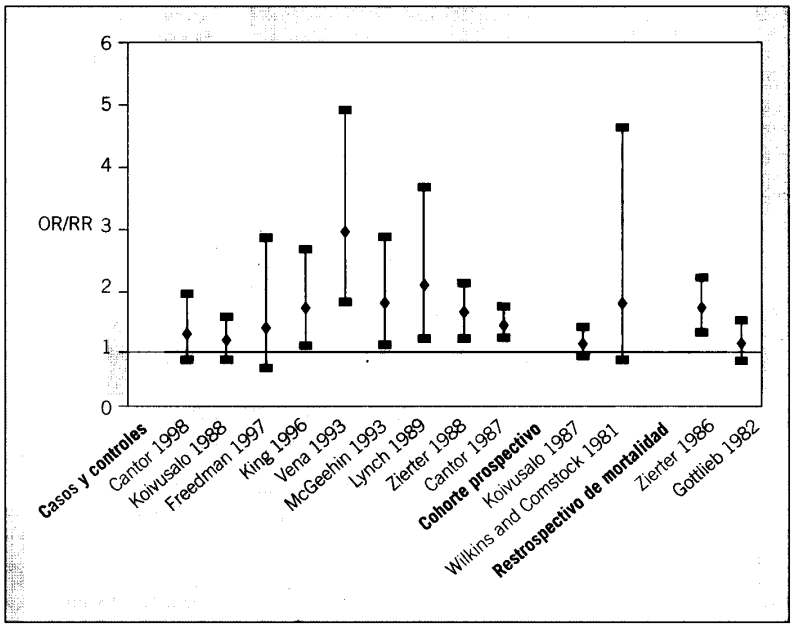

Fig. 1. Riesgos relativos (RR) y odds ratio *(OR) de los estudios que evalúan el riesgo de cáncer de vejiga por exposición a subproducto de la cloración, según el diseño de los estudios.*

Para la revisión de los artículos sobre defectos del nacimiento se seleccionaron estudios que evaluaran estos efectos asociados a la exposición a subproductos de la cloración. Se identificaron 14 estudios con metodologías y diseños de estudio heterogéneos[75-88].

Estudios epidemiológicos

Cáncer de vejiga

Uno de los tumores asociados más uniformemente a esta exposición es el cáncer de vejiga urinaria[45-55]. Es uno de los cánceres más frecuentes en España entre los varones[89]. No se han realizado estudios epidemiológicos en nuestro país con información individual para evaluar el riesgo de cáncer de vejiga asociado a la exposición a subproductos de la cloración. Se han identificado 10 artículos publicados que evalúan de manera individual la exposición a agua clorada y el riesgo de cáncer de vejiga a través de entrevistas personales (tabla 1). En todos estos estudios se ha encontrado una asociación positiva, aunque no en todos los estudios los resultados son estadísticamente significativos (fig. 1). Los estudios utilizan metodologías diferentes en la evaluación de la exposición. La mayoría de los trabajos epidemiológicos evalúan la exposición de la población de estudio utilizando

TABLA 1

Estudios epidemiológicos que han evaluado el riesgo de cáncer de vejiga por exposición a subproductos de la cloración, con información individual extraída de entrevistas personales

Referencia y lugar	N. casos/controles	OR o RR (IC del 95%)	Comentarios
Estudios de casos-controles			
Cantor et al, 1998[45] Iowa (EE.UU.)	732/914	OR = 1,3 (0,9-2,0)	Exposición acumulada durante toda la vida a THM ≥ 2,42 g frente a < 0,04 g (varones)
Koivusalo et al, 1998[46] Finlandia	1.123/1.983	OR = 1,2 (0,9-1,6)	Incremento de 3.000 revertientes/l[a] en la exposición media (ambos sexos)
King et al, 1996[47] Ontario (Canadá)	696/1.545	OR = 1,7 (1,1-2,7)	Exposición > 35 años a ≥ 75 µg/l THM (ambos sexos)
Vena et al, 1993[48] New York Oest (EE.UU.)	351/855	OR = 3,0 (1,8-5,0)	Consumo de agua del grifo > 65 años, cuartil superior de consumo de agua al día frente a cuartil de menor consumo (varones)
McGeehin et al, 1993[49] Colorado (EE.UU.)	327/261	OR = 1,8 (1,1-2,9)	Consumo de agua superficial clorada > 30 años frente a 0 años (ambos sexos)
Lynch et al, 1989[50] Iowa (EE.UU.)	286/658	OR = 2,1 (1,2-3,7)	Expuestos > 50 años a agua clorada frente a expuestos 0 años (ambos sexos)
Zierler et al, 1988[51] Massachusetts (EE.UU.)	614/1.074	MOR[b] = 1,6 (1,2-2,1)	Exposición durante toda la vida clorada frente a cloraminada (ambos sexos)
Cantor et al, 1987[52] EE.UU.	2.805/5.258	OR = 1,4 (1,2-1,7)	Ingestión diaria de ≥ 1,96 litros de agua de grifo frente a 0,8 litros (ambos sexos)
Estudios de cohortes			
Koivusalo et al, 1997[53] Finlandia	(621.431 individuos) 836 casos de cáncer de vejiga	RR = 1,1 (0,9-1,4)	Expuestos frente a no expuestos a agua superficial clorada (ambos sexos)
Wilkins y Comstock, 1981[54] Washington County, Maryland (EE.UU.)	(31.000 individuos) 81 casos de cáncer de vejiga	RR = 1,8 (0,8-4,7)	Expuestos a agua superficial clorada frente a expuestos a agua subterránea (varones)

[a]Actividad mutágena. [b]OR de mortalidad. THM: trihalometanos. OR: *odds ratio*. RR: riesgo relativo.

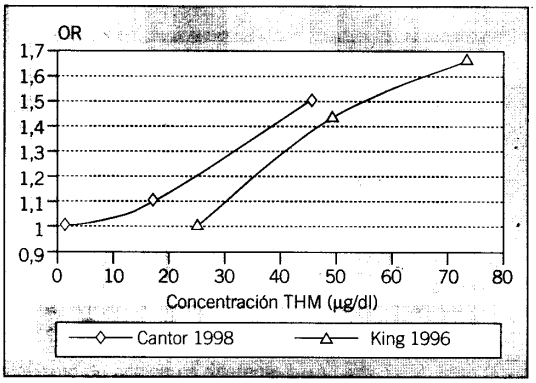

Fig. 2. Variación de la odds ratio *(OR) en función de la concentración media de exposición a trihalometanos (THM) durante la vida.*

