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Case-Control Study of Bladder Cancer and Exposure to Arsenic in Argentina

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Studies have found increased bladder cancer risks associated with high levels of arsenic in drinking water, but little information exists about risks at lower concentrations. Ecologic studies in Argentina have found increased bladder cancer mortality in Córdoba Province, where some wells are contaminated with moderate arsenic concentrations. This population-based bladder cancer case-control study in two Córdoba counties recruited 114 case-control pairs, matched on age, sex, and county, during 1996–2000. Water samples, particularly from wells, were obtained from subjects' current residences and residences in the last 40 years. Statistical analyses showed no evidence of associations with exposure estimates based on arsenic concentrations in drinking water. However, when well-water consumption per se was used as the exposure measure, time-window analyses suggested that use of well water more than 50 years before interview was associated with increased bladder cancer risk. This association was limited to ever smokers (odds ratio = 2.5, 95% confidence interval: 1.1, 5.5 for 51–70 years before interview), and the possibility that this association is due to chance cannot be excluded. This study suggests lower bladder cancer risks for arsenic than predicted from other studies but adds to evidence that the latency for arsenic-induced bladder cancers may be longer than previously thought.

arsenic; bladder neoplasms; case-control studies; water pollutants

Abbreviation: CI, confidence interval.

An association between chronic arsenic ingestion and internal cancers has been established (1–3). However, to date, epidemiologic studies have not produced convincing evidence of risks related to drinking-water concentrations of less than 100 μg As/liter. Estimation of risks associated with lower arsenic concentrations has relied on risk extrapolation, suggesting that the cancer risk at the US maximum contaminant level of 50 μg As/liter may be as high as 1 in 100 (2, 4). To establish scientifically valid arsenic exposure limits, there is a need to better characterize the dose-response relation at lower exposure levels by using studies with exposure measures for individual persons.

In the eastern part of Córdoba Province, Argentina, widespread arsenic contamination of drinking water supplies has been reported (5), as have elevated cancer rates (6–8). When the counties within Córdoba were classified as having "high," "medium," or "low" average levels of arsenic in their water supplies, mortality risks for bladder, lung, and kidney cancers during 1986–1991 were found to increase with increasing exposure (9, 10). The highest risks observed in this and other arsenic studies were for bladder cancer (9, 11,

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12). To follow up the previous ecologic study findings and characterize the dose-response relation between arsenic ingestion and bladder cancer, we conducted a case-control study in Córdoba Province.

The study protocol and informed consent procedures were reviewed and approved by appropriate institutional review boards in the United States and Argentina.

MATERIALS AND METHODS

Recruitment of cases and controls

There is no cancer registry in the study area. Therefore, a case-identification system involving all pathologists and urologists in the study counties and surrounding areas was established. We contacted these specialists regularly, seeking new cases. All identified incident transitional bladder-cell cancer cases aged 20–80 years and living in the contiguous counties of Union from 1996 to 2000 and Marcos Juarez from 1998 to 2000, considered in the previous ecologic study (9) to be "high-" and "medium"-exposure counties, respectively, were invited to participate. All cases of cancer were confirmed histologically. Cases who died before interview were not included in the study.

Controls, matched by county, sex, and year of birth, were selected from computerized voter registration lists by using a systematic method. Voter registration is compulsory in Argentina, and these lists are more than 99 percent complete. Potential cases and controls were sent a letter inviting study participation and were then contacted during residential visits.

Data collection

All cases and controls were administered standardized questionnaires during face-to-face interviews in their homes. Information sought included residential history, water sources at each residence, consumption of beverages (at the time of the interview and 20 and 40 years ago), and smoking, occupational, and medical histories. All subjects signed an informed consent agreement before interview.

Water samples were collected from each subject's current residence and as many of his or her residential sources within the previous 40 years as practicable, particularly from wells. Some wells were inaccessible because they had been closed. In such instances, whenever possible, a sample was collected from a nearby well thought to draw water from the same aquifer (a "proxy well"). Water samples were stored frozen at –20°C, transported to the United States on dry ice, and analyzed for arsenic content by graphite furnace atomic absorption spectroscopy, with a detection limit of 1 μg/liter. Analysts were blind to case or control status. Historical records of arsenic content were obtained for community water supplies.

