Municipal Drinking Water Nitrate Level and Cancer Risk in Older Women: The Iowa Women's Health Study

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Nitrate contamination of drinking water may increase cancer risk, because nitrate is endogenously reduced to nitrite and subsequent nitrosation reactions give rise to N-nitroso compounds; these compounds are highly carcinogenic and can act systemically. We analyzed cancer incidence in a cohort of 21,977 Iowa women who were 55–69 years of age at baseline in 1986 and had used the same water supply more than 10 years (87% >20 years); 16,541 of these women were on a municipal supply, and the remainder used a private well. We assessed nitrate exposure from 1955 through 1988 using public databases for municipal water supplies in Iowa (quartile cutpoints: 0.36, 1.01, and 2.46 mg per liter nitrate-nitrogen). As no individual water consumption data were available, we assigned each woman an average level of exposure calculated on a community basis; no nitrate data were available for women using private wells. Cancer incidence (N = 3,150 cases) from 1986 through 1998 was determined by linkage to the Iowa Cancer Registry. For all cancers, there was no association with increasing nitrate in drinking water, nor were there clear and consistent associations for non-Hodgkin lymphoma; leukemia; melanoma; or cancers of the colon, breast, lung, pancreas, or kidney. There were positive associations for bladder cancer [relative risks (RRs) across nitrate quartiles = 1, 1.69, 1.10, and 2.83] and ovarian cancer (RR = 1, 1.52, 1.81, and 1.84), and inverse associations for uterine cancer (RR = 1, 0.86, 0.86, and 0.55) and rectal cancer (RR = 1, 0.72, 0.95, and 0.47) after adjustment for a variety of cancer risk/protective factors, agents that affect nitrosation (smoking, vitamin C, and vitamin E intake), dietary nitrate, and water source. Similar results were obtained when analyses were restricted to nitrate level in drinking water from 1955 through 1964. The positive association for bladder cancer is consistent with some previous data; the associations for ovarian, uterine, and rectal cancer were unexpected. (Epidemiology 2001;11:327–338)

Keywords: nitrate, N-nitroso compounds, neoplasms, drinking water, cohort study, environmental exposures, water contaminants, gender.

The increasing contamination of municipal and private well drinking water by nitrate, primarily from the widespread use of commercial fertilizers, as well as from human and animal waste, has been documented in many areas of the United States. In Iowa, long-term heavy use of nitrogen fertilizers in both rural and urban areas has resulted in 30–40% of finished public water supplies with nitrate-nitrogen concentrations in excess of 5 mg per liter.[1] The current Environmental Protection Agency maximum contaminant level standard for drinking water, 10 mg per liter nitrate-nitrogen (NO_3-N), was set primarily to prevent methemoglobinemia in infants.[2] Other health effects of nitrate exposure, including cancer risk, have not been fully evaluated. Food (mainly certain vegetables such as lettuce, spinach, celery, turnip greens, etc), contaminated drinking water, cigarette smoking, and certain medications are the main contributors to daily nitrate intake in the U.S. population.[3] Nitrate *per se* does not appear to be carcinogenic in animals or humans in typical exposure scenarios, but a significant portion (~20%) of ingested nitrate is endogenously reduced to nitrite, which can then undergo nitrosation in the stomach with amines and amides to form N-nitroso compounds.[3,4] Nitrosation may also occur in the large intestine and the bladder.[4] N-nitroso compounds are some of the strongest known carcinogens,[2] can act systemically,[5] and have been found to

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induce cancer in a variety of organs in more than 40 animal species including higher primates.[6]

Drinking water can account for a substantial proportion of total nitrate intake. For example, at the Environmental Protection Agency maximum contaminant level of 10 mg per liter nitrate-nitrogen, drinking water accounts for over 50% of total nitrate intake, and at levels over 20 mg per liter it accounts for over 80% of intake.[7] Nitrate in drinking water is positively associated with urine nitrate levels as well as excretion of nitrosoproline, a biomarker of endogenous nitrosation.[8,9] Cigarette smoke accelerates nitrosation reactions, whereas intake of vitamin C or E within 1–2 hours of consumption of a nitrate source inhibits these reactions.[10] The latter observation provides a plausible explanation for the anticancer effect of vegetables, which are a major source of nitrate in the diet but also contain vitamins C and E as well as other antioxidant compounds.[11] In contrast, drinking water is often consumed without any such agents, suggesting a qualitative difference in exposure.

Given the extensive experimental data suggesting a role for nitrate in the formation of carcinogenic N-nitroso compounds and the widespread exposure to nitrate in the population, there is a surprising deficit of epidemiologic data addressing the possible association of nitrate in drinking water with cancer risk. Most of the epidemiologic data are derived from ecologic studies and have focused on gastric cancer, for which the data are extremely mixed.[12] Elevated risk of cancer of the esophagus, nasopharynx, urinary bladder, prostate, and non-Hodgkin lymphoma (NHL) have also been reported.[12,13] In addition to the problem of extrapolating ecologic data to the individual level, most previous ecologic studies had limited nitrate data, relied on cancer mortality (rather than incidence) data, and used residence at the time of death as a surrogate for lifetime residence. In addition, few studies took into account an induction period. Case-control studies have reported mixed results for nitrate in drinking water and gastric cancer,[14–16] null results for brain cancer,[17] and a positive association for NHL,[18] and the only published cohort study[19] reported no association with gastric cancer incidence.

Given this background, we evaluated the role of nitrate in drinking water averaged over approximately 30 years and cancer incidence from 1986 through 1998 in the Iowa Women's Health Study, a large prospective cohort study. We evaluated cancer incidence for all sites as well as individual sites, and we were able to adjust for dietary nitrate intake, factors affecting endogenous nitrosation (vitamin C and E intake and smoking), and other potential confounders.

Subjects and Methods

Iowa Women's Health Study

In 1986, a survey was mailed to 98,030 randomly selected women between the ages of 55 and 69 years who had a valid Iowa driver's license. The survey was completed by 41,836 women. There were only minor demographic differences between respondents and nonrespondents,[20] and compared with nonrespondents, respondents have had somewhat lower cancer incidence and mortality rates for smoking-related cancers.[21] The baseline survey contained demographic data and information on a variety of risk factors for cancer including anthropometric variables, reproductive history, hormone replacement therapy use, family history of cancer, level of physical activity, smoking and alcohol consumption, and prevalent medical conditions.[20] The baseline questionnaire also included a 126-item semiquantitative food frequency questionnaire (including supplement use), which was adapted from the questionnaire used in the 1984 Nurses' Health Study.[22] Nitrate from food was calculated from the Harvard Nutrient Database.[22] The validity and reproducibility of the food frequency questionnaire has been demonstrated in this cohort.[23] Women who had implausible energy intakes (<600 or >5,000 kcal/day) or left more than 30 items blank were assigned missing values for all dietary variables.