variables indirectas que se asocian con la concentración de subproductos de la cloración como, por ejemplo, el origen del agua (superficial frente a subterránea)[53,54], el tipo de desinfectante (cloro frente a cloramina)[51], tiempo de residencia o de consumo de agua clorada del grifo[48-50,55] o cantidad de agua clorada del grifo consumida[52]. Koivusalo et al (1997) y Wilkins y Comstok (1981) evaluaron el riesgo de cáncer de vejiga por consumo a lo largo de la vida de agua superficial clorada respecto a agua subterránea, encontrando una *odds ratio* de 1,1 y 1,8 respectivamente. El grado de actividad mutágena del agua clorada también ha sido utilizado como variable de exposición por Koivusalo et al (1998), que hallaron una *odds ratio* de 1,2 al incrementar la actividad mutágena del agua 3.000 revertientes/l. Solamente los estudios de Cantor et al (1998) y King et al (1996) realizan una evaluación cuantitativa de la exposición a subproductos de la cloración (THM) basada en información extensa sobre las concentraciones de THM. Estos autores encontraron una *odds ratio* de 1,3 para una exposición acumulada a THM durante toda la vida ≥ 2,42 g[45] y de 1,7 para una exposición a THM ≥ 75 µg/l durante > 35 años[47]. En la figura 2 se observa el incremento del riesgo de cáncer de vejiga al aumentar la concentración de exposición a THM, indicando una clara relación dosis-respuesta. Para una concentración media de exposición a THM durante toda la vida de 40 µg/l, la *odds ratio* está entre 1,3 y 1,4.

Los resultados de estudios basados en información extraída de censos y registros poblacionales[55-57], con una evaluación de la exposición más cruda, son acordes con los estudios individuales.

Cáncer de colon, recto y otros cánceres

Los cánceres de colon y recto son los segundos más asociados a la exposición a subproductos de la cloración. Se identificaron 10 estudios sobre riesgo cáncer de colon y recto por exposición a subproductos de la cloración[60]. En la tabla 2 se exponen de forma resumida los estudios epidemiológicos que evalúan individualmente el riesgo de cáncer de colon y recto por la exposición a subproductos de la cloración. La metodología de los diferentes estudios es diversa. Al igual que en el caso de los estudios de cáncer de vejiga, la evaluación de la exposición se realiza mediante la estimación cuantitativa de la exposición a subproductos de la cloración[63-66], de la duración de exposición a agua superficial clorada[57,67], considerando el tipo de desinfectante utilizado[56], y a través de la evaluación de la actividad mutágena del agua[53]. En la mayoría de los estudios se obtienen unos resultados estadísticamente no significativos. La *odds ratio* de cáncer de recto asociado a la exposición a subproductos de la cloración va desde 0,96 del estudio de Zierler et al (1986)[56] hasta 3,18 del estudio de Gottlieb et al (1982)[57]. Las estimaciones de riesgo de cáncer de colon varían entre los estudios, oscilando desde una *odds ratio* de 0,10 (Cragle et al, 1985)[67] hasta 1,67 (Doyle et al, 1997)[64].

Se ha investigado la asociación entre exposición a subproductos de la cloración y otros tipos de cáncer, pero, en general, las evidencias son poco claras.

La asociación entre la exposición a agua clorada y el cáncer de páncreas ha sido evaluada en diversos estudios, con resultados no uniformes. En un estudio ecológico realizado en 1980 se halló una correlación positiva y estadísticamente significativa entre cáncer de páncreas y concentraciones de THM[90]. Kukkula et al (1997)[72] e Ijsselmuiden et al (1992)[73] publicaron 2 estudios en los que se evaluaba la asociación entre la exposición a agua clorada y el cáncer de páncreas. En el primero de ellos se halló una asociación negativa y estadísticamente significativa (OR = 0,2%, IC del 95% 0,04-0,94), mientras que el segundo aporta una asociación positiva y con significación estadística (OR = 2,18; IC del 95% 1,20-3,95). Koivusalo et al (1995) evaluaron, en un estudio de cohortes retrospectivo, la relación entre la actividad mutágena del agua (atribuida a los subproductos de la cloración)

TABLA 2

Estudios epidemiológicos que han evaluado el riesgo de cáncer de colon y recto asociado con la exposición a subproductos de la cloración, con información individual

Referencia y lugar	Evaluacion de la exposición	Tipo de cáncer	OR (IC del 95%)
Hildesheim et al, 1997[63] Iowa (EE.UU.)	Estimación de la exposición media a THM durante la vida	Colon Recto	1,06 (0,7-1,6) 1,66 (1,1-2,6)
Doyle et al, 1997[64] Iowa (EE.UU.)	Exposición a subproductos de la cloración a partir de información de registros históricos	Colon Recto y ano	1,67 (1,07-2,63) 0,88 (0,38-2,06)
Koivusalo et al, 1997[53] Finlandia	Información histórica de actividad mutágena a partir de una ecuación empírica	Recto Colon	1,04 (0,86-1,26) 0,90 (0,77-1,04)
Marrett et al, 1996[65] Canadá	Estimación de la exposición media a THM de 1950 a 1990	Colon y recto	Información no disponible (OR no significativa)
Young et al, 1987[66] Wisconsin (EE.UU.)	Consumo de agua (entrevista) y valor de cloroformo (registros históricos y análisis)	Colorrectal	0,90 (0,60-1,35)
Zierler et al, 1986[56] Massachusetts (EE.UU.)	Residencia en el momento de la muerte en un municipio abastecido con agua tratada con cloro (exposición) frente a agua tratada con cloramina	Recto Colon	0,96 (0,89-1,04) 0,89 (0,86-0,93)
Cragle et al, 1985[67] North Carolina (EE.UU.)	Años de exposición a agua clorada (historial de domicilios e información de compañías de agua)	Colon	0,10 (0,01-0,79)
Gottlieb et al, 1982[57] Louisiana (EE.UU.)	Información del certificado de defunción (ocupación, lugar de nacimiento y dirección). Duración de exposición a agua superficial o subterránea (por la compañía de agua)	Recto Colon	3,18 (1,96-5,19) 0,90 (0,60-1,37)

THM: triahalometanos. OR: *odds ratio*. IC: intervalo de confianza.

y el cáncer de páncreas, encontrando un riesgo relativo entre 1,1 y 1,2 para los que consumían agua mutágena en comparación con los que consumían agua no mutágena[69].
La mayoría de los estudios sobre cáncer de esófago encuentran una asociación positiva, pero no estadísticamente significativa[54,57,70]. En un estudio de casos y controles de 1999[71] se aprecia una asociación positiva y con significación estadística (OR = 2,77, IC del 95%, 1,52-5,03).
El cáncer de mama también ha sido estudiado en relación con la exposición a agua clorada, pero los resultados de los estudios no son concordantes entre sí[54,56-68,70,91]. En tres de estos estudios se obtienen resultados estadísticamente significativos, dos de ellos con asociación positiva[54,57] y uno con asociación inversa[56] entre exposición y efecto. En los otros 2 estudios se encuentra una asociación ligeramente positiva y sin significación estadística[70,91]. En el estudio de Bean et al (1982), de diseño ecológico, se halló una incidencia de cáncer de mama ligeramente superior en comunidades suministradas con agua superficial clorada respecto a las que consumían agua subterránea[68].
En un estudio sobre el riesgo de cáncer de cerebro se encuentra una asociación positiva y estadísticamente significativa[70], mientras que otros dos obtienen resultados sin significación estadística[57,74].

