American Journal of Epidemiology
Vol. 165, No. 2
DOI: 10.1093/aje/kwj364
Advance Access publication November 1, 2006
Copyright © 2006 by the Johns Hopkins Bloomberg School of Public Health
All rights reserved; printed in U.S.A.

Original Contribution

Bladder Cancer and Exposure to Water Disinfection By-Products through Ingestion, Bathing, Showering, and Swimming in Pools

Cristina M. Villanueva[1], Kenneth P. Cantor[2], Joan O. Grimalt[3], Nuria Malats[1], Debra Silverman[2], Adonina Tardon[4], Reina Garcia-Closas[5], Consol Serra[6,7], Alfredo Carrato[8], Gemma Castaño-Vinyals[1], Ricard Marcos[9], Nathaniel Rothman[2], Francisco X. Real[10,11], Mustafa Dosemeci[2], and Manolis Kogevinas[1,12]

[1] Centre for Research in Environmental Epidemiology, Municipal Institute of Medical Research, Barcelona, Spain.
[2] Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD.
[3] Department of Environmental Chemistry, Institute of Chemical and Environmental Research (CSIC), Barcelona, Spain.
[4] Department of Preventive Medicine and Public Health, Universidad de Oviedo, Oviedo, Spain.
[5] Department of Preventive Medicine, Hospital Universitario de Canarias, La Laguna, Tenerife, Spain.
[6] Unit of Research in Occupational Health, Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain.
[7] Corporació Parc Taulí, Sabadell, Spain.
[8] Department of Medical Oncology, Hospital General de Elche, Elche, Spain.
[9] Mutagenesis Group, Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain.
[10] Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain.
[11] Cellular and Molecular Biology Research Unit, Municipal Institute of Medical Research (IMIM), Barcelona, Spain.
[12] Department of Social Medicine, Medical School, University of Crete, Heraklion, Crete, Greece.

Received for publication March 27, 2006; accepted for publication June 8, 2006.

Bladder cancer has been associated with exposure to chlorination by-products in drinking water, and experimental evidence suggests that exposure also occurs through inhalation and dermal absorption. The authors examined whether bladder cancer risk was associated with exposure to trihalomethanes (THMs) through ingestion of water and through inhalation and dermal absorption during showering, bathing, and swimming in pools. Lifetime personal information on water consumption and water-related habits was collected for 1,219 cases and 1,271 controls in a 1998–2001 case-control study in Spain and was linked with THM levels in geographic study areas. Long-term THM exposure was associated with a twofold bladder cancer risk, with an odds ratio of 2.10 (95% confidence interval: 1.09, 4.02) for average household THM levels of >49 versus ≤ 8 μg/liter. Compared with subjects not drinking chlorinated water, subjects with THM exposure of >35 μg/day through ingestion had an odds ratio of 1.35 (95% confidence interval: 0.92, 1.99). The odds ratio for duration of shower or bath weighted by residential THM level was 1.83 (95% confidence interval: 1.17, 2.87) for the highest compared with the lowest quartile. Swimming in pools was associated with an odds ratio of 1.57 (95% confidence interval: 1.18, 2.09). Bladder cancer risk was associated with long-term exposure to THMs in chlorinated water at levels regularly occurring in industrialized countries.

bladder neoplasms; disinfection; drinking; inhalation; skin absorption; trihalomethanes; water supply

Abbreviations: CI, confidence interval; DBP, disinfection by-product; THM, trihalomethane.

Correspondence to Dr. Manolis Kogevinas, Centre for Research in Environmental Epidemiology (CREAL), Municipal Institute of Medical Research (IMIM), 80 Dr Aiguader Road, Barcelona 08003, Spain (e-mail: kogevinas@imim.es).

Drinking water disinfectants encompass highly reactive molecules that generate undesired compounds through reaction with organic matter. These disinfection by-products (DBPs) constitute complex mixtures of chemical species with different properties. Chlorine, the most widely used disinfectant for drinking water, gives rise to trihalomethanes (THMs), usually the most prevalent DBP. Some epidemiologic studies (1–4) have shown an association between long-term exposure to chlorination by-products and increased risk of cancer, which is supported by experimental evidence of carcinogenicity for some of these chemicals (5–8). Many of these compounds have been shown to be genotoxic (6, 7), but the mechanisms of action are not well elucidated, and few studies have evaluated markers of genotoxicity in humans (9). The bladder is one of the cancer sites most consistently associated with exposure to chlorination by-products (10–14). However, methodological limitations and the reported low increased risks require replication of the studies in different settings to better appraise causality of the association.

Although ingestion has been thought to be the most common route of exposure to DBPs, the high volatility and dermal permeability of certain DBPs suggest the potential contribution of the inhalation and dermal absorption pathways. Experimental studies have shown a significant uptake of THMs through these routes when showering, bathing, or swimming in pools (15–18). A simulation study evaluating chloroform uptake through different pathways showed the relevance of swimming in pools and exposure through showers or baths compared with ingested levels (19). However, to date, noningestion routes are not known to have been assessed in relation to cancer risk.

We conducted a multicenter case-control study of bladder cancer in Spain and evaluated the association with DBP exposure. We used THM level as a marker of DBP exposure, and we evaluated bladder cancer risk associated with THM ingestion as well as noningestion routes while showering, bathing, and swimming in pools.

