Nitrogen-nitrate exposure from drinking water and colorectal cancer risk for rural women in Wisconsin, USA

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ABSTRACT

One unintentional result of widespread adoption of nitrogen application to croplands over the past 50 years has been nitrate contamination of drinking water with few studies evaluating the risk of colorectal cancer. In our population-based case-control study of 475 women age 20–74 years with colorectal cancer and 1447 community controls living in rural Wisconsin, drinking water nitrate exposure were interpolated to subjects residences based on measurements which had been taken as part of a separate water quality survey in 1994. Individual level risk factor data was gathered in 1990–1992 and 1999–2001. Logistic regression models estimated the risk of colorectal cancer for the study period, separately and pooled. In the pooled analyses, an overall colorectal cancer risk was not observed for exposure to nitrate-nitrogen in the highest category ($\geq$ 10 ppm) compared to the lowest category ($<$ 0.5 ppm). However, a 2.9 fold increase risk was observed for proximal colon cancer cases in the highest compared to the lowest category. Statistically significant increased distal colon or rectal cancer risk was not observed. These results suggest that if an association exists with nitrate-nitrogen exposure from residential drinking water consumption, it may be limited to proximal colon cancer.

Key words | case-control study, colorectal cancer, nitrogen-nitrate

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INTRODUCTION

Over half a century ago, the first studies linked drinking water and its associated contaminants with cancer risk (Tromp 1955). Nitrogen is the most pervasive groundwater contaminant in the United States (USGS 1999) and in Wisconsin, (Vanden Brook *et al.* 2002) yet few epidemiologic studies have evaluated colorectal cancer risk. Drinking water contaminated with nitrate-nitrogen is a plausible risk factor given the colon's direct exposure to waterborne contaminates (Weyer *et al.* 2001; Gulis *et al.* 2002; De Roos *et al.* 2003). The state of Wisconsin and federal laws set the maximum allowable level of nitrate-nitrogen in public drinking water at 10 parts per million (ppm), although no regulation of private drinking water exists. In Wisconsin, approximately 14% of the private wells servicing the drinking water needs of rural residents has been estimated to exceed 10 ppm nitrate-nitrogen (Vanden Brook *et al.* 2002). To evaluate the relation between nitrate-nitrogen and colorectal cancer risk, we used data from two population-based case-control colorectal studies and an agricultural chemical monitoring study of drinking water quality. The agricultural chemical study, conducted in 1994, sampled water from randomly selected private wells located in rural areas of Wisconsin for nitrate-nitrogen contamination levels (LeMasters *et al.* 1995). We used natural neighbor interpolation to assign exposure values to study participants who were interviewed in 1990–1992 and 1999–2001 and lived in rural areas of Wisconsin at the time of the study.

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METHODS

Identification of cases

Cases were female Wisconsin residents, age 20–74 years, with a new diagnosis of colorectal cancer in two studies conducted during 1990–1992 or 1999–2001. According to a protocol approved by the University of Wisconsin Health Sciences institutional review board, the physician of record for each eligible case subject was contacted by mail to obtain permission to approach the potential participants. Eligibility was limited to case subjects with listed telephone numbers, driver's licenses verified by self-report (if less than 65 years of age), and known dates of diagnosis. Of 2216 women identified as eligible, 129 (5.8%) were not contacted due to physician refusal, 370 (16.6%) were deceased, 211 (9.5%) refused, 26 (1.2%) could not be located, and 4 (0.2%) interviews were deemed unreliable. Overall, 1476 (67%) of eligible women participated in the study; 80% of eligible living cases participated.

Identification of controls

Community controls were enrolled based on random selection from population lists of Wisconsin motor vehicle drivers (women <65 years of age) and Medicare beneficiaries (women age 65 years and older). Data from these participants were used for both a breast cancer and colorectal study, conducted simultaneously and the controls were selected at random to yield an age distribution similar to that of the cases enrolled in a concurrent study of breast cancer. Inclusion criteria required that all control subjects have a listed telephone number, be English speaking and have never been diagnosed with colorectal cancer. Of 5044 women identified as potential controls, 54 (1.1%) were deceased, 598 (11.9%) refused, 93 (1.8%) could not be located, and 2 were unreliable interviews. Overall, 4297 (85%) of population controls participated in the study.