TABLA 3

Resumen de los estudios epidemiológicos sobre subproductos de la cloración y efectos reproductivos adversos en neonatos de madres expuestas más estudiados

Referencia y lugar	Tipo de estudio	Evaluación de la exposición	OR/RR (IC del 95%)
Aborto espontáneo			
Waller et al, 1998[75], California (EE.UU.)	Cohorte	Concentraciones de THM (de empresa de agua) + entrevista personal	1,18 (1,1-3,0)
Swan et al, 1998[75], California (EE.UU.)	Cohorte	Entrevista personal	2,17 (1,22-3,87)
Savitz et al, 1995[81], Central North Carolina (EE.UU.)	Casos y controles poblacional	Concentraciones de THM (de empresas de agua) + entrevista personal	1,2 (0,6-2,4)
Windham et al, 1992[82], Santa Clara, California (EE.UU.)	Casos y controles	Entrevista personal + información sobre origen del agua (superficial/subterráneo)	1,2 (1,0-1,05)
Deane et al, 1992[77], Carolina (EE.UU.)	Cohorte	Entrevista personal	3,4 (0,6-19,4)
Wrensch et al, 1992[78], California (EE.UU.)	Cohorte	Entrevista personal	4,0 (1,8-9,1)
Bajo peso al nacer			
Gallagher et al, 1998[79], Colorado (EE.UU.)	Cohorte	Concentraciones de THM (de empresa de agua) + registros de nacimiento	2,1 (1,0-4,8)
Kanitz et al, 1996[87], Génova (Italia)	Transversal	Registros de nacimiento + información sobre el tipo desinfectante del agua potable	6,0 (0,6-12,6)
Bove et al, 1995[86], New Jersey, (EE.UU.)	Transversal	Registros de nacimiento + concentraciones de THM (de empresas de agua)	1,4 (IC del 50%, 1,2-1,7)
Savtiz et al, 1995[81], Central North Carolina (EE.UU.)	Casos y controles poblacional	Concentraciones de THM (de empresas de agua) + entrevista personal	1,3 (0,8-2,1)
Kramer et al, 1992[83], Iowa (EE.UU.)	Casos y controles poblacional	Certificados de nacimiento + concentraciones de THM (analizados *ad hoc*)	1,3 (0.8-2,2)
Talla pequeña por edad gestacional			
Crecimiento intrauterino retardado			
Bove et al, 1995[86], New Jersey (EE.UU.)	Transversal	Registros de nacimiento + concentraciones de THM (de empresas de agua)	1,5 (IC del 90%, 1,2-1,9)
Kramert et al, 1992[83], Iowa (EE.UU.)	Casos y controles poblacional	Certificados de nacimiento + concentraciones de THM (analizados *ad hoc*)	1,3 (0,8-2,2)
Talla pequeña al nacer			
Dodds et al, 1999[80], Nueva Escocia (Canadá)	Cohorte retrospectivo	Concentraciones de THM (de empresas de agua) + registros	1,08 (0,99-1,18)
Kanitz et al, 1996[87], Génova (Italia)	Transversal	Registros nacimiento + información sobre el tipo de desinfectante del agua potable	2,3 (1,3-4,2)
Defectos del tubo neural			
Dodds et al, 1999[80], Nueva Escocia (Canad')	Cohorte retrospectivo	Concentraciones de THM (de empresas de agua) + registros	1,18 (0,67-2,10)
Klotz et al, 1999[84], New Jersey (EE.UU.)	Casos controles y poblacional	Concentraciones de THM (de empresas de agua) + entrevista personal + certificado nacimiento	2,1 (1,1-4,0)
Magnus et al, 1999[88], Noruega	Transversal	Ecológico + registro nacimientos	1,26 (0,61-2,62)
Bove et al, 1995[8], New Jersey (EE.UU.)	Transversal	Registros de nacimiento + concentraciones de THM (de empresas de agua)	3,0 (IC del 90%, 1,3-6,6)
Muerte fetal tardía			
Dodds et al, 1999[80], Nueva Escocia (Canadá)	Cohorte retrospectivo	Concentraciones de THM (de empresas de agua) + registros	1,66 (1,09-2,52)
Aschengrau et al, 1993[85], Massachusetts (EE.UU.)	Casos y controles	Entrevistas personales + registros de empresas de agua	2,6 (0,9-2,9)
Defectos respiratorios			
Magnus et al, 1999[88], Noruega	Transversal	Registro nacimientos + registros de empresas de agua	1,07 (0,52-2,19)
Aschengrau et al, 1993[85], Massachusetts (EE.UU.)	Casos y controles	Entrevistas personales + registros de empresas de agua	3,2 (1,1-9,5)
Defectos cardíacos mayores			
Magnus et al, 1999[88], Noruega	Transversal	Registro nacimientos + registros de empresas de agua	1,05 (0,76-1,46)
Bove et al, 1995[96], New Jersey, (EE.UU.)	Transversal	Registros de nacimiento + concentraciones de THM (de empresas de agua)	1,8 (1,0-3,3)
Defectos del tracto urinario			
Magnus et al, 1999[88], Noruega	Transversal	Registros nacimientos + registros de empresas de agua	1,99 (1,10-3,57)
Aschengrau et al, 1993[83], Massachusetts (EE.UU.)	Caso y controles	Entrevistas personales + registros de empresas de agua	4,1 (1,2-14,1)

THM: trihalometanos. OR: *odds ratio*. RR: riesgo relativo. IC: intervalo de confianza.

Se ha evaluado la asociación entre cáncer de riñón, hígado y pulmón con la exposición a agua clorada[57,62,68-70,54], pero los resultados no son estadísticamente significativos y no ponen de manifiesto una asociación clara entre exposición y efecto.
La exposición a agua con actividad mutágena atribuida a los subproductos de la cloración ha sido asociada a un incremento del riesgo de linfomas por un estudio de cohorte retrospectivo[69], con un riesgo relativo entre 1,1 y 1,3.

