

6-3-2016

Nitrate from Drinking Water and Diet and Bladder Cancer among Postmenopausal Women in Iowa.

Rena R Jones

Peter J Weyer

Curt T Dellavalle

Maki Inoue-Choi

Kristin E Anderson

See next page for additional authors

Follow this and additional works at: http://hsrc.himmelfarb.gwu.edu/sphhs_exer_facpubs

 Part of the [Nutrition Commons](#), [Oncology Commons](#), and the [Pharmacology, Toxicology and Environmental Health Commons](#)

APA Citation

Jones, R., Weyer, P., Dellavalle, C., Inoue-Choi, M., Anderson, K., Cantor, K., Krasner, S., Robien, K., Beane Freeman, L., Silverman, D., & Ward, M. (2016). Nitrate from Drinking Water and Diet and Bladder Cancer among Postmenopausal Women in Iowa.. *Environmental Health Perspectives*, (). Retrieved from http://hsrc.himmelfarb.gwu.edu/sphhs_exer_facpubs/70

This Journal Article is brought to you for free and open access by the Exercise and Nutrition Sciences at Health Sciences Research Commons. It has been accepted for inclusion in Exercise and Nutrition Sciences Faculty Publications by an authorized administrator of Health Sciences Research Commons. For more information, please contact hsrc@gwu.edu.

Authors

Rena R Jones, Peter J Weyer, Curt T Dellavalle, Maki Inoue-Choi, Kristin E Anderson, Kenneth P Cantor, Stuart Krasner, Kim Robien, Laura E Beane Freeman, Debra T Silverman, and Mary H Ward



ENVIRONMENTAL HEALTH PERSPECTIVES

<http://www.ehponline.org>

Nitrate from Drinking Water and Diet and Bladder Cancer among Postmenopausal Women in Iowa

**Rena R. Jones, Peter J. Weyer, Curt T. Dellavalle,
Maki Inoue-Choi, Kristin E. Anderson, Kenneth P. Cantor,
Stuart Krasner, Kim Robien, Laura E. Beane Freeman,
Debra T. Silverman, and Mary H. Ward**

<http://dx.doi.org/10.1289/EHP191>

Received: 22 June 2015

Revised: 15 December 2015

Accepted: 18 May 2016

Published: 3 June 2016

Note to readers with disabilities: *EHP* will provide a [508-conformant](#) version of this article upon final publication. If you require a 508-conformant version before then, please contact ehp508@niehs.nih.gov. Our staff will work with you to assess and meet your accessibility needs within 3 working days.



Nitrate from Drinking Water and Diet and Bladder Cancer among Postmenopausal Women in Iowa

Rena R. Jones¹, Peter J. Weyer², Curt T. Dellavalle¹, Maki Inoue-Choi^{1,3}, Kristin E. Anderson^{4,5}, Kenneth P. Cantor¹, Stuart Krasner⁶, Kim Robien⁷, Laura E. Beane Freeman¹, Debra T. Silverman¹, and Mary H. Ward¹

¹Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health (NIH), Bethesda, Maryland, USA; ²Center for Health Effects of Environmental Contamination, University of Iowa, Coralville, Iowa, USA; ³National Institute on Minority Health and Health Disparities, NIH, Bethesda, Maryland, USA; ⁴Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, Minnesota, USA; ⁵Prevention and Etiology Research Program, Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota, USA; ⁶Metropolitan Water District of Southern California, La Verne, California, USA; ⁷Department of Exercise and Nutrition Sciences, Milken Institute School of Public Health, George Washington University, Washington, DC, USA

Address correspondence to Rena R. Jones, Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 9609 Medical Center Drive, Room 6E124, Rockville, MD 20850 USA. Telephone: (240) 276-7292. E-mail: rena.jones@nih.gov

Running title: Drinking water nitrate and bladder cancer

Acknowledgments: This work was supported in part by the Intramural Research Program of the National Cancer Institute (NCI) and by NCI extramural grant R01-CA39742. This study was approved by the Institutional Review Boards of the University of Minnesota and the University of Iowa. We thank Jiji Kantamneni of the Center for Health Effects of Environmental Contaminants at the University of Iowa for providing us with the public water supply data, and to Ching-Ping Hong of the University of Minnesota and Matt Butcher at Information Management Services for their programming support on this study.

Competing Financial Interests: The authors declare they have no actual or potential competing financial interests.

Abstract

Background: Nitrate is a drinking water contaminant arising from agricultural sources and a precursor in the endogenous formation of *N*-nitroso compounds (NOC), which are possible bladder carcinogens.

Objectives: To investigate the ingestion of nitrate and nitrite from drinking water and diet and bladder cancer risk in women.

Methods: We identified incident bladder cancers among a cohort of 34,708 postmenopausal women in Iowa (1986-2010). Dietary nitrate and nitrite intakes were estimated from a baseline food frequency questionnaire. Drinking water source and duration were assessed in a 1989 follow-up. For women using public water supplies (PWS) >10 years (N=15,577), we estimated average nitrate (NO₃-N) and total trihalomethanes (TTHM) levels and the number of years exceeding one-half the maximum contaminant level (NO₃-N: 5mg/L, TTHM: 40μg/mL) from historical monitoring data. We computed hazard ratios (HR) and 95% confidence intervals (CI), and assessed nitrate interactions with TTHM and modifiers of NOC formation (smoking, vitamin C).

Results: We identified 258 bladder cancer cases, including 130 among women >10 years at their PWS. In multivariable-adjusted models, we observed non-significant associations among women in the highest versus lowest quartile of average drinking water nitrate concentration (HR=1.48; CI=0.92,2.40; $p_{\text{trend}}=0.11$), and significant associations among those exposed ≥ 4 years to drinking water with >5mg/L NO₃-N (HR=1.62; CI=1.06,2.47; $p_{\text{trend}}=0.03$) compared to women with no years of comparable exposure. TTHM adjustment had little influence on associations, and we observed no modification by vitamin C intake. Relative to a common reference group of never smokers with the lowest nitrate exposures, associations were strongest for current smokers with the highest nitrate exposures (HR=3.67; 95% CI=1.43,9.38 and HR=3.48; 95% CI=1.20,10.06 for average water NO₃-N and ≥ 4 years >5mg/L, respectively). Dietary nitrate and nitrite intakes were not associated with bladder cancer.

Conclusions: Long-term ingestion of elevated nitrate in drinking water was associated with an increased risk of bladder cancer among postmenopausal women.

Introduction

Urinary bladder cancer is the sixth most common malignancy in the United States, with an incidence four-fold higher among men (SEER 2015). Established risk factors include cigarette smoking (Silverman et al. 2006), certain occupational exposures (Silverman et al. 2006), and ingestion of high levels of arsenic in drinking water (IARC 2012; Saint-Jacques et al. 2014). Higher exposure to disinfection by-products (DBP) in public drinking water supplies has also been associated with increased bladder cancer risk in studies in North America and Europe (Cantor 1997; Cantor et al. 2006; Costet et al. 2011). The relationship between bladder cancer risk and other drinking water contaminants has not been as well studied.

Nitrate is a common drinking water contaminant arising primarily from agricultural sources, such as nitrogen fertilizers and manure, and human waste. In the United States, agricultural areas often have elevated levels of nitrate in groundwater and surface waters (Nolan and Stoner 2000; Ward 2009). Nitrate is also found at high levels in certain vegetables. Nitrite sources in the U.S. diet include processed meats, breads, and cereals (IARC 2010). Ingestion of nitrate and nitrite can lead to endogenous formation of *N*-nitroso compounds (NOC) in the presence of nitrosatable precursors such as amines and amides from meat and fish. NOC also have exogenous sources, including cigarette smoke and meats preserved with nitrite and nitrate salts (IARC 2010).