Statistical analysis

For each participant, a year-by-year exposure profile was created from data on residential history, water and total fluid consumption, and water-source arsenic concentrations. In analyses in which only source-well data were used (i.e., no use of proxy-well data), arsenic levels from proxy wells were treated as missing data.

Water from municipal supplies was assumed to contain arsenic concentrations at the medians of the samples taken from each particular supply (collected at homes), unless more relevant historical data were available. Water samples were collected in the two study counties only. Arsenic concentrations in water consumed outside the study counties were assumed to be negligible.

Three arsenic exposure metrics were used. The first was the average concentration of arsenic in domestic water. The underlying assumption was that contrasts in arsenic concentrations would be much greater than contrasts in water consumption volumes and would dominate the associations. This metric was used in two ways. First, some analyses were conducted for the 5 highest years of exposure (whether or not they were continuous), irrespective of the water source. These analyses were restricted to exposures in years 6–40 preceding recruitment. This restriction was based mainly on the a priori assumption that the most recent exposures and exposures that occurred more than 40 years ago were unlikely to have contributed substantially to cancer risk. An alternative metric would have been a measure of cumulative exposure. However, cumulative exposure is correlated with age and is more likely to be affected by periods of missing arsenic data, which would particularly affect analyses in which data for proxy wells were excluded. With cumulative exposure indices, short periods of high exposure may be diluted by long periods of low exposure or by missing data assigned low arsenic values. For stratified analyses, arbitrarily selected cutpoints of 50, 100, and 200 μg/liter were used.

Second, water arsenic concentration was also used in the analysis of 10-year time windows of exposure. In this analysis, average arsenic concentration over the entire time window was used as the basis of exposure. The cutoff point in this analysis was 10 μg/liter, the drinking water standard for arsenic that will become effective in 2006 in the United States.

The second metric involved multiplying the time-weighted water arsenic concentration by a factor representing domestic daily water consumption volume and then dividing by the total daily fluid intake volume. We defined this metric as the fluid-intake-adjusted exposure index. Water and total fluid consumption volumes were calculated from questionnaire responses. The rationale for this metric was that incorporating total fluid intake would better represent bladder exposure because the other fluid consumed would dilute the arsenic in drinking water. We previously used a similar metric (13).

The third exposure metric was based on reported years of well-water consumption and did not involve actual arsenic measurements. We used this metric because we did not collect water samples from sources used only more than 40 years ago. This metric was used to carry out both a cumulative exposure analysis (years of well-water use) and a time-window analysis extending back further than 40 years. For the cumulative exposure analysis, the reference category was subjects reporting no use of well water during their lifetime

TABLE 1. Demographic and descriptive characteristics of bladder cancer cases and controls, Córdoba, Argentina, 1996–2000

Characteristic	Cases (n = 114)	Controls (n = 114)
Sex (no. (%))		
Male	94 (82)	94 (82)
Female	20 (18)	20 (18)
Age in years (mean (SD*))	68.9 (10.7)	68.3 (10.7)
No. of years of education (mean)	4.46	4.49
Smoking status† (no. (%))		
Ever smoked	85 (75)	63 (55)
Never smoked	29 (25)	51 (45)
Present daily fluid consumption in liters (mean (SD))		
Residential water	2.50 (1.04)	2.28 (1.02)
Total fluid	3.25 (1.14)	2.98 (1.08)
Mate con bombilla consumption (ever)‡ (no. (%))		
Yes (present mean daily intake in liters)	109 (96) (0.66)	103 (90) (0.67)
No	5 (4)	11 (10)
Mate cocido consumption (ever) (no. (%))		
Yes (present mean daily intake in liters)	80 (70) (0.23)	82 (72) (0.25)
No	34 (30)	32 (28)
No. of years of well-water consumption (mean (SD))§	25.7 (23.6)	25.6 (23.9)
Use of proxy-well data (% of all well years)	51.6	46.6
Time-weighted arsenic exposure levels (mean, median, range in μg/liter)¶		
Excluding proxy wells	20, 1.3, 0–212	45, 1.2, 0–997
Including proxy wells	38, 8.3, 0–685	60, 7.5, 0–917

* SD, standard deviation.
† Unadjusted odds ratio = 2.37, 95% confidence interval: 1.31, 4.33.
‡ Unadjusted odds ratio = 2.33, 95% confidence interval: 0.71, 8.82.
§ Adjusted for partial use of well water during some years.
¶ During years 6–40 prior to interview; years for which data were missing were excluded from the calculation.

