

# Nitrate in Public Water Supplies and Risk of Bladder Cancer

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**Background.** Nitrate is a precursor compound in the formation of N-nitroso compounds, most of which are potent animal carcinogens. N-nitroso compounds and their precursors have not been extensively evaluated as bladder cancer risk factors.

**Methods.** We conducted a population-based case-control study of bladder cancer in Iowa. Cases were men and women newly diagnosed with bladder cancer in 1986–1989. Nitrate data for Iowa public water supplies were sparse before the 1960s. To reduce misclassification by unknown nitrate levels, we included only those who used public supplies with nitrate data for 70% or more of their person-years since 1960 (808 cases, 1259 controls).

**Results.** Among controls, the median average nitrate level for their Iowa residences with public water supplies was 1.3 mg/

liter nitrate-nitrogen (interquartile range = 0.6–3.0). After adjustment for confounders, we found no increased risk of bladder cancer with increasing average nitrate levels in drinking water; the highest quartile odds ratio for women was 0.8 (95% confidence interval = 0.4–0.8), and for men 0.5 (0.4–0.8). We observed no association among those with high water nitrate exposure (>median) and low (<median) vitamin C intake compared with those who had low water nitrate and high vitamin C intake.

**Conclusions.** Our data suggest that long-term exposure to nitrate in drinking water at levels in this study (90th percentile 5.5 mg/liter nitrate-nitrogen) is not associated with risk of bladder cancer.

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**Key words:** bladder neoplasms, water supply, water pollution, chemical, nitrates, nitrites, case-control studies.

Nitrate is a precursor in the formation of N-nitroso compounds (NOC), most of which are potent animal carcinogens. Schistosomiasis, bilharzia and other chronic bladder infections are associated with increased bladder cancer risk, with formation of NOCs in the bladder suggested as the likely carcinogenic mechanism.<sup>1–4</sup> NOC formation occurs in healthy individuals in the stomach by the chemical interaction of nitrite with amine and amide precursors. Nitrite is predominantly derived from ingested nitrate, although preserved meats and other foods contain nitrite. Vegetables are the major source

of nitrate exposure when drinking water levels are low; however, drinking water contributes substantially to exposure when nitrate levels are at or near the U.S. Environmental Protection Agency Maximum Contaminant Level (MCL) of 10 mg/liter nitrate-nitrogen (nitrate-N). Ingestion of drinking water with nitrate levels above this level has been associated with an increased formation of N-nitrosoproline, a marker for the endogenous formation of NOC.<sup>5</sup>

A recent cohort study of older women in Iowa<sup>6</sup> found an increased risk of bladder cancer among women using public water supplies with >2.5 mg/liter nitrate-N. To our knowledge, no other analytic study has evaluated drinking water nitrate as a bladder cancer risk factor.

Nitrate levels in many Iowa public supplies have been elevated for several decades, attributable primarily to high-nitrogen fertilizer use.<sup>7</sup> We conducted a population-based case-control study of bladder cancer in Iowa originally to evaluate disinfection byproducts and risk of several cancers. The detailed information on drinking water sources, tap water intake and diet allowed us to evaluate nitrate as a risk factor for bladder cancer.

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## Methods

### Study Population

The study population has been described previously.<sup>8</sup> We identified bladder cancer cases through the Iowa State Health Registry. Eligible cases were age 40–85, residents of Iowa, newly diagnosed with histologically confirmed bladder cancer in 1986–1989, and without a previous diagnosis of a malignant neoplasm except for basal or squamous cell carcinoma of the skin. We included cases of *in situ* and invasive bladder cancer.

Controls were selected from state driver's license records for ages <65 and from U.S. Health Care Financing Administrations (HCFA) records for ages ≥65. Controls were frequency-matched to the case distribution at a different ratio in two study periods (1986–1987; 1988–1989) as described previously.<sup>8</sup>

### Interviews

The study was approved by an institutional review board at the University of Iowa. After obtaining informed consent by telephone, we sent participants a postal questionnaire with questions about demographic factors, smoking, occupations and lifetime residences and drinking-water sources. For residences of ≥1 year, we obtained the duration of residence and the primary drinking-water source (private well, public supply, bottled water or another source). Respondents were asked to report the depth of their well in feet (<50, 50–150, 151–250, 251–500, ≥501).