Ingested nitrate and nitrite under conditions that result in the endogenous formation of NOC is classified as a probable human carcinogen by the International Agency for Research on Cancer (IARC 2010) based on animal and mechanistic studies, and limited epidemiologic evidence for stomach and esophagus cancers. Animal data support a role for NOC in causing tumors in numerous organ sites, including the bladder (IARC 2010). In human biomonitoring

studies, ingestion of nitrate in drinking water at the World Health Organization (WHO) acceptable daily dietary intake level (3.7 mg/kg; WHO, 2003) simultaneous with an amine source resulted in NOC excretion in the urine (IARC 2010; Vermeer et al. 1998). Intragastic nitrosation is inhibited by antioxidants, including vitamins C and E (IARC 2010; Mirvish 1986), but it is unclear to what extent modification of endogenous NOC formation occurs outside the gastrointestinal tract.

Despite the carcinogenic potential of NOC, nitrate in drinking water has not been extensively evaluated in relation to bladder cancer risk. Case-control studies in Iowa (Ward et al. 2003) and Spain (Espejo-Herrera et al. 2015) found no association between long-term average nitrate levels in public water supplies (PWS) and bladder cancer. Findings from two prospective studies include a significant positive association in the Iowa Women's Health Study (IWHS) (Weyer et al. 2001) and no association in a Dutch cohort (Zeegers et al. 2006). The Spanish study accounted for concomitant exposure to DBP, which are suspected bladder carcinogens, by adjusting for estimated total trihalomethanes (TTHM) in PWS (Espejo-Herrera et al. 2015). To our knowledge, no U.S. study of drinking water nitrate and bladder cancer has adjusted for potential confounding by DBP. In contrast to the inconclusive findings for drinking water nitrate, positive associations between bladder cancer risk and dietary sources of nitrate and nitrite have been identified in three prospective cohorts (Ferrucci et al. 2010; Michaud et al. 2006) and two case-control studies (Catsburg et al. 2014; Wu et al. 2012). These studies have implicated intake of processed meats or their nitrate/nitrite preservatives as risk factors, but only the studies by Catsburg et al. (2014) and Ferrucci et al. (2010) explored potential interactions with dietary antioxidants.

In the current study, we expanded a prior analysis of drinking water nitrate concentrations in PWS and bladder cancer risk in the IWHS cohort that included 47 cases (Weyer et al. 2001). With an additional 12 years of follow-up, 130 cases with >10 years at their public water source, and an improved drinking water exposure assessment, we estimated the bladder cancer risk associated with drinking water nitrate, adjusting for TTHM levels. We also evaluated dietary sources of nitrate and nitrite and assessed whether these associations were modified by factors that may inhibit or promote endogenous nitrosation.

METHODS

Study Population and Case Ascertainment

Details about the IWHS are available elsewhere (Folsom et al. 1996). Briefly, in 1986, a questionnaire was mailed to 98,030 women 55-69 years old who were selected randomly from Iowa driver's license records. The baseline survey included questions on demographics, anthropometry, reproductive and medical history, tobacco and alcohol consumption, diet, physical activity, and family history of cancer. Informed consent was implied by participants returning the enrollment survey; a total of 41,836 (42%) women responded. The original study and current analyses were approved by the Institutional Review Boards (IRB) of the University of Minnesota and the University of Iowa. The current analyses were additionally approved by the Office of Human Subjects Research at the National Cancer Institute and considered exempt from IRB review.

A 1989 follow-up questionnaire was completed by 36,127 (87.2%) participants, who indicated the primary source of drinking water at their current residence (municipal water system; rural water system; private well; bottled water; other; don't know) and the length of time they drank from this source (<1 year; 1-5 years; 6-10 years; 11-20 years; >20 years; don't know).

A majority of women (76.7%) reported a public (municipal or rural) source, 18.5% used a private well, and fewer than 5% reported using bottled water or other sources. Ninety percent reported using their water source for >10 years; we limited our evaluation to these women.

We identified incident urinary bladder cancers diagnosed between January 1, 1986 and December 31, 2010 by linkage with the State Health Registry (SHR) of Iowa (SHR 2015) and assessed diagnosis date, topography, morphology, and stage. Participant vital status for censoring purposes was determined annually through linkages with the National Death Index [National Center for Health Statistics (NCHS) 2015] and the SHR. Person-years of follow-up were calculated from the date of enrollment until the earliest of the dates of incident bladder cancer diagnosis, death, emigration from Iowa (<0.5% annually), the midpoint between the date of last contact and the date the subject was located outside of Iowa, or December 31, 2010. For deaths outside of Iowa, we censored participants at the midpoint between the dates of last contact and death.

Exposure Assessment

Drinking Water

The exposure assessment for nitrate in drinking water has been described (Inoue-Choi et al. 2015; Weyer et al. 2001). We used historical monitoring data (1955-1988) for finished water samples from Iowa public water utilities to compute annual average levels of nitrate-nitrogen (mg/L NO₃-N; CHEEC 2013). Direct measurements of TTHM were sparse before implementation of their regulation; therefore, most concentrations before 1980 were estimates based on an expert assessment using measurements and information on water source, treatment practices, and other factors (Amy et al. 2005). We obtained estimates of historical levels of two

specific THM (chloroform, bromodichloromethane), TTHM (the sum of chloroform, bromoform, bromodichloromethane, and dibromochloromethane), three specific HAAs (trichloroacetic, dichloroacetic, and bromochloroacetic acid), HAA5 (the sum of trichloroacetic, dichloroacetic, and monochloroacetic acids), and HAA6 (the sum of HAA5 and bromochloroacetic acid); there were little to no occurrence data for other DBP in Iowa. Average levels of chloroform and bromodichloromethane were highly correlated with each other and with TTHM concentration ($\rho=0.95-0.98$; Table S1), and TTHM were correlated with the individual and summed HAAs ($\rho=0.79-0.92$); we therefore used TTHM levels to represent DBP exposure. TTHM were modestly correlated with annual average nitrate concentrations ($\rho=0.24$). Because we had only a categorical duration at the water source as reported on the questionnaire, we estimated the medians within duration categories of 11-20 and >20 years as 16 and 40 years, respectively. These were computed from complete water source histories of female controls of comparable age in a population-based case-control study in Iowa conducted during the same time period (Cantor et al. 1998). For each PWS for which both NO_3 levels and DBP estimates were available, we computed 16- and 40-year averages from the annual averages of $\text{NO}_3\text{-N}$ and TTHM levels, and the number of years within these periods when the annual average exceeded one-half the maximum contaminant level ($\frac{1}{2}$ -MCL; $\text{NO}_3\text{-N}$: 5mg/L and TTHM: 40 $\mu\text{g/mL}$). We also examined two indicators of DBP presence, including whether a PWS was treated by chloramination (yes/no), a disinfection process that may result in *N*-nitrosodimethylamine (NDMA) formation (Krasner et al. 2013; Mitch et al. 2003), and if it was sourced from surface or ground water. We linked the PWS-level drinking water metrics to participants by the 477 Iowa cities where women reported using PWS.

Diet

Dietary intakes were assessed at baseline using an adaptation of the Harvard semi-quantitative food frequency questionnaire (FFQ; Willett et al. 1988), which queried participants regarding their usual intake of 127 food items and dietary supplements over the prior 12 months. A re-administration of the FFQ two years after enrollment in a small sample of the cohort indicated good reproducibility in measuring key macro- and micronutrients, including total energy intake ($r=0.51-0.67$) and vitamin C ($r=0.81-0.84$; Munger et al. 1992). Total vitamin C intake was calculated by multiplying the frequency of consumption of foods and dietary supplements by their vitamin C content and summing across all foods and supplements.

