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Article

# Drinking water nitrate, disinfection byproducts, and prostate cancer incidence in the Agricultural Health Study

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## Abstract

**Background:** Drinking water can be an important source of exposure to nitrate and disinfection by-products, including trihalomethanes (THMs) and haloacetic acids (HAAs). N-nitroso compounds formed endogenously after nitrate ingestion are animal carcinogens, and THM and HAA exposures increase the risk of some cancers. Our objectives were to evaluate associations of drinking water nitrate and disinfection byproducts with total and aggressive (distant stage, poorly differentiated grade, fatal, or Gleason score ≥7) prostate cancer in the Agricultural Health Study cohort.

**Methods:** Male participants who were cancer free and used private wells or public water supplies (PWS) for drinking water at enrollment (1993-1997, $n = 40\,403$) were followed through 2021 (mean = 21.9 years). Average nitrate-nitrogen (nitrate-N) concentrations were estimated for private well users based on state-specific geologic and meteorologic factors. We used monitoring data to compute average nitrate-N, THMs, and HAAs for PWS users. We estimated hazard ratios (HRs, 95% CIs) per doubling and categories of exposure for total ($n = 3625$) and aggressive ($n = 2200$) prostate cancer using Cox proportional hazards regression.

**Results:** Median (interquartile range) average water nitrate-N was 1.49 (0.76-3.01) mg $L^{-1}$; 6% >10 mg $L^{-1}$ (PWS maximum contaminant level). Compared to nitrate-N ≤ 1 mg $L^{-1}$, exposures >10 mg $L^{-1}$ were significantly positively associated with total (1.16, 1.01-1.35; $P = .10$ for trend) and aggressive disease (1.22, 1.02-1.47; $P = .03$ for trend). We observed weak associations between higher nitrate-N (Q4 vs Q1) and total (1.05, 0.95-1.16) and aggressive (1.13, 0.99-1.27) disease. We did not observe associations with total THMs or HAAs.

**Conclusions:** These findings suggest that drinking water nitrate-N exposure, at average levels > 10 mg $L^{-1}$, is a risk factor for prostate cancer, particularly aggressive disease.

## Introduction

Prostate cancer is the second most commonly diagnosed cancer among men globally,[1] and the lifetime risk of developing prostate cancer in the United States is approximately 12.9%.[2] Risk factors are mostly unknown but family history and certain hereditary syndromes have been associated with increased risk,[3-6] and obesity may be a risk factor for aggressive disease.[6,7] There are disparities in the disease burden with the highest incidence and mortality among African American men, and those with low socioeconomic status (SES).[8-10] Elevated rates of prostate cancer have been observed among farmers.[11] While specific pesticides have been associated with increased prostate cancer risk, especially aggressive disease,[12] the role of other environmental exposures, such as drinking water contaminants, is poorly understood. There have been few studies of drinking water nitrate and prostate cancer.[13-15]

The International Agency for Research on Cancer classified nitrate and nitrite as probable human carcinogens when ingested under conditions that result in endogenous formation of N-nitroso compounds (NOCs), most of which are carcinogens.[16] Drinking water can be a major source of exposure to nitrate, particularly in agricultural areas due to runoff from nitrogen

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fertilizer use and animal feeding operations into ground and surface waters.[17,18] Private wells, which serve approximately 10% of the US population, are not federally regulated and can have higher nitrate levels than public water supplies (PWS).[19] The US Environmental Protection Agency (EPA) has established a maximum contaminant level (MCL) of 10 mg $L^{-1}$ for PWS effective in 1992, for nitrate as nitrogen (nitrate-N) based on risk of methemoglobinemia in infants.[20] Epidemiological evidence suggests that exposure to nitrate at levels below the MCL is associated with specific cancers (eg, colorectal, gastric) and birth defects.[15] In addition to drinking water, dietary sources of nitrate/nitrite include processed meats, fruits, and vegetables.[21]

Drinking water can also be a source of exposure to disinfection byproducts (DBPs) created by the interaction of disinfectants with naturally occurring organic compounds. Higher DBP concentrations are observed in PWS reliant on surface water or shallow groundwater supplies due to elevated organic matter.[19] While over 700 DBPs have been identified, the most abundant classes are trihalomethanes (THM: chloroform, dibromochloromethane, bromodichloromethane, bromoform), regulated as the sum total (TTHM; MCL = 80 μg $L^{-1}$), and haloacetic acids (HAA5, regulated as the sum of dichloroacetic acid, trichloroacetic acid, monochloroacetic acid, bromoacetic acid, and dibromoacetic acid; MCL = 60 μg $L^{-1}$).[20,22] Certain DBPs are associated with specific cancers (eg, bladder, colorectal).[23,24] Animal studies have found evidence for cytotoxicity, genotoxicity, and male reproductive toxicity of THMs and HAAs.[25-28]

To our knowledge, only 1 individual-level epidemiologic study, a case-control study in Spain, evaluated drinking water contaminant exposures and prostate cancer risk. With a focus on nitrate and DBPs, waterborne ingested nitrate was associated with increased odds of aggressive prostate cancer (defined as Gleason score ≥8) but there was no association with waterborne ingested THMs.[13] Dietary nitrite/nitrate and heme iron were associated with prostate cancer risk in several prior studies, though evidence is inconsistent.[29,30]

Our objectives were to evaluate prostate cancer risk in relation to drinking water nitrate, TTHM, and HAA5 exposures in the Agricultural Health Study (AHS), a prospective cohort of licensed pesticide applicators (mostly farmers) and their spouses in Iowa (IA) and North Carolina (NC).

# Methods

## Study population

The AHS ($n = 89\,655$) was designed to investigate health risks of pesticides and other agricultural exposures and enrolled individuals applying for restricted-use pesticide licenses and their spouses between 1993 and 1997. Among all males ($n = 55\,966$), we excluded those with prevalent cancer at baseline ($n = 1080$), with no person-time (eg, enrolled out of state and thus not subject to the IA or NC cancer registry follow-up ($n = 336$), and missing information on prostate cancer Gleason score ($n = 109$).[31] At enrollment, participants provided demographic (race and ethnicity, educational attainment), lifestyle (smoking), anthropometric (height and weight used to calculate body mass index [BMI, kg $m^{-2}$]), and pesticide use information.

## Prostate cancer ascertainment

Incident cases of prostate cancer including stage and grade were obtained through linkages to the IA and NC cancer registries through December 2021. We assessed total and aggressive prostate cancer cases, as risk factors often differ.[6,32] Aggressive prostate cancer was defined as having at least one of the following tumor characteristics: distant stage, poorly differentiated grade, Gleason score of ≥7, or fatal prostate cancer (underlying cause of death was prostate cancer). In supplemental analyses, we alternatively evaluated aggressive prostate cancers as a Gleason score ≥8 in combination with the other factors listed above.[12,33] Participants were censored at the earliest time of when they moved out of state, were diagnosed with any cancer, or death. Informed consent was obtained from all participants following procedures approved by the Institutional Review Board.