Information on drinking water use was collected in a follow-up questionnaire mailed in 1989. Participants were asked the main source of their drinking water at their current residence [possible responses: municipal (city) water system, private well, bottled water purchased from a store or dealer, and other] and how long they have been drinking the type of water indicated (possible responses: <1 year, 1–5 years, 6–10 years, 11–20 years, >20 years, and don't know). A total of 36,127 persons responded to the 1989 survey (90% of eligible women): 27,409 (76%) of the respondents reported drinking municipal water, 6,635 (18%) reported drinking private well water, and 2,083 (6%) reported drinking bottled water or water from other sources. Of the 27,409 women using municipal water, 22,375 reported using that water supply for greater than 10 years (3,093 for 11–20 years, and 19,282 for >20 years), and of the 6,635 women using a private well, 5,436 reported using that water supply for greater than 10 years (833 for 10–20 years and 4,603 for >20 years). Data on the volume of tapwater consumed daily and water consumption outside the home were not collected.

Of the 41,836 women in the original cohort, we excluded women who self-reported any cancer at the 1986 baseline (N = 3,881), women who did not respond to or died before the 1989 survey (N = 5,076), and women who had not been on the same municipal or private water supply for more than 10 years (N = 7,143); these exclusions left 25,736 women in the at-risk cohort.

Assessment of Municipal Nitrate Levels

Historical analytical data on Iowa municipal water supplies were used to estimate exposure to nitrate in drinking water; exposure classification was completed before evaluating cancer incidence. Finished water samples were initially collected and analyzed for nitrate during the time periods 1955–1964, 1976–1982, and 1983–1988. All water samples were analyzed at the University of Iowa Hygienic Laboratory (Iowa's state

public health laboratory), and the several thousand individual analytical results were reviewed for consistency and accuracy of information before entry into the electronic database. Utilizing these data, an average nitrate exposure level for the 33-year time frame (1955–1988) was calculated for each community included in the analysis. Of the 25,736 women who reported using a water supply for >10 years, 20,300 resided in a total of 484 communities in 1989; the remainder used a private well. We further restricted these communities to those with a single-source water supply, with "single source" defined as 90% of the water coming from a surface source or a specific aquifer (N = 47 communities excluded) and those with no nitrate analyses for any one of these time periods (N = 41 communities excluded). On the basis of these criteria, a total of 396 communities (16,541 women) were included in the analysis for municipal water supplies.[24] Restricting communities to those with a single source of water was done to increase the validity of the exposure measurement, as contaminant levels can vary between surface and groundwater supplies as well as between depth of water for groundwater supplies.

All women in the cohort who had been on a community water supply >10 years were linked by community name to the database of community nitrate values. All persons in a community were assigned the same level of exposure over the specified time frame. Nitrate data from the period 1955–1964 provided initial values for community water supplies for the start of the 33-year exposure window. The levels for 1976–1982 and 1983–1988 data were more numerous (in many instances, samples were taken and analyzed on a quarterly basis). Thus, three exposure time periods were available for analysis (1955–1964, 1976–1982, and 1983–1988).

Mean and median levels of nitrate were highly correlated within each community and for each time period (all Pearson correlations >0.90), and ranking of women within the cohort on the basis of quartile cutpoints showed little difference whether based on the mean or median. Therefore, we elected to use the mean nitrate level in a community's water supply as the exposure variable. Within each community, mean values from 1955–1964, 1976–1982, and 1983–88 were averaged to obtain an exposure value for the 33-year period. In addition, community drinking water nitrate levels for the 1955–1964 period were used as the exposure variable in a separate analysis to determine what impact baseline exposure levels might have on the relative risks (RRs) for cancers of interest.

Although we had no nitrate data for private well users, in the aggregate, nitrate contamination is higher in private wells relative to community wells in Iowa[25]; thus, this group could be viewed on average as at higher risk. For example, the Iowa Statewide Rural Well Water Survey from 1988–1989 estimated that 18.3% of rural, private drinking water wells in Iowa are contaminated with nitrate in excess of 10 mg per liter nitrate-nitrogen.[26]

Cohort Follow-Up and Data Analysis

The cohort was traced annually for cancer incidence by linkage of personal identifiers to the State Health Registry of Iowa's cancer database, which is part of the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program.[27] The mortality experience of the cohort was determined by linkage to Iowa death certificates, supplemented by linkage to the National Death Index. Each woman in the at-risk cohort was assigned person-years of follow-up from the date of return of the 1986 baseline questionnaire to the date of first cancer diagnosis, date of emigration from Iowa, or date of death; if none of these occurred, follow-up was through December 31, 1998.

RRs with 95% confidence intervals (CIs) were used as the measures of association between nitrate in drinking water and cancer incidence and were estimated using Cox proportional hazards regression.[28] Municipal nitrate exposure was examined as a categorical variable, using quartiles of mean nitrate level within a community over the time period of interest. Private well users were included as a separate category in the main analysis. The dose-response relation between the RR and municipal nitrate levels (excluding private well users) were examined by means of a smoothing spline (3 degrees of freedom) using Cox proportional hazards regression in S-Plus.[29] In the smoothing spline model, nitrate values above 6 mg per liter were recoded to 6 mg per liter and plotted on the square root scale with the corresponding quartile cutpoints indicated.

Total and site-specific cancer incidences were evaluated; site-specific cancers included those previously linked to nitrate exposure (gastrointestinal tract, bladder, and NHL) and other sites with at least 40 cases (hematopoietic, kidney, lung and bronchus, breast, ovary, uterine corpus, pancreas, and skin melanoma). There were too few esophageal (N = 12) or stomach (N = 20) cancers to evaluate these sites individually.

