

Nitrate in Public Water Supplies and the Risk of Colon and Rectum Cancers

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Background: Nitrate is a widespread contaminant of drinking water, but its potential health effects are unclear. In the body, nitrate is reduced to nitrite, which can react with amines and amides by nitrosation to form N-nitroso compounds, known animal carcinogens. N-nitroso compound formation is inhibited by certain nutrients, such as vitamin C, and increased by meat intake.

Methods: We investigated the association of nitrate in public water supplies with incident colon and rectum cancers in a case-control study conducted in Iowa from 1986 to 1989. Nitrate levels in Iowa towns were linked to the participants' water source histories. We focused our analyses on the period from 1960 onward, during which nitrate measurements were more frequent, and we restricted analyses to those persons with public water supplies that had nitrate data (actual or imputed) for greater than 70% of this time period (376 colon cancer cases, 338 rectum cancer cases, and 1244 controls).

Results: There were negligible overall associations of colon or rectum cancers with measures of nitrate in public water supplies, including average nitrate and the number of years with elevated average nitrate levels. For more than 10 years with average nitrate greater than 5 mg/L, the odds ratio (OR) for colon cancer was 1.2 (95% confidence interval [CI] = 0.9–1.6) and for rectum the OR was 1.1 (CI = 0.7–1.5). However, nitrate exposure (>10 years with average nitrate >5 mg/L) was associated with increased colon cancer risk among subgroups with low vitamin C intake (OR = 2.0; CI = 1.2–3.3) and high meat intake (OR = 2.2; CI = 1.4–3.6). These patterns were not observed for rectum cancer.

Conclusions: Our analyses suggest that any increased risk of colon cancer associated with nitrate in public water supplies might occur only among susceptible subpopulations.

Key Words: colon, rectum, nitrate, drinking water, N-nitroso compounds

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Nitrate is a widespread environmental contaminant resulting from the use of nitrogen fertilizers in agricultural areas and from human and animal waste from sewage disposal systems and livestock facilities. Because high exposure can cause methemoglobinemia in infants, nitrate has been regulated under the Safe Drinking Water Act (SDWA) at a maximum contaminant level (MCL) of 10 mg/L nitrate–nitrogen (ie, the total amount of nitrogen in the nitrate form) in public water supplies.¹ However, the effectiveness of this regulatory limit for preventing other health outcomes such as cancer has not been adequately studied.

Ordinarily, most human intake of nitrate is from consumption of vegetables, but more than 50% of nitrate intake can come from drinking water when levels are above the MCL.^{2,3} In the human body, nitrate is reduced to nitrite, primarily by oral bacteria.⁴ Nitrite can then react by nitrosation with amines and amides in the stomach and the gut to form N-nitroso compounds,⁵ many of which are animal carcinogens.^{6,7} Although there is an extensive body of evidence showing the carcinogenicity of N-nitroso compounds in animals, the human data are limited and equivocal.⁷

There is great interindividual variability in the production of N-nitroso compounds mediated by several known factors. Endogenous production of nitrate occurs in inflammatory bowel disease and other immunostimulatory conditions, subsequently resulting in increased production of N-nitroso compounds.^{7–9} A number of different colonic bacteria are able to catalyze formation of N-nitroso compounds,^{10,11} although the significance of the healthy colon as a site for nitrosation is unclear.¹² Nitrosation reactions are inhibited by vitamin C, as well as vitamin E and some carotenoids,^{7,13–15} resulting in lower production of N-nitroso compounds in the stomach. It is unclear, however, whether these compounds are available to inhibit nitrosation in the colon.¹⁴ Meat intake has been positively associated with fecal concentration of

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N-nitroso compounds in controlled experiments,^{16–19} which could be the result of any of several factors, including higher levels of available amines as nitrosatable precursors. Smoking has also been shown to increase nitrosation.²⁰

After administering nitrate to rats via drinking water, concentrations of N-nitroso compounds in the large intestine were higher than in the stomach, and there was a significant positive correlation between formation of N-nitroso compounds and drinking water nitrate level.¹¹ In humans, nitrate levels in drinking water have been associated with excreted nitrate and N-nitrosoproline levels in urine,^{3,21} and nitrate administered via drinking water has been directly related to concentration of total N-nitroso compounds in feces.²² These results demonstrate a contribution of drinking water to overall nitrosation and suggest that nitrate intake can be used as a surrogate measure for exposure of target tissues to N-nitroso compounds.

In Iowa, elevated nitrate levels have been detected in both groundwater and surface drinking water sources. The potential for nitrate contamination of groundwater depends on nitrogen application rates and on aquifer vulnerability, which is determined by soil characteristics and aquifer configuration. Data on these factors indicate that nitrate contamination of groundwater is likely to be high in parts of Iowa.²³ A survey by the United States Geological Survey (USGS) in eastern Iowa found that nitrate levels were greater than the MCL in almost 50% of samples from streams that drain areas with intensive row crop agriculture,²⁴ indicating high nitrogen input into surface water sources. We investigated the association of nitrate in public water supplies with the risk of colon and rectum cancers in a case-control study conducted in Iowa.