## Drinking water exposure assessment

Residential drinking water source at enrollment, and additional details on the drinking water exposure assessment, are described in the Supplementary Material. We excluded participants with no drinking water source information ($n = 12\,561$) or with bottled water ($n = 486$) or some other source ($n = 991$), leaving 40 403 with a private well or PWS source for analysis.

For private well users, nitrate concentrations were previously estimated and validated using state-specific random forest models using well depth, nitrogen inputs, geologic, and meteorologic predictors at the geocoded address at enrollment ($n = 26\,666$) (Supplementary Material).[34,35] Private well users were assumed to have no exposure to TTHM and HAA5 (assigned as 0 μg $L^{-1}$).

Public water supplies monitoring data for nitrate (measured as nitrate-nitrogen) and DBPs were obtained from the Center for Health Effects of Environmental Contamination (CHEEC) at the University of Iowa and the North Carolina Department of Environmental Quality.[31] Public water supplies data were linked to PWS users' enrollment address ($n = 11\,312$) based on town name and other information, including rural water system boundaries.[31] We computed average PWS nitrate (1990-2000, $n = 10\,668$) and TTHM and HAA5 concentrations (1990-2009). We computed alternative average PWS metrics using monitoring data corresponding to the years lived at the enrollment address prior to enrollment (Supplementary Material). We also calculated the daily intake of nitrate from tap water (mg/day) using standard intake rates of daily tap water consumption in US populations.[36]

## Dietary exposure assessment

A subset of participants in the drinking water analyses completed the National Cancer Institute Diet History Questionnaire (DHQ)[37] at Phase 2 (1999-2005, $n = 16\,090$). Nitrate (mg $NO_3$/day), nitrite (mg $NO_2$/day), total heme iron (mg/1000 kilocalories), and vitamin C intake (mg/1000 kilocalories) were estimated using the 144-item DHQ, as previously described[21,38] (Supplementary Material).

## Statistical analyses

We described participant characteristics and drinking water contaminant exposures overall, and for participants diagnosed with prostate cancer separately. We used Cox proportional hazards regression to calculate hazard ratios (HRs) and 95% confidence intervals (CIs), with person-years as the time scale to evaluate associations between drinking water nitrate, TTHM, HAA5, and prostate cancer risk. All statistical analyses were conducted in R version 4.3.1.[39] We evaluated the statistical significance of the associations based on the 95% CIs, and used $P < .05$ (2-sided) as the threshold for tests of statistical trend and interactions.

We modeled drinking water nitrate per 2-fold (log2 transformation) and per 5 mg $L^{-1}$ (half the nitrate MCL) increase, and in quartiles. We tested for linear trend using the median of each

quartile parameterized as a continuous variable. We evaluated regulatory-based thresholds (≤1, >1-10, and >10 mg $L^{-1}$) of drinking water nitrate, and used cubic regression splines to further examine nonlinear associations. We parameterized TTHM and HAA5 exposures as follows: private well users with assumed 0 μg $L^{-1}$ (reference), and tertiles of PWS DBP concentrations. Among PWS users, we also modeled DBPs per 2-fold increase and quartiles. We evaluated individual THMs and haloacetic acids. We adjusted for baseline age, baseline age$^2$, and strata for state (model 1), with further adjustment for potential confounders: smoking status (never, former, current, missing), BMI category (<25 kg $m^{-2}$, 25-<30 kg $m^{-2}$, ≥30 kg $m^{-2}$, or missing), and education (less than high school, high school or equivalent, vocational education or some college, college or above, missing) (model 2). In ad hoc analyses, we evaluated associations per continuous and tertiles of daily contaminant intake (mg/day) from tap water.

In dietary analyses, follow-up started at the phase 2 completion date. We evaluated potential interaction by vitamin C and heme iron intake using stratified models and likelihood ratio tests for interaction. Prior evidence suggests that higher vitamin C intake may inhibit the effect of water nitrate exposure on carcinogenesis,[13,16] while red meat and heme iron intake may promote endogenous nitrosation.[16,29,40-43] We evaluated associations with dietary nitrate and nitrite overall, and separately from plant, animal, and processed meat sources, based on prior limited evidence for dietary nitrate/nitrite and processed meat as potential risk factors for prostate cancer.[29,44] In ad hoc analyses, we evaluated the association between drinking water nitrate and prostate cancer risk among the dietary subpopulation (follow-up starting at phase 2) to confirm our findings were robust in this subgroup.

## Sensitivity analyses

We examined whether the associations were different by state and education. Higher educational attainment is correlated with higher SES, which is associated with prostate cancer incidence likely due to increased access to screening.[9] We also evaluated potential bias by access to screening for prostate-specific antigen (PSA), a protein produced by the prostate gland that is used to detect prostate cancer via a blood test. We used logistic regression to study associations between water nitrate and PSA screening among participants eligible for prostate cancer screening (ie, 50+ years of age) who responded "yes" or "no" to PSA screening at phase 3 (2005-2010) and who did not have a prior prostate cancer diagnosis ($n$ = 14 316). Model covariates were the same as those described above, with additional adjustment for phase 3 completion year, marital status, and family history of prostate cancer.

We evaluated models that separately adjusted for intensity-weighted lifetime days of use of pesticides (aldrin, malathion, fonofos, and terbufos, collected at enrollment) previously associated with aggressive prostate cancer risk.[12] We explored potential confounding by water arsenic, based on evidence that arsenic was associated with prostate cancer incidence rates from ecologic studies in IA and IL.[45,46] We assigned probabilities that private well water arsenic concentrations were >5 and >10 μg $L^{-1}$, generated for 1-km$^2$ grids nationwide using boosted regression tree models.[47] For PWS users in NC, we used population-weighted average concentrations (2006-2011) from the US EPA Contaminants Occurrence Database.[48] For PWS users in IA, we used concentration data (1989-2001) from CHEEC to compute a PWS-specific average concentration.

# Results

Among the 40 403 participants in our drinking water analysis, 3625 men developed prostate cancer, with 2200 aggressive cases and 909 more aggressive (Gleason ≥ 8) cases (mean follow-up = 21.9 years). The overall median baseline age was 46 years, whereas prostate cancer cases were older: 54 years among total prostate cancer cases, and 53 years among aggressive cases (Table 1). Most participants were White (96%), followed by Black (2%), American Indian (<1%), Asian (<1%), and Other (<1%); prostate cancer cases overall followed a similar pattern. Most participants were non-Hispanic (93%), had a BMI <30 kg $m^{-2}$, were never smokers, had a high school education or below, and used private wells, with similar proportions among total and aggressive prostate cancer cases. Median average water nitrate levels were higher among cases compared to overall (Table 1). Participant characteristics were generally similar across quartiles of drinking water nitrate concentration (Table 2) and water source (Table S1). Median water nitrate concentrations were mostly below the MCLs for both private well and PWS users but were higher among private well users; 75th percentile concentrations were higher in IA compared to NC (Figure S1).