RRs were adjusted for a number of common risk or protective factors shared by many cancers including age (continuous), education (less than high school, high school, and postgraduate), smoking status (never, former, and current) and pack-years of smoking (continuous), physical activity (low, moderate, and high), fruit and vegetable consumption (categorical), total energy (continuous), vitamin C intake (categorical), vitamin E intake (categorical), body mass index (categorical), and waist-to-hip ratio (categorical). All continuous variables modeled as categorical used the quartile cutpoints in the at-risk cohort. Additional analyses adjusted for source of municipal drinking water supply (surface water *vs* groundwater), as determined from historical records.[30] Surface water systems were all treated with disinfectants and contained the highest levels of chlorination byproducts. Use of surface supplies has been previously reported to be associated with risk of colon cancer in this cohort.[31]

TABLE 1. Relation between Characteristics of Drinking Water Source and Selected Cancer Risk Factors at Study Baseline (1986), Iowa Women's Health Study

	Private Well	Mean Nitrate Levels (mg/L Nitrate-Nitrogen) in Municipal Water Supplies, 1955–1988			
		<0.36	0.36–1.00	1.01–2.46	>2.46
N	5,436	4,145	4,138	4,135	4,123
Age (years)	61.2	61.8	61.6	61.7	61.6
Body mass index (kg/m^2)	27.5	26.8	26.7	26.7	26.7
Waist-to-hip ratio	0.837	0.838	0.835	0.836	0.833
Total energy (kcal/day)	1,927	1,758	1,739	1,758	1,753
Vitamin C (mg/day)	277	284	301	286	308
Fruits and vegetables (servings/day)	5.5	5.3	5.3	5.3	5.4
Nitrate in diet (mg/day nitrate-nitrogen)	21.5	21.6	21.3	21.4	21.9
Greater than high school education	37%	41%	42%	37%	45%
Cigarette smoking					
Never smoked	79%	63%	61%	60%	62%
Former smoker	12%	21%	23%	23%	23%
Current smoker	9%	16%	16%	17%	15%
Physical activity index					
Low	47%	46%	47%	47%	47%
Moderate	29%	29%	27%	27%	27%
High	24%	25%	26%	26%	26%
Surface source for municipal water supply		4%	13%	36%	47%
Mean nitrate in municipal water, 1955–1988 (mg/liter nitrate-nitrogen)		0.20	0.70	1.91	5.59
Estimated mean ± SD daily nitrate intake from drinking water*		2.6% ± 2.7%	8.6% ± 6.0%	19.9% ± 11.2%	38.4% ± 15.8%

SD = standard deviation.

* Estimated by assuming consumption of 2 liters per day of drinking water.

Results

Of the 21,977 women in this analysis, 75% used a municipal supply (N = 16,541; 88% >20 years) and 25% used a private well (N = 5,436; 85% >20 years). The distribution of common risk factors for cancer among municipal water users showed little association with municipal water nitrate exposure levels (Table 1). Private well users were less likely to have used cigarettes, and they had greater energy intake and body mass index relative to municipal users; otherwise, there were few other notable differences for this group.

Nearly 50% of municipal water supplies with >2.46 mg per liter nitrate-nitrogen were from surface sources, whereas municipal water supplies with the lowest levels of nitrates were almost exclusively (96%) from ground sources (Table 1). With increasing levels of nitrate in the municipal supplies, the estimated mean percentage of daily nitrate intake from drinking water increased from about 3% in the lowest category to about 38% in the highest category.

During 11 years of follow-up (256,709 person-years), 3,150 women developed some type of cancer. As shown in Table 2, there was little evidence for an association between dietary sources of nitrate and risk of cancer, with the possible exception of leukemia and cancer of the kidney or bladder. There was, however, no evidence of a dose-response relation for these sites, and CIs were imprecise.

When municipal water supplies were categorized into ground *vs* surface sources (Table 3), women on a surface source were at a slightly elevated risk of NHL (RR = 1.22) and cancers of the colon (RR = 1.20), ovary (RR = 1.42), uterine corpus (RR = 1.25), and lung and bronchus (RR = 1.23).

RRs for major cancers by quartile of average nitrate in the municipal water supply from 1955–1988, as well as for private well users, are presented in Table 4. For each cancer site in the table, three models are presented: (1) crude RRs; (2) RRs adjusted for age, education, cigarette smoking, cigarette pack-years, level of physical activity, body mass index, waist-to-hip ratio, total energy, fruit and vegetable intake, vitamin C intake (diet and supplements), vitamin E intake (diet and supplements), and dietary nitrate intake ("full-model RR"); and (3) RRs further adjusted for ground *vs* surface water source ("full-model + source RR"). Because most of the RRs did not change substantially in the full models, our discussion will focus on the crude RR, with any deviance from this pattern specifically noted.

There was little association between nitrate levels in municipal water supplies and risk of cancer at all sites, and the cancer risk for private well users was similar to that of municipal water users. There was a weak inverse association between nitrate in water and risk of NHL in the crude model, and after adjustment for water source and other cancer risk factors, this association strengthened. For leukemia there was a U-shaped association, with approximately 50% lower risk for women in the 1.01–2.46 mg per liter nitrate-nitrogen category of municipal nitrate levels compared with the reference category. Women using private wells had a risk of leukemia

TABLE 2. Relative Risks (RR) and 95% Confidence Intervals (in Parentheses) for Selected Cancer Sites in Relation to Dietary Intake of Nitrate

Site	Daily Intake of Nitrate (mg Nitrate-Nitrogen) from Diet			
	<11.6*	11.6–18.0	18.1–27.2	>27.2
All sites				
Person-years	60,111	60,618	60,354	60,231
Cases	775	732	733	737
RR (95% CI)†	1	0.97 (0.87–1.07)	0.98 (0.88–1.08)	0.98 (0.88–1.09)
Non-Hodgkin lymphoma				
Cases	37	34	25	38
RR (95% CI)	1	0.88 (0.55–1.40)	0.62 (0.37–1.04)	0.91 (0.56–1.46)
Leukemia				
Cases	22	28	23	37
RR (95% CI)	1	1.28 (0.73–2.24)	1.07 (0.59–1.94)	1.73 (1.00–3.00)
Digestive organs				
Colon				
Cases	98	78	90	97
RR (95% CI)	1	0.79 (0.59–1.07)	0.93 (0.69–1.24)	1.00 (0.74–1.34)
Rectum				
Cases	28	39	27	28
RR (95% CI)	1	1.42 (0.87–2.31)	1.01 (0.59–1.73)	1.06 (0.61–1.83)
Pancreas				
Cases	19	15	16	19
RR (95% CI)	1	0.79 (0.40–1.56)	0.86 (0.44–1.69)	1.02 (0.52–1.99)
Other digestive				
Cases	17	19	20	15
RR (95% CI)	1	1.18 (0.61–2.27)	1.32 (0.68–2.57)	1.03 (0.50–2.13)
Urinary tract				
Kidney				
Cases	12	15	14	14
RR (95% CI)	1	1.32 (0.62–2.83)	1.32 (0.60–2.89)	1.37 (0.61–3.06)
Bladder				
Cases	9	17	13	14
RR (95% CI)	1	1.88 (0.84–4.24)	1.46 (0.62–3.47)	1.57 (0.66–3.75)
Breast‡				
Cases	253	252	265	254
RR (95% CI)	1	0.98 (0.83–1.17)	1.04 (0.87–1.24)	0.99 (0.83–1.19)
Reproductive organs§				
Ovary				
Cases	24	28	28	22
RR (95% CI)	1	1.12 (0.65–1.94)	1.10 (0.63–1.92)	0.85 (0.47–1.55)
Uterine corpus				
Cases	71	41	51	61
RR (95% CI)	1	0.60 (0.41–0.88)	0.78 (0.54–1.12)	0.97 (0.68–1.39)
Lung and bronchus				
Cases	76	65	66	59
RR (95% CI)	1	0.85 (0.61–1.19)	0.87 (0.62–1.22)	0.78 (0.55–1.11)
Skin (melanoma)				
Cases	22	24	25	17
RR (95% CI)	1	1.11 (0.62–1.99)	1.20 (0.67–2.16)	0.83 (0.43–1.61)