METHODS

We studied nitrate concentrations in public water supplies in relation to the risk of colon and rectum cancers. Our analysis was carried out within a larger case-control study of 6 cancer sites (colon, rectum, bladder, brain, kidney, and pancreas), which was conducted with the primary objective of evaluating drinking water chlorination byproducts as risk factors for cancer. Details of the study design can be found elsewhere.^{25–27}

Study Population

Eligible cases were Iowa residents, age 40 to 85 years, who were newly diagnosed with colon cancer in 1987 or rectum cancer in 1986 or 1987. Cases were identified from the Iowa Cancer Registry (the statewide tumor registry), supplemented by a rapid reporting system during 1987. Of those eligible, we recruited 685 colon cancer cases and 655 rectum cancer cases. A common set of 2434 controls was recruited from 1986 through 1990, identified from driver's license records (for those under age 65) and Health Care

Financing Administration listings (for those age 65 years or older). Controls were frequency-matched to the overall case group (all types of cancers combined) by age (in 5-year age groups) and sex. Participation was 86% for colon and rectum cancers and 80% for controls.

Interviews

We mailed a questionnaire to participants to obtain detailed information on demographic factors, smoking history, diet, occupational history, and other lifestyle and medical factors. We also collected a lifetime residential history, documenting the city and state of each residence since birth and the primary source of drinking water (community supply, private well, bottled water, and other). We asked the amount of tap water consumed, including water used in beverages or foods made with tap water (eg, coffee, tea, iced tea, fruit juices, fruit drinks, soups), for both the usual water source at home and water sources outside the home. For participants who did not respond to the questionnaire, we offered a 15-minute telephone interview with items central to the analysis of water quality and consumption.

Nitrate in Public Water Supplies

Details of the nitrate exposure assessment in public water supplies were described previously.²⁸ Briefly, we obtained data from the Center for Health Effects of Environmental Contaminants on nitrate levels for Iowa towns for the years 1934 through 1988.²⁹ Where data were missing, nitrate levels were imputed by calculating a weighted average of the town's measurements during the period when the water sources were constant; the weights reflected the length of time from the actual measurements. Work and home sources of drinking water were highly correlated; therefore, nitrate intake was estimated using residential nitrate source levels. The yearly average nitrate levels in Iowa towns were linked to participants' residential histories. Participants who reported using bottled water as their primary source for the year were assigned a low value for nitrate (0.5 mg/L nitrate–nitrogen).

Because the frequency of measurement data increased during the study period,²⁸ we focused our analysis of nitrate levels on the time period from 1960 onward. We included in the analysis participants for whom more than 70% of their person-time since 1960 had nitrate data coverage (actual or imputed). Each person's average nitrate level was calculated as the average of actual or imputed annual nitrate levels from 1960 until the year of diagnosis for cases (1986 or 1987) or until 1987 for controls. Other measures of interest were the number of years the average level was greater than 5 mg/L or 10 mg/L nitrate–nitrogen. This analysis included 376 colon cancer cases (55% of total), 338 rectum cancer cases (52% of total), and 1244 controls (51% of total). We excluded participants for whom 30% or more of person-years since 1960 had

any combination of: residing outside of Iowa, residing in Iowa but using private wells, residing in Iowa towns with unknown nitrate levels in the public water supply, and unknown water source history.

Nutrient Intake

A 55-item food frequency questionnaire was used to obtain information on usual adult intake of foods, groups of foods, and beverages, including beer, wine, and liquor. Participants were asked to exclude any changes in their diet during the last couple of years. Data on the frequency of vitamin supplement use was collected for multivitamins and individual vitamin C, A, and E supplements. Dietary intakes of vitamin C, nitrate, and nitrite were calculated as previously described²⁸ by multiplying the frequency of consumption of each food by the nutrient content^{30–35} and the sex-specific portion sizes,³⁰ and summing levels of the nutrient across the various food items. Although the questionnaire was not designed to estimate intake of foods containing nitrate and nitrite, the major sources of nitrate (certain vegetables) and nitrite (preserved meats) were included. Nutrient variables were calculated for participants if they had fewer than 6 missing or “don’t know” responses; data on nutrient intakes were available for 516 colon cancer cases (75%), 485 rectum cancer cases (74%), and 1886 controls (78%).