In fully adjusted models, we observed a positive and borderline significant association with total prostate cancer per two-fold increase in water nitrate (HR = 1.02, 95% CI = 1.00 to 1.03), and per 5 mg $L^{-1}$ increase (HR = 1.04, 95% CI = 0.99 to 1.08) (Table 3). The risk of aggressive prostate cancer was similar per continuous increase in water nitrate. Hazard ratios tended to increase with increasing quartiles of exposure ($HR_{Q4\ vs\ Q1}$ = 1.05, 95% CI = 0.95 to 1.16 [total]; 1.13, 95% CI = 0.99 to 1.27 [aggressive]) but HRs and trends were not statistically significant ($P$ = .16 and .09 for trend, respectively). Comparing water nitrate categories >10-≤1 mg $L^{-1}$ nitrate, we observed a positive and statistically significant association with the risk of total prostate cancer (HR = 1.16, 95% CI = 1.01 to 1.35, $P$ = .10 for trend) and aggressive disease (HR = 1.22, 95% CI = 1.02 to 1.47, $P$ = .03 for trend). Exposure response patterns using cubic splines showed a positive linear association for total and aggressive prostate cancer risk, though the CIs included the null and were wide at the highest concentrations where the numbers exposed were sparse (Figure 1). Nitrate >10 mg $L^{-1}$ (vs ≤1 mg $L^{-1}$) was associated with increased risk of aggressive prostate cancer (HR = 1.23, 95% CI = 1.01 to 1.49, $P$ = .03 for trend) among private well users. We could not evaluate this category in PWS users due to the lower distribution of nitrate exposures. Findings for PWS users were generally similar but attenuated in quartile and categorical analyses (Table 3). We did not observe an association between TTHM and HAA5 concentrations and prostate cancer risk (Table 4). The findings for nitrate, TTHM, and HAA5 were similar in models evaluating more aggressive (Gleason ≥ 8) disease (Tables S2 and S3) and adjusted only for age and state (*model 1*, Tables S4 and S5). Individual DBPs were not associated with prostate cancer risk, except for chloroacetic acid and Gleason ≥ 8 disease ($HR_{log2}$ = 1.22, 95% CI = 1.03 to 1.44) (Table S6). A doubling and higher tertiles of water nitrate intake (mg/day) were positively associated with aggressive prostate cancer risk (Gleason ≥ 7, $HR_{log2}$ = 1.03, 95% CI = 1.01 to 1.05, and $HR_{T3\ vs\ T1}$ = 1.14, 95% CI = 1.02 to 1.27, $P$ = .03 for trend), with weak positive associations for total prostate cancer (Table S7). No association was observed for estimated HAA5 or TTHM intake (Table S8).

In the dietary subpopulation, water nitrate increased total and aggressive prostate cancer risk ($HR_{Q4\ vs\ Q1}$ = 1.21, 95% CI = 1.04 to 1.42, and 1.28, 95% CI = 1.05 to 1.55, respectively;

**Table 1.** Descriptive characteristics of Agricultural Health Study (AHS) participants in drinking water analyses at enrollment, overall, and among incident prostate cancer cases.[a]

| | All AHS participants | All prostate cancers | Aggressive (Gleason ≥7) | Aggressive (Gleason ≥8) |
|---|---|---|---|---|
| No. | 40 403 | 3625 | 2200 | 909 |
| Age (years), median (IQR) | 46 (38-57) | 54 (46-61) | 53 (45-60) | 56 (48-63) |
| Race, No. (%) | | | | |
| White | 38 919 (96) | 3477 (96) | 2126 (97) | 873 (96) |
| Black | 677 (2) | 115 (3) | 54 (2) | 27 (3) |
| American Indian | 158 (<0.1) | 30 (1) | 18 (1) | 8 (1) |
| Asian | 9 (<0.1) | 1 (<0.1) | 1 (<0.1) | 1 (<0.1) |
| Other | 38 (<0.1) | 0 (0) | 0 (0) | 0 (0) |
| Not reported | 602 (1) | 2 (<0.1) | 1 (<0.1) | 0 (0) |
| Ethnicity, No. (%) | | | | |
| Non-Hispanic | 37 689 (93) | 3579 (99) | 2175 (99) | 901 (99) |
| Hispanic | 406 (1) | 31 (1) | 19 (1) | 5 (1) |
| Not reported | 2308 (6) | 15 (<0.1) | 6 (<0.1) | 3 (<0.1) |
| Education, No. (%) | | | | |
| Less than high school | 3531 (9) | 401 (11) | 226 (10) | 108 (12) |
| High school | 18 678 (46) | 1776 (49) | 1080 (49) | 457 (50) |
| Some college | 9627 (24) | 746 (21) | 466 (21) | 180 (20) |
| College and above | 7005 (17) | 546 (15) | 337 (15) | 125 (14) |
| Missing | 1562 (4) | 156 (4) | 91 (4) | 39 (4) |
| Smoking status, No. (%) | | | | |
| Never smoker | 20 786 (51) | 1834 (51) | 1119 (51) | 445 (49) |
| Former smoker | 12 434 (31) | 1297 (36) | 787 (36) | 341 (38) |
| Current smoker | 6034 (15) | 389 (11) | 240 (11) | 96 (11) |
| Missing | 1149 (3) | 105 (3) | 54 (2) | 27 (3) |
| Body mass index (BMI, kg $m^{-2}$), median (IQR) | 27.2 (25.0-29.8) | 27.1 (25.1-29.7) | 27.2 (25.1-29.8) | 27.3 (25.1-30.0) |
| BMI category (kg $m^{-2}$), No. (%) | | | | |
| <25 | 8811 (22) | 769 (21) | 446 (20) | 167 (18) |
| 25-<30 | 18 016 (45) | 1741 (48) | 1037 (47) | 434 (48) |
| ≥30 | 8326 (21) | 699 (19) | 444 (20) | 198 (22) |
| Missing | 5250 (13) | 416 (11) | 273 (12) | 110 (12) |
| State, No. (%) | | | | |
| Iowa | 26 565 (66) | 2335 (64) | 1504 (68) | 578 (64) |
| North Carolina | 13 838 (34) | 1290 (36) | 696 (32) | 331 (36) |
| Water source, No. (%) | | | | |
| Private well | 29091 (72) | 2721 (75) | 1633 (74) | 666 (73) |
| Public water supply | 11312 (28) | 904 (25) | 567 (26) | 243 (27) |
| Water concentration, median (IQR)[b] | | | | |
| Overall ($n = 40\,599$) | | | | |
| Nitrate-N, mg $L^{-1}$ | 1.49 (0.76-3.01) | 1.55 (0.78-3.09) | 1.53 (0.80-3.22) | 1.53 (0.80-3.11) |
| TTHM, μg $L^{-1}$ | 0 (0-1.35) | 0 (0-0) | 0 (0-0) | 0 (0-0.89) |
| HAA5, μg $L^{-1}$ | 0 (0-2.40) | 0 (0-0) | 0 (0-0) | 0 (0-1.20) |
| Private well ($n = 29\,091$) | | | | |
| Nitrate-N, mg $L^{-1}$ | 1.74 (0.98-3.20) | 1.80 (1.00-3.25) | 1.81 (1.02-3.41) | 1.80 (1.05-3.29) |
| PWS ($n = 11\,312$) | | | | |
| Nitrate-N, mg $L^{-1}$ | 0.57 (0.20-2.20) | 0.55 (0.19-2.19) | 0.65 (0.26-2.47) | 0.49 (0.19-2.39) |
| TTHM, μg $L^{-1}$ | 17.00 (5.50-39.61) | 16.21 (5.47-39.25) | 15.70 (5.10-38.61) | 15.70 (4.70-38.33) |
| HAA5, μg $L^{-1}$ | 6.50 (3.77-14.33) | 7.00 (3.80-15.09) | 6.06 (3.80-13.95) | 7.19 (3.77-13.54) |