Efectos reproductivos adversos

Los defectos del nacimiento en neonatos de madres expuestas también se han estudiado para evaluar su posible relación con la exposición a subproductos de la cloración. La investigación epidemiológica de estos efectos es más reciente y se inició en la década de los noventa. De los 14 estudios identificados sobre cloración del agua y defectos en neonatos, seis son de diseño tipo cohorte[75-80], cinco de diseño de casos y controles[81-85] y tres transversales[86-88].
Los principales efectos investigados son aborto espontáneo, peso bajo al nacer, crecimiento intrauterino retardado, talla pequeña al nacer y defectos del tubo neural (tabla 3). También se ha estudiado, aunque en menor medida, la asociación entre la exposición de madres embarazadas con muerte fetal tardía[80,85] y determinadas malformaciones congénitas, como defectos respiratorios[85,88], defectos cardíacos mayores[86,88], defectos del tracto urinario[85,88], malformaciones congénitas mayores[88], defectos del sistema nervioso central[86], labio leporino[86], ictericia al nacer[87], anomalías cromosómicas[80] y muerte neonatal[85].
En la mayoría de los estudios encuentra una asociación positiva entre estos efectos adversos y la exposición de la madre embarazada al agua clorada o a los subproductos de la cloración. El defecto de nacimiento más evaluado ha sido el aborto espontáneo. Los 6 estudios que evalúan este efecto han encontrado una asociación positiva[75-78,81,82]. La *odds ratio* varía entre 1,18[75] y 4,0[78]. Existen 5 estudios que han investigado el peso bajo al nacer asociado a esta exposición, encontrando un incremento del riesgo[79,81,83,86,87]. La *odds ratio* varía entre 1,3[81] y 6,0[87]. La talla pequeña por edad gestacional se ha evaluado en 4 estudios[80,83,86,87], encontrando *odds ratio* de 1,08[80]-2,3[87]. El riesgo de defectos del tubo neural por la exposición a subproductos de la cloración ha sido evaluado por 4 estudios[80,84,86,88], encontrando *odds ratios* desde 1,18[80] hasta 3,0[86].

Discusión

El cáncer de vejiga y determinados defectos del nacimiento en neonatos de madres expuestas son los efectos que más se asocian a la exposición a los subproductos de la cloración. En todos los estudios sobre cáncer de vejiga se encuentra un incremento del riesgo por la exposición a estos compuestos, aunque no siempre los resultados son estadísticamente significativos. Los estudios sobre defectos del nacimiento también son positivos en su mayoría, siendo el aborto espontáneo, el bajo peso al nacer, la talla pequeña por edad gestacional y los defectos del tubo neural los efectos más asociados a dicha exposición. Las evidencias de asociación entre la exposición a subproductos de la cloración y el cáncer de colon y recto, mama, páncreas y otros cánceres no son uniformes.

Consideraciones metodológicas

Muchos de los estudios epidemiológicos revisados tienen limitaciones metodológicas que pueden cuestionar la validez de los resultados. La principal limitación se refiere a la mala evaluación de la exposición, ya que muchos estudios consideran variables cualitativas asociadas indirectamente a la exposición estudiada (origen del agua, desinfectante utilizado, etc.) en lugar de realizar una evaluación cuantitativa de la exposición con datos sobre concentraciones ambientales o individuales de exposición. Otra limitación que conduce a la mala clasificación de la exposición es no considerar valores retrospectivos, asumiendo que la exposición en el momento del estudio es la misma que en el pasado. Esto es especialmente problemático en los estudios sobre efectos que requieren largos períodos de latencia, como el cáncer. Algunos estudios tampoco tienen en cuenta la cantidad de agua ingerida diariamente, ni el origen del agua consumida fuera de casa. Ningún estudio realizado hasta el momento ha considerado otras vías de exposición diferentes de la ingestión de agua.
En conjunto, estas limitaciones implican una mala clasificación de la exposición *a priori* no diferencial (ocurre con la misma proporción entre los casos y los controles) que afectaría a los resultados, atenuando la magnitud de la asociación. Este fenómeno se observa claramente en el estudio de Lynch et al (1989)[50], que encontraba que, para una misma población, la magnitud del riesgo aumentaba a medida que la evaluación de la exposición se ajustaba más a la realidad.

Evaluación del potencial carcinógeno de los subproductos de la cloración por la IARC/OMS

A pesar de que en diversos estudios se ha encontrado una asociación positiva entre consumo de agua clorada y efectos adversos, dicha asociación no ha sido aún aceptada como causal por la Agencia Internacional de Investigación sobre el Cáncer (IARC/OMS). En la evaluación realizada en 1991[92] se concluyó que no había evidencias definitivas para clasificar el agua clorada como cancerígena en humanos, incluyéndola en la categoría 3 de la IARC. Esta evaluación se basaba principalmente en estudios ecológicos y en estudios con información de tipo individual con limitaciones metodológicas que impedían establecer la relación causal. Los estudios sobre cáncer de vejiga posteriores a 1991 han superado en gran parte estas limitaciones metodológicas y todos encuentran una asociación positiva.
El agua clorada contiene una mezcla compleja de subproductos clorados con diferentes propiedades mutágenas y carcinógenas. Existe una fuerte correlación entre las concentraciones de los diferentes compuestos y es difícil aislar los efectos de cada compuesto por separado. Esta cuestión metodológica se plantea en la última revisión de la OMS del cloroformo como cancerígeno humano[93], donde se concluye que, aunque varios estudios han asociado el consumo de agua clorada con cáncer, no se pueden evaluar compuestos individuales.

La calidad de las aguas en España

Cada evaluación de la calidad de las aguas potables debe tener en consideración varios parámetros, entre ellos el nivel de desarrollo de cada sociedad. Algunos de los indicadores utilizados de manera tradicional sobre calidad de las aguas[94] se refieren principalmente a sociedades menos industrializadas que la española, donde no se han solucionado problemas básicos de higiene hídrica. La definición de los niveles aceptables de contaminación por agentes químicos como el plomo, los trihalometanos o los nitratos no sólo depende de un análisis estrictamente cuantitativo sobre riesgos asociados a ciertas concentraciones, sino también de lo que se considera tolerable o adecuado al nivel de calidad de vida esperado por cada sociedad. El suministro de agua potable en España ha solucionado el problema mayor

TABLA 4

Concentraciones medias de THM (μg/l) en el agua potable de diversos países de la Unión Europea[a]

Portugal	< 1-230
España	< 1-210
Bélgica	22-157
Francia	6-135
Irlanda	< 100
Finlandia	< 1-84
Reino Unido	2-73
Italia	< 1-60
Holanda	34
Austria	10-20
Alemania	1-20
Suecia	12
Luxemburgo	3,5-11,5

THM: trihalometanos. [a]Adaptada de: Exposure of the European Population to trihabmethanes (THM) in drinking water. Vol. 2. European Commission 1997.

de epidemias de transmisión hídrica, aunque ocasionalmente aparezcan epidemias de legionelosis y posiblemente otras miniepidemias de síntomas gastrointestinales asociados al agua potable que nunca se identifican[1]. En muchas zonas de España, los problemas derivados de la contaminación de bajo nivel con agentes químicos se encuentran pendientes de solución. También debemos indicar que la contaminación con nitratos, aunque no es un problema general de las aguas en España, puede ser un problema muy serio en áreas rurales. Dicha contaminación ha llegado a concentraciones extremadamente altas debido a la contaminación crónica de las aguas subterráneas, por ejemplo, en las áreas de Cataluña donde se concentra la ganadería porcina.

La sola inspección de la evolución al alza del mercado de aguas envasadas (encuesta de Consumo 1987-1997, Ministerio de Sanidad y Consumo) indica que una parte grande y creciente de la población no encuentra adecuada la calidad del agua disponible por la red de distribución. En varias áreas de España la concentración de agentes organoclorados (trihalometanos y otros) es alta[12]. Aunque sólo en algunas áreas se superan los límites propuestos por la Unión Europea, sí superan los límites aceptados por los consumidores para un agua potable. Se debe tener en cuenta que la exposición a dichos agentes no ocurre sólo por la ingestión de agua sino también por la inhalación y la absorción dérmica. Por tanto, la solución por parte de la población con capacidad adquisitiva para comprar agua envasada soluciona sólo parte del problema.