Statistical Analyses

To estimate the association between each nitrate measure in public water supplies and colon or rectum cancer, we calculated odds ratios (OR) and 95% confidence intervals (CI) using unconditional logistic regression adjusting for the matching factors of age and sex. Nitrate–nitrogen measures in drinking water were examined by the following categories: average level (≤ 1 mg/L, >1 to 3 mg/L, >3 to 5 mg/L, >5 mg/L), years with average level above 5 mg/L (<1 year, 1 to 10 years, >10 years), and years with average level above 10 mg/L (<1 year, 1 or more years). We evaluated several factors as potential confounders of the association between drinking water nitrate levels and colon and rectum cancers based on previous associations with either cancer in our study^{36,37} or elsewhere. These potential confounders included education, body mass index, current and past smoking status, self-reported history of colitis or other bowel inflammation, first-degree relative with cancer, physical activity level, duration served by chlorinated surface water, average population size of residences, dietary vitamin C intake, and total servings per year of fruit, vegetables, high-fiber foods, meat, and red meat. Only duration of chlorinated surface water proved to confound the nitrate–rectum cancer associations; therefore, indicator variables for increasing categories of duration of chlorinated surface water were included in all models for rectum cancer.

We evaluated modification of the estimated effects of nitrate in public water supplies by factors known to increase nitrosation, namely, low vitamin C intake (below the median intake among controls), high meat intake (above the median intake among controls), smoking (ever smoked), and a self-reported history of bowel inflammation. We also evaluated effect modification by factors related to longer fecal transit time, namely, low volume of total beverages consumed per day (below the median amount among controls) and low intake of high-fiber foods (below the median intake among controls). Multiplicative interaction was tested using interaction terms for the categorical variables in logistic regression models; *P* values for interaction terms less than 0.10 were considered indicative of effect modification on the multiplicative scale.

We conducted further analyses to determine whether certain subgroups influenced the results. Nitrate levels are often high in water from private wells in agricultural areas.³⁸ However, we did not have data on nitrate levels in private wells, resulting in potential misclassification of nitrate intake among participants with long-term well use. Participants included in the drinking water nitrate analyses reported an average of 13.7 years of private well use. All analyses were rerun after excluding participants who used private wells for longer than 20 years over their lifetime. Participants who were residents of Des Moines during the entire analysis period of 1960 onward (approximately 9% of the nitrate analysis population) comprised 44% to 58% of the high exposure categories. Because long-term Des Moines residence was associated with elevated colon and decreased rectum cancer risks, we conducted subanalyses excluding these participants to evaluate potential confounding of our results.

We assessed dietary intake of nitrate and nitrite as risk factors for colon and rectum cancers by comparing the highest quartile (calculated using the control distribution) with the lowest quartile for dietary nitrate, dietary nitrite, dietary nitrite from animal sources, dietary nitrite from vegetable sources, and the percentage of total nitrate intake (both dietary and drinking water intake) from public water supplies. In addition, we estimated the risks associated with use of a private well or use of a shallow private well (<50 feet deep) for longer than 20 years.

RESULTS

As expected, participants included in the analyses of nitrate in public water supplies differed from the total study population in having a greater number of years at residences served by public water supplies, fewer years at residences using private wells, and a greater number of years with chlorinated surface water (Table 1). On average, controls included in drinking water nitrate analyses ($N = 1244$) were more likely than controls in the total

TABLE 1. Characteristics of the Study Population and of the Subgroup Included in Analyses of Public Water System Nitrate*