[a] Aggressive prostate cancer was defined as having at least one of the following tumor characteristics: distant stage, poorly differentiated grade, Gleason score of ≥7, or fatal prostate cancer. Aggressive (Gleason ≥8) was alternatively defined as the aforementioned characters, and a Gleason score of ≥8.
[b] Water contaminant exposures were linked by address at enrollment and were assigned by the self-reported water source; 37 334 participants had water nitrate-N estimates; concentrations were modeled for private well users and were computed as 1990-2000 average concentrations for public water supply (PWS) users[31]; 39 959 participants had total trihalomethane (TTHM) estimates, and 39 910 participants had total haloacetic acids (HAA5) estimates; concentrations were assigned as 0 μg $L^{-1}$ for private well users and were computed as 1990-2009 average concentrations for PWS users.

$P < .05$ for trend) (Table S9). Water nitrate increased aggressive prostate cancer risk in tertiles 2 and 3 of heme iron intake ($HR_{Q4\ vs\ Q1} = 1.72$, 95% CI = 1.21 to 2.45, and 1.36, 95% CI = 0.99 to 1.86, respectively; $P < .05$ for trend, $P = .002$ for interaction) (Table 5). In contrast to expectation, total and aggressive prostate cancer risk was strongest in the third tertile of vitamin C intake ($HR_{Q4\ vs\ Q1} = 1.38$, 95% CI = 1.07 to 1.79, and 1.67, 95% CI = 1.19 to 2.35), with statistical interaction for total and aggressive prostate cancer risk ($P < .05$ for interaction) (Table 6). Higher quartiles of water nitrate were associated with more aggressive (Gleason ≥ 8) disease in the top 2 tertiles of heme iron ($P < .05$ for trend), with no clear pattern by tertile of vitamin C (Table S10).

We did not observe any associations for total intakes of dietary nitrate or nitrite, or for nitrite from plant, animal, and processed meat sources separately, with total or aggressive prostate cancer (Table S11). Higher tertiles of heme iron intake were associated with increased risk of total and aggressive (Gleason ≥7) prostate cancer risk ($P < .05$ for trend); we observed no associations between vitamin C and prostate cancer risk (Table S12).

## Sensitivity analyses

Results adjusting for use of fonofos and terbufos were consistent with the main findings (Table S13). Adjusting for aldrin or malathion use made the HRs stronger, particularly for aggressive

**Table 2.** Descriptive characteristics of Agricultural Health Study participants at enrollment, by quartile of drinking water nitrate-N concentration.[a]