* Reference category.
† Adjusted for age and total energy.
‡ Excludes women with a mastectomy at baseline (N = 210).
§ Excludes women with bilateral oophorectomy (N = 3,778) or hysterectomy (N = 7,244) at baseline for ovarian and uterine cancer analyses, respectively.

similar to that of the reference (<0.36 mg per liter) category.

There was no consistent pattern of associations for the digestive organs. Although there was no evidence for a dose response between nitrate levels and colon cancer, RRs were elevated in the second (RR = 1.49; 95% CI = 1.07–2.08) and third (RR = 1.61; 95% CI = 1.16–2.24) quartiles of exposure even after multivariate adjustment. Risk of cancer of other digestive organs (mainly upper digestive tract organs as well as liver, gallbladder, or peritoneum) showed a positive association with nitrate levels, although these RRs were estimated with low precision. There was no consistent association with pancreatic cancer. For rectal cancer there was an inverse association that was mainly restricted to the highest quartile of exposure (RR = 0.49; 95% 0.27–0.89); private wells users also had a lower risk of rectal cancer (RR = 0.52; 95% CI = 0.31–0.89) compared with the lowest category of municipal water nitrate levels. When nitrate was modeled as a continuous variable, the inverse association was also apparent (Figure 1A).

There was a weak positive association with kidney cancer that attenuated with multivariate adjustment. There was a stronger positive association between municipal water nitrate levels and bladder cancer risk (Table 4 and Figure 1B). Compared with women drinking water with <0.36 mg per liter nitrate-nitrogen, women drinking water with >2.46 mg per liter were at increased risk of bladder cancer (RR = 2.59; 95% CI = 1.08–6.21). Adjustment for water source further strengthened the association. Women using private well water were only at a slightly elevated risk of bladder cancer com-

TABLE 3. Relative Risks (RR) and 95% Confidence Intervals for Selected Cancer Sites in Relation to Surface (Compared with Ground) Municipal Water Source

Site	Municipal Water Source*		Crude RR	95% CI
	Ground (Cases)	Surface (Cases)		
All sites	1,806	614	1.04	0.94–1.13
Non-Hodgkin lymphoma	75	30	1.22	0.80–1.86
Leukemia	75	19	0.77	0.47–1.28
Digestive organs				
Colon	215	85	1.20	0.94–1.55
Rectum	82	24	0.89	0.57–1.40
Pancreas	50	11	0.67	0.35–1.29
Other digestive	45	10	0.68	0.34–1.34
Urinary tract				
Kidney	35	10	0.87	0.43–1.75
Bladder	34	13	1.16	0.62–2.21
Breast†	617	193	0.95	0.81–1.11
Reproductive organs‡				
Ovary	56	26	1.42	0.89–2.25
Uterine corpus	118	50	1.25	0.90–1.75
Lung and bronchus	169	68	1.23	0.93–1.63
Skin (melanoma)	51	17	1.01	0.59–1.75
Person-years	144,781	47,652		

* Analysis includes only municipal water users (N = 16,541).
† Excludes women with a mastectomy at baseline (N = 170).
‡ Excludes women with bilateral oophorectomy (N = 2,866) or hysterectomy (N = 5,361) at baseline for ovarian and uterine cancer analyses, respectively.

pared with women in the lowest category of municipal water nitrate levels (multivariate RR = 1.31), although this estimate lacked precision (95% CI = 0.48–3.55).

There was little association with breast cancer, whereas opposite results were found for cancers of the ovary and uterine corpus. For ovarian cancer, there was a positive association with nitrate levels that was somewhat attenuated after multivariate adjustment (Table 4 and Figure 1C). Compared with women drinking water with <0.36 mg per liter nitrate-nitrogen, women drinking water with >2.46 mg per liter were at a twofold increased risk (95% CI = 1.05–3.96) of ovarian cancer; this estimate was slightly lower after adjusting for water source and potential confounders (RR = 1.84; 95% 0.88–3.84). Further adjustment for parity did not affect these results (data not shown). Women drinking private well water were also at elevated risk of ovarian cancer (RR = 1.44; 95% CI = 0.74–2.81) compared with women using municipal water with <0.36 mg per liter nitrate-nitrogen. In contrast, there was a suggestive inverse association between municipal nitrate levels and risk of cancer of the uterine corpus, which further strengthened after multivariate adjustment (Table 4 and Figure 1*D*). After multivariate adjustment that included water source, women consuming the highest levels of municipal water nitrate had an RR of 0.55 (95% CI = 0.33–0.92). Private well users, however, showed no difference in incidence relative to women in the lowest quartile of municipal nitrate water exposure.

There was no evidence for a dose response between lung cancer or melanoma with municipal water nitrate levels.

During the exposure period, nitrate levels increased and then leveled off. For the women on municipal supplies in this analysis, the mean (median) level of nitrate (mg per liter nitrate-nitrogen) for 1955–1964 was 1.31 (0.82), for 1976–1982 it was 2.73 (1.11), and for 1983–1988 it was 2.35 (1.56). The correlations between mean levels within a community for 1955–1964 and 1976–1982 and for 1955–1964 and 1983–1988 were r = 0.58 and r = 0.65, respectively. To evaluate the role of a longer induction period, we analyzed the association between nitrate levels in 1955–1964 with cancer incidence from the period 1986–1998 among women who were on the same municipal supplies for more than 20 years (Table 5). The quartile cutpoints for this time period were slightly lower than those for the overall analysis, reflecting the rising levels of nitrate over this time period. The pattern of association between nitrate levels and risk at individual cancer sites was similar to the results from the full analysis.