We used a 55-item food-frequency questionnaire to obtain usual adult intake of foods and beverages. We asked about the frequency of intake of beverages and soups made with tap water consumed at home and away. A small percent of water was consumed away from home, and this percent was similar for cases and controls. For men the median among controls was 10% (interquartile range [IQR] = 0–21) and for women the median was 3% (IQR = 0–11). Work and residential water sources were highly correlated; therefore, water nitrate intake was computed using total intake and residential nitrate levels.

If respondents were hesitant to participate they were offered an alternative 15-minute telephone interview that included questions essential to the water analyses. If key information about water supplies, cigarette smoking and fluid intake was missing on postal questionnaires, we retrieved additional data by telephone.

Of 1716 eligible cases identified, 1,452 (85%) participated by completing a mail survey (N = 1,309), a full-length telephone interview (N = 87), or an abbreviated telephone interview (N = 56). Of the 999 controls from state driver's license listings, 817 (82%) participated. Of the 2034 selected HCFA controls, 1,617 (80%) participated. A total of 2164 controls completed

a mail questionnaire, 102 a full telephone interview, and 168 an abbreviated telephone interview.

Based on the information from the initial telephone contact, we mailed a self- or proxy-questionnaire. Among cases, we sent 156 proxy questionnaires (11%); among controls, we sent only 2. At the end of both questionnaire types, respondents were asked to indicate who assisted them in completing the questionnaire. On the self-questionnaire, "self" was also listed. On some of the self-questionnaires, the "self" response was not circled but another choice was indicated. It is possible that some self-respondents neglected to circle the self-response. If we make the conservative assumption that all of these questionnaires were completed by proxies, the number of proxy interviews would be 317 (22%) among cases and 243 (10%) among controls.

### Drinking-Water Nitrate Exposure Assessment

The Iowa Center for Health Effects of Environmental Contaminants maintains a database of historical public water supply and treatment data for utilities serving towns with populations of at least 400. Data include the specific sources of the water (aquifers, rivers, reservoirs, etc.), the years sources operated and the estimated percent of the water from each source by year.<sup>9</sup> Historical water quality data were compiled for utilities in the database and were linked to specific water sources whenever possible. For smaller utilities (serving populations of at least 25), the Iowa Center maintains nitrate data collected under the Safe Drinking Water Act beginning in the late 1970s.

We used nitrate measurement data for samples taken in the public water supply distribution system. These are samples taken after treatment at a faucet served by the public water supply. Distribution system samples are more likely to reflect the water quality at the taps of homes compared with samples taken at individual sources such as wells or intakes from rivers.

Nitrate levels from multiple samples taken in the same year were averaged and linked to an individual's public water supply history by year and town. We could match utility names to towns because of the linkage established by the Iowa Center.

Nitrate measurements were available for some Iowa public water supplies beginning in 1934; however, measurements were sparse before the 1960s. Of the 610 Iowa towns where bladder cancer cases and controls lived, only 55 had two or more years with measurements from 1934 through 1959. For years when a town had no nitrate measurements, an average level was imputed using a weighted average of the annual averages in adjacent years. Greater weight was given to years closer in time to the year with no measurements. The imputation weights were 1 for 1–2 years from a year with no data, 0.75 for 3–4 years, 0.5 for 5–6 years and 0.25 for

**TABLE 1. Median Number of Years with Nitrate Measurements\* and Interquartile Range (IQR) for Residences with Iowa Public Water Supplies, by Decade, for Bladder Cancer Cases and Controls**

Decade or Time Period	Cases		Controls	
	Median	IQR	Median	IQR
1950–1959	1	0–5	1	0–5
1960–1969	2	1–9	2	1–8
1970–1979	3	2–4	3	2–4
1980–1988	3	2–5	3	2–6

\* Measurements from finished water samples only.

7–9 years. If there were no data within 10 years, the nitrate level was considered to be unknown. Levels were imputed only for the period of years when a city’s sources and the percent each supplied did not vary by more than 10%. We imputed average nitrate levels using slightly different weights and also calculated average nitrate levels based on measurement data only. All methods gave results similar to those for the imputation method described, and only these results are presented.

Cases and controls had a similar number of person-years with nitrate measurements from 1950 onwards (Table 1). If a participant reported using bottled water as their primary drinking-water source, we counted the years and assigned a low-nitrate level (0.5 mg nitrate-N/liter). We computed person years by type of water source (eg, Iowa private wells, Iowa public supplies or bottled water) from the year of birth to the year of diagnosis for cases (1986–1989). For controls we computed person-years to the year of interview or 1989, whichever date was earlier.