España es el segundo país de la Unión Europea después de Portugal con las concentraciones más altas de trihalometanos, según un informe de la Comisión Europea publicado en 1997[95] (tabla 4). Se ha calculado que alrededor del 20% de la mortalidad por cáncer de vejiga en áreas españolas de exposición intermedia-alta a THM se puede atribuir a la exposición a subproductos de la cloración[96]. Se ha encontrado un porcentaje similar para la población de Nueva Zelanda con valores de THM parecidos a los de España[97].

Alternativas al tratamiento del agua potable con cloro

El proceso de potabilización actualmente más extendido en España se caracteriza por el uso de cloro como principal desinfectante. Esto conlleva la generación de subproductos organoclorados, en especial en aguas superficiales de mala calidad que contienen concentraciones elevadas de carga orgánica.

Existen alternativas al tratamiento convencional con cloro que, manteniendo la desinfección de las aguas, reducen la formación de subproductos clorados. El dióxido de cloro, el ozono, las cloraminas, la radiación ultravioleta y el peróxido de hidrógeno son desinfectantes alternativos al cloro que generan menos subproductos clorados. No obstante, al ser compuestos altamente reactivos también generan subproductos orgánicos e inorgánicos[98]. Por tanto, una buena alternativa al tratamiento convencional con cloro debería combinar la eliminación previa de precursores orgánicos de los subproductos de la desinfección con un desinfectante menos agresivo que el cloro. Algunos de estos tratamientos se realizan actualmente en países del norte de Europa como, por ejemplo, Alemania, con una considerable reducción de las concentraciones de trihalometanos. Algunos de los desinfectantes alternativos pueden ser incluso más efectivos que el cloro. Un estudio realizado en hospitales de los EE.UU. pone de manifiesto que el uso de cloramina como desinfectante de las aguas potables reduce significativamente la incidencia de brotes de *legionella*[2] de origen nosocomial. Con frecuencia se considera que los procesos utilizados hoy día para la desinfección del agua potable son necesarios para evitar el riesgo de infecciones hídricas. Como consecuencia se considera que se debe aceptar el menor de los riesgos frente un riesgo mayor: el de las infecciones. En este sentido, hay que indicar que la prevención química y bacteriológica de las aguas no son antagónicas, especialmente para un país del nivel socioeconómico de España. El riesgo de la cloración del agua sobre la salud humana es evitable, ya que existen métodos de desinfección y líneas de potabilización alternativos al cloro y a los procedimientos actuales, con igual capacidad desinfectante y menor formación de compuestos clorados y bromados.

Conclusiones

La cloración del agua, junto con el proceso de desinfección y eliminación de compuestos indeseables, genera una mezcla compleja de subproductos clorados a niveles de concentración traza con propiedades mutágenas, cancerígenas, espermatotóxicas y teratógenas. La exposición a estos contaminantes a través del agua potable clorada durante largos períodos de la vida puede originar efectos adversos sobre la salud. Las evidencias más importantes de estos posibles daños se han encontrado en relación con el cáncer de vejiga. También se han descrito recientemente hallazgos que asocian la cloración del agua, en concreto las concentraciones de trihalometanos, con defectos del nacimiento en neonatos de madres expuestas. Es incuestionable que la desinfección de las aguas potables constituye una etapa esencial y necesaria para la potabilización de las aguas. Existen alternativas al tratamiento tradicional con cloro que supondrían una menor generación de subproductos clorados. Es necesario llevar a cabo una gestión integral y racional del ciclo del agua que permita que la calidad de las aguas en el punto de capta-ción para su potabilización sea la óptima. En países desarrollados como España, no se debería considerar que la desinfección y la minimización de los subproductos de la cloración sean objetivos antagónicos.

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Article 7

Cloración del agua potable en España y cáncer de vejiga

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(Chlorination of drinking water in Spain and bladder cancer)

Resumen

Objetivos: La cloración del agua potable genera trihalometanos y otros subproductos con propiedades mutágenas y cancerígenas en experimentos con animales. Los trihalometanos se han asociado en estudios epidemiológicos con un incremento del riesgo de cáncer de vejiga urinaria. Evaluamos los niveles de trihalometanos en 4 áreas de España y calculamos el riesgo de cáncer de vejiga atribuible a dicha exposición.

Métodos: Se han analizado los niveles de trihalometanos en 111 muestras de agua potable en 4 áreas de España utilizando cromatografía de gases. Se ha contactado con las potabilizadoras de agua y se ha analizado información sobre hábitos de consumo de agua en España. Se ha hecho una revisión de los estudios epidemiológicos que evalúan el riesgo de cáncer de vejiga asociado a la exposición a subproductos de la cloración. Se ha calculado el riesgo atribuible de cáncer de vejiga a partir de estos niveles, los datos de mortalidad por área y las estimaciones del riesgo extraídas de la bibliografía.

Resultados: Los niveles de trihalometanos más altos se encuentran en la franja mediterránea, con niveles medios de 81, 80, 61 y 52 µg/l en Sabadell, Alicante, Barcelona y Manresa, respectivamente. Los valores más bajos se encuentran en Tenerife y Asturias, con 7 y 20 µg/l, respectivamente. En las áreas con niveles altos de trihalometanos el riesgo de cáncer de vejiga atribuible a los subproductos de cloración puede ser, en promedio, de un 20%.

Conclusiones: Los niveles de trihalometanos identificados son altos en comparación con otros países de la Unión Europea. En las áreas de exposición alta la cloración del agua puede dar lugar a un número considerable de casos de cáncer de vejiga. Estas estimaciones se tienen que interpretar con cautela y verificarse con estudios más extensos.

Palabras clave: Cáncer de vejiga. Epidemiología. Agua clorada. Trihalometanos.

Summary

Objectives: Drinking water chlorination generates trihalomethanes and other by-products with mutagenic and carcinogenic properties in animal experiments. Epidemiological studies have associated trihalomethanes to an increased risk of bladder cancer. We evaluate trihalomethane levels in four Spanish areas and calculate the bladder cancer risk attributable to this exposure.

Methods: Trihalomethanes have been analysed in 111 drinking water samples from four Spanish areas using gas chromatography. Water utilities were contacted and information on drinking water consumption in Spain has been collected. We reviewed the epidemiological studies that assess the association between bladder cancer risk and exposure to chlorination by-products. Attributable risk was calculated on the basis of these levels, mortality data per area and risk estimates obtained from the literature.

Results: Mediterranean areas present the highest levels of trihalomethanes with 81, 80, 61 and 52 µg/l in Sabadell, Alicante, Barcelona and Manresa respectively. Lower levels are found in Tenerife and Asturias with 7 and 20 µg/l respectively. The bladder cancer attributable risk in high trihalomethane exposure areas may be, on average, around 20%.