Subjects for analysis

Analysis was limited to women who lived in rural areas of Wisconsin, defined as their residential city, village, or town with no public water system (475 cases and 1447 controls). Rural participants' street mailing addresses were assigned latitude/longitude coordinates to the address point location with an 80 percent spelling and overall sensitivity score. If there was not a match at this level, the nine-digit ZIP code line segment centroid was used as the geocode, and, lastly, the zip code centroid was used. For cases, the participant's mailing address at time of colorectal cancer diagnosis was used. For controls, the participant's mailing address approximately one year before interview was used. See McElroy *et al*. (2003) for more details on the geocoding methods.

Interview data

Both cases and controls completed a structured 30–40 minute telephone interview covering established and suspected risk factors for colorectal cancer. Interviews were administered by trained interviewers concurrently for both case and control participants. Questions regarding personal and family histories of cancer and cancer screening were asked toward the end of the interview to maintain blinding of interviewers to the disease status of participants. Risk factor information was assessed for women approximately two years prior to interview; for cases this was about one year prior to diagnosis. No residential drinking water quality or quantity questions were asked.

Definitions of risk factors

For each case, a reference date was defined as the registry-provided date of colorectal cancer diagnosis. For comparability, the control subjects interviewed contemporaneously with cases were assigned an individual reference date approximately one year before their interview date which reflects the normal reporting length from cancer diagnosis to availability of data for research for the cases. This was done to maintain comparability between cases and controls, and to maintain interviewer blinding to case-control disease status. Reference age was defined as the woman's age at the reference date. First degree family history of colorectal cancer was defined as having a parent or sibling diagnosed with colorectal cancer (absent, present, unknown). Recent beer consumption was computed as the total number of drinks of beer usually consumed per week approximately 2 years before the inter-

view (3 categories). Recent alcohol consumption other than beer was computed similarly as the total number of drinks of wine and hard liquor usually consumed per week approximately 2 years before the interview (3 categories). A woman was classified as premenopausal if she reported still having periods and was not using hormone replacement therapy and postmenopausal if she reported an oophorectomy or natural menopause (no menstrual periods for at least six months) before the reference date. Women who reported currently taking postmenopausal hormones and still having periods, and women who reported hysterectomy alone were classified as premenopausal if their reference ages were in the first decile of age at natural menopause among the controls (approximately <41 years of age for current smokers and approximately $<$ 43 years of age for non-smokers), and postmenopausal if their reference ages were in the highest decile for age at natural menopause in the control group (approximately $\geq$ 54 years of age for current smokers and approximately $\geq$ 56 years of age for non-smokers). For women in the intermediate ages (second to ninth decile), menopausal status was considered unknown. Other covariates in the analyses were age at reference (7 categories), body mass index (BMI) (kg/m^2; approximate quartiles based on the distribution among controls), age at menopause (5 categories), colorectal screening (never, ever), education (5 categories), and smoking (never, former, current). All risk factors were coded as indicator variables for the different categories.

Nitrate-nitrogen well water data

We obtained publicly available data on nitrate-nitrogen contamination of groundwater from the Wisconsin Department of Agriculture, Trade and Consumer Protection (WDATCP). As part of the Atrazine Rule Evaluation Study, WDATCP randomly sampled 289 wells to analyze the water for various herbicides and nitrate-nitrogen in 1994. This study's purpose was to quantify agricultural chemicals levels in Wisconsin groundwater servicing the rural population and to compare these levels over time (Baldock 1993; LeMasters *et al*. 1995; LeMasters & Baldock 1997; Vanden Brook *et al*. 2002). Wells were selected using a stratified random sampling procedure. The nine Agricultural Statistical Districts in Wisconsin, which are groups of adjoining counties, formed the sampling strata. The number of wells sampled for each stratum was based on 1) the number of farms and 2) atrazine (a common corn herbicide) concentration variability from previous water quality studies. To select the wells, a random sample of the public land survey system (PLSS) sections was drawn (excluding those sections covered by water or publicly owned). The PLSS typically divides land into 36 square mile parcels called townships (6-mile by 6-mile); townships are further divided into 36 one-mile square sections. In each PLSS section, a random 10-acre parcel was selected and the well nearest its center was identified. PLSS sections were used because no comprehensive current list of locations of private wells exists. The parcel was visited to determine if a private well existed within the 10-acres. If a private well existed, three attempts were made to make contact with the owner to determine their willingness to participate in the survey. If no acceptable well or willing owner was found within the entire section, a replacement section and random 10-acre plot was selected. The contact protocol was repeated until a well water sample was obtained. Number of wells varied by agricultural district with the lowest number of wells ($n = 7$) in the Southwest district and the highest number of wells ($n = 85$) in the Southcentral district (Figure 1). Samples were collected from May to October of 1994. Water was collected through the cold water faucet after letting the water run for 10 minutes. These samples were analyzed using gas chromatography for nitrate-nitrogen by the WDATCP's Bureau of Laboratory Science.