As described in detail previously (Ward et al. 2003; Ward et al. 2006), we reviewed published data for U.S. and Canadian populations to identify the nitrate and nitrite contents of foods and estimated food-specific mean nitrate and nitrite values, accounting for the preparation method (e.g., raw, cooked, and canned) and weighting by the number of samples analyzed. To assign the food-specific nitrate and nitrite levels to the IWHS FFQ line items (e.g., raw carrots), we weighted the contributing food-specific values (e.g., raw carrots, cooked carrots) by female-specific intakes of each food from the 1994-1996 Continuing Survey of Food Intake by Individuals (Subar et al. 2000). For each participant, we estimated overall dietary intakes of nitrate and nitrite, and source-specific intakes from plant, animal, and processed meat (e.g., sausage, salami, bologna, bacon, and hot dogs) sources.

Statistical Analysis

Of the 41,836 women enrolled in the IWHS, we excluded those who reported a prior cancer (other than non-melanoma skin cancer) or previous cancer chemotherapy (N=3,830), had

implausible dietary intakes (<600 or >5000 kcal/day) or responded to fewer than 30 dietary questions (N=2,751), or were still menstruating at enrollment (N=547), to be consistent with the cohort's target composition of postmenopausal women. After these exclusions, made for consistency with the previous study by Weyer et al. (2001), data for 34,708 women were available for dietary analyses.

For drinking water analyses, we excluded women who reported using their water source for ≤ 10 years in 1989 (N=5,168 on PWS, N=624 on private wells), and those not reporting a duration (N=4,705 on PWS, N=13 on private wells). We also excluded women on PWS with no nitrate or TTHM measurements during the time period they were drinking from the PWS (N=1,638). To reduce variability in contaminant levels within a PWS due to changes in its sources over time, we excluded women on supplies that either lacked detail on sources or that had a sole surface water source or aquifer for less than 75% of the study period (<30 years; N=1,615). This left 15,910 women on PWS and 5,035 on private wells for analysis.

We used Cox regression to estimate hazard ratios (HR) and 95% confidence intervals (95% CI). In drinking water analyses, we compared average nitrate exposure quartiles to the lowest exposure category (Q1). We divided years $> \frac{1}{2}$ -MCL at the median (4 years) and compared to those with no years of exposure at this level. We evaluated the linearity of these relationships by modeling the exposures as continuous variables and by including cubic splines and quadratic exposure terms. The non-parametric analyses showed no statistically significant evidence of non-linear relationships (data not shown), so we present only natural log-transformed (ln-transformed) results for continuous models in addition to those from categorical analyses. Because no measurement data were available for private wells, we compared women on private well water to women on PWS in Q1 of average nitrate exposure. In dietary analyses,

we estimated relative risks for quartiles of dietary nitrate and nitrite overall and by nitrite intakes from plant, animal, and processed meat sources. We evaluated a number of potential confounders ascertained from the baseline questionnaire in models of both diet and water exposures. Covariates were selected for inclusion in a stepwise fashion and retained based on a $\geq 10\%$ change in the exposure parameter estimate, and included sociodemographic and lifestyle characteristics and history of various site-specific cancers among first-degree female relatives. Smoking status (never, former, current smoker) was assessed at baseline and in 1992, 1997, and 2004 follow-up surveys, with participation rates of 82.6, 79.1, and 68.3%, respectively. We modeled the most recent smoking status available and pack-years of smoking (0, ≤ 1 to 19, 20 to 39, ≥ 40) reported at baseline, the only survey in which smoking intensity and duration were assessed. We evaluated separate adjustments for smoking intensity and duration in all models. We computed age-adjusted associations (Model 1), ran multivariable models further adjusted for smoking status and baseline pack-years (Model 2), and then additionally adjusted these models for ln-transformed TTHM concentration (Model 3). Dietary models were adjusted for smoking, age, and total calorie intake, and mutually adjusted for ln-transformed continuous dietary nitrate or nitrite intake.

We tested for linear trend by modeling continuous variables derived from the median value within each exposure category. We assessed effect modification by TTHM ($<$ median or \geq median) and smoking status (never, former, or current) by estimating HRs relative to common jointly low-exposed reference groups of those with the lowest levels of average nitrate or 0 years > 5 mg/L and the lowest level of the modifier ($<$ median TTHM and nonsmokers, respectively). The common reference group for vitamin C analyses was low nitrate and \geq median vitamin C, the group of women with *a priori* assumed lowest risk, given that vitamin C is known to inhibit

intragstric nitrosation under certain conditions. We used likelihood ratio tests comparing the fit of models with and without product interaction terms to derive an overall interaction p-value for each potential effect modifier.

We used several approaches to assess consistency with prior investigations and sources of bias. The initial analysis by Weyer et al. (2001) was restricted to utilities where $\geq 90\%$ of the water in the supply came from a single source. Because we relaxed this criterion to $\geq 75\%$, we also compared risks estimated using the original criterion. The Environmental Protection Agency's 1992 Phase II rule minimally requires annual nitrate monitoring under the Safe Drinking Water Act. However, monitoring frequency prior to this rule depended on the size of the population served by the PWS, and additional testing may have been implemented where previous measurements were elevated (EPA 2012); therefore, our nitrate exposure metrics were derived from a variable number of measurements per PWS. We computed the coefficient of variation (CV) between years for annual mean nitrate levels and repeated analyses after excluding women in the top 10% of between-year exposure variation. To further evaluate the influence of data availability on our results, we also repeated analyses after restricting to women with long duration at their water source (>20 years) whose average nitrate level was based on at least the median number of years of data available (≥ 8 years). We conducted all analyses in SAS® (9.3), with $p \leq 0.05$ as the criterion for statistical significance.

RESULTS

We observed 263 urinary bladder cancers among 34,708 postmenopausal women over an average 21 years (median=25) of follow-up, including 170 cases among the women who used a private well (N=5,035) or PWS (N=15,910) as their primary drinking water source >10 years.

Most women with private wells lived on a farm or in non-farm rural areas, while most PWS users lived in towns (Table 1). The fraction of women on PWS sourced from surface water varied by nitrate quartile, with 46% in the highest category. Conversely, women with the highest average PWS nitrate levels had the lowest proportion of PWS disinfected by chloramination. Other factors did not differ appreciably by nitrate level. Compared with private well users, current smoking was more prevalent among PWS users, who also tended to have lower BMI, consume fewer calories, and were more highly educated. Average nitrate and TTHM levels varied across the 10 most populated cities and towns in our analysis (37% of the cohort using PWS) with no pattern, and the most populated city, Des Moines, accounted for only 8% of the women in our analysis (Table S2).

In multivariable-adjusted models, women in the highest quartile of mean drinking water nitrate exposure had a non-significantly higher relative risk of bladder cancer (Model 2 HR_{Q4 vs. Q1}=1.48, 95%CI=0.92, 2.40; $p_{\text{trend}}=0.11$); the association was very similar following additional adjustment for TTHM (Model 3; Table 2) and for chloramination status (HR_{Q4 vs. Q1}=1.43, 95%CI=0.87, 2.33). Risk among women exposed to ≥ 4 years above the $\frac{1}{2}$ -MCL level was significantly greater than for women with no years of exposure to levels above this threshold (Model 2 HR=1.62, 95%CI=1.06, 2.47; $p_{\text{trend}}=0.03$), and associations were also similar after further adjustment for TTHM (Model 3; Table 2). Positive associations were also present with exposures analyzed as continuous variables, significantly so for the $\frac{1}{2}$ -MCL metric (Table 2). We observed no association in multivariable-adjusted models comparing N=4,930 private well users (N=36 cases) to women on PWS in the lowest NO₃-N quartile (Model 2 HR=1.16, 95%CI=0.70,1.91). We observed no significant associations with TTHM and bladder cancer for

either the TTHM long-term average (Model 2 $HR_{Q4 \text{ vs. } Q1}=0.93$, 95%CI= 0.55,1.58) or the $\frac{1}{2}$ -MCL exposure metric (Model 2 $HR_{\geq 4 \text{ years vs. } 0}=0.80$, 95%CI=0.47,1.38).