(30 percent of cases and controls combined). Data for subjects who had used well water were dichotomized at the median duration of exposure (36 years). A time-window analysis compared subjects who had any well-water exposure within a time window with those who had none within that same window.

The main method of analysis was conditional logistic regression. All analyses were adjusted for factors shown in the preliminary analysis to have some association with bladder cancer: smoking, consumption of *mate con bombilla* (a beverage made from the herb *Ilex paraguariensis*; ever or never), and having more than an elementary school education. Some evidence exists that *mate* is a carcinogen (14), and it has been associated with bladder cancer (15). There are two main ways in which *mate* is consumed in Argentina: *mate con bombilla*, in which the infusion is consumed at near boiling temperature through a metal straw; and *mate cocido,* which is consumed more like ordinary tea. For most analyses, smoking was adjusted according to whether the subject was a current, former, or never smoker.

To investigate possible interactions between smoking and arsenic, separate analyses were carried out for ever and never smokers. For these analyses, to prevent loss of study power from elimination of case and control pairs discordant on smoking status, the match was broken and unconditional logistic regression, including adjustment for matching variables, was used. In these analyses, ever smokers were classified according to the maximum daily number of cigarettes they reported ever having smoked: 1–10, 11–20, or more than 20 cigarette equivalents per day. Cigars and pipes were treated as cigarette equivalents.

Time-window analyses for 10-year exposure periods were conducted according to previously described methods (13).

RESULTS

Of the 122 potential study cases still living when contact was made, eight were unwilling or were too unwell to participate, giving a participation rate of 93 percent. Twenty-eight of the potential controls sought were not located at the residential address recorded in the voter registration lists. Potentially, these persons had moved outside the study area and would not have been eligible as cases in the study even if diagnosed with bladder cancer. Of the remainder still living, 39 were unwilling or unable to participate. On that basis, the control participation rate was 75 percent. Thirteen bladder

TABLE 2. Multivariate-adjusted odds ratios, from conditional logistic regression analysis, for bladder cancer associated with average arsenic concentration in water over the 6–40 years prior to interview, bladder cancer case-control study, Córdoba, Argentina, 1996–2000

Exposure	Model 1: Excluding proxy wells*				Model 2: Including proxy wells			
	Cases (no.)	Controls (no.)	OR†	95% CI†	Cases (no.)	Controls (no.)	OR	95% CI
Arsenic (μg/liter)‡								
0–50	87	80	1.00		70	62	1.00	
51–100	8	8	1.11	0.3, 3.7	13	18	0.88	0.3, 2.3
101–200	13	13	0.81	0.3, 2.0	22	19	1.02	0.5, 2.3
>200	3	10	0.28	0.1, 1.4	9	15	0.60	0.2, 1.7
Smoking								
Never	28	50	1.00		29	51	1.00	
Current	28	23	2.75	1.1, 6.9	29	23	2.61	1.1, 6.3
Former	55	38	3.82	1.6, 9.3	56	40	3.41	1.4, 7.9
Mate con bombilla consumption								
Never	5	11	1.00		5	11	1.00	
Ever	106	100	2.04	0.6, 7.5	109	103	2.24	0.6, 8.1
Education§								
No	93	97	1.00		96	100	1.00	
Yes	18	14	1.37	0.5, 4.1	18	14	1.46	0.5, 4.3
Water consumption¶								
Below median	49	64	1.00		49	65	1.00	
Above median	62	47	1.50	0.8, 2.7	65	49	1.46	0.8, 2.7

* For two cases and one control, arsenic exposure data were derived from proxy wells only.
† OR, odds ratio; CI, confidence interval.
‡ Average arsenic concentration of 5 years of highest exposure during the period 6–40 years before interview.
§ Whether subjects had more than an elementary school education (up to 6–7 years).
¶ Refers to home tap-water consumption (based on reports of most recent consumption).

cancer cases and 13 potential controls were no longer alive when they were sought for study participation.

Table 1 shows descriptive characteristics of the 114 matched case-control pairs. Cases and controls were similar with respect to number of years of education, *mate cocido* consumption, and mean number of years of well-water consumption. Cases were more likely to have been smokers, to have consumed *mate con bombilla*, and, at the time of interview, to have consumed higher estimated volumes of fluid and water. Mean time-weighted arsenic concentrations in residential wells were higher for controls than for cases. However, median arsenic levels were similar. Although years of well-water use were similar for both cases and controls, there were slightly more years of proxy-well measurements for cases than for controls. Cases had lived in an average of 5.1 residences and controls in 4.7.