Discussion

We found positive trends between municipal water nitrate levels and risk of bladder and ovarian cancers, and inverse trends for cancer of the uterine corpus and rectum. Adjustment for common cancer risk factors, modulators of nitrosation (smoking, vitamin C, and vitamin E), dietary nitrate, and water source generally had little influence on these associations. In contrast, we found little or no evidence for a clear and consistent trend between municipal water nitrate levels and cancer risk for all sites; leukemia; NHL; melanoma; or cancers of the colon, kidney, breast, or lung. Strengths of exposure measurement in this study include the availability of a database with historical data on nitrate levels in Iowa communities dating back to 1955, with all samples analyzed at a single laboratory; quality-control checks on the nitrate data put into the database; and knowledge of the water source(s), ground or surface, of all water supplies in this study. Other strengths of this study include

TABLE 4. Relative Risks (RR) and 95% Confidence Intervals (in Parentheses) for Selected Cancer Sites in Relation to Nitrate Levels in Municipal Drinking Water Supplies

Cancer Site	Private Wells*	Mean Nitrate Levels (mg/L Nitrate-Nitrogen) in Municipal Water Supplies, 1955–1988			
		<0.36†	0.36–1.00	1.01–2.46	>2.46
All sites					
Person-years	64,276	48,438	48,163	47,821	48,011
Cases	730	586	620	630	584
RR‡	0.94 (0.84–1.04)	1	1.06 (0.95–1.19)	1.09 (0.97–1.22)	1.01 (0.90–1.13)
Full-model RR§	1.01 (0.90–1.14)	1	1.07 (0.95–1.20)	1.11 (0.99–1.25)	0.97 (0.86–1.09)
Full-model + source RR‖		1	1.07 (0.95–1.21)	1.11 (0.98–1.25)	0.97 (0.85–1.10)
Non-Hodgkin lymphoma					
Cases	38	31	27	23	24
RR	0.92 (0.57–1.48)	1	0.88 (0.52–1.47)	0.75 (0.44–1.29)	0.78 (0.46–1.33)
Full-model RR	0.88 (0.52–1.47)	1	0.91 (0.52–1.57)	0.87 (0.50–1.51)	0.71 (0.39–1.28)
Full-model + source RR		1	0.85 (0.49–1.47)	0.71 (0.39–1.29)	0.55 (0.29–1.04)
Leukemia					
Cases	27	27	24	12	31
RR	0.86 (0.51–1.44)	1	0.89 (0.52–1.55)	0.45 (0.23–0.89)	1.16 (0.69–1.94)
Full-model RR	0.82 (0.47–1.43)	1	0.83 (0.46–1.49)	0.38 (0.18–0.81)	0.92 (0.52–1.63)
Full-model + source RR		1	0.86 (0.48–1.55)	0.44 (0.20–0.95)	1.12 (0.61–2.06)
Digestive organs					
Colon					
Cases	85	58	86	92	64
RR	1.10 (0.79–1.54)	1	1.49 (1.07–2.08)	1.61 (1.16–2.24)	1.11 (0.78–1.59)
Full-model RR	1.14 (0.80–1.62)	1	1.54 (1.09–2.17)	1.58 (1.13–2.23)	1.01 (0.70–1.48)
Full-model + source RR		1	1.53 (1.09–2.16)	1.54 (1.08–2.19)	0.98 (0.66–1.46)
Rectum					
Cases	23	33	25	32	16
RR	0.52 (0.31–0.89)	1	0.76 (0.45–1.28)	0.98 (0.61–1.60)	0.49 (0.27–0.89)
Full-model RR	0.65 (0.37–1.12)	1	0.72 (0.42–1.26)	0.98 (0.59–1.63)	0.50 (0.27–0.93)
Full-model + source RR		1	0.72 (0.41–1.25)	0.95 (0.56–1.62)	0.47 (0.24–0.92)
Pancreas					
Cases	14	17	13	20	11
RR	0.62 (0.30–1.25)	1	0.77 (0.37–1.58)	1.20 (0.63–2.28)	0.65 (0.31–1.39)
Full-model RR	0.66 (0.31–1.41)	1	0.84 (0.41–1.76)	1.25 (0.64–2.43)	0.52 (0.22–1.22)
Full-model + source RR		1	0.88 (0.42–1.84)	1.45 (0.73–2.88)	0.64 (0.27–1.56)
Other digestive tract¶					
Cases	21	11	12	16	16
RR	1.43 (0.69–2.97)	1	1.10 (0.48–2.49)	1.48 (0.69–3.18)	1.47 (0.68–3.16)
Full-model RR	1.69 (0.75–3.82)	1	1.31 (0.55–3.11)	1.55 (0.67–3.58)	1.70 (0.74–3.88)
Full-model + source RR		1	1.39 (0.58–3.30)	1.83 (0.78–4.31)	2.09 (0.88–4.94)
Urinary Tract					
Kidney					
Cases	13	9	11	13	12
RR	1.09 (0.47–2.55)	1	1.23 (0.51–2.97)	1.46 (0.63–3.43)	1.35 (0.57–3.19)
Full-model RR	1.07 (0.45–2.57)	1	1.32 (0.54–3.18)	1.26 (0.52–3.04)	1.06 (0.42–2.68)
Full-model + source RR		1	1.34 (0.55–3.25)	1.38 (0.56–3.41)	1.20 (0.46–3.16)
Bladder					
Cases	10	7	14	8	18
RR	1.07 (0.41–2.81)	1	2.01 (0.81–4.98)	1.16 (0.42–3.19)	2.59 (1.08–6.21)
Full-model RR	1.31 (0.48–3.55)	1	1.65 (0.65–4.19)	1.00 (0.35–2.85)	2.43 (1.01–5.88)
Full-model + source RR		1	1.69 (0.66–4.30)	1.10 (0.38–3.20)	2.83 (1.11–7.19)
Breast#					
Cases	275	208	209	185	208
RR	0.99 (0.83–1.18)	1	1.01 (0.83–1.22)	0.90 (0.74–1.09)	1.00 (0.83–1.22)
Full-model RR	1.01 (0.83–1.22)	1	1.02 (0.84–1.25)	0.95 (0.77–1.17)	1.00 (0.82–1.23)
Full-model + source RR		1	1.03 (0.84–1.26)	0.97 (0.78–1.20)	1.03 (0.83–1.28)
Reproductive organs**					
Ovary					
Cases	25	13	19	24	26
RR	1.44 (0.74–2.81)	1	1.47 (0.73–2.98)	1.87 (0.95–3.68)	2.04 (1.05–3.96)
Full-model RR	1.55 (0.77–3.13)	1	1.52 (0.73–3.15)	1.94 (0.96–3.90)	2.03 (1.01–4.07)
Full-model + source RR		1	1.52 (0.73–3.17)	1.81 (0.88–3.74)	1.84 (0.88–3.84)
Uterine Corpus					
Cases	70	44	44	48	32
RR	1.16 (0.79–1.69)	1	1.01 (0.66–1.52)	1.08 (0.72–1.63)	0.73 (0.46–1.15)
Full-model RR	1.09 (0.74–1.61)	1	0.90 (0.58–1.39)	1.01 (0.66–1.54)	0.65 (0.40–1.06)
Full-model + source RR		1	0.86 (0.55–1.35)	0.86 (0.55–1.36)	0.55 (0.33–0.92)
Lung and bronchus					
Cases	43	56	57	77	47
RR	0.58 (0.39–0.86)	1	1.02 (0.71–1.48)	1.40 (0.99–1.97)	0.85 (0.58–1.25)
Full-model RR	0.92 (0.59–1.42)	1	1.00 (0.68–1.48)	1.49 (1.04–2.14)	0.83 (0.55–1.26)
Full-model + source RR		1	1.00 (0.67–1.47)	1.49 (1.02–2.17)	0.83 (0.53–1.30)
Skin (melanoma)					
Cases	25	18	17	18	15
RR	1.04 (0.57–1.91)	1	0.95 (0.49–1.84)	1.01 (0.53–1.95)	0.84 (0.42–1.67)
Full-model RR	1.09 (0.58–2.06)	1	0.95 (0.48–1.89)	0.98 (0.49–1.94)	0.81 (0.40–1.64)
Full-model + source RR		1	0.94 (0.47–1.87)	0.94 (0.46–1.91)	0.75 (0.35–1.61)