MATERIALS AND METHODS

Study design and population

We conducted a multicenter, hospital-based case-control study of bladder cancer between June 1998 and June 2001. Cases and controls were identified in 18 participating hospitals from five geographic areas of Spain: Barcelona, Vallès/Bages (including the cities of Sabadell and Manresa), Alicante, Tenerife, and Asturias. Cases were identified through the hospital urologic services at diagnosis and were defined as patients with a histologically confirmed diagnosis of a primary bladder cancer, aged 20–80 years, and living in the catchment geographic area of the participating hospitals. In addition to registers from urologic services, complete case ascertainment was secured by regular and frequent evaluations of hospital discharge records, pathology records, and local cancer registries. Controls were patients admitted to the participating hospitals with diagnoses thought to be unrelated to the main risk factors for bladder cancer, such as tobacco use. They were matched individually 1:1 to cases by gender, age group (5-year strata), and geographic area of residence. Controls were admitted to hospitals for the following reasons: hernias (37 percent), other abdominal surgery (11 percent), fractures (23 percent), other orthopedic problems (7 percent), hydrocele (12 percent), circulatory disorders (4 percent), dermatologic disorders (2 percent), ophthalmologic disorders (1 percent), and other diseases (3 percent). The study proposal and the manner in which informed consent was obtained from subjects were approved by the review board of the participating institutions.

Individual data

After informed consent was obtained, trained interviewers administered a comprehensive computer-assisted personal interview to participants during their hospital stay. Collected information included sociodemographic characteristics; smoking habits; occupational, residential, and medical histories; and familial history of cancer. A food frequency questionnaire was self-administered. We identified 1,457 eligible cases and 1,465 eligible controls. Among them, 84 percent of cases ($n = 1{,}219$) and 87 percent of controls ($n = 1{,}271$) responded to the questionnaire. Among respondents, subjects who refused to answer the computer-assisted personal interview were administered a reduced interview of critical items (21 percent of cases and 19 percent of controls).

Information on water-related habits included the following: residential history from birth (all residences of at least 1 year); drinking water source at each residence (municipal/bottled/private well/other); average daily consumption, including water-based fluids (e.g., coffee, tea, and water); average frequency and duration of showering and bathing; and lifetime swimming in pools. The questionnaire of critical items covered residential history, drinking water source by residence (municipal/bottled/private well/other), frequency of showering and bathing, and whether the subject ever swam in indoor and outdoor pools and the number of times per year doing so.

To evaluate the reproducibility of responses about showering, bathing, and swimming, a subset of 200 controls (representative of the general population in the study base) was interviewed by telephone two times within a 6-week span. The response rate was 70 percent. The agreement rate for the reported type of personal hygiene (shower or bath) was 97.8 percent. Frequencies of showering and bathing (times per week) reported in both telephone calls were correlated with 0.73 and 0.95 Pearson coefficients, respectively. The agreement rate for having ever swum in pools was 89.6 percent. For those responding positively on both occasions, the Pearson coefficient of frequency of swimming in pools was 0.94.

Micronuclei were analyzed in exfoliated urine cells of a subset of study subjects. Urine samples were collected from 92 female controls ($n = 72$ with adequate samples) 1 year after hospital discharge. For those women, information on THM exposure was complete for 44. Cells were stained with a DNA-specific stain (1 μg/ml 4′,6-diamino-2-phenylindole dihydrochloride). A total of 2,000 cells per donor were scored on coded slides by one scorer under an Olympus

BX50 fluorescence microscope (Olympus America Inc., Melville, New York).

Exposure data

We contacted approximately 200 local authorities and 150 water companies in the study's geographic areas and used a structured questionnaire to collect data on water parameters in the past. For 123 study municipalities, covering 78.5 percent of the total study person-years, we obtained annual average THM levels, water source history since 1920 (proportion of surface and ground source over the years), and year that chlorination began. In addition, THMs (chloroform, bromodichloromethane, dibromochloromethane, and bromoform) were measured in a central laboratory in 113 tap water samples from the study's geographic areas between September and December 1999.

Historical THM levels were estimated by municipality under the assumption that THM level remained unchanged for a constant water source. Average THM levels in recent years were extrapolated approximately back to 1920. In the event of water source changes, the proportion of surface water was used as a weight for this average. THM level before chlorination started was assumed to be zero. Details of the exposure assessment are available elsewhere (20).

Lifetime individual exposure indices

Individual and municipal databases were merged by year and municipality, obtaining individual year-by-year average THM levels, water source, and chlorination status. We created several individual exposure indices for the period from age 15 years until the time of interview. This exposure window minimized missing exposure data in the population because of better recall of residences after early ages (20). The five indices are described below.

Residential THM exposure (μg/liter): time-weighted average municipal THM level at all residences since age 15 years.

Duration of exposure to chlorinated surface water: number of years living in residences supplied with chlorinated surface water since age 15 years.

Ingestion THM exposure (μg/day): average THM level based on the reported drinking water source at each residence since age 15 years and amount of water consumed. Level of THM ingestion was assumed to be zero for water from private well, bottle, or other nonmunicipal sources. Municipal THM level was attributed when subjects reported drinking municipal water. For each individual, a time-weighted average was calculated and multiplied by the daily amount of total tap water consumed. Total tap water included consumption of tap water and beverages made with tap water (coffee, tea, etc.).