	Total Study Population			Subset Included in Analyses of Public Water Supply Nitrate†		
	Colon Cancer Cases (n = 685)	Rectum Cancer Cases (n = 655)	Controls (n = 2434)	Colon Cancer Cases (n = 376)	Rectum Cancer Cases (n = 338)	Controls (n = 1244)
Age (years); mean (SD)	69.0 (9.3)	67.4 (9.9)	68.3 (9.9)	68.7 (9)	68.2 (9)	68.1 (10)
Years at public water supplies; mean (SD)	40.3 (24.8)	37.9 (24.7)	37.7 (24.4)	54.1 (15)	52.9 (15)	52.9 (15)
Years with chlorinated surface water; mean (SD)	20.7 (18.5)	22.8 (20.4)	19.8 (18.3)	23.4 (19)	26.5 (21)	23.5 (19)
Years at private well; mean (SD)	27.4 (25.0)	28.6 (25.0)	29.0 (25.4)	13.6 (14)	14.8 (15)	13.7 (15)
Years used bottled water as primary drinking water source; mean (SD)	0.1 (0.8)	0.02 (0.3)	0.1 (1.6)	0.1 (1)	0.04 (1)	0.2 (2)
Years used unknown drinking water source; mean (SD)	1.5 (6.9)	1.1 (6.1)	0.5 (3.8)	1.5 (6)	0.9 (4)	0.4 (2)
Sex						
Male	347 (51)	388 (59)	1601 (66)	193 (51)	194 (57)	775 (62)
Female	338 (49)	267 (41)	833 (34)	183 (49)	144 (43)	469 (38)
Educational level						
Less than high school	242 (35)	249 (38)	829 (34)	126 (34)	123 (36)	377 (30)
High school graduate	281 (41)	237 (36)	922 (38)	154 (41)	121 (36)	465 (37)
Some college or more	153 (22)	159 (24)	668 (27)	90 (24)	88 (26)	393 (32)
Average population size of residences						
<2500	267 (39)	261 (40)	947 (39)	75 (20)	72 (21)	248 (20)
2500 to <10,000	151 (22)	163 (25)	590 (24)	86 (23)	85 (25)	313 (25)
10 to <50,000	156 (23)	135 (21)	551 (23)	120 (32)	106 (31)	402 (32)
≥50,000	111 (16)	95 (15)	344 (14)	95 (25)	75 (22)	281 (23)
Body mass index (kg/m ²)						
<20	30 (4)	33 (5)	126 (5)	19 (5)	22 (7)	80 (6)
20 to <25	235 (34)	239 (37)	921 (38)	135 (36)	122 (36)	486 (39)
25 to <30	260 (38)	228 (35)	924 (38)	135 (36)	120 (36)	447 (36)
≥30	79 (12)	84 (13)	264 (14)	38 (10)	40 (12)	132 (11)
Cigarette smoking						
Never	325 (47)	295 (45)	1107 (45)	151 (40)	125 (37)	531 (43)
Ever	360 (53)	360 (55)	1327 (55)	225 (60)	213 (63)	713 (57)
History of bowel inflammation						
No	479 (70)	418 (64)	1995 (82)	260 (69)	226 (67)	1150 (92)
Yes	131 (19)	172 (26)	254 (10)	71 (19)	82 (24)	138 (11)
Beverage consumption						
<Median (2.6 L/day)	347 (51)	297 (45)	1190 (49)	193 (51)	159 (47)	581 (47)
≥Median	278 (41)	313 (48)	1190 (49)	153 (41)	154 (46)	630 (51)
Vitamin C intake from diet						
≤Median (131.8 mg/day)	294 (43)	246 (38)	943 (39)	160 (43)	129 (38)	495 (40)
≥Median	222 (32)	239 (37)	943 (39)	120 (32)	120 (36)	487 (39)
High-fiber foods intake						
<Median (1.4 servings/day)	298 (44)	245 (37)	949 (39)	161 (43)	129 (38)	508 (41)
≥Median	220 (32)	244 (37)	948 (39)	120 (32)	121 (36)	478 (38)
Meat intake						
<Median (1.5 servings/day)	261 (38)	234 (36)	975 (40)	149 (40)	126 (37)	576 (46)
≥Median	281 (41)	275 (42)	975 (40)	147 (39)	135 (40)	438 (35)

*Number (%), except where otherwise indicated. Percentages do not total to 100% where there are missing data.

†Included participants with public water system nitrate data covering >70% of their person-years.

study population (N = 2434) to be female, more highly educated, and to have lived in more densely populated areas over their lifetimes. In addition, they had lower body mass index, lower meat consumption, and were more likely to have ever smoked. Average nitrate levels in public water supplies since 1960 were generally low (Fig.

1). Almost half of the control participants had average nitrate–nitrogen levels lower than 1 mg/L, and only 5 control participants (<1%) had average nitrate–nitrogen levels greater than the MCL of 10 mg/L.

Average drinking water nitrate level since 1960 was negligibly associated with colon and rectum cancers (Table

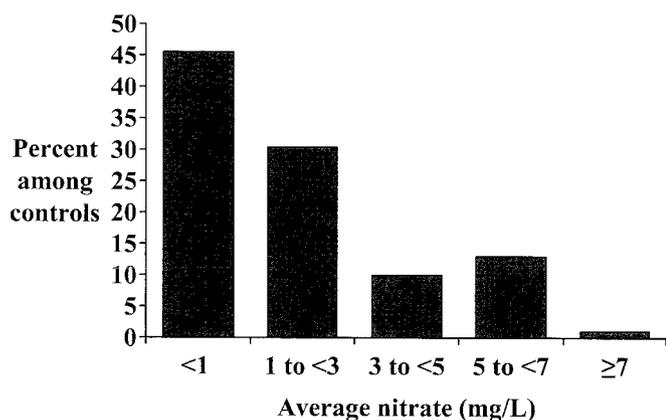


FIGURE 1. Average public water supply nitrate levels since 1960 among control participants.

2). There were slight decreased risks associated with levels in the middle-range values of average nitrate–nitrogen (from 3 to 5 mg/L). There were similar observations in the analysis of number of years with average nitrate–nitrogen levels greater than 5.0 mg/L. Average nitrate–nitrogen level greater than 10 mg/L for 1 year or longer was not associated with either cancer, and there was insufficient variability to evaluate the risk associated with longer time periods with levels above the MCL.