There is no regulatory requirement to monitor nitrate levels in private wells and levels can be substantially higher than public supplies. To reduce misclassification, we limited the public water-supply nitrate analyses to those who had public supplies with an estimated nitrate level (included bottled water) for 70% or more of their person-years since 1960 (808 cases, 1259 controls). We chose 1960 because nitrate measurements were sparse previously and nitrate contamination of public water supply was uncommon before the beginning of widespread nitrogen fertilizer use in the 1950s.<sup>7</sup> Those excluded from the analyses were those with more than 30% of their person-years from 1960 in the following categories: 1) residing outside of Iowa; 2) residing in Iowa but using private wells; 3) residing in Iowa towns with unknown nitrate levels in the public supplies; and 4) having an unknown water source history. Any combination that summed to more than 30% of their person-years from 1960 resulted in exclusion from the analyses. Restricting the analysis to those who had 80% and 90% of their person-years with an estimated nitrate level did not change our results.

We computed an average public water-supply nitrate level from 1960. We also computed the number of years that an individual had an Iowa public water supply with nitrate levels  $\geq 10$  mg/liter nitrate-N; all years  $\geq 10$  mg/liter were after 1960.

Nitrate levels in shallow private wells in Iowa (less than 50 feet deep) can be substantially higher than deeper wells.<sup>10</sup> We evaluated the duration of private well use in Iowa overall and duration of use of wells less than 50 feet deep.

**Dietary Analysis**

Nutrient intakes were calculated by multiplying the frequency of consumption of each food by the nutrient contents and the sex-specific portion sizes derived from the second National Health and Nutrition Evaluation Survey (NHANES II).<sup>11</sup> The nitrate and nitrite contents of the questionnaire food items were determined from the literature.<sup>12–16</sup>

The food-frequency questionnaire was not designed to estimate levels of nitrite and nitrate in foods. However, the major sources of nitrite (preserved meats) and nitrate (certain vegetables) were included. Exceptions were the high nitrate vegetables: beets, celery, rhubarb and radishes; however, these were not likely to have been eaten frequently based on data from another Midwestern population.<sup>17</sup> For questions that included multiple foods with differing nitrate or nitrite levels (eg, hot dogs, bratwurst, lunch meats) a weighted average of the median or mean levels for each food was computed using weights based on the age- and sex-specific intakes as reported in NHANES II.

We included in the dietary analyses the 1118 cases (77%) and 1886 controls (77%) who had fewer than six missing or “don’t know” responses. The frequency of intake for the missing foods was imputed using the sex-specific median intake values for controls.

**Data Analysis**

We computed maximum likelihood estimates of the odds ratios (ORs) using unconditional multiple logistic regression to estimate the association between the exposure metrics and bladder cancer.<sup>18</sup> We adjusted the ORs for known or suspected risk factors for bladder cancer including age (categories: 40–54, 55–64, 65–74, 75–85 years), cigarette smoking (never, ex-smoker, current smokers), years of education (5 categories) and duration of chlorinated surface water use (5 levels). Because the ratio of controls to cases differed by study period, we included a variable for period in all models. Further adjustment for smoking using a six-level variable, employment in a high risk occupation (determined from a review of the literature<sup>19</sup>), and ever having a bladder or kidney infection did not change the risk estimates, and so results adjusted for these factors are not

TABLE 2. Characteristics of the Study Population Included and Excluded from the Public Water Supply Nitrate Analysis\*