Conclusions: The trihalomethane levels found are high compared to those of other European Union countries. In the high exposure areas, drinking water chlorination may generate a considerable number of bladder cancer cases. These estimations have to be carefully interpreted and verified with more extensive studies.

Key words: Bladder cancer. Epidemiology. Clorinated drinking water. Trihalomethane.

Introducción

La contaminación del agua potable se puede dividir en dos grandes categorías: contaminación microbiológica y contaminación química. La contaminación microbiológica provoca efectos agudos (enfermedades infecciosas como cólera, tifus, malaria, fiebre amarilla, síntomas gastrointestinales, etc.). La contaminación química se puede asociar a efectos crónicos como el cáncer, efectos neurológicos o efectos reproductivos. En nuestro medio la contaminación microbiológica es una cuestión en general superada gracias a los procesos de potabilización y desinfección de las aguas. La contaminación química no se ha considerado aún en España como uno de los problemas mayores en salud pública, básicamente con el argumento de que la calidad de las aguas de consumo sigue, en promedio, las normas de la legislación española[1] y europea[2]. Los contaminantes químicos se pueden clasificar en grandes grupos como metales, nitratos, pesticidas, isótopos radiactivos, flúor, asbesto y los subproductos de la cloración. El presente trabajo se centra en uno de estos contaminantes químicos, los subproductos de la cloración, generados en el proceso de desinfección para eliminar la contaminación microbiológica.

La cloración del agua supuso un avance en salud pública a principios del siglo XX al eliminar patógenos del agua reduciendo la incidencia de enfermedades infecciosas. A pesar de este beneficio, el cloro reacciona con precursores orgánicos del agua generando una mezcla compleja de subproductos organoclorados y organobromados con propiedades mutágenas y cancerígenas[3,4]: trihalometanos (THM), ácidos acéticos halogenados, acetonitrilos halogenados, etc. (tabla 1). Los THM son los subproductos de la cloración generados en mayor cantidad y se utilizan como indicadores del nivel total de subproductos de la cloración de un agua. La ley vigente de aguas potables[1] no impone un nivel máximo admisible de trihalometanos, pero existe una nueva directiva europea[2] que propone un nivel de 100 µg/l. En los últimos años diversos estudios epidemiológicos han evaluado la asociación entre la exposición a subproductos de la cloración y efectos sobre la salud humana. Básicamente hay dos efectos asociados a esta exposición: alteraciones de la reproducción y del desarrollo[5,6] y diversos tipos de cáncer[7], siendo el de vejiga el más consistentemente asociado.

El presente trabajo pretende estimar el riesgo de cáncer de vejiga que se puede atribuir poblacionalmente a la exposición a los subproductos de la cloración en 4 zonas de España.

Tabla 1. Evaluaciones de la IARC y niveles recomendados por la OMS para los diferentes subproductos de la cloración

	Clasificación IARC[a]	Nivel recomendado por la OMS (µg/l)[b]
Trihalometanos		
Cloroformo ($CHCl_3$)	2b (1999)[c]	200
Bromodiclorometano ($CHBrCl_2$)	2b (1999)	60
Dibromoclorometano ($CHBr_2Cl$)	3 (1999)	100
Bromoformo ($CHBr_3$)	3 (1999)	100
Ácidos acéticos halogenados ($C_2O_2HX_3$)		
Ácido monocloroacético	–	NAD[d]
Ácido dicloroacético	3 (1995)[e]	50
Ácido tricloroacético	3 (1995)	100
Acetonitrilos halogenados (C_2NX_3)		
Cloroacetonitrilo	3 (1999)	–
Dicloroacetonitrilo	3 (1999)	90
Dibromoacetonitrilo	3 (1999)	100
Bromocloroacetonitrilo	3 (1999)	NAD
Tricloroacetonitrilo	3 (1999)	1
Bromoacetonitrilo	–	–
Hidrato de cloral (tricloroacetoaldehído $C_2H_3O_2Cl_3$)	3 (1995)	10
Haloacetonas ($C_3H_3OX_3$)		
Cloroacetona	–	NAD
Cloropicrina (CCl_3NO_2)	–	NAD
Cloruro de cianógeno ($CNCl$)	–	70
MX (3-cloro-4-(diclorometil)-5-hidroxi-2(5H)-furanona)	–	NAD

[a]Categoría 2b: posiblemente cancerígeno. Categoría 3: datos inadecuados para su clasificación como cancerígeno o no cancerígeno.
[b]Las evaluaciones del año 1999 corresponden a la monografía núm. 71 de la IARC: Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide.
[c]Las evaluaciones del año 1995 corresponden a la monografía núm. 63 de la IARC: Dry cleaning, Some Chlorinated Solvents and Other Industrial Chemicals.
[d]NAD: no hay datos adecuados que permitan una recomendación de nivel guía basado en criterios de salud.

Métodos

Áreas de estudio

El presente trabajo se engloba en el estudio multicéntrico español de cáncer de vejiga, integrado a su vez en el proyecto europeo EPICURO (EPIdemiology of the Cancer of UROthelium). Las áreas de estudio están delimitadas por los 18 hospitales que participan aportando casos y controles en 4 provincias españolas: Asturias, Alicante, Barcelona y Tenerife. En Alicante el área de estudio se concentra en la ciudad de Elche y su área de influencia; en Barcelona se distinguen 3 áreas de estudio: área metropolitana de Barcelona, Sabadell, Manresa y las respectivas áreas de influencia.

El área de estudio en Asturias incluye todo el principado y en Tenerife toda la isla.

Análisis de trihalometanos

Distribución de las muestras. Se han tomado 111 muestras de agua potable distribuidas en las áreas de estudio entre septiembre y diciembre de 1999. Se han tomado 36 muestras en el principado de Asturias, 27 en el área metropolitana de Barcelona, 19 en la provincia de Alicante, 10 en la ciudad de Sabadell, 10 en la isla de Tenerife y 9 en la ciudad de Manresa.

Se realizó un protocolo para estandarizar el procedimiento de toma, conservación y envío de las muestras. La elevada volatilidad de los compuestos a analizar requería precaución en la recogida, conservación y envío de las muestras. La determinación analítica se hacía lo antes posible, no dejando pasar más de 15 días desde el muestreo.

Todas las muestras se han tomado por duplicado en viales de 40 ml con tapa de rosca y *septum* de teflón. Se añadieron 3 mg de tiosulfato de sodio para evitar la posterior formación de trihalometanos. Las muestras se toman del grifo, con agua fría, vigilando que no se formen burbujas y que no quede aire entre el agua y el tapón. Los viales son identificados con etiquetas donde consta la fecha del muestreo, la persona que toma la muestra, la dirección y si el agua es directa o de depósito. La muestra era enviada por mensajero hasta el centro de análisis.