In our sensitivity analyses applying a more conservative source criterion ($\geq 90\%$ of the PWS served from the same source, $N=14,775$, 121 cases), or excluding women whose nitrate exposures represented the top 10% of the variance across estimates ($N=14,029$, 110 cases), did not materially change these associations (data not shown). We observed somewhat stronger associations among the subset of women with >20 years of use of their PWS whose average nitrate exposure was estimated from 8 or more years of measurements ($N=8,032$; Model 2 $HR_{Q4 \text{ vs. } Q1}=2.38$, 95%CI=1.08-5.22; $p_{\text{trend}}=0.02$; Table S3); the number of years of available data were weakly correlated with nitrate levels ($\rho=0.11$).

Nitrate models stratified by smoking status suggested a multiplicative interaction (global p -value for interaction=0.03) on bladder cancer risk (Table 3), with the strongest association among current smokers consuming the highest average nitrate level compared to never smokers with low average nitrate levels ($HR=3.67$, 95%CI=1.43,9.38; $p_{\text{interaction}}=0.03$). We observed a somewhat similar pattern for the $\frac{1}{2}$ -MCL exposure metric ($p_{\text{interaction}}=0.01$), with the strongest association among current smokers with ≥ 4 years drinking water at $>\frac{1}{2}$ -MCL, as well as elevated relative risks among former smokers, compared to never smokers with no years $>\frac{1}{2}$ -MCL.

In models stratified by TTHM level, we observed a non-significant positive association among women drinking from PWS with high average nitrate (>2.97 mg/L) and \geq median TTHM levels ($HR_{Q4 \text{ vs. } Q1}=1.64$, 95%CI=0.94, 2.86) compared with women $<$ median TTHM and low NO_3 -N, but not among women with $<$ median TTHM and high NO_3 -N ($HR_{Q4 \text{ vs. } Q1}=1.12$, 95%CI=0.53,2.37; $p_{\text{interaction}}=0.07$; Table S4). We found little evidence for interaction between either PWS nitrate exposure metric and vitamin C intake (Table S5). Compared to those with the

lowest average intake of NO₃-N and > median vitamin C, we observed associations among women with high NO₃-N and ≤ vitamin C (HR=3.05, 95%CI= 1.37,6.79) as well as elevated risk among women with high NO₃-N and > median vitamin C ($p_{\text{interaction}}=0.27$; Table S5). Similar patterns were observed with the ½-MCL metric. However, we observed a significant positive association with private well use and bladder cancer among women with ≥ median vitamin C intake compared to women on PWS with low (Q1) average nitrate levels (HR=2.38,95%CI=1.03,5.51). In contrast, there was no association with private well use among women with < median vitamin C intake (HR=0.69,95%CI=0.36,1.35; $p_{\text{interaction}}=0.02$).

Dietary nitrate intakes came almost exclusively from plant sources (median proportion=97.0%) and thus only total nitrate is presented, whereas nitrite came from plant (62.3%) and animal sources (37.7%), including 4.4% from processed meats. Nitrate and nitrite intakes overall were moderately correlated ($\rho=0.49$) and strongly so for processed meats ($\rho=0.99$). We observed no overall association between dietary nitrate intake and bladder cancer risk, and no association for dietary nitrite overall or from separate sources (Table 4). There was no pattern of different risk estimates by vitamin C intake group comparing to common reference groups of ≥ median vitamin C and either low nitrate or nitrite, and global tests for interaction were not statistically significant (Table S6). Compared to a common reference group of never smokers with Q1 of dietary nitrite intake, risk of bladder cancer among current smokers with the highest nitrite intake was significantly increased (HR=2.66,95% CI=1.05,6.75); whereas, there was a non-significant positive association among former smokers (HR=1.49, 95%CI=0.70,3.21) and no association with high nitrite intake among never smokers (HR=0.75,95% CI=0.38,1.49; $p_{\text{interaction}}=0.06$; Table S7). Non-significant associations with dietary nitrate intake

were apparent across intake quartiles among current smokers only ($p_{\text{interaction}}=0.10$; Table S7).

DISCUSSION

Consistent with an earlier report on this cohort (Weyer et al. 2001), we estimated a greater relative risk of bladder cancer among postmenopausal women in Iowa whose drinking water source had higher long-term average nitrate levels, with statistically significant associations for those with four or more years at levels $>1/2$ -MCL. These associations were generally unchanged by adjustment for TTHM level. Our analyses suggested possible modification of the nitrate-bladder cancer association among women with a history of smoking and among those simultaneously exposed to high TTHM levels in their drinking water. Dietary nitrate and nitrite were not associated with bladder cancer. Vitamin C, a known inhibitor of intragastric NOC formation, did not significantly modify observed associations.

Our finding of a bladder cancer association with long-term average drinking water nitrate exposure is generally consistent with a prior analysis in the IWHS, which found positive associations among women at their water source for >10 years ($RR_{Q4\text{vs}.Q1}=2.43$ 95%CI=1.01,5.88) based on 47 cases (Weyer et al. 2001). However, the prior exposure assessment assigned a single 33-year average PWS nitrate level to all participants. In our analysis of nearly three times as many cases using a duration-based average exposure, we found a similar pattern but an overall weaker association among women with >10 years at their water source, which strengthened when we restricted analyses to women with an estimated >20 years of exposure.

Other epidemiologic evidence from case-control or cohort studies to support this association is limited. A large population-based bladder cancer case-control study in Iowa

(N=808 cases) with comparable PWS nitrate exposure levels found no relationship with bladder cancer overall, nor among the 186 female cases specifically (Ward et al. 2003). Another population-based case-control analysis of exposure to chlorinated DBP in Colorado (N=327 cases) reported no association or confounding effects of concurrent nitrate levels (McGeehin et al. 1993). In a Spanish case-control study of over 1,400 cases, average nitrate levels in drinking water were not associated with bladder cancer (Espejo-Herrera et al. 2015). A large prospective study in the Netherlands with over 800 cases also reported no overall or gender-specific associations (Zeegers et al. 2006). The average long-term nitrate level in the Dutch cohort (1.68 mg/L) was comparable to ours (median=1.1 mg/L). However, their exposure metric was based on samples from a single year, which may not reflect usual exposure over time. Inconsistent associations between drinking water nitrate and bladder cancer risk across studies could also be due to regional variation in the mixtures and relative abundance of water contaminants resulting from differences in disinfection practices (Costet et al. 2011), agricultural inputs, and the prevalence of naturally occurring chemicals. The extent of exposure misclassification and subsequent ability to detect associations may also differ between studies.

The MCL for nitrate in drinking water (10 mg/L NO₃-N) was established based on evidence of health risks from short-term exposure, such as methemoglobinemia (blue baby syndrome) in infants (EPA 2012). Bladder cancer development associated with chronic intake of levels below the MCL has only been evaluated in a small number of studies (Cantor et al. 2006; Villanueva et al. 2014). To our knowledge, our finding of significantly increased bladder cancer risk associated with four or more years above ½-MCL exposure is the first such epidemiologic finding in a prospective cohort with quantitative exposure assessment. The Spanish study similarly reported elevated bladder cancer ORs associated with longer duration of exposure (>20

years) to higher levels (>9 mg/L NO_3^- , i.e. >2 mg/L $\text{NO}_3\text{-N}$) of drinking water nitrate (Espejo-Herrera et al. 2015). While our categorical drinking water exposure metrics were correlated ($\rho=0.74$), only 15% of women were exposed to both high (Q4; >2.97 mg/L) average levels and ≥ 4 years >5 mg/L, indicating these metrics capture different features of nitrate exposure.