Analyses of the joint and individual effects of drinking water nitrate and factors associated with increased nitrosation suggested potential effect modification of the nitrate associ-

ations with colon cancer, but not rectum cancer (Tables 3 and 4). The results for years with nitrate–nitrogen greater than 5 mg/L are presented here; similar results were obtained in these analyses for the other measures of drinking water nitrate. There was a suggested interaction ($P < 0.10$ for multiplicative interaction term) between drinking water nitrate and dietary vitamin C intake, in that participants with longer than 10 years average nitrate–nitrogen level greater than 5 mg/L and dietary vitamin C intake below the median had a 2-fold increased risk of colon cancer (OR = 2.0; CI = 1.2–3.3), whereas those with similar nitrate history whose dietary vitamin C intake was above the median had no increased risk (OR = 1.1; CI = 0.7–1.9). When we included information on consumption of vitamin C supplements, similar results were observed (results not shown). A positive interaction was also observed ($P < 0.05$) between high meat intake and longer duration nitrate–nitrogen levels greater than 5 mg/L. There was no indicated effect modification with having ever smoked. Participants with a history of bowel inflammation were at increased risk of colon cancer; there was a greater risk among those with 0 to 10 years duration nitrate–nitrogen levels above 5 mg/L (OR = 2.1; CI = 1.1–3.9) compared with those with no years elevated PWS nitrate levels (OR = 1.5; CI = 1.0–2.3); however, there was no indication of interaction for the highest-duration group.

Lower total beverage consumption was associated with slightly increased risks of colon and rectum cancers, and intake of high-fiber foods was associated with colon cancer (Tables 3 and 4). There was some indication of interaction of these factors

TABLE 2. Association of Public Water System Nitrate Level Since 1960 and the Risk of Cancers of the Colon and Rectum*

	Colon Cancer (n = 376)		Rectum Cancer (n = 338)		Controls (n = 1244)
	No.	OR† (CI)	No.	OR† (CI)	No.
Average nitrate					
≤1 mg/L‡	172	1.0	154	1.0	566
>1 to ≤3 mg/L	116	1.0 (0.8–1.3)	98	0.8 (0.6–1.1)	380
>3 to ≤5 mg/L	27	0.7 (0.4–1.1)	30	0.7 (0.5–1.2)	124
>5 mg/L	61	1.2 (0.8–1.7)	56	1.2 (0.8–1.8)	174
Number of years with average nitrate >5 mg/L					
0 years‡	240	1.0	222	1.0	783
1 to ≤10 years	65	0.8 (0.6–1.1)	57	0.8 (0.6–1.1)	263
>10 years	71	1.2 (0.9–1.6)	59	1.1 (0.7–1.5)	198
Number of years with Average nitrate >10 mg/L					
0 years‡	300	1.0	272	1.0	1007
1 year or longer	76	1.1 (0.8–1.4)	66	1.0 (0.7–1.4)	237

*Nitrate measured as nitrate–nitrogen. Included participants with public water system nitrate data covering >70% of their person-years.

†All estimates are adjusted for the frequency-matched factors of age and sex, and estimates for rectum cancer are additionally adjusted for years served with chlorinated surface water.

‡Reference category.

TABLE 3. Colon Cancer Risk Associated With Joint Exposures of Average Public Water System Nitrate Level >5 mg/L Since 1960 and Various Dietary or Medical Risk Factors*

	Years With Nitrate Level >5 mg/L					
	0		1–10		>10	
	Cases/ Controls	OR [†] (CI)	Cases/ Controls	OR [†] (CI)	Cases/ Controls	OR [†] (CI)
Vitamin C intake from diet						
Above median (131.8 mg/day)	74/302	1.0 [‡]	22/98	0.9 (0.5–1.5)	24/87	1.1 (0.7–1.9)
Below median	97/310	1.4 (1.0–1.9)	28/109	1.1 (0.7–1.9)	35/76	2.0 (1.2–3.3) [§]
Meat intake						
Below median (1.5 servings/day)	98/354	1.0 [‡]	25/117	0.7 (0.5–1.2)	26/105	0.9 (0.6–1.5)
Above median	82/277	1.2 (0.8–1.6)	30/99	1.2 (0.7–1.9)	35/62	2.2 (1.4–3.6)
Ever smoked						
No	94/342	1.0 [‡]	32/110	1.0 (0.6–1.6)	25/79	1.2 (0.7–1.9)
Yes	146/441	1.5 (1.1–2.0)	33/153	0.9 (0.6–1.5)	46/119	1.7 (1.1–2.7)
History of bowel inflammation						
No	172/630	1.0 [‡]	41/218	0.7 (0.5–1.0)	47/167	1.1 (0.7–1.5)
Yes	39/88	1.5 (1.0–2.3)	17/27	2.1 (1.1–3.9) [§]	15/23	2.2 (1.1–4.3)
Beverage consumption [¶]						
Above median (2.6 L/day)	100/407	1.0 [‡]	28/124	0.9 (0.6–1.5)	25/99	1.0 (0.6–1.7)
Below median	118/360	1.3 (0.9–1.7)	34/130	1.0 (0.6–1.5)	41/91	1.8 (1.2–2.8)
High-fiber food intake						
Above median (4.6 servings/day)	75/294	1.0 [‡]	23/104	0.8 (0.5–1.4)	22/80	1.1 (0.6–1.9)
Below median	97/321	1.3 (0.9–1.8)	27/103	1.1 (0.7–1.9)	37/84	1.8 (1.1–2.9)

*Nitrate measured as nitrate–nitrogen. Included participants with public water system nitrate data covering >70% of their person-years and sufficient data on each of the dietary or medical risk factors.