Showers and baths (minutes/day × μg/liter): average duration of showers or baths (in minutes per day) was calculated and multiplied by average residential THM level since age 15 years. Showers and baths were given the same weight. This exposure index combined duration and intensity of exposure to THMs through inhalation and dermal absorption while showering and bathing.

Swimming in pools: derived from questions asked about indoor and outdoor pool use during an adult's lifetime. Swimming in indoor and outdoor pools was treated similarly. Lifetime duration of swimming in pools was calculated (in hours).

Statistical analysis

Subjects were grouped by using quartiles as category boundaries for the different exposure indices. We used unconditional logistic regression to calculate odds ratios and 95 percent confidence intervals. Odds ratios were adjusted for age (continuous), gender, smoking status (never/former/current), size of the municipality of longest residence until age 18 years (reported by the participant from four options provided: metropolis/city, small city, village, farm), education (level of formal education grouped in three strata, as reported by the participant: less than primary school, less than high school, high school or more), geographic area (six groups: Barcelona, Vallès/Bages (split into two areas: Sabadell and Manresa), Alicante, Tenerife, and Asturias), and overall quality of the interview (reported by the interviewer from four options provided: unsatisfactory, questionable, reliable, and high quality). Analyses in which more detailed information on smoking was used (duration, number of cigarettes, type of tobacco, pack-years) gave similar results and are not reported here. Missing data for covariates (indicated in table 1) were coded in a separate category for each variable and were included in the models.

The analyses of residential THM levels were restricted to subjects for whom exposure information for at least 70 percent of the exposure window was available ($n = 1{,}572$). Of these subjects, we excluded 93 whose overall quality of interview was considered unsatisfactory or questionable, leaving data for a total of 707 cases and 772 controls for analysis. In the analysis of ingestion THMs, of the 1,770 subjects for whom information covering at least 70 percent of the exposure window was available, we excluded 579 because amount of water consumed was missing. Of the remaining 1,191 subjects, we excluded 44 whose quality of interview was questionable or unsatisfactory, resulting in a total of 577 cases and 570 controls for analysis. In the analysis of duration of exposure to chlorinated surface water, information on water source and chlorination status that covered at least 70 percent of the exposure window was available for 1,573 subjects. Of these, we excluded 93 because of unsatisfactory or questionable interviews, leaving 707 cases and 773 controls in the analysis.

Questions on showering, bathing, and swimming in pools were added to the personal interview 6 months after the study started, and subjects interviewed during this period were excluded from these analyses ($n = 105$). During the first 6 months of subject recruitment, more cases than controls were interviewed through the critical items interview. To avoid bias, we excluded data from this interview ($n = 500$), leaving data for 1,885 subjects to analyze. In the models on THM exposure during showering and bathing, we included subjects with known THM levels for at least 70 percent of the exposure window ($n = 1{,}351$). Of those subjects, we excluded respondents with unsatisfactory or

TABLE 1. Description of the population included in a hospital-based case-control study conducted in Spain, 1998–2001

	Cases ($n = 1,219$)		Controls ($n = 1,271$)		OR*	95% CI*
	No.	%	No.	%		
Gender						
Men	1,067	87.5	1,105	86.9		
Women	152	12.5	166	13.1		
Age (years)						
≤65.4	472	38.7	564	44.4		
>65.4	747	61.3	707	55.6		
Geographic area						
Barcelona	229	18.8	247	19.4		
Vallès	188	15.4	190	14.9		
Alicante	88	7.2	84	6.6		
Tenerife	219	18.0	226	17.8		
Asturias	495	40.6	524	41.3		
Smoking status						
Never	218	18.0	464	36.8	1.00	
Former	499	41.2	510	40.5	3.05	2.37, 3.92
Current	495	40.8	286	22.7	6.41	4.89, 8.41
Missing	7		11			
Education						
<Primary school	563	46.4	592	46.9	1.00	
<High school	470	38.7	491	38.9	1.10	0.92, 1.32
≥High school	166	13.7	164	13.0	1.19	0.92, 1.53
Other	15	1.2	15	1.2	1.19	0.57, 2.50
Missing	5		9			
Size of municipality of longest residence until age 18 years†						
Metropolis/city	388	38.9	366	34.3	1.00	
Small city	156	15.6	156	14.6	0.94	0.72, 1.23
Village	451	45.2	543	50.9	0.75	0.62, 0.92
Farm	3	0.3	2	0.2	1.44	0.24, 8.75
Missing	221		204			

* Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by logistic regression, adjusting for age, gender, and geographic area.

† As reported by study subjects from the four options provided.

questionable interviews ($n = 71$) and missing data on showering or bathing ($n = 74$), leaving information on 546 cases and 660 controls for analysis. In the analysis of ever swimming in pools, 44 subjects were excluded because data on swimming in pools were missing, and 118 were excluded because of a questionable or unsatisfactory interview, leaving data on 787 cases and 963 controls to analyze. The analyses by duration of swimming in pools excluded 115 subjects missing data on duration and 105 whose interviews were questionable or unsatisfactory, leaving information on 750 cases and 915 controls for analysis.