A key finding of the analysis conducted by Weyer et al. (2001) was a stronger water nitrate-bladder cancer association when models were adjusted for whether a PWS used surface versus ground water; the former is a crude surrogate for higher DBP levels. Although DBP, often represented as TTHM, have been associated with bladder cancer (Cantor et al. 2006; Costet et al. 2011; Villanueva et al. 2014), our associations with drinking water $\text{NO}_3\text{-N}$ were upheld with adjustment for TTHM, which were not independently associated with bladder cancer. We were underpowered to further examine the suggestive finding of stronger nitrate associations for women with simultaneously higher TTHM exposures, but the Spanish study identified a similar risk pattern (Espejo-Herrera et al. 2015). Estimated TTHM levels in our study (median=4.6 $\mu\text{g/L}$, interquartile range 0.9-14.3) were generally below the MCL and levels associated with bladder cancer in other studies (>5 $\mu\text{g/L}$; Costet et al. 2011). Non-regulated and therefore non-measured DBP, such as NDMA, could also have driven our observed nitrate associations (Richardson et al. 2007), although adjustment for PWS chloramination did not meaningfully change our results.

We found no consistent association between dietary nitrate overall or nitrite intake from processed meat and bladder cancer. A similar lack of relationship was reported from a prospective European study of over 1,000 bladder cancer cases that examined associations with intakes of red or processed meats or dietary nitrosamines (Jakszyn et al. 2011). In contrast, unlike in the prospective Nurses' Health Study cohort (N=304 incident cases; Michaud et al. 2006) and population-based case-control studies in California (N=1,660 incident cases; Catsburg

et al. 2014) and New England (N=1,068 incident cases; Wu et al. 2012), we did not find associations with nitrate or nitrite intakes from processed meat sources. Processed meat intake in our cohort was considerably lower than intakes reported in these studies. This may in part reflect lower intakes in women; dietary intakes of certain NOC, such as the rodent bladder carcinogen NDMA, have been reported as up to three times higher among men (Dich et al. 1996; Jakszyn et al. 2006). Our ability to compare findings is limited, as associations for women specifically were reported only from the Nurses' Health Study (Michaud et al. 2006). Catsburg et al. (2014) reported no effect modification by gender, and the study in New England did not report a gender-specific evaluation (Wu et al. 2012).

Human feeding studies have demonstrated endogenous formation of NOC after ingestion of nitrate at or above the MCL (IARC 2010; Vermeer et al. 1999; Vermeer and van Maanen 2001). About 5% of ingested nitrate is reduced to nitrite by bacteria in the mouth, which can further react with secondary and some tertiary amines or amides to form NOC in the stomach (Mirvish and Ramm 1987). NOC, such as dibutyl nitrosamine, are metabolized by the liver and ultimately pass through the urinary bladder to be excreted (Mirvish 1995). Less is known about the influence of intrinsic or extrinsic factors on NOC formation within the bladder. We evaluated interactions between nitrate and vitamin C on bladder cancer risk based on the capacity for antioxidants to inhibit intragastric NOC formation (Bartsch et al. 1993), a mechanism thought to explain the elevated nitrate-associated gastrointestinal malignancies observed among individuals with low vitamin C intake (Dellavalle et al. 2014; IARC 2010; Loh et al. 2011). Our results provide no compelling support for an interaction between vitamin C and nitrate on bladder cancer risk, consistent with previous studies of drinking water nitrate (Ward et al. 2003; Espejo-Herrera et al. 2015) and two recent analyses that reported no modification of dietary nitrate-

bladder cancer associations by vitamin C from diet or supplementation (Ferrucci et al. 2010) or from estimated from fruit and vegetable intakes (Wu et al. 2012). The interpretation of an interaction between vitamin C intake and private well use is unclear, as we had no quantitative exposure estimates for these women and the precision of the associations was limited.

Cigarette smoking was a potential confounder in our analysis, as it is a known risk factor for bladder cancer and a source of NOC exposure. Smoking was independently associated with bladder cancer risk in our data, as reported previously in the cohort (Tripathi et al, 2002). Thiocyanate in cigarette smoke is a potential catalyst for intragastric NOC formation (Preston-Martin and Correa 1989), and greater endogenous nitrosation could explain stronger associations with drinking water nitrate among smokers. However, the results of these interaction analyses should be interpreted with caution due to their small numbers. We assessed smoking status at both baseline and a more recent time period for many women, which likely reduced misclassification of this variable in our analyses. Smoking intensity was moderately but not linearly correlated with average drinking water nitrate concentrations (data not shown), and we saw no change in estimated associations from adjustment for smoking, including our main models with intensity and duration combined as pack-years, or in analyses separating these characteristics (data not shown). Therefore, residual confounding from smoking is also unlikely to explain the observed risks.

Our study remains among the few prospective analyses of drinking water nitrate exposure and bladder cancer risk. To our knowledge, it is also the first to assess this association in a cohort study with simultaneous assessment of TTHM. Our PWS-level exposure estimates reflected duration-specific average levels as well as the duration of exposure to elevated levels. The residential stability of the cohort (inferred by the large proportion of women reporting >20 years

at their drinking water source) likely reduced drinking water exposure misclassification due to changes in drinking water sources over time. Sensitivity analyses with more stringent exposure assessment criteria indicated that our results may indeed underestimate the true drinking water nitrate-bladder cancer association.

We acknowledge some limitations, including an inability to adjust for high-risk occupation. Iowa is predominantly agricultural, and unmeasured occupational or environmental exposures could have contributed to the observed associations. Our data suggest that most participants were not employed in occupations likely to have these exposures, and that occupational categories did not differ with drinking water nitrate levels. Moreover, measured lifestyle and other characteristics were not different among women in the top categories of average nitrate exposure. Importantly, we lacked a measure of total fluid intake, which would influence exposure to nitrate from drinking water (IARC 2010). Private well users are potentially exposed to nitrate, but not to DBP, in their drinking water. A limitation of our study was the fact that we had no nitrate exposure estimates for the women using private wells nor did we have information on well depth, an important predictor of nitrate concentrations. We addressed other limitations of our exposure assessment, confirming a lack of correlation between sampling frequency and nitrate concentration, and identifying stronger associations with exposure metrics derived from more years of measurements. The latency of bladder cancer following nitrate exposure is unknown. However, findings from studies of arsenic, another drinking water contaminant, suggest that the latency of arsenic-related bladder cancer is 40 or more years (Baris et al. 2016; Steinmaus et al. 2013). Thus, although we had no water measurements after 1988, a long latency for bladder cancer and our retrospective assessment should reduce the impact of this lack of exposure information on our results. Misclassification of dietary intakes is also possible

due to our use of a FFQ and our inability to identify some factors contributing to nitrate levels in vegetables and NOC formation in processed meats, such as regional variation in growing conditions and cooking methods, respectively. Further, whether these findings are specific to the postmenopausal status or homogeneous racial/ethnic makeup of our female study population or to drinking water sources in Iowa should be further evaluated.

Conclusion

Our results indicate that ingested nitrate from drinking water may play a role in bladder cancer etiology. We assessed both nitrate and TTHM exposures and bladder cancer risk and found that the previously observed associations with drinking water nitrate remained with additional follow-up. We observed an interaction between drinking water nitrate and smoking status on bladder cancer risk that requires replication, and a suggested interaction between nitrate and TTHM that also deserves evaluation in future studies. These results should be interpreted in the context of very limited epidemiologic data on drinking water nitrate exposures and bladder cancer risk, and with regard to our population of predominantly non-Hispanic white, postmenopausal women.