The highest arsenic concentrations were found in private wells. The concentration range for the 389 well-water samples was 0–3,033 μg/liter, with a mean of 164 μg/liter and a median of 101 μg/liter. For the 11 springwater samples, the range was 9–70 μg/liter, with a mean of 40 μg/liter and a median of 37 μg/liter. Results for rainwater, bottled water, and public water supplies were generally at or below the detection limit.

Table 2 presents results for two separate multivariate logistic regression models, based on average arsenic concentrations in water. In the first model, we used arsenic levels from source wells only (no proxy wells); the second model included proxy wells. Expected associations between bladder cancer risk and smoking occurred (16). Increased risks were found for *mate* consumption, but the confidence intervals were wide, reflecting the fact that there were few never users of *mate*. For arsenic exposure, no clear risk pattern emerged.

Table 3 shows adjusted odds ratios for the categories of arsenic exposure used in table 2 but with subjects stratified as ever smokers and never smokers. Again, no clear pattern of arsenic risk emerged. Surprisingly, there were reduced odds ratios in the highest exposure categories shown in both tables 2 and 3, but the confidence intervals were generally broad because of small numbers.

Table 4 shows associations found by using the fluid-intake-adjusted exposure metric. Exposure quartiles were defined on the basis of the frequency distribution of all participants, without regard to case or control status (17). Again, no clear patterns were apparent, and we found discrepancies depending on whether proxy-well results were included. When proxy wells were excluded, there was some evidence of an increasing risk for never smokers, although it

TABLE 3. Multivariate-adjusted odds ratios, from unconditional logistic regression analysis, for bladder cancer associated with average arsenic concentration in water over the 6–40 years prior to interview, according to smoking status, bladder cancer case-control study, Córdoba, Argentina, 1996–2000

Arsenic (μg/liter)*	Subjects who never smoked				Subjects who ever smoked			
	Cases (no.)	Controls (no.)	OR†,‡	95% CI†	Cases (no.)	Controls (no.)	OR‡,§	95% CI
Excluding proxy wells								
0–50	22	37	1.00		65	45	1.00	
51–100	2	4	1.05	0.2, 6.9	7	4	1.29	0.3, 5.0
101–200	3	5	1.10	0.2, 6.3	10	8	0.96	0.3, 3.0
>200	1	4	0.58	0.1, 6.2	2	6	0.17	0.0, 1.0
Including proxy wells								
0–50	19	25	1.00	0.1,2.3	51	37	1.00	
51–100	4	12	0.53	0.1, 3.1	9	6	1.22	0.4, 4.0
101–200	5	8	0.64	0.0, 2.7	17	11	1.27	0.5, 3.6
>200	1	6	0.25		8	9	0.57	0.2, 1.8

* Average arsenic concentration of 5 years of highest exposure during the period 6–40 years before interview.
† OR, odds ratio; CI, confidence interval.
‡ Adjusted for *mate con bombilla* consumption, education, and home tap-water consumption (as per table 2).
§ Adjusted for the highest daily number of cigarettes subjects reported ever having smoked.

disappeared when proxy-well measurements were included. However, the estimates were imprecise because of small numbers of never smokers.

Table 5 contains results for a time-window analysis based on average water arsenic concentration, in which the four decades immediately preceding interview were used as windows. A cutpoint of 10 μg/liter for average arsenic concentration in water over each of the time periods was used because of the limited number of subjects consuming water containing arsenic at the higher levels. For the analysis that excluded proxy wells, missing values were excluded from the calculation of mean arsenic concentration.

Whether or not proxy-well results were included, a similar pattern emerged. Risks, particularly for exposures in the period 20–40 years before interview, were elevated for nonsmokers but were reduced for smokers.

A cumulative data analysis was carried out by using number of years of well-water use as the exposure variable.