* No data on nitrate levels.

† Reference category.

‡ Unadjusted.

§Relative risk adjusted for age, education, smoking (never/former/current and pack-years), physical activity, body mass index, waist-to-hip ratio, total energy, intakes of vitamin C, vitamin E, dietary nitrate, and fruits and vegetables.

‖ Relative risk further adjusted for water source (ground *vs* surface).

¶ Includes esophagus (N = 12), stomach (N = 14), small intestine (N = 6), liver and bile ducts (N = 18), gallbladder (N = 16), peritoneum and retroperitoneum (N = 9), and other ill-defined digestive organs (N = 1).

Excludes women with a mastectomy at baseline (N = 210).

** Excludes women with bilateral oophorectomy (N = 3,778) or hysterectomy (N = 7,244) at baseline for ovarian and uterine cancer analyses, respectively.

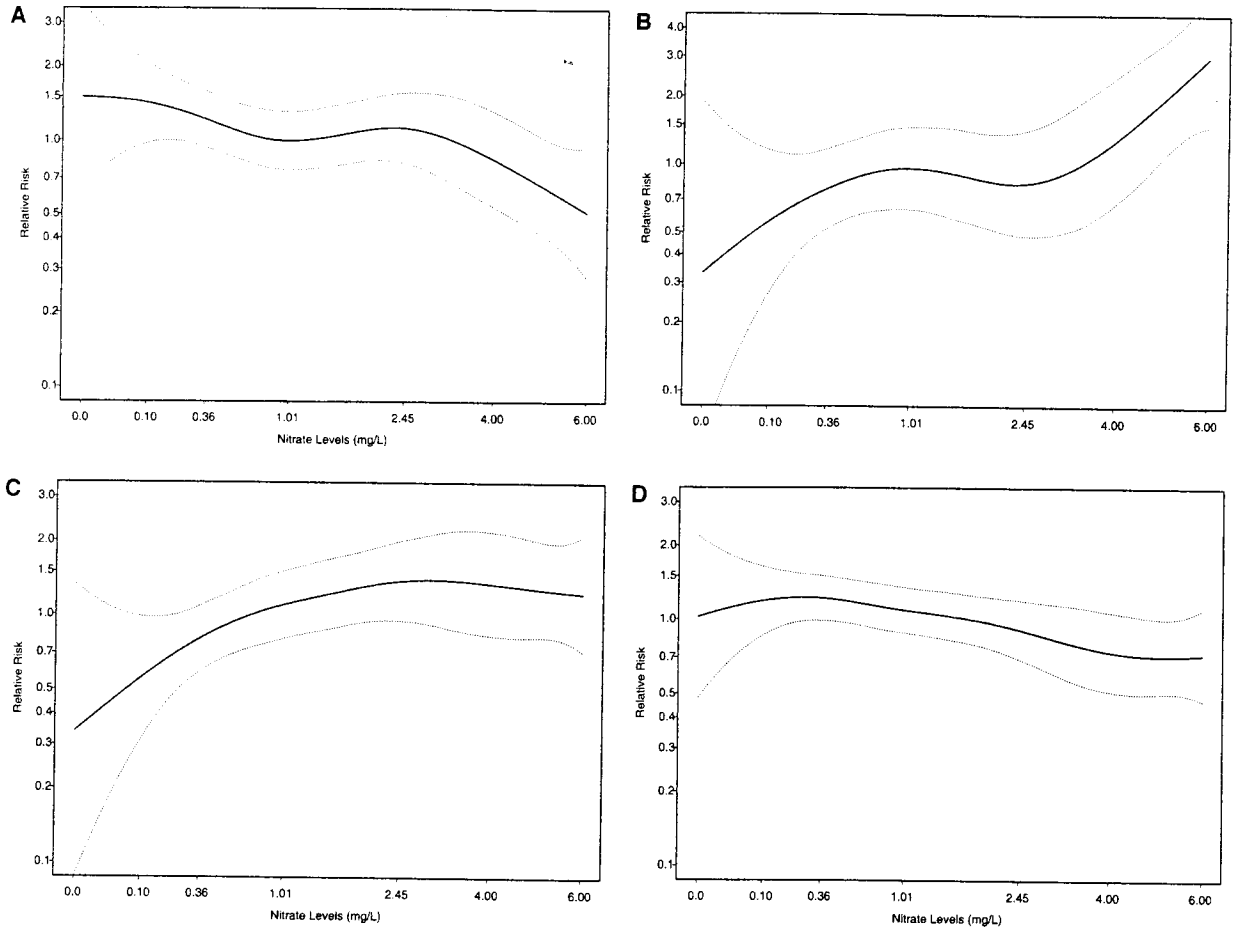

FIGURE 1. Estimated relative risk and 95% confidence bands for rectal (*A*), bladder (*B*), ovarian (C), and uterine (*D*) cancer by nitrate levels (mg per liter) based on a multivariate Cox proportional hazards model.

the prospective design, case ascertainment using a SEER registry, low population mobility of this group of women, availability of a variety of potential confounders, and a meaningful range of nitrate exposures within the cohort.