| | Nitrate-N concentration, range within quartiles (mg $L^{-1}$) | | | | |
|---|---|---|---|---|---|
| | **Overall 0-46.0** | **Quartile 1 0-0.76** | **Quartile 2 0.76-1.49** | **Quartile 3 1.49-3.01** | **Quartile 4 3.01-46.0** |
| No. | 37 334 | 9334 | 9333 | 9330 | 9337 |
| All prostate cancers, No. | 3367 | 815 | 825 | 864 | 863 |
| Aggressive (Gleason ≥7), No.[b] | 2042 | 477 | 527 | 485 | 553 |
| Aggressive (Gleason ≥8), No.[b] | 845 | 197 | 218 | 208 | 222 |
| Age (years), median (IQR) | 46 (38-57) | 45 (37-56) | 46 (38-57) | 47 (38-58) | 45 (38-56) |
| Race, No. (%) | | | | | |
| White | 36 076 (97) | 8976 (96) | 9070 (97) | 8893 (95) | 9137 (98) |
| Black | 548 (1) | 184 (2) | 95 (1) | 214 (2) | 55 (1) |
| American Indian | 139 (<0.1) | 54 (1) | 14 (<0.1) | 50 (1) | 21 (<0.1) |
| Asian | 8 (<0.1) | 2 (<0.1) | 1 (<0.1) | 1 (<0.1) | 4 (<0.1) |
| Other | 31 (<0.1) | 5 (<0.1) | 9 (<0.1) | 7 (<0.1) | 10 (<0.1) |
| Not reported | 532 (1) | 113 (1) | 144 (2) | 165 (2) | 110 (1) |
| Ethnicity, No. (%) | | | | | |
| Non-Hispanic | 34 901 (93) | 8772 (94) | 8700 (93) | 8561 (92) | 8868 (95) |
| Hispanic | 370 (1) | 84 (1) | 94 (1) | 99 (1) | 93 (1) |
| Not reported | 2063 (6) | 478 (5) | 539 (6) | 670 (7) | 376 (4) |
| Education, No. (%) | | | | | |
| Less than high school | 3119 (8) | 692 (7) | 757 (8) | 1020 (11) | 650 (7) |
| High school | 17 375 (47) | 4061 (44) | 4331 (46) | 4275 (46) | 4708 (50) |
| Some college | 8991 (24) | 2386 (26) | 2321 (25) | 2052 (22) | 2232 (24) |
| College and above | 6459 (17) | 1871 (20) | 1574 (17) | 1546 (17) | 1468 (16) |
| Missing | 1390 (4) | 324 (3) | 350 (4) | 437 (5) | 279 (3) |
| Smoking status, No. (%) | | | | | |
| Never smoker | 19 367 (52) | 4823 (52) | 4950 (53) | 4413 (47) | 5181 (55) |
| Former smoker | 11 467 (31) | 2821 (30) | 2836 (30) | 3043 (33) | 2767 (30) |
| Current smoker | 5495 (15) | 1464 (16) | 1284 (14) | 1557 (17) | 1190 (13) |
| Missing | 1005 (3) | 226 (2) | 263 (3) | 317 (3) | 199 (2) |
| Body mass index (BMI, kg $m^{-2}$), median (IQR) | 27.2 (25.0-29.8) | 27.2 (25.1-29.9) | 27.1 (25.0-29.8) | 27.2 (25.0-29.8) | 27.2 (25.1-29.8) |
| BMI category (kg $m^{-2}$), No. (%) | | | | | |
| <25 | 8111 (22) | 1932 (21) | 2120 (23) | 2099 (22) | 1960 (21) |
| 25-<30 | 16 831 (45) | 4106 (44) | 4191 (45) | 4317 (46) | 4217 (45) |
| ≥30 | 7804 (21) | 1944 (21) | 1944 (21) | 1971 (21) | 1945 (21) |
| Missing | 4588 (12) | 1352 (14) | 1078 (12) | 943 (10) | 1215 (13) |
| State, No. (%) | | | | | |
| Iowa | 25 321 (68) | 6483 (69) | 6744 (72) | 3995 (43) | 8099 (87) |
| North Carolina | 12 013 (32) | 2851 (31) | 2589 (28) | 5335 (57) | 1238 (13) |
| Water source, No. (%) | | | | | |
| Private well | 26 666 (71) | 3560 (38) | 7797 (84) | 8160 (87) | 7149 (77) |
| Public water supply | 10 668 (29) | 5774 (62) | 1536 (16) | 1170 (13) | 2188 (23) |
| Water concentration, median (IQR) | | | | | |
| Overall | | | | | |
| TTHM, μg $L^{-1}$ | 0 (0-1.62) | 1.79 (0-24.64) | 0 (0-0) | 0 (0-0) | 0 (0-0) |
| HAA5, μg $L^{-1}$ | 0 (0-2.73) | 3.40 (0-8.50) | 0 (0-0) | 0 (0-0) | 0 (0-0) |
| Private well | | | | | |
| Nitrate-N, mg $L^{-1}$ | 1.74 (0.98-3.20) | 0.64 (0.57-0.70) | 1.06 (0.89-1.26) | 2.04 (1.75-2.44) | 6.42 (3.97-11.03) |
| PWS | | | | | |
| Nitrate-N, mg $L^{-1}$ | 0.57 (0.20-2.20) | 0.23 (0.09-0.39) | 1.00 (0.86-1.19) | 2.06 (1.68-2.53) | 4.75 (3.83-5.94) |
| TTHM, μg $L^{-1}$ | 16.65 (5.47-38.81) | 13.88 (3.06-41.71) | 35.31 (5.54-50.62) | 17.45 (5.33-29.65) | 15.17 (6.58-29.50) |
| HAA5, μg $L^{-1}$ | 6.00 (3.74-13.81) | 5.98 (3.78-14.14) | 10.31 (4.05-24.60) | 5.05 (3.58-14.00) | 5.31 (3.73-9.32) |
| Iowa | | | | | |
| Nitrate-N, mg $L^{-1}$ | 1.38 (0.75-4.17) | 0.54 (0.34-0.65) | 0.99 (0.86-1.19) | 2.05 (1.70-2.48) | 6.19 (4.41-10.25) |
| TTHM, μg $L^{-1}$ | 0 (0-2.34) | 0 (0-7.00) | 0 (0-0) | 0 (0-2.34) | 0 (0-2.26) |
| HAA5, μg $L^{-1}$ | 0 (0-3.42) | 0 (0-4.46) | 0 (0-0) | 0 (0-2.90) | 0 (0-2.41) |
| North Carolina | | | | | |
| Nitrate-N, mg $L^{-1}$ | 1.60 (0.82-2.28) | 0.12 (0.03-0.30) | 1.19 (1.01-1.35) | 2.04 (1.76-2.43) | 3.50 (3.20-4.00) |
| TTHM, μg $L^{-1}$ | 0 (0-0) | 26.22 (3.98-54.14) | 0 (0-0) | 0 (0-0) | 0 (0-0) |
| HAA5, μg $L^{-1}$ | 0 (0-0) | 9.52 (2.43-28.88) | 0 (0-0) | 0 (0-0) | 0 (0-0) |

[a] Water exposures were linked by address at enrollment and reported water source; 37 334 participants had water nitrate-N estimates; concentrations were modeled for private well users and were computed as 1990-2000 average concentrations for public water supply (PWS) users[31]; 39 959 participants had total trihalomethane (TTHM) estimates, and 39 910 participants had total haloacetic acids (HAA5) estimates; concentrations were assigned as 0 μg $L^{-1}$ for private well users and were computed as 1990-2009 average concentrations for PWS users.

[b] Aggressive prostate cancer was defined as having at least one of the following tumor characteristics: distant stage, poorly differentiated grade, Gleason score of ≥7, or fatal prostate cancer. Aggressive (Gleason ≥8) was alternatively defined as the aforementioned characters of aggressive, and a Gleason score of ≥8.

cancers. We observed similar and slightly stronger associations in analyses adjusted for private well or PWS arsenic, respectively (Table S14). In analyses that assigned a residential duration weighted average exposure to PWS users (rather than the decadal average), the observed associations were generally consistent (Table S15).

**Table 3.** Adjusted hazard ratios (95% CIs) for the associations of prostate cancer with continuous and categories of water nitrate,[a] among participants in the Agricultural Health Study (AHS).