Estimation approach

Natural neighbor interpolation (Sibson 1981) was used to estimate nitrate-nitrogen levels in groundwater across the entire state. Natural neighbor interpolation uses a weighted moving average of concentrations of nitrate-nitrogen residues in residential drinking water in surrounding or neighboring observed wells. Neighboring points and the corresponding weights are based on the Voronoi diagram of the data points (Okabe & Okabe 2000). The Voronoi diagram of a set of points is a partitioning of the plane into regions associated with each point such that every point in a given partition is closer to the generating point than any other point. As a secondary analysis, nearest neighbor interpolation was used based on the concentration of nitrate-nitrogen residues in the well nearest to the study

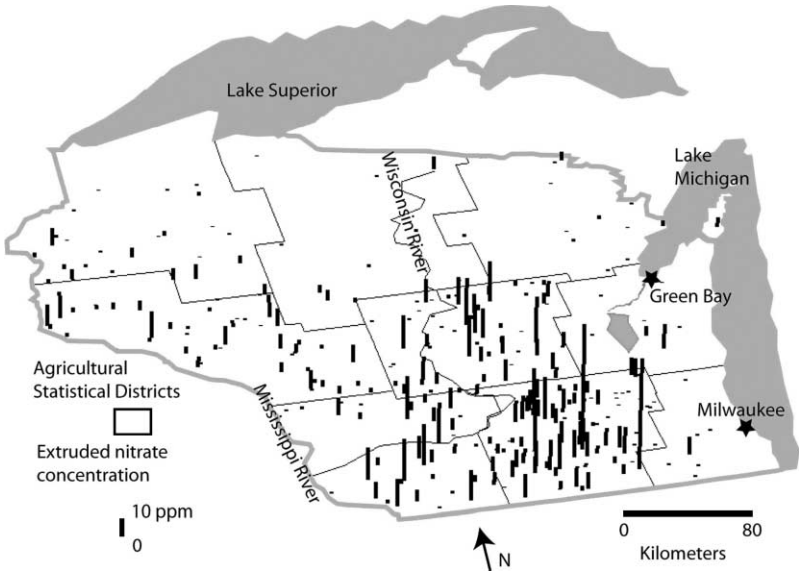

Figure 1 | Extruded nitrate-nitrogen concentration values of well water samples in 1994 ($n = 289$).

participant's residence. Interpolation was performed using ArcGIS 9.0 spatial analysis tool (Environmental Systems Research Inc, Redwood CA).

Statistical analysis

We modeled the association between the risk of colorectal cancer and nitrate-nitrogen levels in drinking water using 5 groups with the highest category at the Wisconsin's statutory action level (≥ 10 ppm) and the lowest category below the level of detection (< 0.5 ppm). Models contained terms for factors with known or suspected associations with colorectal cancer and/or nitrate-nitrogen contamination. We used odds ratios (OR) and 95% confidence intervals (CI) from logistic regression models to estimate relative risks of colorectal cancer incidence (Breslow & Day 1980). We analyzed the risk of colon (proximal/transverse and distal) and rectal cancer, combined and stratified, associated with nitrate-nitrogen contamination of drinking water for women interviewed in 1990–1992 and women interviewed in 1999–2001, separately and pooled. Tests for heterogeneity in the odds ratio for nitrate-nitrogen concentration levels according to the study years were conducted by comparing the change in log likelihoods in models with and without cross-product terms. Propensity weighting adjustment for study non-response was performed at the county level to assess the influence of non-response on the results (Cochran 1968). Given the geographic nature of the exposure assessment, differential response rates for cases and controls within counties could bias the results in any direction. For example, if there was no true association between drinking water nitrate exposure and colorectal cancer, but response rates for cases were higher than response rates for controls in counties with high nitrate levels (and similar in counties with low nitrate levels), there would be an apparent association between nitrate levels and colorectal cancer in the standard unweighted analysis. On the other hand, we would correctly observe no association between nitrate levels and colorectal cancer in the weighted analysis. If there were substantial differences between the results of the two analyses, the weighted analysis would be more reliable. Separate models stratified by the type of geocode (street-level geocode, ZIP + 4 line segment geocode, ZIP code centroid geocode) were also constructed to check for differential associations of colorectal cancer risk with nitrate-nitrogen contamination.