Descripción del procedimiento analítico. Se ha seguido la técnica analítica previamente optimizada por Amaral (1996)[6]. Se realiza un pretratamiento de la muestra denominado *purga y trampa*, donde la muestra (5 ml) es inyectada en un recipiente mientras la atraviesa una corriente de helio de 40 ml/min durante 11 minutos. Durante este tiempo los compuestos volátiles son arrastrados de la muestra acuosa al material adsorbente donde quedan retenidos. Posteriormente el material adsorbente se pasa a la fase automatizada del análisis: desorción térmica automatizada (ATD) acoplada a un cromatógrafo de gases con detector de captura electrónica.

Validación de la técnica analítica. Se realizó una validación de la técnica analítica a través de la comparación de resultados propios con los de un laboratorio de referencia. Se tomaron 20 muestras duplicadas distribuidas entre las áreas de estudio. Una de las muestras se envió al laboratorio de referencia y la otra se analizó en los laboratorios propios. La primera validación mostró que se estaban infraestimando niveles. Después de introducir cambios en el procedimiento analítico, se realizó una segunda validación mostrando que el procedimiento que se seguía era correcto.

Revisión bibliográfica

Se ha realizado una revisión bibliográfica de los estudios epidemiológicos con información individual sobre exposición a subproductos de la cloración y cáncer de vejiga urinaria. Para la identificación de los estudios se ha utilizado la base de datos MEDLINE, los archivos de la IARC/OMS y los archivos personales de los autores, y se evaluó la bibliografía de los estudios identificados. Se seleccionaron solamente artículos publicados de estudios con información individual sobre consumo de agua. De éstos, se seleccionaron los artículos con información sobre exposición a THM. Todos menos dos eran de tipo casos y controles y presentaban estimadores del riesgo obtenidos en base a modelos multivariados.

Estimación del riesgo atribuible

La proporción de cánceres de vejiga de ámbito poblacional que se pueden atribuir a la exposición a subproductos de la cloración del agua se ha calculado para las 4 provincias españolas (Barcelona, Asturias, Alicante y Tenerife). Las áreas de Sabadell y Manresa quedan incluidas en la provincia de Barcelona. Se obtuvieron los datos de mortalidad por provincias y para toda España correspondientes al año 1996, publicados por el Instituto de Salud Carlos III (Servicio de Epidemiología del Cáncer del Área de Epidemiología Aplicada). Se ha calculado el riesgo atribuible utilizando la fórmula AR_p = P(OR-1)/1 + P(OR-1), donde P es la proporción de personas expuestas en la población (personas que beben agua del grifo) y OR es el riesgo *(odds ratio)* de ser caso asociado a dicha exposición. Se han estimado los riesgos atribuibles por duplicado aplicando los valores de OR de los 2 estudios con información más detallada sobre niveles de trihalometanos[9,10]. La información sobre el porcentaje de personas que bebe agua del grifo y agua embotellada se ha extraído de las estadísticas de la parte española del estudio EPICURO, que en la actualidad incluye datos de aproximadamente 1.400 casos y controles. Sobre la base de los resultados del estudio EPICURO, la proporción de personas que consume actualmente agua del grifo en las 4 provincias del estudio es del 61,6%. Un 10,3% consume agua embotellada; un 6,4%, agua de pozo y el 18,6% de las personas, agua de otros orígenes o no beben agua.

Resultados

Niveles de trihalometanos

Las áreas con los niveles más altos se concentran en la costa mediterránea: Sabadell y Alicante, con ni-

veles de 81,0 y 79,7 µg/l, respectivamente. Los niveles más bajos se sitúan en Tenerife y Asturias (7,5 y 20,5 µg/l, respectivamente). Manresa y Barcelona presentan unos niveles intermedios-altos (52,4 y 60,8 µg/l, respectivamente). La tabla 2 muestra estos resultados. Se han definido tres categorías de exposición: áreas de exposición baja (Tenerife y Asturias), áreas de exposición intermedia (Barcelona ciudad y Manresa) y áreas de exposición elevada (Alicante y Sabadell).

Revisión bibliográfica

Se identificaron 10 estudios que evalúan individualmente el riesgo de cáncer de vejiga asociado a la exposición a subproductos de la cloración[5-14]. Todos los estudios encuentran un incremento del riesgo de cáncer de vejiga por exposición a agua clorada o subproductos de la cloración en personas que han consumido agua clorada durante décadas. Los cálculos de riesgo atribuible se basaron en los 2 trabajos que presentaban información de niveles medios de exposición a trihalometanos a lo largo de la vida[9,10] (fig. 1).

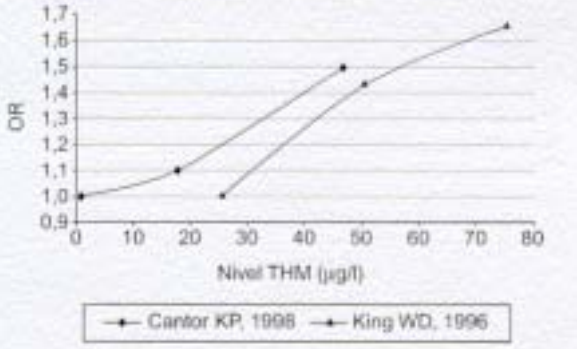
Riesgo atribuible

Los niveles de trihalometanos en Tenerife y Asturias corresponden a un incremento del riesgo del orden del 10% (OR, 1,1), en Manresa y Barcelona a un aumento del riesgo entre el 43% y 50% (OR, 1,43, 1,5) y en Alicante y Sabadell a un riesgo entre 50% y 66% (OR, 1,5, 1,66). El riesgo atribuible en las áreas evaluadas varía entre 0 y el 28,9% (tabla 3) dependiendo de las cifras de trihalometanos presentes. En promedio, un 20% de los casos de cáncer de vejiga en Barcelona, Manresa, Sabadell y Alicante se podrían atribuir a la cloración. Entre todas estas provincias, el número de muertes anuales por cáncer de vejiga atribuible a esta exposición en varones es de alrededor de 95-98 muertes.

Tabla 2. Niveles medios de trihalometanos (µg/l) en las áreas de estudio

	Media geométrica	Media aritmética (desviación estándar)	Mínimo	Máximo	Número muestras
Sabadell	81,0	82,5 (16,5)	64,1	100,9	9
Alicante	79,7	85,9 (30,6)	35,2	125,3	19
Barcelona	60,8	63,6 (20,6)	34,6	121,7	25
Manresa	52,4	56,1 (22,2)	28,3	101,8	9
Asturias	20,5	22,2 (8,5)	6,4	16,3	34
Tenerife	7,5	8,0 (3,4)	5,1	44,5	10

Figura 1. Variación de la OR en función del grado de exposición a THM en los 2 estudios epidemiológicos tomados como referencia para el cálculo del riesgo atribuible.