REFERENCES

- Amy G, Graziano N, Craun G, Krasner S, Cantor KP, Hildesheim M, et al. 2005. Improved exposure assessment on existing cancer studies. American Water Works Association (AWWA) Research Foundation: Denver, CO.
- Baris D, Waddell R, Beane Freeman LE, Schwenn M, Colt JS, Ayotte JD, et al. 2016. Elevated bladder cancer in Northern New England: the role of drinking water and arsenic. *J Natl Cancer Inst* doi: 10.1093/jnci/djw099 [Online 2 May, 2016]
- Bartsch H, Pignatelli B, Calmels S, Ohshima H. 1993. Inhibition of nitrosation. *Basic Life Sci* 61:27-44.
- Cantor KP. 1997. Drinking water and cancer. *Cancer Cause Control* 8:292-308.
- Cantor KP, Lynch CF, Hildesheim ME, Dosemeci M, Lubin J, Alavanja M, et al. 1998. Drinking water source and chlorination byproducts. I. Risk of bladder cancer. *Epidemiology* 9:21-28.
- Cantor KP, Ward MH, Moore LE, Lubin JE. 2006. Water contaminants. In: *Cancer Epidemiology and Prevention*, (Schottenfeld D, Fraumeni JF, eds). New York: Oxford University Press, 382-404.
- Catsburg CE, Gago-Dominguez M, Yuan JM, Castelao JE, Cortessis VK, Pike MC, et al. 2014. Dietary sources of n-nitroso compounds and bladder cancer risk: Findings from the Los Angeles Bladder Cancer Study. *Int J Cancer* 134:125-135.
- CHEEC (Center for Health Effects of Environmental Contamination). 2013. Historical community water supply and treatment data for the state of Iowa, 6th ed. University of Iowa: Iowa City, IA.
- Costet N, Villanueva CM, Jaakkola JJ, Kogevinas M, Cantor KP, King WD, et al. 2011. Water disinfection by-products and bladder cancer: Is there a European specificity? A pooled and meta-analysis of European case-control studies. *Occup Environ Med* 68:379-385.
- Dellavalle CT, Xiao Q, Yang G, Shu XO, Aschebrook-Kilfoy B, Zheng W, et al. 2014. Dietary nitrate and nitrite intake and risk of colorectal cancer in the Shanghai Women's Health Study. *Int J Cancer* 134:2917-2926.
- Dich J, Jarvinen R, Knekt P, Penttila PL. 1996. Dietary intakes of nitrate, nitrite and NDMA in the Finnish Mobile Clinic Health Examination Survey. *Food Addit Contam* 13:541-552.

Environmental Protection Agency (EPA). 2012. Basic information on the chemical contaminant rules. Available:
<http://water.epa.gov/lawsregs/rulesregs/sdwa/chemicalcontaminantrules/basicinformation.cfm>
[accessed 15 May 2016]

Espejo-Herrera N, Cantor KP, Malats N, Silverman DT, Tardon A, Garcia-Closas R, et al. 2015. Nitrate in drinking water and bladder cancer risk in Spain. *Environ Res* 137:299-307.

Ferrucci LM, Sinha R, Ward MH, Graubard BI, Hollenbeck AR, Kilfoy BA, et al. 2010. Meat and components of meat and the risk of bladder cancer in the NIH-AARP Diet and Health Study. *Cancer* 116:4345-4353.

International Agency for Research on Cancer (IARC). 2010. Ingested nitrate and nitrite and cyanobacterial peptide toxins. *IARC Monogr Eval Carcinog Risk Hum* 94: 9-464.

International Agency for Research on Cancer (IARC). 2012. IARC Arsenic, metals, fibres, and dusts. *IARC Monogr Eval Carcinog Risk Hum* 100C: 11-465.

Inoue-Choi M, Jones RR, Anderson KE, Cantor KP, Cerhan JR, Krasner S, et al. 2015. Nitrate and nitrite ingestion and risk of ovarian cancer among postmenopausal women in Iowa. *Int J Cancer* 137:173-182.

Jakszyn P, Agudo A, Berenguer A, Ibanez R, Amiano P, Pera G, et al. 2006. Intake and food sources of nitrites and n-nitrosodimethylamine in Spain. *Public Health Nutr* 9:785-791.

Jakszyn P, Gonzalez CA, Lujan-Barroso L, Ros MM, Bueno-de-Mesquita HB, Roswall N, et al. 2011. Red meat, dietary nitrosamines, and heme iron and risk of bladder cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Cancer Epidem Biomar* 20:555-559.

Krasner SW, Mitch WA, McCurry DL, Hanigan D, Westerhoff P. 2013. Formation, precursors, control, and occurrence of nitrosamines in drinking water: A review. *Water Res* 47:4433-4450.

Loh YH, Jakszyn P, Luben RN, Mulligan AA, Mitrou PN, Khaw KT. 2011. N-nitroso compounds and cancer incidence: The European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk study. *Am J Clin Nutr* 93:1053-1061.

McGeehin MA, Reif JS, Becher JC, Mangione EJ. 1993. Case-control study of bladder cancer and water disinfection methods in Colorado. *American J Epidemiol* 138:492-501.

Michaud DS, Holick CN, Giovannucci E, Stampfer MJ. 2006. Meat intake and bladder cancer risk in 2 prospective cohort studies. *Am J Clin Nutr* 84:1177-1183.

Mirvish SS. 1986. Effects of vitamins C and E on N-nitroso compound formation, carcinogenesis, and cancer. *Cancer* 58:1842-1850.

Mirvish SS, Ramm MD. 1987. Demonstration of in vivo formation of the nitrosamine n-nitroso-n-methylaniline from amyl nitrite. *Cancer Lett* 36:125-129.

Mirvish SS. 1995. Role of n-nitroso compounds (NOC) and n-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC. *Cancer Lett* 93:17-48.

Mitch WA, Sharp JO, Rhoades Trussell R, Valentine RL, Alvarez-Cohen L, DSedlak DL. 2003. N-nitrosodimethylamine (NDMA) as a drinking water contaminant: A review. *Environ Engineering Sci* 20:389-404.

Munger RG, Folsom AR, Kushi LH, Kaye SA, Sellers TA. 1992. Dietary assessment of older Iowa women with a food frequency questionnaire: Nutrient intake, reproducibility, and comparison with 24-hour dietary recall interviews. *Am J Epidemiology* 136:192-200.

National Center for Health Statistics (NCHS) 2015. National Death Index. Available: <http://www.cdc.gov/nchs/ndi/index.htm> [accessed 15 May 2016]

Nolan BT, Stoner JD. 2000. Nutrients in groundwaters of the conterminous United States, 1992-1995. *Environ Sci Technol* 34:1156-1165.

Preston-Martin S, Correa P. 1989. Epidemiological evidence for the role of nitroso compounds in human cancer. *Cancer Surv* 8:459-473.

Richardson SD, Plewa MJ, Wagner ED, Schoeny R, Demarini DM. 2007. Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. *Mutation Res* 636:178-242.

Saint-Jacques N, Parker L, Brown P, Dummer TJ. 2014. Arsenic in drinking water and urinary tract cancers: A systematic review of 30 years of epidemiological evidence. *Environ Health* 13:44.

National Cancer Institute Surveillance, Epidemiology, and End Results Program (SEER). 2015. SEER Stat fact sheets: Bladder cancer. Available: <http://seer.cancer.gov/statfacts/html/urinb.html> [accessed 15 May 2016]

Silverman DT, Devesa SS, Moore LE, Rothman N. 2006. Bladder cancer. In: *Cancer Epidemiology and Prevention*, (Schottenfeld D, Fraumeni JF, eds). New York: Oxford University Press, 1101-1127.

State Health Registry of Iowa. Iowa Cancer Registry. Available: <http://www.public-health.uiowa.edu/shri/> [accessed 15 May 2016]

Steinmaus C, Ferreccio C, Romo JA, Yuan Y, Cortes S, Marshall G, et al. 2013. Drinking water arsenic in Northern Chile: high cancer risks 40 years after exposure cessation. *Cancer Epidemiol Biomarkers Prev.* 22: 623-630

Subar AF, Midthune D, Kuhlthorn M, Brown CC, Thompson FE, Kipnis V, et al. 2000. Evaluation of alternative approaches to assign nutrient values to food groups in food frequency questionnaires. *Am J Epidemiol* 152:279-286.