[†]All estimates are adjusted for the frequency-matched factors age and sex

[‡]Reference category.

[§]*P* value for interaction on the multiplicative scale <0.10.

^{||}*P* value for interaction on the multiplicative scale <0.05.

[¶]Usual fluid intake, including drinking water (from any source), coffee, tea, fruit juices/drinks, soups, milk, soft drinks, and alcoholic beverages.

with drinking water nitrate for colon cancer, in that increased risks were only observed in the subgroups hypothesized to be associated with longer fecal transit time; however, these interactions were not statistically significant. Statistically significant interactions were observed for rectum cancer only for the midlevels of duration (1 to 10 years) of nitrate >5 mg/L; however, the directions of these associations were not consistent with our *a priori* hypothesis of longer fecal transit time causing increased risk of nitrate in public water supplies.

Fifty-five percent of control participants included in the drinking water nitrate analyses had used a private well of any depth for longer than 20 years over their lifetime, whereas 18% had used a shallow well (<50 feet deep) for this duration. Long-term use of a private well was not associated with either cancer, but use of a shallow private well for longer than 20 years was associated with decreased colon cancer risk (OR = 0.7; CI = 0.6–1.0). Exclusion of long-term private well users or shallow well users did not change any associations we observed with drinking water nitrate measures (results not shown).

Associations between rectum cancer and the highest categories of drinking water nitrate exposure were increased on exclusion of long-term Des Moines residents (those residing in Des Moines for the entire analysis period), but results for colon cancer were not changed (Table 5). Exclusion of long-term Des Moines residents slightly attenuated the observed associations of drinking water nitrate measures and colon cancer among susceptible subpopulations; however, the patterns of effect modification remained (eg, nitrate >5 mg/L longer than 10 years and high meat intake: OR = 1.7; CI = 0.9–3.3 [*P* value for multiplicative interaction = 0.13]). As in our full analysis, no consistent patterns of effect modification were found for rectum cancer between nitrate measures and potentially susceptible subgroups (results not shown).

Dietary nitrate intake, which came primarily from vegetable sources, was inversely associated with colon cancer but not rectum cancer (Table 6); those in the highest quartile of intake had a 30% reduced risk of colon cancer. Dietary nitrite intake was positively associated with colon and rectum cancers, with

TABLE 4. Rectum Cancer Risk Associated With Joint Exposures of Average Public Water System Nitrate Level >5 mg/L Since 1960 and Various Dietary or Medical Risk Factors*

	Years With Nitrate Level >5 mg/L					
	0		1–10		>10	
	Cases/Controls	OR [†] (CI)	Cases/Controls	OR [†] (CI)	Cases/Controls	OR [†] (CI)
Vitamin C intake from diet						
Above median (131.8 mg/day)	87/302	1.0 [‡]	15/98	0.5 (0.3–1.0)	18/87	0.7 (0.4–1.3)
Below median	82/310	0.9 (0.7–1.3)	24/109	0.8 (0.5–1.3)	23/76	1.1 (0.6–1.8)
Meat intake						
Below median (1.5 servings/day)	84/354	1.0 [‡]	23/117	0.8 (0.5–1.4)	19/105	0.8 (0.4–1.4)
Above median	92/277	1.5 (1.1–2.1)	20/99	0.9 (0.5–1.6)	23/62	1.6 (0.9–2.9)
Ever smoked						
No	87/342	1.0 [‡]	22/110	0.8 (0.5–1.3)	16/79	0.8 (0.4–1.5)
Yes	135/441	1.3 (1.0–1.8)	35/153	1.0 (0.7–1.6)	43/119	1.6 (1.0–2.6)
History of bowel inflammation						
No	152/630	1.0 [‡]	37/218	0.7 (0.5–1.1)	37/167	0.9 (0.6–1.4)
Yes	53/88	2.4 (1.7–3.6)	13/27	1.9 (0.9–3.8)	16/23	2.9 (1.4–5.6)
Beverage consumption [§]						
Above median (2.6 L/day)	93/407	1.0 [‡]	33/124	1.2 (0.8–1.9)	28/99	1.2 (0.7–2.0)
Below median	115/360	1.4 (1.0–1.9)	18/130	0.6 (0.3–1.0)	26/91	1.2 (0.7–2.0)
High-fiber food intake						
Above median (4.6 servings/day)	82/294	1.0 [‡]	14/104	0.5 (0.3–0.9)	25/80	1.1 (0.6–1.9)
Below median	87/321	1.0 (0.7–1.4)	26/103	0.9 (0.6–1.6) [¶]	16/84	0.7 (0.4–1.3)

*Nitrate measured as nitrate–nitrogen. Included participants with public water system nitrate data covering >70% of their person-years and sufficient data on each of the dietary or medical risk factors.

[†]All estimates are adjusted for the frequency-matched factors age and sex, as well as years served with chlorinated surface water.