TABLE 4. Multivariate-adjusted odds ratios for bladder cancer associated with quartiles of the fluid-intake-adjusted arsenic exposure index over the 6–40 years prior to interview, according to smoking status, bladder cancer case-control study, Córdoba, Argentina, 1996–2000

Arsenic exposure quartile (μg/liter)*	All subjects†,‡				Subjects who never smoked†,§				Subjects who ever smoked†,§,¶			
	Cases (no.)	Controls (no.)	OR#	95% CI#	Cases (no.)	Controls (no.)	OR	95% CI	Cases (no.)	Controls (no.)	OR	95% CI
Excluding proxy-well measurements												
0–0.5	26	31	1.00		6	14	1.00		20	17	1.00	
0.6–1.2	28	28	0.97	0.4, 2.3	5	13	2.15	0.4, 11	23	15	1.19	0.5, 3.1
1.3–35	31	25	1.46	0.7, 3.3	11	11	4.03	0.9, 18	20	14	1.06	0.4, 2.8
>35	27	29	0.97	0.4, 2.3	6	12	2.27	0.4, 12	21	17	1.05	0.4, 2.8
Including proxy-well measurements												
0–1.0	34	23	1.00		9	10	1.00		25	13	1.00	
1.1–17	21	36	0.35	0.1, 0.9	4	14	0.36	0.1, 1.7	17	22	0.29	0.1, 0.8
18–80	32	25	0.90	0.3, 2.3	10	14	0.95	0.2, 3.9	22	11	0.88	0.3, 2.7
>80	27	30	0.46	0.2, 1.3	6	13	0.59	0.1, 2.9	21	17	0.46	0.2, 1.4

* Average of 5 years of highest exposure during the 6–40 years before interview multiplied by the estimated daily tap-water concentration divided by the estimated daily fluid consumption.
† Adjusted for ever consumption of *mate con bombilla* (ever, never) and whether subjects had more than an elementary school education.
‡ Conditional logistic regression analysis, adjusted for smoking (never, current, former), was used.
§ Unconditional logistic regression analysis was used.
¶ Adjusted for the highest daily number of cigarettes subjects reported ever having smoked.
# OR, odds ratio; CI, confidence interval.

TABLE 5. Multivariate-adjusted odds ratios for bladder cancer associated with arsenic exposure* during 10-year time windows for the 40 years prior to interview, according to smoking status, bladder cancer case-control study, Córdoba, Argentina, 1996–2000

Exposure time window†	All subjects‡,§				Subject who never smoked‡,¶				Subject who ever smoked‡,¶,#			
	Cases (no.)	Controls (no.)	OR**	95% CI**	Cases (no.)	Controls (no.)	OR	95% CI	Cases (no.)	Controls (no.)	OR	95% CI
Excluding proxy-well measurements												
1–10 years												
0–10 μg/liter	91	82	1.00		23	34	1.00		68	48	1.00	
>10 μg/liter	23	32	0.64	0.3, 1.3	6	17	0.52	0.2, 1.7	17	15	0.81	0.3, 1.9
11–20 years												
0–10 μg/liter	84	77	1.00		18	35	1.00		66	42	1.00	
>10 μg/liter	25	30	0.72	0.3, 1.5	9	13	1.63	0.5, 5.7	16	17	0.48	0.2, 1.2
21–30 years												
0–10 μg/liter	72	70	1.00		16	32	1.00		56	38	1.00	
>10 μg/liter	29	29	1.00	0.4, 2.3	11	12	1.94	0.6, 6.6	18	17	0.71	0.3, 1.8
31–40 years												
0–10 μg/liter	71	66	1.00		14	31	1.00		57	35	1.00	
>10 μg/liter	28	30	0.78	0.4, 1.7	11	10	2.98	0.8, 11	17	20	0.42	0.2, 1.1
Including proxy-well measurements												
1–10 years												
0–10 μg/liter	86	79	1.00		21	33	1.00		65	46	1.00	
>10 μg/liter	28	35	0.75	0.4, 1.4	8	18	0.65	0.2, 2.0	20	17	0.79	0.4, 1.8
11–20 years												
0–10 μg/liter	77	67	1.00		18	29	1.00		59	38	1.00	
>10 μg/liter	36	47	0.62	0.3, 1.3	10	22	0.66	0.2, 2.4	26	25	0.58	0.2, 1.4
21–30 years												
0–10 μg/liter	68	65	1.00		15	29	1.00		53	36	1.00	
>10 μg/liter	44	49	0.74	0.4, 1.5	13	22	1.46	0.5, 4.7	31	27	0.68	0.3, 1.6
31–40 years												
0–10 μg/liter	61	62	1.00		13	28	1.00		48	34	1.00	
>10 μg/liter	52	50	1.15	0.6, 2.2	15	22	1.83	0.6, 5.9	37	28	0.85	0.4, 1.9

* Average well-water arsenic concentration of >10 μg/liter vs. ≤10 μg/liter.
† Average arsenic concentration in drinking water during the time window.
‡ Adjusted for sex, year of birth, consumption of *mate con bombilla* (ever, never), and education.
§ Conditional logistic regression analysis, adjusted for smoking (never, current, former), was used.
¶ Unconditional logistic regression analysis was used.
# Adjusted for the highest daily number of cigarettes subjects reported ever having smoked.
** OR, odds ratio; CI, confidence interval.