Risk of micronuclei was evaluated through logistic regression as a dichotomous variable and in a linear regression as a continuous variable, adjusting for age, smoking, and geographic region. Linear p trends were calculated by using a likelihood ratio test comparing the model with and without the exposure variable with each quartile coded numerically (0, 1, 2, 3).

RESULTS

Median age at interview was 67 years, and 87.5 percent of study subjects were men. After we adjusted for age, gender, and geographic area, excess risks were found for former and current smokers. Subjects who reported that their longest

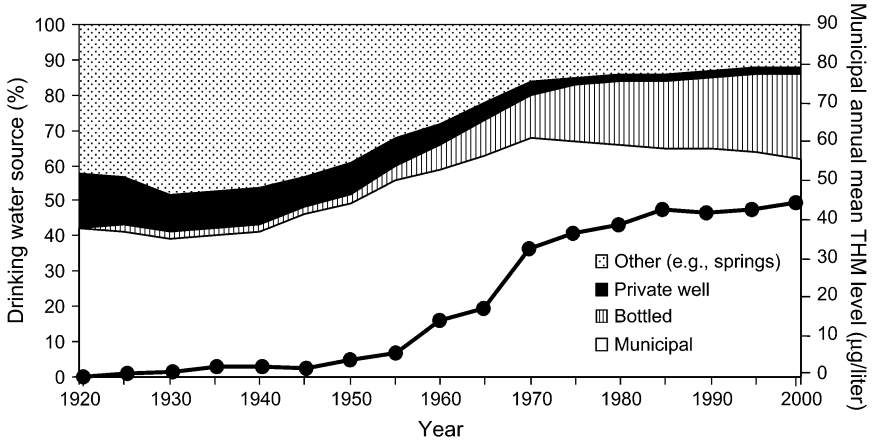

FIGURE 1. Trends in drinking water source over the years in all geographic study areas for controls in a hospital-based case-control study conducted in Spain, 1998–2001. The line with points indicates municipal annual mean trihalomethane (THM) levels (μg/liter). For each residence, the authors asked for one water source. Since subjects lived in their last residence on average for decades (about 30 years) and tended to report their most recent water source, the observed increase in bottled water probably occurred more recently than shown. Municipalities included in the THM figure are the main cities from the geographic study areas (Barcelona: Barcelona, Badalona, Santa Coloma; Vallès/Bages: Sabadell; Asturias: Oviedo, Gijón, Avilés, Mieres, Valdés; Tenerife: Santa Cruz de Tenerife, La Laguna, Güimar). The main municipality of Alicante (Elche) was not included because of comparatively limited retrospective data.

residence until age 18 years was in a village had a lower risk of bladder cancer compared with those who lived in a metropolitan area. Cases and controls had a similar educational level (table 1).

The proportion of subjects consuming bottled water increased substantially in all geographic areas over the years, especially in geographic areas with high THM levels (figures 1 and 2). Figures 1 and 2 include controls, to represent the general population of the study base. The proportions of bottled water consumption among controls who left residences in the 1960s, 1970s, 1980s, and 1990s were 4 percent, 9 percent, 13 percent, and 22 percent, respectively, with higher use in geographic regions with higher THM levels (20). Consumption of municipal water decreased

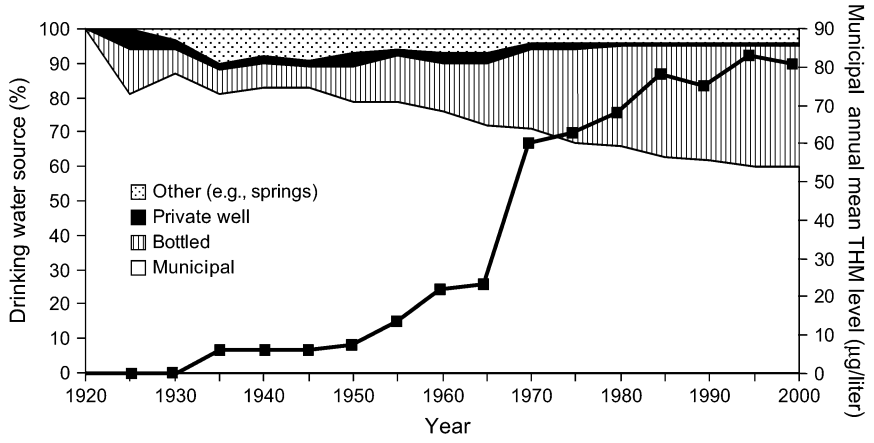

FIGURE 2. Trends in drinking water source over the years in geographic areas with high trihalomethane (THM) levels for controls in a hospital-based case-control study conducted in Spain, 1998–2001. The line with points indicates municipal annual mean THM levels (μg/liter). For each residence, the authors asked for one water source. Since subjects lived in their last residence on average for decades (about 30 years) and tended to report their most recent water source, the observed increase in bottled water probably occurred more recently than shown. Municipalities included in the THM figure are the main cities from the geographic study areas (Barcelona: Barcelona, Badalona, Santa Coloma; Vallès/Bages: Sabadell; Asturias: Oviedo, Gijón, Avilés, Mieres, Valdés; Tenerife: Santa Cruz de Tenerife, La Laguna, Güimar). The main municipality of Alicante (Elche) was not included because of comparatively limited retrospective data. The municipalities included in the "high-THM areas" were selected because their current THM level was >60 μg/liter (Barcelona, Sabadell, Badalona, Santa Coloma).