	Population in Nitrate Analysis		Population Excluded	
	Cases (N = 808)	Controls (N = 1,259)	Cases (N = 644)	Controls (N = 1,175)
Water supply use, years: Mean $\pm$ SD				
Public water supply in Iowa	50 $\pm$ 15	50 $\pm$ 15	20 $\pm$ 20	18 $\pm$ 20
Private well in Iowa	13 $\pm$ 15	14 $\pm$ 15	42 $\pm$ 26	46 $\pm$ 25
Bottled water years	0.11 $\pm$ 1	0.23 $\pm$ 2.2	0.06 $\pm$ 0.6	0.08 $\pm$ 0.8
Chlorinated surface water use, no. of years: No. and (%)				
None	386 (48)	628 (50)	523 (81)	944 (80)
1-19	234 (29)	348 (28)	109 (17)	200 (17)
20-39	95 (12)	140 (11)	12 (2)	28 (2)
$\geq$ 40	93 (12)	143 (11)	0	3 (0.3)
Age, years: No.				
40-54	86 (11)	122 (10)	67 (10)	120 (10)
55-64	182 (22)	291 (23)	134 (21)	257 (22)
65-74	312 (39)	473 (38)	240 (37)	432 (37)
75-85	228 (28)	373 (30)	203 (32)	366 (31)
Sex: No. and (%)				
Male	622 (77)	788 (63)	513 (80)	813 (69)
Female	186 (23)	471 (37)	131 (20)	362 (31)
Study period: No. and (%)				
1986-1987	319 (40)	792 (63)	239 (37)	711 (60)
1988-1989	489 (60)	467 (37)	405 (63)	464 (40)
Cigarettes, packs/day: No. and (%)				
Never-smoker	161 (20)	540 (43)	171 (27)	567 (48)
Former, <1	103 (13)	139 (11)	72 (11)	154 (13)
Former, $\geq$ 1	231 (29)	303 (24)	170 (27)	263 (22)
Current, <1	67 (8)	87 (7)	48 (8)	54 (5)
Current, 1-<2	168 (21)	147 (12)	127 (20)	104 (9)
Current, $\geq$ 2	69 (9)	41 (3)	52 (8)	32 (3)
Missing	9	2	4	1
Years as farmer: No. and (%)				
None	682 (84)	1108 (88)	357 (55)	635 (54)
1-<10	73 (9)	89 (7)	61 (10)	68 (6)
10+	53 (7)	62 (5)	226 (35)	472 (40)
High-risk occupations, ever: No. and (%)				
Men	351 (56)	207 (40)	318 (40)	220 (27)
Women	33 (18)	53 (11)	23 (18)	38 (11)
Bladder/kidney infection, ever: No. and (%)				
No	380 (47)	898 (71)	313 (49)	859 (73)
Yes	383 (47)	268 (21)	292 (45)	226 (19)
Missing	45 (6)	93 (7)	39 (6)	90 (8)
Dietary intake (mg/day): Mean $\pm$ SD				
Nitrate	97 $\pm$ 75	98 $\pm$ 63	92 $\pm$ 62	100 $\pm$ 66
Nitrite	1.11 $\pm$ 0.60	0.97 $\pm$ 0.48	1.17 $\pm$ 0.57	1.13 $\pm$ 0.58
Vitamin C	141 $\pm$ 101	141 $\pm$ 97	138 $\pm$ 93	144 $\pm$ 91

\* Percentages do not always total 100 because of missing information.

presented. Adjustment for respondent type did not change the results nor did the exclusion of proxies from the analyses.

We evaluated the association of drinking water nitrate levels and bladder cancer risk stratified by dietary vitamin C intake and smoking status, which are factors known to affect endogenous nitrosation. We stratified the analysis by ever having a bladder or kidney infection to evaluate a possible interaction between this bladder cancer risk factor and drinking water nitrate exposure.

## Results

Table 2 shows characteristics of the study population included in and excluded from the PWS nitrate analyses. Cases and controls included in the PWS nitrate analyses

had used Iowa public water supplies for an average of 50 years, compared with 20 and 18 years for excluded cases and controls, respectively. Conversely, the average duration of private well use among those included in the analysis was substantially less than among those excluded. The mean number of years of bottled water use was low (<1) among cases and controls. A greater proportion of those in the public water-supply analysis group had used chlorinated surface water supplies compared with excluded respondents. Compared with those excluded, the analysis group smoked slightly more, were less likely to be farmers and were more likely to have held a high risk occupation (men only).