Tripathi A, Folsom AR, Anderson KE. 2002. Risk factors for urinary bladder carcinoma in postmenopausal women. *Cancer* 95:2316-2323.

Vermeer IT, Pachon DM, Dallinga JW, Kleinjans JC, van Maanen JM. 1998. Volatile N-nitrosoamine formation after intake of nitrate at the ADI level in combination with an amine-rich diet. *Env Health Perspect* 106:459-63.

Vermeer IT, Moonen EJ, Dallinga JW, Kleinjans JC, van Maanen JM. 1999. Effect of ascorbic acid and green tea on endogenous formation of n-nitrosodimethylamine and n-nitrosopiperidine in humans. *Mutation Res* 428:353-361.

Vermeer IT, van Maanen JM. 2001. Nitrate exposure and the endogenous formation of carcinogenic nitrosamines in humans. *Rev Environ Health* 16:105-116.

Villanueva CM, Kogevinas M, Cordier S, Templeton MR, Vermeulen R, Nuckols JR, et al. 2014. Assessing exposure and health consequences of chemicals in drinking water: Current state of knowledge and research needs. *Environ Health Perspect* 122:213-221.

Ward MH, Cantor KP, Riley D, Merkle S, Lynch CF. 2003. Nitrate in public water supplies and risk of bladder cancer. *Epidemiology* 14:183-190.

Ward MH, Cerhan JR, Colt JS, Hartge P. 2006. Risk of non-hodgkin lymphoma and nitrate and nitrite from drinking water and diet. *Epidemiology* 17:375-382.

Ward MH. 2009. Too much of a good thing? Nitrate from nitrogen fertilizers and cancer. *Rev Environ Health* 24:357-363.

Weyer PJ, Cerhan JR, Kross BC, Hallberg GR, Kantamneni J, Breuer G, et al. 2001. Municipal drinking water nitrate level and cancer risk in older women: The Iowa Women's Health Study. *Epidemiology* 12:327-338.

Willett WC, Sampson L, Browne ML, Stampfer MJ, Rosner B, Hennekens CH, et al. 1988. The use of a self-administered questionnaire to assess diet four years in the past. *Am J Epidemiol* 127:188-99.

World Health Organization (WHO). 2003. Nitrate and Nitrite in Drinking Water. Background Document for Development of WHO Guidelines for Drinking-water Quality. WHO/SDE/WSH/03.04.56. Geneva, Switzerland: WHO. Available: http://www.who.int/water_sanitation_health/dwq/chemicals/nitratenitrite2ndadd.pdf [Accessed 15 May 2016]

Wu JW, Cross AJ, Baris D, Ward MH, Karagas MR, Johnson A, et al. 2012. Dietary intake of meat, fruits, vegetables, and selective micronutrients and risk of bladder cancer in the New England region of the United States. *Brit J Cancer* 106:1891-1898.

Zeegers MP, Selen RF, Kleinjans JC, Goldbohm RA, van den Brandt PA. 2006. Nitrate intake does not influence bladder cancer risk: The Netherlands Cohort Study. *Environ Health Perspect* 114:1527-1531.

Table 1. Characteristics of Iowa Women’s Health Study participants with >10 years at their drinking water source, by private well use and nitrate-nitrogen (NO₃-N) levels in public water.

Characteristic	Private well (N=5,035)	Mean ^a NO ₃ -N (mg/L) levels in public water (N=15,910)			
		<0.47	0.47-1.07	1.08-2.97	>2.97
Length of follow-up, years (mean ± SD)	21.3 ± 5.5	20.7 ± 5.9	20.5 ± 5.9	20.6 ± 5.9	20.6 ± 6.0
Age at baseline, years (mean ± SD)	61.2, ± 4.1	61.7 ± 4.2	61.7 ± 4.2	61.6 ± 4.2	61.6 ± 4.2
White race [<i>n</i> (%)]	4,971 (99.6)	3,999 (99.5)	3,849 (99.2)	4,146 (99.2)	3,645 (98.7)
Missing (<i>n</i>)	46	46	34	31	29
Surface water as source for PWS [<i>n</i> (%)]	-	146 (3.6)	1,101 (28.1)	668 (15.9)	1,716 (46.1)
PWS chloraminated [<i>n</i> (%)]	-	721 (17.7)	1,483 (37.9)	1,614 (38.3)	273 (7.3)
Nitrate in diet, mg/day (median) ^b	59.1	61.2	61.3	61.8	62.0
Nitrite in diet, mg/day (median) ^b	0.66	0.64	0.64	0.65	0.65
Vitamin C in diet, mg/day (median) ^b	99.9	108.0	111.8	108.7	112.9
Total caloric intake, kcal/day (median)	1,829	1,696	1,683	1,693	1,682
Smoking status ^c [<i>n</i> (%)]					
Never	3,882 (78.3)	2,499 (62.4)	2,393 (61.7)	2,487 (59.7)	2,275 (62.1)
Former	850 (17.2)	1,174 (29.3)	1,157 (29.7)	1,329 (31.9)	1,079 (29.4)
Current	223 (4.5)	334 (8.3)	325 (8.4)	347 (8.3)	311 (8.4)
Missing (<i>n</i>)	46	57	38	48	57
Pack-years of smoking ^d [<i>n</i> (%)]					
1-19	468 (45.0)	594 (40.6)	604 (41.5)	624 (38.2)	526 (39.1)
20-39	358 (34.4)	488 (33.3)	489 (33.6)	576 (35.2)	461 (34.3)
≥40	214 (20.6)	383 (26.1)	363 (24.9)	435 (26.6)	358 (26.6)
Missing (<i>n</i>)	80	34	22	33	44
Occupation [<i>n</i> (%)]					
Homemaker	2,643 (52.5)	1322 (32.5)	1309 (33.5)	1366 (32.4)	1221 (32.8)

Professional	604 (12.0)	670 (16.5)	657 (16.8)	636 (15.1)	618 (16.6)
Clerical/craft	1,293 (25.7)	1995 (49.1)	1883 (48.1)	2139 (50.8)	1834 (49.3)
Farmer	473 (9.4)	60 (1.5)	46 (1.2)	41 (1.0)	34 (0.9)
Other	22 (0.4)	17 (0.4)	18 (0.5)	41 (0.7)	34 (0.4)
Residence [n(%)]					
Farm	3,592 (71.5)	129 (3.2)	129 (3.3)	90 (2.2)	98 (2.7)
Rural area (non-farm)	971 (19.3)	73 (1.8)	86 (2.2)	61 (1.5)	106 (2.9)
Towns \geq 1000 residents	460 (9.2)	3,844 (95.0)	3,660 (94.5)	4,035 (96.4)	3,494 (94.5)
Missing (<i>n</i>)	12	18	38	25	24
BMI (kg/m ²) [<i>n</i> (%)]					
<25	1,797 (35.7)	1,658 (40.8)	1,661 (42.5)	1,801 (42.8)	1,551 (41.7)
25-29.9	1,914 (38.0)	1,514 (37.3)	1,418 (36.2)	1,511 (35.9)	1,364 (36.7)
\geq 30	1,324 (26.3)	892 (22.0)	834 (21.3)	899 (21.4)	807 (21.7)
Education [<i>n</i> (%)]					
Less than high school	505 (10.1)	275 (6.8)	237 (6.1)	276 (6.6)	202 (5.4)
High school	2,621 (52.2)	2,163 (53.3)	2,030 (52.0)	2,268 (54.0)	1,828 (49.1)
More than high school	1,900 (37.8)	1,619 (39.9)	1,640 (42.0)	1,658 (39.5)	1,690 (45.4)
Missing (<i>n</i>)	9	7	6	9	2

^a Exposure assigned to individuals based on their reported duration at drinking water source.