There are several important limitations to our exposure assessment that require comment. We only ascertained usual source of water in the home for the residence in 1988, and not for prior residences. This limitation forced us to exclude 18% of the municipal users because they had been on a supply for 10 or fewer years. We also did not have exact length of time on the water source, because it was ascertained as a categorical variable, and our upper category was ">20 years." Some of the women in the latter category may not have been on the municipal water supply for much more than 20 years, and this possibility could introduce misclassification of our exposure variable. Given the low residential mobility of women in this cohort, however, this situation is unlikely to have occurred frequently, and therefore it is unlikely to have greatly biased our results. We also had to make the assumption that the majority of an individual's drinking water came from the community of residence. An unknown proportion may have been consuming some portion of their drinking water from another source, particularly if they were employed in another town. Although only 33% of these women were employed outside the home at baseline in 1986, the amount and type of misclassification are difficult to estimate, although this factor would mainly be expected to be nondifferential and therefore is most likely to attenuate any association.

We also did not have individual water consumption data, which prevented us from evaluating interpersonal variation in water consumption habits; again, the net effect is likely to be nondifferential misclassification. Finally, there were no accessible computerized data available on nitrate levels in Iowa municipal finished water supplies during 1965–1975, which also limits exposure assessment, although, as we have shown, communities tended to retain their rank order in terms of nitrate levels over the time frame 1955–1988.

Other limitations of this study include a relatively small sample size for some of the cancer sites (for example, bladder cancer and NHL) and too small a size for meaningful analysis for other sites of *a priori* interest (for example, esophagus, nasopharynx, stomach, and brain). We also could not adequately evaluate interactions between nitrate levels and vitamin C or other factors

TABLE 5. Relative Risks (RR) and 95% Confidence Intervals (in Parentheses) for Selected Cancer Sites in Relation to Nitrate Levels in Municipal Drinking Water Supplies from 1955–1964*

Site	Mean Nitrate Levels (mg/L Nitrate-Nitrogen) in Municipal Water Supplies, 1955–1964			
	<0.33†	0.33–0.81	0.82–2.22	>2.22
All sites				
Person-years	41,679	41,277	42,389	43,260
Cases	505	548	540	565
RR (95% CI)‡	1	1.13 (0.99–1.29)	1.07 (0.94–1.22)	1.05 (0.92–1.21)
Leukemia				
Cases	23	17	15	26
RR (95% CI)	1	0.79 (0.40–1.56)	0.54 (0.26–1.13)	1.20 (0.64–2.27)
Non-Hodgkin lymphoma				
Cases	28	16	27	19
RR (95% CI)	1	0.59 (0.30–1.13)	0.97 (0.55–1.71)	0.55 (0.28–1.09)
Digestive organs				
Colon				
Cases	51	85	58	65
RR (95% CI)	1	1.75 (1.21–2.55)	1.20 (0.81–1.78)	1.17 (0.77–1.76)
Rectum				
Cases	32	20	22	21
RR (95% CI)	1	0.61 (0.34–1.10)	0.58 (0.33–1.03)	0.57 (0.31–1.06)
Pancreas				
Cases	14	11	12	16
RR (95% CI)	1	1.06 (0.47–2.40)	0.93 (0.42–2.09)	1.35 (0.61–2.99)
Other digestive organs				
Cases	11	11	12	13
RR (95% CI)	1	1.32 (0.54–3.26)	1.37 (0.57–3.53)	1.42 (0.57–3.53)
Urinary tract				
Kidney				
Cases	9	13	8	12
RR (95% CI)	1	1.46 (0.60–3.56)	0.71 (0.25–2.01)	1.24 (0.48–3.21)
Bladder				
Cases	6	9	15	14
RR (95% CI)	1	1.44 (0.49–4.22)	2.22 (0.84–5.87)	2.27 (0.82–6.28)
Breast§				
Cases	177	170	187	198
RR (95% CI)	1	1.01 (0.80–1.26)	1.10 (0.88–1.36)	1.12 (0.89–1.41)
Reproductive organs‖				
Ovary				
Cases	9	17	26	22
RR (95% CI)	1	1.66 (0.73–3.80)	2.32 (1.07–5.05)	1.86 (0.82–4.26)
Uterine corpus				
Cases	37	45	37	28
RR (95% CI)	1	1.00 (0.62–1.61)	0.82 (0.50–1.34)	0.60 (0.34–1.05)
Lung and bronchus				
Cases	46	58	50	59
RR (95% CI)	1	1.38 (0.90–2.12)	1.22 (0.79–1.88)	1.20 (0.77–1.87)
Skin (melanoma)				
Cases	15	20	14	14
RR (95% CI)	1	1.24 (0.60–2.56)	0.88 (0.41–1.89)	0.81 (0.36–1.82)

* Based on 19,103 women on a municipal water supply >20 years.

† Reference category.

‡ Relative risk and 95% confidence interval adjusted for age, education, smoking (never/former/current and pack-years), physical activity, body mass index, waist-to-hip ratio, total energy, intakes of vitamin C, vitamin E, dietary nitrate, and fruits and vegetables.

§ Excludes women with a mastectomy at baseline (N = 178).

‖ Excludes women with bilateral oophorectomy (N = 3,248) or hysterectomy (N = 6,053) at baseline for ovarian and uterine cancer analyses, respectively.

owing to a paucity of subjects for many of the sites of interest. This study was also restricted to older women, and the results may not be generalizable to men. Finally, although the exposure of interest was nitrate in drinking water, we were not able to evaluate other potential contaminants in drinking water, including disinfection byproducts, pesticides, heavy metals, and so forth, that may be of etiologic importance. We were, however, able to adjust for type of water supply (surface *vs* ground), which is a reasonable surrogate for chlorination byproduct contamination in this cohort.[31]

There are few epidemiologic data evaluating nitrate intake from water and bladder cancer risk. Ecologic studies conducted in Canada[32] and Spain[33] both found no association with nitrate levels in drinking water and bladder cancer incidence or mortality, respectively, whereas another study from Spain found a positive association for bladder cancer incidence, although the estimates were based on small numbers.[34] Only one study, however, accounted for an induction period,[33] and the mortality study from Spain[33] had only a limited number of bladder cancer cases, particularly among women. Follow-up studies of workers involved in the manufacture of nitrogen-based fertilizers have found no association with bladder cancer,[35–37] although the number of exposed cases was generally small. Chlorination byproducts in drinking water have been linked to bladder cancer in some studies,[12] but a recent case-control

study conducted in Iowa showed no association for women,[38] and data from this cohort also showed no association.[31] Adjustment for groundwater *vs* surface water source (a strong surrogate for higher levels of disinfection byproducts in these data[31]) if anything strengthened the association with nitrate.