[‡]Reference category.

[§]Usual fluid intake, including drinking water (from any source), coffee, tea, fruit juices/drinks, soups, milk, soft drinks, and alcoholic beverages.

^{||}*P* value for interaction on the multiplicative scale <0.05.

[¶]*P* value for interaction on the multiplicative scale <0.10.

50% to 70% increased risk at levels in the highest quartile; this increased risk was associated primarily with nitrite intake from animal sources rather than vegetable sources (for increasing quartiles versus the lowest quartile of dietary nitrite from animal sources: colon, ORs = 1.3, 1.6, 1.6; rectum, ORs = 1.2, 1.8, 2.0). The percentage of total nitrate intake from public water supplies was not associated with either cancer.

DISCUSSION

In this analysis of nitrate in public water supplies and risk of colon and rectum cancers, we observed negligible overall associations of either cancer with average nitrate level and with the number of years with average nitrate–nitrogen level greater than 5 or 10 mg/L. There was some suggestion of increased colon cancer incidence associated with drinking water nitrate among subpopulations expected to have increased nitrosation such as those with low vitamin C intake, high meat intake, or chronic bowel inflammation.

We studied nitrate intake from drinking water as a precursor of endogenous production of N-nitroso compounds.

Our hypothesis derives from animal experiments in which rats³⁹ and hamsters⁴⁰ had higher rates of intestinal tumors after administration of N-nitroso compounds, either in drinking water or by injection. Among humans, data on the health effects of nitrate intake have been limited. Dietary nitrate intake was not associated with colon or rectum cancer risk in 2 previous studies,^{41,42} but was associated with decreased risk of colon cancer in our analysis. The primary source of dietary nitrate in our study population was from vegetable sources, which contain other nutrients, including fiber and folate, that might be protective for colon cancer.^{43,44}

Epidemiologic evidence for a link between drinking water nitrate and colorectal cancers is mixed. A recent ecologic study conducted in Slovakia found a strong positive correlation between drinking water nitrate and colorectal cancer incidence.⁴⁵ Other ecologic studies of high drinking water nitrate levels found somewhat elevated digestive organ and peritoneum cancer incidences,^{46,47} decreased colon cancer mortality,⁴⁸ and no association with colon cancer mortality.⁴⁹ In addition to the limitations of the ecologic study

TABLE 5. Association of Average Public Water Supplies Nitrate Level Since 1960 With the Risk of Colon and Rectum Cancers, Excluding Participants With Residence in Des Moines Since 1960*

	Colon Cancer (n = 330)		Rectum Cancer (n = 321)		Controls (n = 1137)
	No.	OR [†] (CI)	No.	OR [†] (CI)	No.
Average nitrate					
≤1 mg/L [‡]	172	1.0	154	1.0	566
>1 to ≤3 mg/L	116	1.0 (0.8–1.3)	98	0.8 (0.6–1.1)	380
>3 to ≤5 mg/L	27	0.7 (0.4–1.1)	30	0.7 (0.5–1.2)	124
>5 mg/L	15	0.8 (0.4–1.4)	39	2.1 (1.4–3.3)	67
Number of years with average nitrate >5 mg/L					
0 years [‡]	240	1.0	222	1.0	783
1 to 10 years	65	0.8 (0.6–1.1)	57	0.8 (0.6–1.1)	263
>10 years	25	0.9 (0.6–1.5)	42	1.6 (1.1–2.4)	91

*Nitrate measured as nitrate–nitrogen. Included participants with public water system nitrate data covering >70% of their person years.

[†]All estimates are adjusted for the frequency-matched factors age and sex, and estimates for rectum cancer are additionally adjusted for years served with chlorinated surface water.

[‡]Reference category.

design, these studies were especially vulnerable to misclassification of exposure, because participants were classified based on residence at one time.

A prospective cohort study of Iowa women found that municipal drinking water nitrate levels were associated with an elevated risk of colon cancer that did not consistently increase with exposure and a decreased risk of rectum cancer across exposure groups.⁴¹ However, the highest quartile of drinking water nitrate that was examined in the cohort represented relatively low levels, with a cutpoint of 2.22 mg/L nitrate–nitrogen. In our study, we observed an increase in risk of colon cancer only for certain susceptible subpopulations with exposure levels greater than 5 mg/L. However, we did observe some suggestion of weak decreased rectum cancer risk associated with nitrate levels at these lower levels of exposure, somewhat consistent with the Iowa cohort study results.