Discusión

La calidad de las aguas en origen es el principal determinante de la cantidad de subproductos de la cloración generados y explica las diferencias observadas entre las áreas de España estudiadas. Las aguas subterráneas (pozos y minas), con menor cantidad de precursores orgánicos y menor cloro requerido respecto a las superficiales, darán niveles bajos de subproductos de la cloración. Esto se refleja en los niveles de trihalometanos en las diferentes áreas de España. En Tenerife, donde el agua potable es de origen subterráneo, se encuentran los niveles más bajos. El resto de áreas utilizan agua básicamente superficial, pero se observa una diferencia significativa entre los niveles de Asturias y los de la franja mediterránea (Sabadell, Alicante, Barcelona y Manresa), donde la formación de trihalometanos es mayor debido a la peor calidad de las aguas superficiales.

El cálculo del riesgo atribuible para el cáncer de vejiga está basado en determinadas asunciones y por eso

Tabla 3. Número de muertes anuales por cáncer de vejiga en varones atribuibles a la exposición a subproductos de la cloración, estimadas por áreas de estudio

	Riesgo atribuible (%)[a]	Muertes anuales por cáncer de vejiga (varones)	Muertes anuales atribuibles a la exposición a subproductos de la cloración (varones)[a]
Alicante	23,5-28,9	111	26-32
Asturias	5,8-0	86	5-0
Barcelona[b]	20,9-23,5	280	58-66
Tenerife	5,8-0	46	3-0
Total		253	95-98

[a]El primer valor corresponde a las estimaciones en base al estudio de Cantor KP et al, 1998, y el segundo al estudio de King W et al, 1996.
[b]Las áreas de Sabadell y Manresa están incluidas en la provincia de Barcelona.

se tiene que considerar con cautela. En primer lugar, la OR aplicada está basada en estudios realizados en América del Norte. Aunque lo ideal sería aplicar una OR correspondiente a la población española, en muchas ocasiones se ha observado que la OR de un nivel de exposición a un factor de riesgo es una medida del riesgo sólida y repetible en distintas poblaciones genéticamente similares[19]. Se tomaron los estudios de Cantor et al (1998)[9] y King et al (1996)[10] por ser los únicos que disponían de OR asociadas a la exposición durante toda la vida a distintos niveles medios de THM. La OR del estudio de Cantor et al corresponde a la población masculina, mientras en la población femenina no se encuentra un aumento del riesgo. Hay que tener en cuenta que en su conjunto los 10 estudios que evalúan cáncer de vejiga asociado a la exposición a subproductos de la cloración encuentran estimaciones de riesgo consistentemente positivas. Sin embargo, los riesgos para estratos específicos de las poblaciones evaluadas (fumadores/no fumadores, varones/mujeres) no son siempre consistentes. En parte esto se puede atribuir al azar, particularmente dado que el cáncer de vejiga es infrecuente en mujeres y el número de casos femeninos es a menudo muy bajo.

En segundo lugar, el cáncer es una enfermedad con un largo período de latencia, por lo que la exposición realmente asociada al efecto no es la actual sino la exposición durante varias décadas antes de la manifestación clínica. En el análisis asumimos que la exposición histórica a los subproductos de la cloración es la misma que la actual, con relación a los niveles de trihalometanos y la prevalencia de consumo de agua del grifo (61,6%). Los registros históricos disponibles de niveles de trihalometanos en agua potable en el área de Barcelona, donde se dispone de esta información desde hace más tiempo, muestran una ligera tendencia al incremento de los niveles a lo largo de las últimas décadas[20]. Esto sugiere que la población habría estado menos expuesta en el pasado que en la actualidad. Por otro lado, el consumo de agua embotellada ha aumentado en los últimos años de 23 litros per cápita anuales en 1987 a 50 litros per cápita al año en 1997 (Encuesta de Consumo, Ministerio de Agricultura, Pesca y Alimentación). Por tanto, la prevalencia de la exposición actual (proporción de personas que beben agua del grifo) es menor que en el pasado. El efecto de estas dos tendencias se complementa en parte.

Hemos utilizado la prevalencia de consumo de agua clorada correspondiente a casos y controles juntos extraída del estudio de casos y controles EPICURO Español, que está aún en fase de recogida de datos. La inclusión de los casos en los cálculos de la prevalencia probablemente resulta en una ligera sobrestimación de los riesgos atribuibles. Utilizando una fórmula alternativa para la estimación del riesgo atribuible (AR = p [casos] * [OR-1]/OR) se puede calcular que esta sobrestimación puede ser, en promedio, del orden del 2-3%.

Asumiendo una exposición intermedia en el ámbito español, el riesgo atribuible sería superior al 20% y el de muertes anuales en España por cáncer de vejiga atribuible a esta exposición ambiental se situaría alrededor de 600 fallecimientos aproximadamente. Este riesgo atribuible estaría entre los más elevados de los descritos en la literatura científica. Un grupo neozelandés[21] estima en un 25% los cánceres de vejiga que se pueden atribuir a esta exposición ambiental. Estimaciones correspondientes a la población de Ontario (Canadá) atribuyen a esta exposición un 14-16% de los casos de cáncer de vejiga[10], mientras que la Agencia de Protección Ambiental de Estados Unidos (US EPA)[22] calcula que un 2-17% de los cánceres de vejiga en Estados Unidos se podrían atribuir a la exposición a subproductos de la cloración.

La estimación del riesgo atribuible se ha centrado en el efecto por el cual hay datos cuantitativos válidos, a pesar de que el cáncer colorrectal y defectos en neonatos de madres expuestas han sido también asociados a esta exposición. Un análisis reciente de Nueva Zelanda[21] sobre el riesgo de cáncer colorrectal y efectos reproductivos concluyó que alrededor del 25% de estos efectos podían ser atribuibles a la exposición a los subproductos de la cloración. Resulta imperativo llevar a cabo estudios extensos sobre estos efectos aplicando métodos de evaluación de la exposición detallados.

Existen grandes diferencias en la prevalencia del cáncer de vejiga en España. El patrón geográfico no necesariamente corresponde con los niveles de exposición a trihalometanos ya que, por ejemplo, el tabaco es un factor de riesgo claramente más importante que puede enmascarar en comparaciones ecológicas los efectos de la exposición a subproductos de la cloración.

La cloración del agua y el tratamiento del agua potable más habitual en España pueden representar un riesgo sobre la salud de las personas, incluso estando por debajo de las exigencias de la nueva directiva europea. España es el segundo país de la Unión Europea después de Portugal con los niveles más elevados de trihalometanos[23]. Frecuentemente se considera que los procesos utilizados actualmente para la desinfección del agua potable son necesarios para evitar el riesgo de infecciones hídricas. Como consecuencia se considera que el menor de los riesgos se tiene que aceptar frente a un riesgo mayor, el de las infecciones. En este sentido hay que indicar que la prevención química y bacteriológica de las aguas no son antagónicas, especialmente para un país del nivel socioeconómico como España. El riesgo sobre la salud humana de la cloración del agua es evitable, ya que hay métodos de desinfección y líneas de potabilización alternativos al cloro y a los procedimientos actuales, con igual capa-

cidad desinfectante y menor formación de compuestos clorados y bromados.

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