Table 3 shows ORs for bladder cancer by quartile of average nitrate in Iowa public water supplies from 1960. Compared with the lowest quartile, increasing quartiles

TABLE 3. Association of Quartiles of Average Nitrate in Public Water Supplies from 1960 Onwards with Bladder Cancer, by Sex\*

Average Nitrate Mg/L Nitrate-N	Men				Average Nitrate Mg/L Nitrate-N	Women			
	Cases	Controls	OR	95% CI		Cases	Controls	OR	95% CI
<0.6†	171	197	1.0		<0.67	57	117	1.0	
0.6–<1.40	171	196	0.9	0.6–1.2	0.67–<1.18	44	118	0.7	0.4–1.2
1.40–<3.09	164	195	0.8	0.6–1.1	1.18–<2.48	38	118	0.6	0.3–1.1
≥3.09	116	200	0.5	0.4–0.8	≥2.48	47	118	0.8	0.4–1.3

\* Analysis only included those with ≥70% of their residential person-years from 1960 onwards with public water nitrate estimates. Adjusted for age, education, cigarette smoking, years with chlorinated surface water and study period.  
 † Reference category.

were not associated with an increased bladder cancer risk among men or women. The distribution of nitrate levels was slightly lower among women than among men. Men in the highest quartile of average nitrate had a substantially lower risk of bladder cancer. Comparing those in the 90th percentile of average nitrate with the lowest quartile showed no inverse trend (≥5.5 mg/liter: OR = 0.7; 95% confidence interval [CI] = 0.4–1.0). Average nitrate metrics were also computed without counting the last 5 or 10 years of person-years and the results were similar to those for the metrics computed over the entire exposure period. We evaluated average nitrate levels stratified by age (<60 years, ≥60 years). The association with bladder cancer risk was the same in both age groups.

We evaluated exposure to public water supply water nitrate levels of ≥10 mg/liter nitrate-N (the MCL) (Table 4). Few subjects were ever exposed at this level and total years of exposure were few. There was no association of years at a public water supply of ≥10 mg/liter with risk of bladder cancer.

There was no evidence of interaction when we stratified the average nitrate level by the median intake of dietary vitamin C (Table 5). Stratification by tap water intake among controls (median: 2.1 liters/day), ever having a bladder or kidney infection, or smoking status (never, past, current) showed no evidence of interaction (data not shown).

We multiplied the estimated tap-water intake by the average nitrate level to estimate the daily nitrate intake from water. Among men, the median intake was 2.6-mg/

day nitrate-N (IQR = 1.2–6.3) and among women the median was 2.4 mg/day (IQR = 1.2–5.4). Quartiles of daily nitrate intake from drinking water showed a similar association with risk of bladder cancer as the average nitrate level (data not shown). We computed the contribution of drinking water nitrate to the usual nitrate intake from water and dietary sources. Water nitrate contributed a small percent of total nitrate intake in men (median among controls = 13%; IQR = 6–26) and women (median among controls = 11%; IQR = 6–23).

We had no information about nitrate levels in private wells; however, we evaluated the duration of private well use and the duration of shallow well use. Shallow wells in Iowa tend to have higher nitrate levels.<sup>10</sup> Compared with those with no private well use, increasing years of well use overall and years of shallow well use showed a weak inverse association with risk among men, whereas there was no association among women (data not shown).

Dietary nitrate and nitrite intakes showed no association with risk of bladder cancer (Table 6). We evaluated intakes from animal and plant sources separately because animal sources lack nitrosation inhibitors and contain amines, which are NOC precursors (data not shown). Among men, the highest quartile of nitrite from plant sources was associated with a modest elevated risk (OR = 1.3; CI = 1.0–1.6) but there was no trend with increasing intake and there was no association for animal nitrite, the greater contributor to dietary nitrite intake. Among women, there was no association for animal or plant sources of nitrite. Among men, plant

TABLE 4. Association of Number of Years with a Public Water Supply at or Above 10 mg/L Nitrate-N after 1934 with Bladder Cancer, by Sex\*

Years	Men				Cases	Women			
	Cases	Controls	OR	95% CI		Controls	OR	95% CI	
0†	513	618	1.0		146	388	1.0		
1	26	34	0.8	0.4–1.3	12	21	1.8	0.8–4.3	
2	69	104	0.7	0.5–1.1	22	48	1.0	0.5–1.9	
3+	14	32	0.5	0.3–1.0	6	14	0.9	0.3–2.8	

\* Analysis only included those with ≥70% of their residential person-years from 1960 onwards with public water nitrate estimates. Adjusted for age group, cigarette smoking, years with chlorinated surface water, education and study period.  
 † Reference category.