For all subjects, the odds ratio for up to 36 years of well-water exposure (relative to none) was 1.05 (95 percent confidence interval (CI): 0.5, 2.2); for more than 36 years of exposure, the odds ratio was 1.26 (95 percent CI: 0.5, 3.1). For smokers, the corresponding odds ratios were 1.25 (95 percent CI: 0.5, 3.0) and 1.64 (95 percent CI: 0.5, 5.0).

Table 6 shows the results of an exposure time-window analysis in which we used well-water consumption during seven 10-year periods. Subjects were categorized as consuming any or no well water during each of the seven decades. For ever smokers, there was an upward trend in the odds ratios with increasing time before interview, peaking for exposures 51–60 years earlier (odds ratio = 2.65, 95 percent CI: 1.2, 5.8) but with a similarly high odds ratio for exposures 61–70 years ago (odds ratio = 2.54, 95 percent CI: 1.0, 6.4). Combining these periods yielded an odds ratio of 2.49 (95 percent CI: 1.1, 5.5) for exposure during the prior 51–70 years. For never smokers, no clear patterns emerged.

DISCUSSION

To date, only a few case-control studies have investigated associations between arsenic in water supplies and bladder cancer (11, 13, 18, 19). A clear association with arsenic exposure was found in the study from Taiwan (11) and possible associations in the other three, from the United States and Finland.

Previously, an ecologic study showed a relation between bladder cancer mortality and estimated arsenic levels in water supplies across Córdoba counties (9). Standardized mortality ratios for males in low-, medium-, and high-exposure counties were 0.80, 1.42, and 2.14, respectively. The corresponding standardized mortality ratios for females were 1.21, 1.58, and 1.82. If a true causal relation between bladder cancer and arsenic exposure were assumed, the relative risk estimates (odds ratios) obtained by using incident cases of bladder cancer and individual-level exposure data

TABLE 6. Multivariate-adjusted odds ratios for bladder cancer associated with years of well-water use, in 10-year time windows, by smoking status, bladder cancer case-control study, Córdoba, Argentina, 1996–2000

Consumption of well water*	All subjects†,‡				Subjects who never smoked†,§				Subjects who ever smoked†,§,¶			
	Cases (no.)	Controls (no.)	OR#	95% CI#	Cases (no.)	Controls (no.)	OR	95% CI	Cases (no.)	Controls (no.)	OR	95% CI
0–10 years												
No	83	78	1.00		20	34	1.00		63	44	1.00	
Yes	31	36	0.85	0.4, 1.7	9	17	1.00	0.3, 3.3	22	19	0.74	0.3, 1.7
11–20 years												
No	74	66	1.00		19	28	1.00		55	38	1.00	
Yes	40	48	0.75	0.3, 1.5	10	23	0.57	0.2, 2.0	30	25	0.77	0.3, 1.8
21–30 years												
No	65	58	1.00		16	24	1.00		49	34	1.00	
Yes	49	56	0.78	0.4, 1.6	13	27	1.00	0.3, 3.1	36	29	0.82	0.4, 1.8
31–40 years												
No	54	52	1.00		13	22	1.00		41	30	1.00	
Yes	59	61	1.02	0.5, 1.9	15	28	1.04	0.3, 3.1	44	33	0.84	0.4, 1.8
41–50 years												
No	47	48	1.00		12	19	1.00		35	29	1.00	
Yes	65	64	1.11	0.6, 2.1	15	30	0.77	0.3, 2.3	50	34	1.21	0.5, 2.7
51–60 years												
No	47	56	1.00		12	17	1.00		35	39	1.00	
Yes	63	54	1.59	0.8, 3.0	15	31	0.73	0.2, 2.2	48	23	2.65	1.2, 5.8
61–70 years												
No	52	61	1.00		15	26	1.00		37	35	1.00	
Yes	40	31	1.68	0.8, 3.4	10	16	1.28	0.4, 4.1	30	15	2.54	1.0, 6.4