TABLE 2. Odds ratios and 95% confidence intervals* for bladder cancer associated with average residential exposure to THMs† and duration of supply of chlorinated surface water from a hospital-based case-control study conducted in Spain, 1998–2001

	Men				Women				All	
	Cases (no.)	Controls (no.)	OR	95% CI	Cases (no.)	Controls (no.)	OR	95% CI	OR	95% CI
Average residential THM level (μg/liter)‡										
≤8.0	137	172	1.00	1.00	24	25	1.00		1.00	
>8.0–26.0	140	158	1.53	0.95, 2.48	18	33	0.40	0.13, 1.27	1.25	0.80, 1.93
>26.0–49.0	183	160	2.34	1.36, 4.03	23	22	1.14	0.31, 4.10	1.98	1.21, 3.24
>49.0	158	180	2.53	1.23, 5.20	24	22	1.50	0.26, 8.61	2.10	1.09, 4.02
p-trend			<0.01				0.61		<0.01	
Duration of chlorinated surface water in the residence (years)‡										
0–3	135	173	1.00		19	26	1.00		1.00	
>3–25	156	155	2.26	1.19, 4.29	20	22	2.72	0.56, 13.26	2.17	1.21, 3.89
>25–30	116	110	2.58	1.33, 5.01	13	18	2.32	0.44, 12.13	2.36	1.29, 4.31
>30	211	233	2.21	1.17, 4.20	37	36	2.33	0.51, 10.55	2.13	1.19, 3.79
p-trend			0.20				0.62		0.17	

* Odds ratios (ORs) and 95% confidence intervals (CIs) were obtained from logistic regression, adjusting for smoking status, age, gender, education, urbanicity of longest residence until age 18 years, overall quality of the interview, and geographic area.

† THMs, trihalomethanes.

‡ From the time window between age 15 years and the time of interview.

correspondingly, particularly in high-THM geographic areas. Average residential THM level in the population included in the analysis was 33 μg/liter (standard deviation, 28). Subjects lived in a residence with chlorinated surface water for 21.9 years on average (standard deviation, 14.4). Average ingestion THM level ranged from 0 to 240 μg/day in the study population, with a mean of 24 μg/day (standard deviation, 34). The vast majority of subjects took showers (81 percent). Swimming in pools at least once per year was reported by 13.6 percent of the study subjects.

Residential THM level was associated with a statistically significant increased risk of bladder cancer with a dose-response trend, particularly for men (table 2). Subjects exposed to an average level of 50 μg/liter or higher had twice the risk of those nonexposed or those exposed to less than 8 μg/liter. Habitation in residences supplied with chlorinated surface water was associated with an increased risk of bladder cancer, but a linear dose-response pattern was not found (table 2).

Exposure to THMs through ingestion was associated with a non-statistically significant increased risk of bladder cancer for the highest versus the lowest quartile of exposure (odds ratio = 1.35, 95 percent confidence interval (CI): 0.92, 1.99) (table 3). Odds ratios for men and women exposed to more than 35 μg/day were 1.61 (95 percent CI: 1.06, 2.44; *p*-trend = 0.02) and 0.47 (95 percent CI: 0.15, 1.51; *p*-trend = 0.21), respectively, relative to those unexposed (subjects who never drank municipal water). Subjects were asked to report the water source at each residence: tap water, bottled, and so forth. Since subjects lived in the last residence on average 30 years and bottled water consumption increased after the 1980s, a shift from municipal to bottled water probably occurred in the population, which likely led to an underestimation of exposure level. The possible effects of misclassification on risk estimates were evaluated by sensitivity analysis using two scenarios. First, we assumed that all subjects reporting consumption of bottled water in their last residence actually drank municipal water before 1990 and bottled water thereafter. In the second scenario, subjects reporting use of bottled water in their last residence were assumed to drink municipal water before 1980 and bottled water after that date. The odds ratio for the first scenario for exposure to THMs through ingestion of more than 35 μg/day compared with unexposed subjects was 1.82 (95 percent CI: 0.91, 3.66). For the 1980 (less extreme) scenario, the odds ratio was 1.51 (95 percent CI: 0.90, 2.52).

Duration of shower or bath was not associated with an increased risk of bladder cancer (results not shown). However, duration of shower or bath weighted by average residential THM level was associated with a statistically significant twofold increased risk for both men and women in the highest category of exposure versus the lowest and a linear dose-response pattern for men (table 3). The odds ratio for men exposed to more than 333 μg/liter × minutes per day was 2.01 (95 percent CI: 1.23, 3.28) relative to exposure to less than 50 μg/liter × minutes per day (*p*-trend = 0.01). For women, the odds ratio for the same exposure category was 2.26 (95 percent CI: 0.58, 8.90; *p*-trend = 0.38). Subjects who had ever swum in a pool showed an increased risk of bladder cancer compared with those who had never swum in pools. Odds ratios were similar for men and women, and the overall odds ratio was 1.57 (95 percent CI: 1.18, 2.09). Available data on duration suggested a duration-response relation for cumulative time spent in

TABLE 3. Odds ratios and 95% confidence intervals* for bladder cancer associated with different indices of exposure to disinfection by-products from a hospital-based case-control study conducted in Spain, 1998–2001