RESULTS

For the pooled sample, women with colorectal cancer (cases) were slightly older with mean age of 62 years

compared to 55 years for women without colorectal cancer (controls). Case women were more likely to have a family history of colorectal cancer and less likely to have colorectal screening procedure compared to controls. Participants had differences in several of the covariates between the study years (1990–1992 and 1999–2001) reflecting secular changes. For example, 18% of the case women in the first time period had a BMI $\geq 29.3\,kg/m^2$ whereas almost twice as many case women (28%) were in this category in the later time period; 6% of case women in the first time period had a college degree compared to 16% in the second time period (Table 1). The women with colorectal cancer who lived in urban and suburban areas were serviced by community water supplies, and therefore eliminated from this study.

Assignment of latitude/longitude coordinates was accomplished for 42% of the mailing addresses (street number and name). For the remaining 58% that did not geocode to the mailing address, 46% were geocoded to the nine digit ZIP code line segment centroid. For rural areas in Wisconsin, this line segment is typically less than half a mile. Finally, 12% were assigned a geocode corresponding to the ZIP code centroid (Figure 2).

Of the 289 drinking water wells sampled in 1994, 69% ($n = 199$) had detectable nitrate-nitrogen concentration levels and 18% ($n = 52$) exceeded the enforcement standard of 10 ppm. A clear north-south spatial pattern of detects was notable (Figure 2).

The odds ratio of colorectal cancer for women in the pooled analyses, who were exposed to nitrate-nitrogen concentrations of 10 ppm or higher as compared to women in the lowest exposure category (<0.5 ppm) was 1.52 (95% CI: 0.95, 2.44) after adjustment for age. When stratified by site: proximal and transverse colon, distal colon and rectal, an increased risk was observed for proximal colon cancer for women in the highest category (10 ppm or higher) (OR = 2.91; 95% CI: 1.52, 5.56) compared to women in the lowest exposure category (<0.5 ppm) in the age-adjusted model. These odds ratios did not change after adjustment of known and suspected colorectal cancer risk factors (Table 2). The age-adjusted odds ratio using nearest neighbor estimation technique produced similar results as the natural neighbor estimation technique (data not shown). Results were similar to the whole group for a subset of participants whose street mailing addresses were used to assign the residential location (data not shown). No significant interaction term was discerned between any of the risk factors shown in Table 1 and nitrate-nitrogen exposure (data not shown) for either study period.

Results from the propensity weighting analysis for colorectal cancer, comparing the highest exposure category of ≥ 10 ppm nitrate-nitrogen to the lowest category (OR = 3.20; 95% CI: 1.25, 8.15 for women interviewed 1990–1992; OR = 1.03; 95% CI: 0.48, 2.22 for women interviewed 1999–2001) were similar to the results from the unweighted analysis (Table 2).

DISCUSSION

Our study investigated the risk of colorectal cancer for rural Wisconsin women from nitrate-nitrogen contamination in residential drinking water. For our population-based study, nitrate-nitrogen exposure from drinking water was not significantly associated with a colorectal cancer risk overall. However, there was a suggestion that exposure to nitrate-nitrogen contaminated drinking water may increase the risk of proximal and transverse cancer. Both study years (1990–1992 and 1999–2001) analyzed separately produced similar results. Furthermore, both natural neighbor and nearest neighbor exposure estimation techniques produced similar results increasing our confidence in our estimation techniques.

Although several epidemiologic studies have assessed the risks of cancers associated with nitrate-nitrogen contamination of drinking water, including stomach, bladder, esophageal, and brain (Preston-Martin & Correa 1989), only a few studies have evaluated the risk of colorectal cancer and nitrate-nitrogen contamination of drinking water (Weyer *et al.* 2001; Gulis *et al.* 2002; De Roos *et al.* 2003). In a cohort study, an average nitrate level from each of the municipal water supplies in Iowa over a 33 year period was calculated and assigned to the 396 communities included in the analysis. For the 16,541 participants in this cohort who reported using a municipal water supply for more than 10 years, the community mean nitrate level was assigned. An inverse association with rectal cancer and a null association with colon cancer across nitrate quartiles was observed (Weyer *et al.* 2001). A case-control study also assigned a yearly average nitrate level computed from respondents' municipal water supply from 1960 to year of

Table 1 | Characteristics of rural Wisconsin women with colorectal cancer and community controls. Aged 20–74 Interviewed 1990–1992 and 1999–2001