^b Adjusted for 1,000 kcal per day of total energy intake.

^c Determined based on most recent follow-up participation; otherwise from baseline report.

^d Among current or former smokers.

Table 2. Association between drinking water nitrate-nitrogen (NO₃-N) in public water supplies and bladder cancer in the Iowa Women’s Health Study (N=15,577^a)

Drinking water nitrate	Cases	N	Model 1 ^b	Model 2 ^c	Model 3 ^d
			HR (95% CI)	HR (95% CI)	HR (95% CI)
Average NO₃-N (mg/L)					
<0.47	29	3,973	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
0.47-1.07	32	3,853	1.16 (0.70,1.91)	1.16 (0.70,1.92)	1.14 (0.68,1.90)
1.08-2.97	30	4,130	1.00 (0.60,1.67)	0.98 (0.59,1.64)	0.97 (0.58,1.62)
>2.97	39	3,621	1.49 (0.92,2.41)	1.48 (0.92,2.40)	1.47 (0.91,2.38)
<i>p</i> _{trend} ^e			0.10	0.11	0.11
Continuous ^f	130	15,577	1.13 (0.96,1.32)	1.12 (0.95,1.32)	1.12 (0.95,1.32)
Years ½–MCL (>5 mg/L NO₃-N)					
0	83	10,947	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
< 4	18	2,295	1.04 (0.62,1.73)	1.05 (0.63,1.75)	1.06 (0.63,1.76)
≥ 4	29	2,335	1.66 (1.09,2.53)	1.62 (1.06,2.47)	1.61 (1.05,2.47)
<i>p</i> _{trend} ^e			0.02	0.03	0.03
Continuous ^g	130	15,577	1.07 (1.01,1.13)	1.06 (1.00,1.12)	1.06 (1.00,1.12)

^a After excluding 333 women with missing covariate data.

^b Adjusted for age.

^c Adjusted for age, smoking status, and pack-years of smoking.

^d Adjusted for age, smoking status, pack-years of smoking, and ln-transformed TTHM level.

^e Estimated by modeling a continuous variable derived from the median value within each exposure category.

^f HR per one natural log increase in concentration (mg/L).

^g HR per one year increase in number of years >½-MCL.

Table 3. Association between drinking water nitrate-nitrogen (NO₃-N) in public water supplies and bladder cancer in the Iowa Women’s Health Study, stratified by smoking status (N=15,577)

Drinking water nitrate	Never smokers			Former smokers			Current smokers			<i>p</i> _{interaction} ^b
	Cases	N	HR ^a (95% CI)	Cases	N	HR ^a (95% CI)	Cases	N	HR ^a (95% CI)	
Average NO₃-N (mg/L)										
<0.47	17	2,499	1.00 (Ref.)	8	1,149	0.70 (0.28,1.76)	4	325	1.24 (0.37,4.13)	
0.47-1.07	13	2,393	0.81 (0.39,1.67)	16	1,142	1.43 (0.66,3.14)	3	318	1.00 (0.26,3.78)	
1.08-2.97	14	2,487	0.83 (0.41,1.68)	11	1,305	0.83 (0.35,1.95)	5	338	1.57 (0.51,4.82)	
>2.97	15	2,275	0.97 (0.49,1.95)	14	1,048	1.30 (0.58,2.94)	10	298	3.67 (1.43,9.38)	0.03
	59	9,654		49	4,644		22	1,279		
Years >½-MCL (>5 mg/L NO₃-N)										
0	39	6,784	1.00 (Ref.)	34	3,268	1.25 (0.68,2.27)	10	895	1.39 (0.59,3.30)	
< 4	9	1,475	1.06 (0.51,2.19)	2	631	0.38 (0.09,1.67)	7	189	4.69 (1.79,12.27)	
≥ 4	11	1,395	1.39 (0.71,2.71)	13	745	1.99 (0.94,4.22)	5	195	3.48 (1.20,10.06)	0.01
	59	9,654		49	4,644		22	1,279		

^a Adjusted for age and pack-years of smoking.

^b Derived from a likelihood ratio test comparing fit of models with and without a cross-product term for smoking status and nitrate exposure.

Table 4. Association between dietary nitrate and nitrite and bladder cancer in the Iowa Women's Health Study (N=33,964^a)

	Cases	N	Age-adjusted HR (95% CI)	Fully adjusted ^c HR (95% CI)
Dietary nitrate (mg NO₃-N/day^b)				
<u>All sources</u>				
<16.2	67	8,467	1.00 (Ref.)	1.00 (Ref.)
16.2-23.9	68	8,489	1.00 (0.72,1.41)	1.01 (0.72,1.43)
24.0-34.2	64	8,506	0.92 (0.66,1.30)	0.94 (0.66,1.35)
>34.2	59	8,502	0.86 (0.60,1.22)	0.85 (0.58,1.26)
<i>p</i> _{trend} ^d			0.32	0.34
Continuous ^e	258	33,964	0.85 (0.69,1.04)	0.83 (0.65,1.05)
Dietary nitrite (mg/day)				
<u>All sources</u>				
<0.86	63	8,450	1.00 (Ref.)	1.00 (Ref.)
0.86-1.12	66	8,514	1.01 (0.71,1.42)	1.15 (0.78,1.70)
1.13-1.43	73	8,487	1.12 (0.80,1.56)	1.38 (0.89,2.16)
>1.43	56	8,513	0.85 (0.60,1.22)	1.15 (0.65,2.03)
<i>p</i> _{trend}			0.44	0.69
Continuous	258	33,964	0.95 (0.70,1.29)	1.59 (0.89,2.84)
<u>Plant sources</u>				
<0.51	62	8,461	1.00 (Ref.)	1.00 (Ref.)
0.51-0.67	75	8,507	1.17 (0.84,1.64)	1.33 (0.92,1.91)
0.68-0.90	69	8,490	1.08 (0.76,1.52)	1.32 (0.88,1.99)
>0.90	52	8,506	0.81 (0.56,1.17)	1.05 (0.64,1.72)
<i>p</i> _{trend}			0.15	0.82
Continuous	258	33,964	0.86 (0.66,1.12)	1.10 (0.74,1.63)
<u>Animal sources</u>				
<0.29	67	8,474	1.00 (Ref.)	1.00 (Ref.)
0.29-0.40	54	8,443	0.79 (0.55,1.14)	0.86 (0.59,1.25)
0.41-0.56	64	8,533	0.92 (0.66,1.30)	1.03 (0.70,1.53)
>0.56	73	8,514	1.06 (0.76,1.47)	1.24 (0.79,1.95)
<i>p</i> _{trend}			0.53	0.22
Continuous	258	33,964	1.10 (0.86,1.39)	1.31 (0.93,1.86)
<u>Processed meats</u>				
<0.01	69	8,718	1.00 (Ref.)	1.00 (Ref.)
0.01-0.03	75	8,707	1.00 (0.72,1.40)	0.95 (0.68,1.33)
0.04-0.06	54	8,107	0.89 (0.63,1.26)	0.86 (0.60,1.22)
>0.06	60	8,432	0.95 (0.67,1.33)	0.88 (0.61,1.26)
<i>p</i> _{trend}			0.68	0.46
Continuous	258	33,964	0.99 (0.95,1.02)	0.98 (0.94,1.02)

^a After excluding 744 women with missing covariate data.

^b NO₃ converted to NO₃-N. Nitrate intakes came almost exclusively from plant sources.

^c Adjusted for age, smoking status, pack-years of smoking, and ln-transformed total energy intake. Nitrate models were also adjusted for total ln-transformed dietary nitrite from all sources, and nitrite models were adjusted for total ln-transformed dietary nitrate from all sources.

^d Estimated by modeling a continuous variable derived from the median value within each exposure category.

^e HR per one natural log increase in intake (mg/day).