| | | All prostate cancers | | Aggressive (Gleason ≥7) | |
|---|---|---|---|---|---|
| Nitrate-N (mg $L^{-1}$) | No. | No. of cases | HR (95% CI)[b] | No. of cases | HR (95% CI)[b] |
| Overall | | | | | |
| Log2 | 37 334 | 3367 | 1.02 (1.00 to 1.03) | 2042 | 1.02 (1.00 to 1.05) |
| Per 5 mg $L^{-1}$ | 37 334 | 3367 | 1.04 (0.99 to 1.08) | 2042 | 1.05 (0.99 to 1.10) |
| Quartiles (range) | | | | | |
| <25% (0-0.76) | 9334 | 815 | 1.0 (reference) | 477 | 1.0 (reference) |
| 25-<50% (0.76-1.49) | 9333 | 825 | 0.98 (0.89 to 1.07) | 527 | 1.07 (0.94 to 1.21) |
| 50-<75% (1.49-3.01) | 9330 | 864 | 1.01 (0.91 to 1.11) | 485 | 1.01 (0.89 to 1.15) |
| ≥75% (3.01-46.0) | 9337 | 863 | 1.05 (0.95 to 1.16) | 553 | 1.13 (0.99 to 1.27) |
| *P* for trend[c] | | | 0.16 | | 0.088 |
| Categorical | | | | | |
| ≤1 mg $L^{-1}$ | 13 409 | 1156 | 1.0 (reference) | 691 | 1.0 (reference) |
| >1-10 mg $L^{-1}$ | 21 830 | 1989 | 1.02 (0.95 to 1.10) | 1207 | 1.07 (0.97 to 1.17) |
| >10 mg $L^{-1}$ | 2095 | 222 | 1.16 (1.01 to 1.35) | 144 | 1.22 (1.02 to 1.47) |
| *P* for trend[d] | | | .103 | | .031 |
| Private well users | | | | | |
| Log2 | 26 666 | 2511 | 1.02 (0.99 to 1.05) | 1511 | 1.04 (1.00 to 1.07) |
| Quartiles (range) | | | | | |
| <25% (0.14-0.76) | 3560 | 348 | 1.0 (reference) | 199 | 1.0 (reference) |
| 25-<50% (0.76-1.49) | 7797 | 697 | 0.90 (0.79 to 1.03) | 439 | 1.04 (0.88 to 1.23) |
| 50-<75% (1.49-3.01) | 8160 | 779 | 0.95 (0.83 to 1.09) | 437 | 1.04 (0.87 to 1.24) |
| ≥75% (3.01-46.0) | 7149 | 687 | 0.99 (0.87 to 1.13) | 436 | 1.12 (0.95 to 1.32) |
| *P* for trend[c] | | | .301 | | .146 |
| Categorical | | | | | |
| ≤1 mg $L^{-1}$ | 6859 | 626 | 1.0 (reference) | 371 | 1.0 (reference) |
| >1-10 mg $L^{-1}$ | 17 728 | 1664 | 1.01 (0.92 to 1.11) | 996 | 1.10 (0.97 to 1.24) |
| >10 mg $L^{-1}$ | 2079 | 221 | 1.13 (0.97 to 1.32) | 144 | 1.23 (1.01 to 1.49) |
| *P* for trend[d] | | | .203 | | .03 |
| PWS users | | | | | |
| Log2 | 10 668 | 856 | 1.01 (0.98 to 1.05) | 531 | 1.03 (0.99 to 1.08) |
| Quartiles (range) | | | | | |
| <25% (0-0.75) | 5774 | 467 | 1.0 (reference) | 278 | 1.0 (reference) |
| 25-<50% (0.77-1.49) | 1536 | 128 | 1.08 (0.88 to 1.32) | 88 | 1.20 (0.93 to 1.54) |
| 50-<75% (1.51-3.01) | 1170 | 85 | 0.96 (0.75 to 1.23) | 48 | 0.88 (0.64 to 1.21) |
| ≥75% (3.01-18.0) | 2188 | 176 | 1.08 (0.89 to 1.30) | 117 | 1.15 (0.91 to 1.46) |
| *P* for trend[c] | | | .521 | | .371 |
| Categorical | | | | | |
| ≤1 mg $L^{-1}$ | 6550 | 530 | 1.0 (reference) | 320 | 1.0 (reference) |
| >1-5 mg $L^{-1}$ | 3250 | 257 | 1.02 (0.87 to 1.20) | 166 | 1.05 (0.86 to 1.28) |
| >5 mg $L^{-1}$ | 868 | 69 | 1.04 (0.80 to 1.36) | 45 | 1.08 (0.78 to 1.49) |
| *P* for trend[d] | | | .72 | | .575 |

Total person-years = 821 178.

[a] Water exposures were linked by address at enrollment and were assigned by the self-reported water source. Nitrate-N concentrations were modeled for private well users and represent 1990-2000 average concentrations for public water supply (PWS) users. For private well users, total person-years = 585 068. For PWS users, total person-years = 236 110.

[b] Model adjusts for age at enrollment, age at enrollment$^2$, strata for state, smoking status (never, former, current, missing), education (less than high school, high school or equivalent, vocational education or some college, college or above, missing), body mass index (<25 kg $m^{-2}$, 25-<30 kg $m^{-2}$, ≥30 kg $m^{-2}$, missing).

[c] *P* for trend was evaluated using the median of each quartile parametrized as a continuous variable.

[d] *P* for trend was evaluated using each category as a numeric variable.

The association between water nitrate and total and aggressive prostate cancer risk did not differ substantially by state and education, with no statistical interaction ($P > .05$) (Table S16). We found an inverse association between water nitrate level (log2 transformed) and having reported a prior PSA screening at phase 3 (odds ratio = 0.97, 95% CI = .95 to 1.00) (Table S17). This suggests that participants with greater nitrate exposure were slightly less likely to undergo PSA screening.

## Discussion

To our knowledge, this is the first prospective study to investigate the associations of drinking water nitrate and DBPs with incident prostate cancer in the US population. Although we did not observe clear evidence of an association with total prostate cancer in continuous and quartile analyses, restricting to aggressive prostate cancer generally strengthened associations. We observed positive and statistically significant associations between average drinking water nitrate >10 mg $L^{-1}$ and total and aggressive prostate cancer risk. These findings were robust in sensitivity analyses that accounted for additional environmental risk factors of prostate cancer (such as pesticide use), and some dietary factors involved in endogenous nitrosation. Drinking water TTHM and HAA5 were not associated with prostate cancer risk.

Our findings are consistent with evidence from the only other study with individual-level exposure assessment, a case-control study in Spain, which found positive associations between lifetime average waterborne ingested nitrate in public water (at slightly lower levels) and aggressive prostate cancer (Gleason scores ≥8).[13] There is biological plausibility, including animal and cell evidence that DNA damage caused by NOCs can cause prostate cancer.[16,49] We found no association between DBPs and prostate cancer risk, similar to no association for total