* Indicates whether or not a well was the source of water for consumption any time during the time window before interview.
† Adjusted for sex, year of birth, consumption of *mate con bombilla* (ever, never), and education.
‡ Conditional logistic regression analysis, adjusted for smoking (never, current, former), was used.
§ Unconditional logistic regression analysis was used.
¶ Adjusted for the highest daily number of cigarettes subjects reported ever having smoked.
# OR, odds ratio; CI, confidence interval.

would have been expected to be higher than the standardized mortality ratios in the ecologic study, because only a proportion of the populations of the two counties would have been exposed to high levels of arsenic. Therefore, it is perhaps surprising that the present study found no association between estimated arsenic exposures and bladder cancer risk for cases diagnosed during 1996–2000. In fact, there was even a suggestion of a risk reduction at high arsenic levels (tables 2 and 3). However, a protective effect of high arsenic concentrations is not plausible, and these results are likely to be due to random variation associated with the small number of subjects for whom exposure levels were high.

One of the first considerations in interpreting any case-control study is selection bias. Controls were selected from voter registration lists, which are very complete. All cases in this study were found on the lists, providing confidence that controls represented the source population for the cases.

Controls had a lower participation rate compared with cases. For this difference to explain the results, controls who had lower historical arsenic exposures would need to be less likely to participate than cases with low exposures. We know of no reason why this might occur.

The other selection bias possibility involves cases. Without a tumor registry, the completeness of case ascertainment was uncertain, with potential bias if ascertainment were related to residential location and hence the likelihood of drinking from contaminated wells. Wells are located predominantly in rural areas. We contacted all pathologists and urologists in the area regularly throughout the study to identify cases. In addition, attempts were made to determine whether some patients went directly to major centers in the province, bypassing local medical services. We found no such evidence but cannot exclude the possibility. It seems likely that any such cases would have been wealthier and lived in urban areas. If so, they would have been less likely to have been exposed to arsenic, and the bias would have increased the association with arsenic rather than reducing it.

Several other reasons are possible for the discrepancy in findings between this study and the previous ecologic study (9). First, the carcinogenic effects of arsenic may no longer be occurring in the study population because of improvements in water quality and the passage of time. Between the ecologic and case-control studies, there was approximately a decade of difference in the periods in which outcomes occurred. Since bladder cancer cases often live some time after diagnosis, mortality in 1986–1990 reflects bladder cancer incidence further back (perhaps 5–10 years) in time, thus lengthening the true comparison period between the two

studies. In males at least, we found some evidence of decreased mortality in the two high-exposure counties of Córdoba (including Union) after 1991. The standardized mortality ratio for bladder cancer in males changed from 2.14 (95 percent CI: 1.78, 2.53) in 1986–1991 to 1.71 (95 percent CI: 1.34, 2.16) in 1992–1995, the latest years for which data were available. The corresponding standardized mortality ratios for females were 1.81 (95 percent CI: 1.19, 2.64) and 1.91 (95 percent CI: 1.15, 2.99) (C. Hopenhayn, unpublished results).

Second, although this study's attempts to characterize individual arsenic exposure were more elaborate than those used in most other studies, some exposure misclassification was inevitable because of the unavoidable reliance on current water measurements. These uncertainties apply particularly to the wells, which provided the main exposure contrasts. The uncertainties increased the further back in time that the sampled well was used as a water source and are of three main types. First, there is the possibility that arsenic levels in well water have changed over time. If so, recent measures would not accurately reflect arsenic levels in water consumed decades ago. Second, although it was assumed, for practical reasons, that arsenic exposures outside the study area would have been negligible, some of these exposures could have been high because the study area did not encompass all of the Córdoba counties with raised arsenic levels. However, only 10 percent of lifetime years for cases and 14 percent for controls were spent outside the two study counties. Third, it is possible that some recent water sampling may have been from the wrong wells. At interview, subjects were asked to recount their residential histories, including addresses. Efforts were made to locate former addresses and to obtain water samples from the original source wells. However, because study subjects did not accompany the sampling team, the possibility cannot be excluded that some incorrect wells were sampled. In analyses in which proxy wells were used, there is additional uncertainty regarding the assumption that arsenic levels in water from nearby wells would have been comparable. We did not have data to confirm this, although efforts were made to use proxy wells that accessed the same aquifers.