An association between nitrate intake and bladder cancer is biologically plausible.[39] Under normal circumstances, approximately 70% of orally ingested nitrate is excreted in the urine,[3] and endogenous nitrosation occurs in the bladder.[4] In addition, nitrosation byproducts rapidly appear in the urine after oral ingestion of nitrate in drinking water,[9] and N-nitroso compounds are carcinogenic in the bladder in animal models.[40] Bladder infection also greatly increases endogenous nitrosation,[40] and the formation of N-nitroso compounds is one of the leading hypotheses to explain the strong association between schistosomiasis infection and bladder cancer risk.[39] Unfortunately, we did not collect data on occurrence of bladder infections in these women.

Our finding of no positive association between municipal nitrate levels and NHL (and possibly an inverse association in the multivariate model) is in contrast to a limited number of published reports. Ecologic studies conducted in Iowa[41] and Nebraska[42] found positive results, whereas a study from Denmark[43] found no association for lymphatic or hematopoietic cancers (separate results not reported). In the population-based case-control study (157 cases and 527 controls) conducted by Ward *et al*,[18] long-term consumption of community water (1947–1979) with average nitrate levels ≥4 mg per liter nitrate-nitrogen, compared with <1.6 mg per liter, was positively associated with NHL risk (OR = 2.0; 95% CI = 1.1–3.6), and there was evidence of a dose response with increasing nitrate levels. The association was similar in men and women and was somewhat stronger among persons consuming lower levels of vitamin C and carotene. Although our quartile cutpoints were lower than those of Ward *et al*,[18] reanalysis of our data using the same cutpoints did not change our results (data not shown). Although we only had 105 NHL cases among municipal water users, the 95% CIs of the point estimates suggest that we were unlikely to miss an RR of greater than 2.0. We had too few cases to evaluate adequately an interaction between vitamin C use and nitrate levels in municipal water users. At this point, it is not clear why our results are discrepant with those of Ward *et al*,[18] and further studies will be required to clarify this issue.

We found a U-shaped association between nitrate and leukemia and no elevation in risk among the private well users. Of two ecologic studies, one study in China found a positive correlation between urinary levels of nitrate and N-nitroso compounds and mortality rates from leukemia,[44] whereas another study in Denmark[43] found no association with lymphatic and hematopoietic cancer incidence and nitrate levels in municipal water.

The gastrointestinal tract has long been a focus of interest for the carcinogenic effects of nitrate and N-nitroso compounds[13]; this research initially focused on the stomach, but these compounds are also biologically relevant to the rest of the gastrointestinal tract owing in part to the ability of endogenous nitrosation to occur in the large bowel. N-nitroso compounds also induce colon cancer in animal models.[13] Our finding of no important association for colon cancer, at least at the highest level of nitrate, is consistent with ecologic data.[32,33,43,45] An inverse association between nitrate and rectal cancer has not been previously reported. In addition, most[36,37,46] but not all[47] studies of nitrate-based fertilizer workers report no association with colorectal cancer.

We had too few cases of esophageal or stomach cancer to evaluate these sites individually; when we evaluated all "other gastrointestinal sites" (which included those sites), there was a suggestive positive association.

The positive association of municipal levels of nitrate in drinking water and ovarian cancer risk was an unexpected finding. Adjustment for other cancer risk factors did not alter this association. Risk was also elevated in private well users (RR = 1.44; 95% CI = 0.74–2.81), a group expected on average to have a higher level of nitrate exposure. When source of water supply was added to the multivariate model, there was a slight attenuation of the association. Women on a surface source of water were also at elevated risk of ovarian cancer (RR = 1.44; 95% CI = 0.74–2.81), and chlorination byproducts may be important. Chloroform concentration in municipal water supplies was not associated with ovarian cancer in this cohort in a dose-response manner[31]; however, there are many other disinfection byproducts to consider.

Of two previous ecologic studies to evaluate nitrate levels in drinking water and ovarian cancer, both found null associations.[32,43] The etiology of ovarian cancer (mainly epithelial ovarian cancer in this cohort) is not well known. The most consistent risk factors are age, a family history of the disease, parity, and use of oral contraceptives, whereas the limited evaluation of dietary factors has not shown consistent results.[48] In this cohort, parity and total vegetable intake (particularly leafy green vegetables) were inversely associated with risk, whereas a family history of ovarian cancer in a first-degree relative; body fat distribution; higher physical activity; and greater intakes of cholesterol, eggs and lactose were positively associated with risk.[49,50] Many of these findings are difficult to reconcile with our current limited understanding of the pathogenesis of ovarian cancer. Nevertheless, the fivefold international variation in ovarian incidence and mortality rates[48] and the increase in ovarian cancer rates in migrants from Japan to the United States[51] strongly suggest a role for environmental exposures.

An inverse association between cancer of the uterine corpus and levels of nitrate in municipal drinking water was also an unexpected finding. A potential argument against the association being due to nitrate *per se* is that there was no reduction in risk among private well users, who as a group would be expected to have higher levels of nitrate in their drinking water. In contrast to our findings, a positive association between municipal nitrate levels and uterine cancer was reported in ecologic

studies conducted in Canada[32] and Denmark.[43] The etiology of endometrial cancer is most consistent with the "unopposed" estrogen hypothesis,[52] and risk factors include exogenous estrogen use without a progestin, obesity, early age at menarche, late age at menopause, and parity, and these are risk factors for endometrial cancer in this cohort.[53,54] In this cohort, smoking is inversely associated with risk,[53] whereas there are few strong dietary correlates,[55] which is consistent with the literature.[52] Cigarette smoking is also a major source of nitrate and nitrites; however, the protective effect of smoking on endometrial cancer has been attributed to its modulation of steroid hormones by nicotine, carbon monoxide, and polycyclic aromatic hydrocarbons, although the exact mechanism is unknown.[56] To our knowledge, nitrate and N-nitroso compounds are not known to modulate hormonal systems, and thus the inverse association with water nitrate levels and uterine cancer risk in this cohort has no obvious biologic plausibility, and may be a chance finding.

In summary, the positive association for bladder cancer is consistent with prior epidemiologic evidence, and has a supporting biologic rationale. The positive association with ovarian cancer and the inverse associations with uterine and rectal cancer were unexpected.

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