	Men				Women				All	
	Cases (no.)	Controls (no.)	OR	95% CI	Cases (no.)	Controls (no.)	OR	95% CI	OR	95% CI
Average ingestion THM† exposure (μg/day)‡										
0	119	141	1.00		27	19	1.00		1.00	
>0–10	118	124	0.97	0.65, 1.45	16	18	0.42	0.13, 1.38	0.88	0.61, 1.27
>10–35	132	119	1.32	0.88, 1.98	14	16	0.55	0.17, 1.77	1.17	0.80, 1.71
>35	134	114	1.61	1.06, 2.44	17	19	0.47	0.15, 1.51	1.35	0.92, 1.99
p-trend			0.02				0.21		0.09	
Duration of shower and bath × average residential THM level (minutes/day) × (μg/liter)‡										
<50	86	133	1.00		18	16	1.00		1.00	
50–<167	142	167	1.63	1.09, 2.45	17	33	0.41	0.15, 1.09	1.30	0.90, 1.87
167–<333	103	117	1.79	1.11, 2.88	11	24	0.38	0.11, 1.24	1.38	0.90, 2.13
≥333	146	157	2.01	1.23, 3.28	23	13	2.26	0.58, 8.90	1.83	1.17, 2.87
p-trend			0.01				0.38		<0.01	
Swimming in pools										
Never	539	684	1.00		99	130	1.00		1.00	
Ever	138	112	1.62	1.20, 2.19	11	10	1.53	0.58, 4.06	1.57	1.18, 2.09
Lifetime hours										
>0–165	53	45	1.67	1.06, 2.62	2	5	0.61	0.11, 3.45	1.50	0.98, 2.31
>165	52	46	1.59	1.01, 2.51	5	5	1.19	0.30, 4.72	1.52	0.99, 2.34
p-trend			<0.01				0.97		0.02	

* Odds ratios (ORs) and 95% confidence interval (CIs) were obtained from logistic regression, adjusting for smoking status, age, gender, education, urbanicity of longest residence until age 18 years, overall quality of the interview, and geographic area.

† THM, trihalomethane.

‡ From the time window between age 15 years and the time of interview.

swimming pools (table 3). The odds ratio for swimming up to 165 hours was 1.50 (95 percent CI: 0.98, 2.31) and for swimming more than 165 hours was 1.52 (95 percent CI: 0.99, 2.34) relative to never swimming in a pool (p-trend = 0.02).

We found limited evidence of multiplicative interaction between THM exposure and smoking. The odds ratio for average residential THM exposure of more than 49 relative to ≤8 μg/liter was 2.20 (95 percent CI: 0.31, 15.92) for male never smokers and 4.77 (95 percent CI: 1.30, 17.59) for current smokers. The p value of the interaction term between smoking (never vs. current) and residential THM exposure (below vs. above the median: 26 μg/liter) was 0.127.

Frequency of micronuclei was associated with THM exposure among the study controls, although, in most analyses, these results were not statistically significant. Women exposed to residential THM levels above the median (>26 μg/liter) had a 70 percent increased probability of having a frequency of micronuclei above the median (9/1,000) compared with those exposed to THM levels below 26 μg/liter. These results were adjusted for age and smoking status. Adjustment also for geographic region resulted in a higher, but unstable risk estimate (odds ratio = 4.77, 95 percent CI: 0.41, 54.96). We observed even higher associations for THM exposure through showering and bathing. The odds ratio adjusted for age and smoking status was 3.34 (95 percent CI: 0.90, 12.39) for exposure above 200 (μg/liter THMs) × (minutes/day), relative to below 200, during showering or bathing. Additional adjustment for geographic study area led to an odds ratio of 13.7 (95 percent CI: 1.39, 135). Similar results were observed for an analysis of micronuclei as a continuous variable (results not shown).

DISCUSSION

We found an increased risk of bladder cancer associated with estimates of DBP exposure from ingestion of drinking water, dermal absorption, and inhalation while showering, bathing, and swimming in pools. A doubling of the risk for bladder cancer was associated with exposure to DBP levels of about 50 μg/liter, commonly found in industrialized societies. Risks tended to be higher for exposure through showering, bathing, and swimming in pools compared with

drinking of water, but differences were small. Exposure misclassification is certainly the most important problem in retrospectively evaluating levels of drinking water contaminants. Sensitivity analyses using alternative exposure scenarios and analyses of current genotoxicity markers in exfoliated urothelial cells suggest that the observed increased risks are unlikely due to bias.

Extensive data on THMs and related variables were collected in the geographic study areas, as well as comprehensive individual data on lifetime water consumption and water-related habits. Doing so enabled us to estimate several exposure indices and to evaluate the robustness of the findings. The high repeatability of the questions on showering, bathing, and swimming supports the reliability of measuring these activities through interview. However, the assumptions used to model past THM levels certainly oversimplified the temporal and spatial variability of exposure within municipalities. This type of exposure misclassification is likely to be nondifferential and to bias risks toward the null. Inclusion of an ingestion exposure index at work (results not presented) did not add to the overall model possibly because we collected more limited information on water habits at work, or perhaps because exposure is more limited during time at work compared with home. Omitting the use of filters probably introduced a minor measurement error in the ingestion THM metric since the prevalence of filter use is expected to be very low (approximately 2.5 percent), as shown by preliminary data from a new study we are conducting in one of the study areas in Spain. Despite the available literature, the complexity of THM chemistry in hot beverages, including additional organic matter such as coffee and tea, complicates the modeling of THM variation. The impact on the measurement error of treating tea and coffee as plain water is difficult to predict.