	Interviewed 1990–1992				Interviewed 1999–2001				Pooled (1990–1992 & 1999–2001)			
Risk factors	Cases (n = 201) %	Controls (n = 659) %*	OR†	95% CI†	Cases (n = 274) %	Controls (n = 788) %*	OR†	95% CI†	Cases (n = 475) %	Controls (n = 1447) %*	OR‡	95% CI‡
Family history of colorectal cancer												
Absent	80	88	1.00		72	81	1.00		76	85	1.00	
Present	18	9	2.20	1.36–3.56	19	10	2.08	1.39–3.11	19	10	2.12	1.58–2.92
Body Mass Index												
< 22.8	29	33	1.00		20	22	1.00		24	27	1.00	
22.8–25.7	21	29	0.89	0.56–1.42	27	27	1.23	0.80–1.89	25	27	1.08	0.79–1.48
25.8–29.2	28	20	1.61	1.02–2.52	23	25	0.96	0.62–1.49	25	23	1.20	0.88–1.64
≥ 29.3	18	18	1.24	0.74–2.08	28	23	1.17	0.76–1.79	24	21	1.20	0.87–1.66
Smoking												
Never	62	65	1.00		54	60	1.00		57	62	1.00	
Ever	38	35	1.17	0.82–1.67	46	40	1.18	0.88–1.58	42	37	1.18	0.94–1.47
Former	22	19	1.22	0.80–1.88	28	24	1.20	0.86–1.69	25	21	1.21	0.93–1.58
Current	16	16	1.06	0.66–1.70	18	16	1.15	0.77–1.71	17	16	1.13	0.84–1.53
Colorectal cancer screening												
No	64	53	1.00		68	61	1.00		66	58	1.00	
Yes	33	45	0.54	0.38–0.78	30	34	0.84	0.60–1.16	32	39	0.68	0.54–0.87
Education												
< 12th grade	30	30	0.97	0.63–1.47	11	8	1.37	0.82–2.30	19	19	1.14	0.83–1.57
High school graduate	44	43	1.00		47	49	1.00		46	46	1.00	
At least some college	18	18	0.92	0.58–1.46	24	22	1.13	0.78–1.62	21	20	1.06	0.79–1.41
College graduate	6	9	0.65	0.33–1.28	16	19	1.01	0.67–1.51	12	14	0.88	0.63–1.25

*Percentages are adjusted to the age distribution in the cases. Percentages do not add up to 100 due to missing data and rounding.
†Adjusted for age.
‡Adjusted for age and interview period.

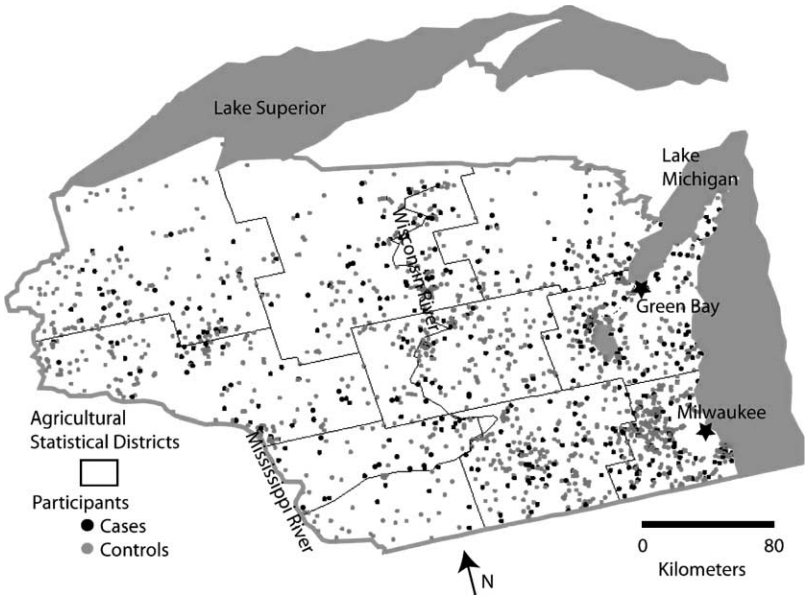

Figure 2 | Spatial distribution of colorectal cancer cases and control participants living in rural areas for 1990–1992 and 1999–2001 studies.