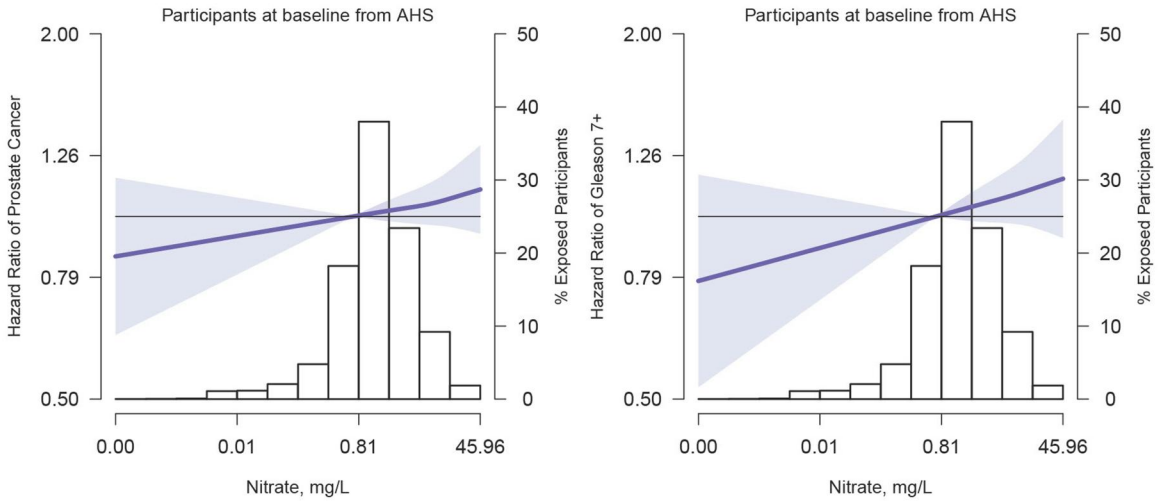


**Figure 1.** Exposure-response associations for prostate cancer cases by nitrate-N concentrations in the Agricultural Health Study (AHS). Lines with shaded areas represent the hazard ratios (95% confidence interval [CI]), based on cubic splines for Cox proportional hazards models using log2-transformed concentrations with knots at the 25th (reference), 75th, and 90th percentiles of nitrate concentrations. A horizontal line is at HR = 1. The histogram represents the frequency distribution of nitrate estimates in the study sample. Aggressive prostate cancer was defined as having at least one of the following tumor characteristics: distant stage, poorly differentiated grade, Gleason score of ≥7, or fatal prostate cancer. Nitrate concentrations were modeled for private well users around the time of enrollment. Lines (shaded areas) represent the estimated hazard ratios in models adjusted for age at baseline, age$^2$, strata for state, smoking status (never, former, current, missing), education (less than high school, high school or equivalent, vocational education or some college, college or above, missing), body mass index (<25 kg m$^{-2}$, 25-<30 kg m$^{-2}$, ≥30 kg m$^{-2}$, missing).

**Table 4.** Adjusted hazard ratios (95% CIs) for the associations of prostate cancer with total trihalomethanes (TTHM) and the sum of 5 haloacetic acids (HAAs).[a]

| | | All prostate cancers | | Aggressive (Gleason ≥7) | |
|---|---|---|---|---|---|
| | No. | No. of cases | HR (95% CI)[b] | No. of cases | HR (95% CI)[b] |
| Overall | | | | | |
| Private well HAA5 (0-0) | 29 091 | 2721 | 1 (reference) | 1633 | 1 (reference) |
| <33% HAA5 (0.66-4.22) | 3541 | 288 | 0.95 (0.84 to 1.07) | 184 | 0.97 (0.84 to 1.14) |
| 33-<60% HAA5 (4.22-9.88) | 3512 | 271 | 0.91 (0.81 to 1.04) | 170 | 0.91 (0.78 to 1.07) |
| ≥60% HAA5 (10-81.11) | 3766 | 307 | 0.92 (0.81 to 1.03) | 184 | 0.93 (0.80 to 1.08) |
| *P* for trend[c] | | | .053 | | .184 |
| Private well TTHM (0-0) | 29091 | 2721 | 1 (reference) | 1633 | 1 (reference) |
| <33% TTHM (0.37-7.46) | 3510 | 273 | 0.91 (0.80 to 1.03) | 175 | 0.93 (0.80 to 1.09) |
| 33-<60% TTHM (7.5-32) | 3647 | 302 | 0.96 (0.85 to 1.08) | 189 | 0.96 (0.83 to 1.12) |
| ≥60% TTHM (32.14-184.2) | 3711 | 295 | 0.91 (0.81 to 1.03) | 175 | 0.91 (0.78 to 1.07) |
| *P* for trend[c] | | | .078 | | .201 |
| PWS users | | | | | |
| Log2 HAA5 | 10 819 | 866 | 0.99 (0.94 to 1.04) | 538 | 0.98 (0.92 to 1.05) |
| <25% HAA5 (0.66-3.77) | 2704 | 198 | 1 (reference) | 126 | 1 (reference) |
| 25-<50% HAA5 (3.77-6.43) | 2703 | 227 | 1.15 (0.95 to 1.40) | 144 | 1.14 (0.90 to 1.46) |
| 50-<75% HAA5 (6.5-14.29) | 2703 | 223 | 1.13 (0.93 to 1.37) | 136 | 1.10 (0.86 to 1.40) |
| ≥75% HAA5 (14.33-81.11) | 2709 | 218 | 1.01 (0.83 to 1.23) | 132 | 1.01 (0.78 to 1.30) |
| *P* for trend[d] | | | .464 | | .616 |
| Log2 TTHM | 10 868 | 870 | 0.99 (0.96 to 1.03) | 539 | 0.99 (0.94 to 1.03) |
| <25% TTHM (0.37-5.47) | 2711 | 219 | 1 (reference) | 141 | 1 (reference) |
| 25-<50% TTHM (5.5-16.85) | 2681 | 227 | 1.03 (0.86 to 1.24) | 141 | 1.01 (0.80 to 1.27) |
| 50-<75% TTHM (17-39.48) | 2736 | 208 | 0.91 (0.75 to 1.10) | 133 | 0.91 (0.72 to 1.16) |
| ≥75% TTHM (39.61-184.2) | 2740 | 216 | 0.93 (0.77 to 1.14) | 124 | 0.87 (0.68 to 1.12) |
| *P* for trend[d] | | | .275 | | .187 |

Analyses of all participants were categorized as private well users (reference), and by tertiles of TTHM and HAA5 concentrations among public water supply (PWS) users. Analyses restricted to PWS users modeled TTHM and HAA5 exposures per doubling and quartiles. Units = μg L$^{-1}$.

[a] Water exposures were linked by address at enrollment and were assigned by the self-reported water source. TTHM and HAA5 concentrations were assigned as 0 for private well users and represent 1990-2009 average concentrations for PWS users. Among all participants, total person-years among participants with HAA5 and TTHM data are 874 531 and 875 551, respectively. Among PWS users, total person-years among participants with HAA5 and TTHM data are 239 246 and 240 265, respectively.

[b] Model adjusts for age at enrollment, age at enrollment$^2$, strata for state, smoking status (never, former, current, missing) + education (less than high school, high school or equivalent, vocational education or some college, college or above, missing) + body mass index (<25 kg m$^{-2}$, 25-<30 kg m$^{-2}$, ≥30 kg m$^{-2}$, missing).

[c] *P* for trend was evaluated using each category as a numeric variable.

[d] *P* for trend was evaluated using the median of each quartile parametrized as a continuous variable.