The suggestion of an increased bladder cancer risk not only with ingestion but also through inhalation and dermal absorption is consistent with toxicologic data. Many of the most prevalent DBPs have been shown to have genotoxic and carcinogenic effects in animals (5–8) at high exposure levels. All chronic bioassays on carcinogenicity have administered these compounds via the digestive tract, and, to date, we know of no long-term study that has evaluated alternative exposure routes. However, toxicologic studies in animals and experimental studies in humans have shown that the most prevalent compounds (THMs) are absorbed primarily through the lungs or the skin (18, 19). Inhalation or dermal absorption may lead to a higher concentration directly in target organs (e.g., kidney, bladder, or colon), bypassing efficient detoxification steps in the liver that occur upon ingestion (21). Some enzymes, including GSTT1 (that metabolize brominated and chlorobrominated DBPs into biologically active metabolites), are expressed in these target organs.

The positive association observed between micronuclei frequency and THM levels provides evidence of an intermediate marker of effect for THM exposure and concurs with experimental data (22). The identification of an increased micronuclei frequency with high THM levels could be expected because many of the DBPs, including some THMs, haloacetic acids, and others, have been repeatedly shown to have genotoxic action (6, 7). These results are limited, however, by small numbers. A similar analysis in Australia did not find an association between micronuclei and chloroform levels (9), but models used to evaluate THM exposure were not comparable. In the Spanish study, the highest micronuclei frequency was associated with exposure from baths and showers rather than ingestion.

In this hospital-based case-control study, controls were matched to cases by age group, gender, and geographic area of residence. Since geographic area of residence was related to THM exposure but not to disease status, matching on this factor probably reduced statistical efficiency (widening confidence intervals) but did not introduce bias (23). Reporting bias is not likely to have occurred. Study subjects were unlikely to have had knowledge of possible links between water-related exposures and the disease; therefore, differential response to these questions from cases and controls was improbable. In the statistical analysis of THMs and water source, we included subjects with known exposure for at least 70 percent of the exposure window as a quality criterion of the exposure metrics. There was no evidence of bias due to selection of this subsample. A comparison of the included and excluded populations showed no statistically significant different proportion of exclusion among cases and controls, and the odds ratio for the main risk factor (smoking) was similar in both groups.

This study is one of the few evaluating the risk of bladder cancer associated with DBP exposure in non-North-American populations. We found results comparable with previous studies evaluating THM exposure in individuals (10, 11), and our results add considerably to the experimental and epidemiologic evidence showing that THMs and other DBPs are associated with an increased risk of cancer. In addition, our findings suggest for the first time that, besides ingestion, inhalation and dermal absorption of DBPs from household activities and swimming in pools may be associated with development of bladder cancer. The finding of increased risks associated with exposure through all routes is consistent with recent toxicologic data and animal bioassays. If confirmed elsewhere, this observation has significant public health implications in relation to preventing exposure to these water contaminants.

ACKNOWLEDGMENTS

This research was primarily supported by the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Division of Cancer Epidemiology and Genetics (NCI contract NO2-CP-11015). This project was also funded by the Spanish Ministry of Health (FIS/Spain 00/0745 and ISIII-GO3/174) and the European Union (BMH4-98-3243).

The authors thank Natalia Blanco and Marta Huguet for their help in collecting THM data from municipalities and the technical officers in the municipalities and treatment plants who completed the questionnaires. They also thank Robert C, Saal from Westat, Rockville, Maryland, and Leslie Carroll and Eric Boyd from IMS, Silver Spring, Maryland.

Participants from study centers in Spain: M. Sala, F. Fernández, A. Amorós, M. Torà, D. Puente, C. Murta, J. Fortuny, E. López, S. Hernández, and R. Jaramillo (Institut Municipal d'Investigació Mèdica and la Universitat Pompeu Fabra, Barcelona); J. Lloreta, S. Serrano, L. Ferrer, A. Gelabert, J. Carles, O. Bielsa, and K. Villadiego (Hospital del Mar and Universitat Autònoma de Barcelona, Barcelona); L. Cecchini, J. M. Saladié, and L. Ibarz (Hospital Germans Tries i Pujol, Badalona, Barcelona); M. Céspedes (Hospital de Sant Boi, Sant Boi, Barcelona); D. García, J. Pujadas, R. Hernando, A. Cabezuelo, C. Abad, A. Prera, and J. Prat (Centre Hospitalari Parc Taulí, Sabadell, Barcelona); M. Domènech, J. Badal, and J. Malet (Centre Hospitalari i Cardiològic, Manresa, Barcelona); J. Rodríguez de Vera and A. I. Martín (Hospital Universitario, La Laguna, Tenerife); J. Taño and F. Cáceres (Hospital La Candelaria, Santa Cruz, Tenerife); F. García-López, M. Ull, A. Teruel, E. Andrada, A. Bustos, A. Castillejo, and J. L. Soto (Hospital General Universitario de Elche, Universidad Miguel Hernández, Elche, Alicante); J. L. Guate, J. M. Lanzas, and J .Velasco (Hospital San Agustín, Avilés, Asturias); J. M. Fernández, J. J. Rodríguez, and A. Herrero (Hospital Central Covadonga, Oviedo, Asturias); R. Abascal and C. Manzano (Hospital Central General, Oviedo, Asturias); M. Rivas and M. Argüelles (Hospital de Cabueñes, Gijón, Asturias); M. Díaz, J. Sánchez, and O. González (Hospital de Jove, Gijón, Asturias); A. Mateos and V. Frade (Hospital de Cruz Roja, Gijón, Asturias); P. Muntañola and C. Pravia (Hospital Alvarez-Buylla, Mieres, Asturias); A. M. Huescar and F. Huergo (Hospital Jarrio, Coaña, Asturias); and J. Mosquera (Hospital Carmen y Severo Ochoa, Cangas, Asturias).