TABLE 5. Odds Ratios for Bladder Cancer by Average Nitrate Level in Public Water Supplies and Level in Vitamin C Intake\*

Drinking Water Nitrate (mg Nitrate-N/L)	Vitamin C							
	≥130 mg/Day				<130 mg/Day			
	Cases	Controls	OR	95% CI	Cases	Controls	OR	95% CI
<1.25†	169	232	1.0		171	251	0.8	0.6–1.1
≥1.25	134	265	0.7	0.5–1.0	158	246	0.7	0.5–0.9

\* Included only those with an adequate dietary history (<6 missing foods) and with ≥70% of their residential person-years from 1960 onwards with public water nitrate estimates. Adjusted for age, gender, cigarette smoking, duration chlorinated surface water, education and study period.

† Reference category.

and animal nitrate intakes were not associated with risk. Among women, intake of animal nitrate was associated with elevated ORs (for the highest quartile: OR = 1.5; CI = 0.9–2.4), but showed no trend with increasing intake.

## Discussion

We found no increased risk of bladder cancer with increasing average nitrate levels in Iowa public water supplies. Only a small percentage of the study population had more than a few years of exposure to nitrate levels above the maximum contaminant level (10 mg/liter nitrate-N) and this measure of exposure was not associated with risk. The results were not modified by the level of intake of vitamin C (an inhibitor of endogenous nitrosation), the amount of tap water, or ever having had a bladder infection.

Ecologic studies in Valencia, Spain<sup>20</sup> and Ontario, Canada<sup>21</sup> found no association between bladder cancer rates and nitrate levels in water supplies. Another ecologic analysis in Spain<sup>22</sup> found a positive association with nitrate levels, based on small numbers. All of these studies had limitations including the ecologic study design, use of mortality data, or exposure estimates concurrent with the period of cancer incidence.

A cohort study of older women in Iowa by Weyer *et al.*<sup>6</sup> found a significant 2.8-fold elevated risk of bladder

cancer among those with public water supply nitrate levels above 2.5 mg/liter nitrate-N; however, there was not a monotonic trend with increasing nitrate level. The distribution of public water-supply nitrate levels in that study was almost identical to ours; however, there were several differences in our methods of computing average nitrate exposure. Weyer *et al.*<sup>6</sup> computed an average based only on the woman's residence in 1989, after excluding women with residences of less than 10 years. We computed an average using all Iowa residences from 1960 onwards. Weyer *et al.* excluded 47 public water supplies for which greater than 10% of the water supply came from more than one groundwater aquifer, whereas we did not.

To determine if differences in our metrics could explain the differences between our findings, we computed a metric in the same way as Weyer<sup>6</sup> did (*ie*, among those who lived at their last residence at least 10 years and excluding public water supplies analogous to the 47 they excluded). The metrics were strongly correlated and our ORs computed using the Weyer metric were almost identical to our original results. Thus, differences between our exposure assessment methods cannot explain the differences in our findings.

The use of monitoring data for assessing exposure to nitrate has several limitations. Distribution samples taken at one or more taps quarterly or annually (as is required) may not adequately reflect exposure if there is

TABLE 6. Odds Ratios for Bladder Cancer by Quartiles of Dietary Intakes of Nitrate and Nitrite, by Sex\*

Men 867 cases, 1,247 controls			Women 251 cases, 639 controls		
Mg/Day	OR	95% CI	Mg/Day	OR	95% CI
Dietary nitrate (mg/day)			Dietary nitrate (mg/day)		
<59	1.0		<62	1.0	
59–<84	0.8	0.7–1.1	62–<90	1.2	0.8–1.9
84–<119	0.9	0.7–1.2	90–<127	0.9	0.5–1.4
≥119	0.9	0.7–1.1	≥127	0.8	0.5–1.3
Dietary nitrite (mg/day)			Dietary nitrite (mg/day)		
<0.81	1.0		<0.58	1.0	
0.81–<1.06	1.1	0.9–1.4	0.58–<0.75	1.0	0.6–1.5
1.06–<1.39	1.2	0.9–1.5	0.75–<0.98	0.8	0.5–1.3
≥1.39	1.2	0.9–1.6	>0.98	1.0	0.7–1.6

\* Analysis only included those with an adequate dietary history (<6 missing foods). Adjusted for age, cigarette smoking, education, duration of chlorinated surface water use study period.

† Reference category.

substantial seasonal or spatial variation. Spatial variation across a distribution system can occur when wells with different nitrate levels are pumped directly into the system at different locations. Wells are more likely to have differing nitrate levels if they come from different sources (*ie*, well fields of varying depth). Our exclusion of towns for which less than 90% of water came from a single source (47 public water supplies) should have reduced misclassification of nitrate exposure.