We observed an increased risk of colon cancer associated with drinking water nitrate among certain subgroups expected to have high rates of nitrosation, but we did not observe the same patterns for rectum cancer. The colon and rectum have similar epithelial tissues, but the 2 cancer types have somewhat different risk factors. Although N-nitroso compounds formed in the digestive tract would be expected to pass through the rectum, contractile activities in the rectum cause fecal matter to pass through quickly, resulting in less contact time with the rectum than with the colon.⁵⁰

Our population-based case-control study included a random sample of Iowa residents and had high response rates. However, analyses of drinking water nitrate included only the subset (52%) of participants with a large proportion of time

on Iowa public water supplies to reduce exposure misclassification. This subset is not fully representative of the Iowa population in several respects. By restricting our analyses to persons with over 70% of their person-years in residences serviced by public water systems having nitrate data, we restricted our analyses to a more urban population. A major reason for exclusion was missing data as a result of private well use, which is associated with rural residence. Levels of nitrate contamination in private wells in agricultural areas are frequently high, because they are often located in areas of heavy nitrogen fertilizer use or animal feedlots and are unregulated. Indeed, a survey in the late 1980s, a time period relevant to this study, found that 18% of private drinking water wells in Iowa had nitrate–nitrogen levels greater than 10 mg/L.³⁸ Exclusion of these potentially highly exposed participants from our analyses limited our exposure range to largely below the MCL.

Higher average drinking water nitrate levels were strongly associated with residence in the city of Des Moines in our study. Measurements taken in Des Moines since the late 1960s indicate that average nitrate–nitrogen levels were usually higher than 5 mg/L during our analysis period (since 1960) and were higher than the MCL for a few years in the late 1970s. For the people included in our drinking water nitrate analyses, the 170 participants (9%) who resided in Des Moines for the entire analysis period were categorized in the highest level of each nitrate measure. Our subanalysis excluding long-term Des Moines residents indicates that some positive confounding for colon cancer and negative confounding for rectum cancer could have occurred as a result of unknown factors associated with Des Moines residence.

TABLE 6. Associations of Dietary Nitrate, Nitrite, and Percent Nitrate From Public Water Supplies With the Risk of Colon and Rectum Cancers*

	Colon Cancer		Rectum Cancer		Controls No. (%)
	No. (%)	OR [†] (CI)	No. (%)	OR [†] (CI)	
Dietary nitrate intake					
Quartile 1 (≤ 59.3 mg/day) [‡]	89 (32)	1.0	56 (22)	1.0	261 (27)
Quartile 2 (cutoff >59.3 mg/day)	68 (24)	0.8 (0.6–1.2)	67 (27)	1.3 (0.9–1.9)	241 (25)
Quartile 3 (cutoff >86.6 mg/day)	68 (24)	0.8 (0.5–1.1)	66 (27)	1.2 (0.8–1.8)	246 (25)
Quartile 4 (cutoff >122.0 mg/day)	55 (20)	0.7 (0.4–1.0)	60 (24)	1.1 (0.8–1.7)	234 (24)
Dietary nitrite intake					
Quartile 1 (≤ 0.705 mg/day) [‡]	90 (32)	1.0	74 (30)	1.0	311 (32)
Quartile 2 (cutoff >0.705 mg/day)	73 (26)	1.1 (0.8–1.6)	62 (25)	1.1 (0.7–1.6)	251 (26)
Quartile 3 (cutoff >0.940 mg/day)	48 (17)	0.9 (0.6–1.3)	43 (17)	0.9 (0.6–1.4)	220 (22)
Quartile 4 (cutoff >1.26 mg/day)	69 (25)	1.5 (1.0–2.1)	70 (28)	1.7 (1.1–2.5)	200 (20)
Percent nitrate intake from public water supply [§]					
Quartile 1 (≤ 5.96 %) [‡]	68 (26)	1.0	66 (28)	1.0	254 (27)
Quartile 2 (cutoff >5.96 %)	60 (23)	1.0 (0.6–1.4)	56 (24)	0.9 (0.6–1.3)	232 (24)
Quartile 3 (cutoff >12.53 %)	61 (23)	0.9 (0.6–1.4)	59 (25)	0.8 (0.5–1.2)	239 (25)
Quartile 4 (cutoff >24.76 %)	76 (29)	1.2 (0.8–1.8)	55 (23)	0.8 (0.6–1.3)	233 (24)

*Included participants with public water system nitrate data covering $>70\%$ of their person-years and sufficient data on each of the dietary factors.

[†]All estimates are adjusted for the frequency-matched factors age and sex, and estimates for rectum cancer are additionally adjusted for years served with chlorinated surface water.

[‡]Reference category.

[§]As a percentage of total nitrate intake from diet and public water supply. Data are missing for persons with missing information on tap water consumed at home per day.

However, the magnitude of confounding was not sufficient to explain the positive associations of drinking water nitrate and colon cancer among susceptible subpopulations.

In view of the suggestive results presented here and in other recent studies, further exploration of the association between colon cancer incidence and nitrate in drinking water is worthwhile, both in epidemiologic studies and in controlled experiments. In particular, little is known about the potential for drinking water nitrate to modulate the endogenous synthesis of N-nitroso compounds that reach the colon, and controlled studies with measurements of N-nitroso compounds in feces would be contributory. Studies of populations with potentially high exposures such as private well users, and of susceptible subpopulations such as those with a history of inflammatory bowel disease, will be critical in gaining a full understanding of the risk associated with nitrate in drinking water supplies.

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