Conflict of interest: none declared.

REFERENCES

1. Cantor KP. Drinking water and cancer. Cancer Causes Control 1997;8:292–308.
2. Koivusalo M, Pukkala E, Vartiainen T, et al. Drinking water chlorination and cancer—a historical cohort study in Finland. Cancer Causes Control 1997;8:192–200.
3. Doyle TJ, Zheng W, Cerhan JR, et al. The association of drinking water source and chlorination by-products with cancer incidence among postmenopausal women in Iowa: a prospective cohort study. Am J Public Health 1997;87: 1168–76.
4. Cantor KP, Hoover R, Hartge P, et al. Bladder cancer, drinking water source, and tap water consumption: a case-control study. J Natl Cancer Inst 1987;79:1269–79.
5. Some chemicals that cause tumours of the kidney or urinary bladder in rodents, and some other substances. IARC monographs on the evaluation of carcinogenic risks to humans. Vol 73. Lyon, France: International Agency for Research on Cancer, 1999.
6. Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide. IARC monographs on the evaluation of carcinogenic risks to humans. Vol 71. Lyon, France: International Agency for Research on Cancer, 1999.
7. Some drinking water disinfectants and contaminants, including arsenic. IARC scientific publications. Vol 84. Lyon, France: International Agency for Research on Cancer, 2002.
8. Dry cleaning, some chlorinated solvents and other industrial chemicals. IARC monographs on the evaluation of carcinogenic risks to humans. Vol 63. Lyon, France: International Agency for Research on Cancer, 1995.
9. Ranmuthugala G, Pilotto L, Smith W, et al. Chlorinated drinking water and micronuclei in urinary bladder epithelial cells. Epidemiology 2003;14:617–22.
10. King WD, Marrett LD. Case-control study of bladder cancer and chlorination by-products in treated water (Ontario, Canada). Cancer Causes Control 1996;7:596–604.
11. Cantor KP, Lynch CF, Hildesheim ME, et al. Drinking water source and chlorination byproducts. I. Risk of bladder cancer. Epidemiology 1998;9:21–8.
12. Lynch CF, Woolson RF, O'Gorman T, et al. Chlorinated drinking water and bladder cancer: effect of misclassification on risk estimates. Arch Environ Health 1989;44:252–9.
13. Koivusalo M, Hakulinen T, Vartiainen T, et al. Drinking water mutagenicity and urinary tract cancers: a population-based case-control study in Finland. Am J Epidemiol 1998;148: 704–12.
14. Villanueva CM, Cantor KP, Cordier S, et al. Disinfection byproducts and bladder cancer. A pooled analysis. Epidemiology 2004;15:357–67.
15. Levesque B, Ayotte P, LeBlanc A, et al. Evaluation of dermal and respiratory chloroform exposure in humans. Environ Health Perspect 1994;102:1082–7.
16. Backer LC, Ashley DL, Bonin MA, et al. Household exposures to drinking water disinfection by-products: whole blood trihalomethane levels. J Expo Anal Environ Epidemiol 2000; 10:321–6.
17. Aggazzotti G, Fantuzzi G, Righi E, et al. Environmental and biological monitoring of chloroform in indoor swimming pools. J Chromatogr A 1995;710:181–90.
18. Nuckols JR, Ashley DL, Lyu C, et al. Influence of tap water quality and household water use activities on indoor air and internal dose levels of trihalomethanes. Environ Health Perspect 2005;113:863–70.
19. Whitaker H, Nieuwenhuijsen MJ, Best N. The relationship between water concentrations and individual uptake of chloroform: a simulation study. Environ Health Perspect 2003; 111:688–94.
20. Villanueva CM, Cantor KP, Grimalt JO, et al. Assessment of lifetime exposure to trihalomethanes through different routes. Occup Environ Med 2006;63:273–7.
21. Landi S, Naccarati A, Ross MK, et al. Induction of DNA strand breaks by trihalomethanes in primary human lung epithelial cells. Mutat Res 2003;538:41–50.
22. Torti VR, Cobb AJ, Wong VA, et al. Induction of micronuclei in wild-type and p53(+/−) transgenic mice by inhaled bromodichloromethane. Mutat Res 2002;520:171–8.
23. Rothman KJ, Greenland S, eds. Modern epidemiology. 2nd ed. Philadelphia, PA: Lippincott-Raven, 1998.