cancer diagnosis for cases (1986 or 1987) or to 1987 for controls. Participants who had more than 70% of their person-time since 1960 with actual or imputed nitrate data were included in the analysis. In this study, a positive association was observed in a subpopulation of high meat or low vitamin C consumers (De Roos *et al.* 2003). The third study also used municipal water supply data but rather than assign a mean value, this study assigned a low (0–10 mg/L), medium (10.1–20 mg/L) or high (20.1–50 mg/L) nitrate value to participants based on data collected over a 20-year period. An overall positive association for colorectal cancer with the standardized incidence ratios increasing from villages with low nitrate levels in their public drinking water supply in Trnava District, Slovakia to high nitrate levels (Gulis *et al.* 2002). The mean concentration of nitrate-nitrogen exposure in the highest category from these studies was >2.46 ppm in the inverse association study (Weyer *et al.* 2001), >2.46 ppm and >5 ppm in the null finding study (Weyer *et al.* 2001) and >5 ppm and 20–50 ppm in the positive association studies (Gulis *et al.* 2002; De Roos *et al.* 2003). In contrast to these three epidemiologic study, our study used randomly sampled individual well water data. We assigned the nitrogen-nitrate value to a participant based on proximity to the well using an interpolation technique–not on a mean value from municipal water supplies.

Several surveillance studies have reported different incidence rates by colon subsite with a shift of colon carcinomas to a more proximal location as people age, as well as females having an increased proximal colon cancer risk compared with males (Nelson *et al.* 1997; Saltzstein *et al.* 1998; Wu Cheng *et al.* 2001, 2004). For exposure to nitrate-nitrogen from drinking water, the risk may vary by site with the colon having the possibility of experiencing a greater risk than the rectum (Weyer *et al.* 2001; De Roos *et al.* 2003). When we stratified by colon subsite, we observed an increased risk for proximal colon cancer, even though we had few proximal and transverse cancer cases. The effect was statistically similar in the 1990–1992 study compared to the 1999–2001 study.

The design of our study had potential limitations. We used well water samples from randomly selected wells in an environmental monitoring study conducted in 1994. An exposure surface was estimated and these surface values were assigned to participants' residences. Nitrate-nitrogen application to agricultural land has a long history in Wisconsin with roughly 10% of the total nitrate application leaching into groundwater (Chern *et al.* 1999) where its half life ranges from approximately 500 days in zones with the presence of organic substances and 2750 days in zones where organic matter is absent (Uffink 2003).

In sequential follow-up studies of rural well water quality in Wisconsin conducted in 1996 and 2001 (with 50% repeat samples in the follow-up years), the proportion

Table 2 | Multivariate odds ratio for colon and rectal cancer for rural Wisconsin women according to nitrate-nitrogen exposure from drinking water