Private wells often have higher nitrate levels than public supplies. A survey of private well-water users in Iowa found that 35% of wells less than 50 feet deep had nitrate levels exceeding the maximum contaminant level.<sup>10</sup> Years of shallow well use showed a weak inverse association with risk among men but not women in our study. The duration of private well use was strongly correlated with the duration of farming and it was not possible to adjust for farming duration. Occupational surveys indicate that farmers have a lower risk of bladder cancer compared with the general population, which has been hypothesized to be attributable to lower rates of smoking among farmers.<sup>23,24</sup> Adjustment for smoking did not appreciably change the association we observed; however, uncontrolled residual confounding by smoking or other factors associated with farming may explain the association we observed.

A case-control study in Canada<sup>25</sup> found an approximately two-fold increased risk of bladder cancer among men who used nonpublic water sources, but no association among women. In the cohort study in Iowa<sup>6</sup> private well use was associated with a 30% elevated risk of bladder cancer.

We found no consistent associations with animal or plant sources of nitrate and nitrite and risk of bladder cancer. In contrast, Weyer *et al.*<sup>6</sup> found elevated risks of bladder cancer among women for all intake quartiles of dietary nitrate intake compared with the lowest quartile. Wilkens *et al.*<sup>26</sup> found a positive association between intake of nitrite and NOC among Japanese men but not other ethnic groups. A case-control study by Howe *et al.*<sup>25</sup> in Canada found no association between intake of preserved meats and bladder cancer risk.

Vegetables are the main sources of dietary nitrate; however, most vegetables contain vitamin C and polyphenols, inhibitors of endogenous nitrosation. Therefore, dietary nitrate may not result in substantial formation of NOC. Dietary sources of nitrite usually contribute a small percentage (approximately 10%) of gastric nitrite; the large majority comes from endogenous reduction of ingested nitrate to nitrite.<sup>12</sup> Drinking water can contribute the majority of nitrate intake when levels are close to the maximum contaminant level.<sup>27</sup> In our study, drinking water nitrate contributed a fairly small percent of total nitrate intake for most individuals.

Strengths of our analysis include the high response rates among cases and controls, the large sample size (including both men and women), a lifetime water-source history, the availability of historical nitrate levels for Iowa public water supplies and our ability to evaluate effect modification by vitamin C intake, smoking and bladder infections.

Our analysis of PWS nitrate levels in the public water supply was limited to the most recent 3 decades of nitrate exposure because of infrequent measurements earlier. Our results should be interpreted with caution because of the lengthy induction period often observed for bladder cancer.<sup>19</sup> Longer exposure periods may be associated with bladder cancer risk such as has been observed for chlorination byproducts.<sup>8,28,29</sup> Our study could not evaluate risk at nitrate levels above the maximum contaminant level because of the lack of exposure data for private wells and the infrequent exposure above this level among public water-supply users. Further, the large majority of the population using public water supplies had average nitrate levels that were <5 mg/liter, half the maximum contaminant level.

In contrast to previous studies, we had a lifetime history of well use and information about well depth. Well depth is an important predictive factor for nitrate levels in Iowa and other areas of the United States.<sup>10,30</sup> However, other factors can also be important determinants of water quality, including the well construction, location with respect to nitrogen point sources (such as septic tanks and animal feedlots) and characteristics of the aquifer in which the well is located.<sup>31,32</sup> Lack of information about these factors likely resulted in substantial misclassification of nitrate exposure in our analysis of private well duration.

Bacterially mediated endogenous nitrosation occurs in the bladder<sup>2-4</sup> and specific NOCs are bladder carcinogens in animals.<sup>33</sup> However, in healthy individuals NOCs are primarily formed in the stomach. Nitrosamines must be metabolized before they are biologically active and nitrosamides are chemically unstable, acting at the site of formation. Nitrosamides, formed in the bladder under conditions of bacterial or other infections, may be more relevant to bladder cancer etiology. In summary, although it is biologically plausible that drinking-water nitrate intake is a risk factor for bladder cancer, we found no evidence that nitrate levels below about 5 mg/liter were associated with risk. Our study had limited power to evaluate higher exposure levels. Because of the few analytic studies that have evaluated drinking water nitrate and bladder cancer risk, more research is needed to further evaluate this hypothesis. Studies in populations with higher exposure levels are warranted.

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