It is also possible that, by the time water samples were collected, wells with higher arsenic levels had been sealed. This could have happened if there had been prior arsenic measurements for the wells. If so, we may have obtained samples from wells with lower arsenic levels, which would also have biased odds ratios toward the null value. However, data in table 1 indicate that proxy wells tended to have higher arsenic levels than original source wells, arguing against such a bias.

Considering all of the potential sources of exposure misclassification, the overall effect would have been to reduce associations between arsenic exposure and bladder cancer.

An advantage to using well-water consumption as an indirect measure of arsenic exposure is that it was possible to explore associations with exposures further back in time; water sample collection was focused on the 40 years immediately preceding study recruitment. At study outset, it seemed likely that the latency for arsenic-induced cancers would be less than 40 years, although this period has not yet been well characterized (3). A time-window analysis based on well-water use suggested an association in the period 50–70 years before diagnosis (table 6).

We cannot exclude the possibility that this result was a consequence of the number of statistical comparisons carried out in this study (20), which might also account for the apparently elevated risks for never smokers shown in tables 4 and 5. However, the limitation of the association shown in table 6 to smokers is consistent with findings from previous case-control studies of arsenic and bladder cancer (13, 18), one of which found evidence for latencies of more than 30 years (13). In addition to these older studies, a recently completed bladder cancer case-control study in areas of California and Nevada with elevated arsenic levels in drinking water also found increased risks for smokers only for those arsenic exposures that occurred more than 40 years ago (odds ratio = 3.7, 95 percent CI: 1.4, 9.4) (19). Corresponding risks were not elevated for never smokers.

Although a cancer latency of more than 50 years seems long, it is not unprecedented. In an investigation of the association between chlorinated water source consumption and bladder cancer, Cantor et al. (21) found the highest odds ratios to be associated with 60 years or more of consumption of chlorinated surface water. Latencies of arsenic-induced bladder cancers longer than 50 years would provide a further possible reason why associations with arsenic exposures in the four decades before diagnosis were not seen in this study. Studies from Taiwan provide evidence that arsenic-induced bladder cancer latencies may be greater than 40 years (11, 22).

As previously hypothesized, limitation of the association to smokers provides a possible reason why arsenic administered alone does not seem to cause cancer in animal toxicology studies (13).

If arsenic-associated bladder cancers cause death more rapidly than bladder cancers not associated with arsenic exposure, this could also explain the apparent incompatibility between the results of the earlier ecologic study (9) and this study. However, even if this were so, the incidence (and odds ratios from case-control studies) of bladder cancer would also need to be increased, although not as much as if the level of aggressiveness of arsenic-induced bladder cancers was similar to that of bladder cancers with other causes. In this study, we excluded patients who had died. If arsenic caused a more aggressive form of bladder cancer, resulting in early death, a selection bias would have been introduced that pushed odds ratios toward the null value. There is limited, but indirect evidence for an increased level of aggressiveness of arsenic-associated bladder cancers. An investigation of the chromosomal stability of tumor specimens from cases in this study and bladder cancer specimens from Chilean patients exposed to water with much higher levels of arsenic (~500 μg/liter) found a progressive increase in the number of chromosomal changes associated with increasing arsenic exposure, particularly in grade 2 and 3 tumors (23). Whether such changes cause increased mortality is presently unknown. However, a study from Taiwan suggested that arsenic-associated bladder cancer

might have a lower fatality rate than bladder cancers with other causes (24).

A final possible reason why we may not have found an association with arsenic exposure level is that the associations found in the ecologic study were confounded by some other exposure. However, this possibility seems unlikely. Such an exposure would need to be very strongly correlated at a county level with water arsenic concentrations and also cause the cancers associated with arsenic. If such a confounder exists, it is not clear what it could be.

Considered overall, the findings in this study are perhaps surprising, but they suggest lower bladder cancer risks than predicted from other studies. The most likely explanation may be a longer latency for arsenic-induced bladder cancer than previously appreciated. To test this hypothesis, it would be necessary to collect exposure information for a longer period of subjects' history. We are currently conducting a lung cancer case-control study in the same region of Argentina and, to the extent practicable, are extending our collection of exposure data into the period more than 40 years before interview.

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