	Interviewed 1999–2001						Interviewed 1999–2001						Pooled (Interviewed 1990–1992 and 1999–2001)					
	Cases	Controls					Cases	Control					Cases	Controls				
Nitrate exposure (in ppm)	%	%	OR*	95 % CI*	OR†	95% CI†	%	%	OR*	95% CI*	OR†	95% CI†	%	%	OR‡	95% CI‡	OR§	95% CI§
Colorectal Cancer	$n = 201$‖	$n = 659$					$n = 274$‖	$n = 788$					$n = 475$‖	$n = 1447$				
<0.5	31	41	1.00		1.00		31	36	1.00		1.00		31	38	1.00		1.00	
0.5–1.9	23	19	1.49	0.94–2.36	1.51	0.94–2.41	21	19	1.29	0.86–1.94	1.31	0.86–1.98	22	19	1.38	1.02–1.87	1.39	1.02–1.89
2.0–5.9	26	23	1.47	0.94–2.30	1.45	0.91–2.28	31	27	1.28	0.89–1.85	1.22	0.84–1.77	29	25	1.37	1.03–1.81	1.32	0.99–1.76
6.0–9.9	11	12	1.18	0.67–2.10	1.11	0.61–2.01	12	11	1.33	0.80–2.19	1.34	0.81–2.22	12	11	1.27	0.88–1.85	1.28	0.88–1.88
≥10.0	8	5	2.88	1.42–5.86	2.95	1.43–6.11	5	7	0.97	0.51–1.87	1.00	0.52–1.94	7	6	1.52	0.95–2.44	1.57	0.97–2.52
Proximal Colon Cancer	$n = 71$	$n = 659$					$n = 80$	$n = 788$					$n = 151$	$n = 1447$				
<0.5	30	41	1.00		1.00		29	36	1.00		1.00		29	38	1.00		1.00	
0.5–1.9	21	19	1.30	0.63–2.68	1.23	0.59–2.57	19	19	1.40	0.69–2.84	1.48	0.71–3.08	20	19	1.35	0.82–2.24	1.35	0.81–2.26
2.0–5.9	28	23	1.52	0.78–2.98	1.38	0.70–2.73	29	27	1.32	0.70–2.49	1.31	0.68–2.53	28	25	1.43	0.91–2.27	1.36	0.85–2.17
6.0–9.9	8	12	0.86	0.32–2.28	0.81	0.30–2.17	15	11	1.78	0.80–3.93	1.85	0.82–4.16	12	11	1.33	0.73–2.44	1.34	0.73–2.47
≥10.0	13	5	4.88	1.90–12.50	4.53	1.72–11.93	9	7	2.01	0.79–5.09	1.89	0.72–4.92	11	6	2.91	1.52–5.56	2.76	1.42–5.38
Distal Colon Cancer	$n = 84$	$n = 659$					$n = 118$	$n = 788$					$n = 202$	$n = 1447$				
<0.5	29	41	1.00		1.00		30	36	1.00		1.00		29	38	1.00		1.00	
0.5–1.9	27	19	1.80	0.96–3.39	1.83	0.96–3.49	22	19	1.44	0.82–2.52	1.43	0.81–2.51	24	19	1.60	1.05–2.43	1.58	1.03–2.40
2.0–5.9	24	23	1.42	0.74–2.71	1.34	0.68–2.63	33	27	1.41	0.85–2.34	1.36	0.82–2.27	29	25	1.42	0.96–2.11	1.38	0.92–2.06
6.0–9.9	14	12	1.56	0.72–3.35	1.54	0.70–3.41	11	11	1.27	0.63–2.57	1.29	0.64–2.62	12	11	1.41	0.85–2.37	1.43	0.85–2.41
≥10.0	6	5	2.06	0.70–6.08	2.19	0.73–6.58	4	7	0.82	0.30–2.22	0.87	0.32–2.36	5	6	1.18	0.57–2.44	1.23	0.59–2.56
Rectal Cancer	$n = 36$	$n = 659$					$n = 60$	$n = 788$					$n = 96$	$n = 1447$				
<0.5	33	41	1.00		1.00		33	36	1.00		1.00		33	38	1.00		1.00	
0.5–1.9	22	19	1.31	0.51–3.32	1.29	0.50–3.31	23	19	1.36	0.66–2.81	1.33	0.63–2.79	23	19	1.32	0.74–2.33	1.29	0.73–2.31

Table 2 | *(continued)*

Nitrate exposure (in ppm)	Interviewed 1999–2001					Interviewed 1999–2001					Pooled (interviewed 1990–1992 and 1999–2001)					
	Cases	Controls				Cases	Control				Cases	Controls				
	%	%	OR*	95 % CI*	OR†	95% CI†	%	%	OR*	95% CI*	OR†	95% CI†	%	%	OR‡	95% CI‡	OR§	95% CI§
2.0–5.9	25	23	1.34	0.55–3.30	1.29	0.52–3.20	30	27	1.14	0.58–2.25	1.14	0.57–2.26	28	25	1.21	0.70–2.07	1.19	0.69–2.06
6.0–9.9	11	12	1.14	0.35–3.68	0.92	0.25–3.42	10	11	0.95	0.36–2.52	1.01	0.37–2.73	10	11	1.03	0.49–2.17	1.11	0.52–2.36
≥ 10.0	8	5	2.77	0.71–10.84	2.92	0.72–11.78	3	7	0.60	0.13–2.66	0.68	0.15–3.08	5	6	1.14	0.43–3.05	1.26	0.47–3.43

The pooled analysis has 26 cases of unknown subsite.
*Logistic regression models adjusted for age only.
†Logistic regression models adjusted for age, family history of colorectal cancer, colorectal screening, and smoking status (never/ever).
‡Logistic regression models for the pooled data adjusted for age and interview period.
§Logistic regression models for the pooled data adjusted for age and interview period, as well as the aforementioned covariates.
‖10 cases interviewed between 1990–1992 have unknown subsite. 16 cases interviewed between 1999–2001 have unknown subsite.

of nitrate-nitrogen detects in well water did not change (Vanden Brook *et al.* 2002). Due to the stability of the proportion of wells with detectable nitrate-nitrogen concentration levels (over 2 ppm), we are reassured in our use of 1994 well data to assign exposure to women interviewed up to 4 years before or 6 years after the wells were sampled.

Other limitations in this study may have affected the results. For this analysis, the exposure classification system may not accurately reflect a woman's actual exposure to nitrate-nitrogen and as described by Vineis, non-differential misclassification of exposure may attenuate the odds ratio (Vineis 2004). For example only home water supply at the time of the interview was considered as the potential source of nitrate-nitrogen exposure and we did not obtain information on the quality of this water. A significant amount of water could have been consumed outside the home (Shimokura *et al.* 1998). The formation of the carcinogenic *N*-nitroso compounds is the result of complex interaction of the amount of nitrate-nitrogen ingested from both water and foods, the concomitant ingestion of nitrosation cofactors and precursors, such as preserved meats and fish, beer, certain occupational exposures, consumption of tobacco products, and specific medical conditions including inflammatory bowel disease and periodontal disease (Tricker 1997). Besides our estimated nitrate-nitrogen exposure from residential drinking water, we were unable to adjust for these potential confounders in this study.

As well as assuming exposure from home-tap water, we were not able to control for population migration. However, Wisconsin has a fairly stable population. Approximately 70 percent of women enrolled in a separate case-control population-based cancer study in which we gathered residential history information had not moved in the previous 10 years and 23 percent had moved only once in the previous 10 years (McElroy *et al.* 2004).

Another possible source of misclassification is reflected in the potential for a sampling, location and/or response bias, and this is important where the exposure of interest is spatially distributed. To adjust for a potential bias in the distribution of cases and controls, a propensity weighting scheme was used. The similarity of the results from the weighted and unweighted analyses suggests that the

associations reported in this paper cannot be explained by response bias. Similar results for the highest exposure category of ≥10 ppm nitrate-nitrogen compared to the lowest exposure category was observed, ameliorating the possibility of a systemic spatial sampling or response bias in these data. Although participation proportions have been declining over time, for these two populations the proportions were reasonably similar (first and second study respectively for cases was 83% and 79% and for controls was 91% and 81%). Further, when we stratified by type of geocode, results from women whose geocodes used mailing address (the most accurate assignment of location) were similar to those using the full sample. Finally, the target for the 1994 environmental monitoring study was described as groundwater in rural areas where private well use predominates (Vanden Brook *et al*. 2002). Because we limited our analyses to rural women in Wisconsin (33% of our study population), who would very likely use private well water, not a community water system, these results cannot be generalized to the urban population.

Experimental studies provide a putative mechanistic basis to support an association between nitrate-nitrogen intake and cancer risk. After gastrointestinal resorption and recirculation of ingested nitrate from foods and water, up to 20% of ingested nitrate is reduced to nitrite in the oral cavity by bacterial activity (Tannenbaum *et al*. 1976; Eisenbrand *et al*. 1980). This conversion can also occur in the colon (Ward *et al*. 2005). Nitrite can interact with secondary amines and amides in the stomach and possibly the colon to form N-nitrosamines and *N*-nitrosoamides, which are among the strongest known carcinogens (Bartsch 1991; Donnelly *et al*. 2004). Dietary intake, particularly of vegetables, is considered the significant contributor to nitrate-nitrogen exposure in humans. However, with 10 ppm or more of nitrate-nitrogen in groundwater wells, drinking water becomes an important contributor to exposure in humans (Chilvers *et al*. 1984; Moller *et al*. 1989). Additionally, the formation of *N*-nitroso compounds (NOC), a class of genotoxic compounds, may more readily occur from drinking water sources since nitrate-nitrogen exposure from dietary food sources may be counterbalanced by ascorbate and polyphenols from food, which inhibits formation of NOCs (Bartsch & Frank 1996).

CONCLUSIONS

In conclusion, two different study populations were used to evaluate nitrate-nitrogen levels in drinking water and an overall risk for colorectal cancer was not found. However, when we stratified by colon subsite, we did observe a statistically increased risk for proximal colon cancer. Although these results are interesting, we encourage more research in this area. Research on the biology and measurements of *N*-nitroso compounds formation in the colon by subsite from nitrate-nitrogen contaminated drinking water would be beneficial. Further, prospective studies of susceptible populations, such as those with inflammatory diseases or low micronutrient intakes, would contribute to our understanding of the influence of nitrate-nitrogen contaminated drinking water and colorectal cancer risk. Due to increasing demands on our water, water quality will only grow in public health importance. Even a small increased cancer risk from contaminated drinking water could translate into a very large public health problem.

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