**Table 5.** Adjusted hazard ratios (95% CIs) for the associations of prostate cancer with a doubling and quartiles of water nitrate[a] in the Agricultural Health Study (AHS), stratified by tertile of heme iron intake (mg 1000 kilocalories per day$^{-1}$).[b]

| | | All prostate cancers | | Aggressive (Gleason ≥7) | |
|---|---|---|---|---|---|
| | No. | No. of cases | HR (95% CI)[c] | No. of cases | HR (95% CI)[c] |
| Per log2 nitrate-N | | | | | |
| Heme iron tertile 1 (0.06-188.98) | 4607 | 384 | 1.02 (0.96 to 1.08) | 230 | 1.01 (0.94 to 1.08) |
| Heme iron tertile 2 (188.99-307.1) | 4607 | 424 | 1.08 (1.02 to 1.14) | 272 | 1.09 (1.02 to 1.17) |
| Heme iron tertile 3 (307.1-3233.9) | 4747 | 450 | 1.03 (0.98 to 1.09) | 287 | 1.05 (0.99 to 1.12) |
| *P* for interaction[d] | | | .30 | | .209 |
| Per quartile nitrate-N | | Tertile 1 heme iron (0.06-189.0 mg 1000 kilocalories per day$^{-1}$) | | | |
| <25% | 1098 | 88 | 1.0 (reference) | 47 | 1.0 (reference) |
| 25-<50% | 1224 | 110 | 1.11 (0.84 to 1.47) | 75 | 1.42 (0.99 to 2.05) |
| 50-<75% | 1189 | 103 | 1.08 (0.80 to 1.44) | 67 | 1.39 (0.95 to 2.03) |
| ≥75% | 1096 | 83 | 0.91 (0.67 to 1.23) | 41 | 0.81 (0.53 to 1.24) |
| *P* for trend[e] | | | .281 | | .044 |
| Per quartile nitrate-N | | Tertile 2 heme iron (189.0-307.1 mg 1000 kilocalories per day$^{-1}$) | | | |
| <25% | 1081 | 76 | 1.0 (reference) | 49 | 1.0 (reference) |
| 25-<50% | 1222 | 118 | 1.38 (1.03 to 1.84) | 72 | 1.31 (0.91 to 1.89) |
| 50-<75% | 1087 | 97 | 1.28 (0.94 to 1.74) | 60 | 1.28 (0.87 to 1.89) |
| ≥75% | 1217 | 133 | 1.64 (1.24 to 2.19) | 91 | 1.72 (1.21 to 2.45) |
| *P* for trend[e] | | | .003 | | .004 |
| Per quartile nitrate-N | | Tertile 3 heme iron (307.1-3234 mg 1000 kilocalories per day$^{-1}$) | | | |
| <25% | 1151 | 104 | 1.0 (reference) | 64 | 1.0 (reference) |
| 25-<50% | 1218 | 104 | 0.90 (0.69 to 1.19) | 69 | 0.98 (0.70 to 1.38) |
| 50-<75% | 1075 | 100 | 1.00 (0.75 to 1.32) | 51 | 0.85 (0.58 to 1.23) |
| ≥75% | 1303 | 142 | 1.15 (0.89 to 1.49) | 103 | 1.36 (0.99 to 1.86) |
| *P* for trend[e] | | | .091 | | .012 |
| *P* for interaction[d] | | | .055 | | .002 |

[a] Water exposures were linked by address at enrollment and were assigned by the self-reported water source. Nitrate-N concentrations were modeled for private well users and represent 1990-2000 average concentrations for public water supply (PWS) users. Analyses were conducted among participants in the dietary subpopulation, with follow-up beginning at phase 2. Total person-years = 240 349.

[b] Zero values of heme iron intake were assigned as the lowest non-zero value of heme iron intake divided by 10, 2.91 mg/10 = 0.291. These values were then converted to milligrams per 1000 kilocalories per day: (heme intake/total kilocalories)×1000.

[c] Model adjusts for age at enrollment, age at enrollment$^2$, natural log transformed total kilocalories per day, strata for state, smoking status (never, former, current, missing), education (less than high school, high school or equivalent, vocational education or some college, college or above, missing), body mass index (<25 kg m$^{-2}$, 25-<30 kg m$^{-2}$, ≥30 kg m$^{-2}$, missing).

[d] *P* value for statistical interaction was calculated using the likelihood ratio test, $\chi^2$ test statistic.

[e] *P* for trend was evaluated using the median of each quartile parameterized as a continuous variable.

waterborne ingested THMs in the Spanish study; however, they observed a positive association for residential tap water chloroform.[13]

We observed a pattern of stronger associations between drinking water nitrate and aggressive prostate cancer risk among participants in the second and to a lesser extent in the third tertiles of dietary heme iron intake. Prior evidence supports that heme iron promotes endogenous NOC formation and our results generally support this potential biologic mechanism.[43] We observed stronger associations among participants with higher than the median dietary vitamin C intake, in contrast to the role of antioxidants in the suppression of endogenous NOC formation. The Spanish case-control study found associations between waterborne ingested nitrate and prostate cancer risk among men with lower (<130 mg day$^{-1}$) but not higher (≥130) vitamin C intake, but found no statistical interaction (*P*>.05 for interaction).[13] Although we do not have a biological explanation for these findings, we acknowledge that our reliance on self-reported DHQ data may have introduced measurement error and biased estimates of vitamin C intake.

We found no association of dietary nitrate or nitrite with prostate cancer risk. Prior evidence on associations between dietary nitrate/nitrite, heme iron, and/or processed meat has been inconsistent.[29,40,50,51] This study suggests that drinking water, rather than diet, is an important route of exposure to nitrate that may be related to prostate cancer risk.

## Strengths and limitations

Strengths of this study include our drinking water exposure assessment, which relied on self-reported information to assign participant water source.[31] We used historical water quality monitoring data for PWS users, and estimated nitrate concentrations in private wells using robust, state-specific models that were previously validated. Although we were able to investigate associations with dietary nitrate/nitrite and interactions between drinking water nitrate with vitamin C and heme iron using data collected via a standardized and validated DHQ,[37] dietary intakes were only available for a subcohort. We had a long duration of prospective follow-up (mean = 21.9 years) and detailed information on potential confounders including occupational pesticide use and water arsenic; associations were stronger adjusting for arsenic, aldrin, and malathion. We also assessed the relationship between water nitrate and a prior PSA screening in over 14 000 men (phase 3). The small inverse association between screening and nitrate concentrations may have biased associations with total prostate cancer risk toward the null, as more highly exposed participants were less likely to undergo PSA screening and be diagnosed with nonaggressive disease.[52]

Limitations of our study include no information on usual intake of tap water (L day$^{-1}$) and potential measurement error in water nitrate estimates. We expect that this error will be nondifferential by prostate cancer diagnosis and may have attenuated the observed associations. An additional limitation is the limited racial and ethnic diversity of the study population, comprised of mainly White and non-Hispanic participants. Future research including more heterogeneous study populations is needed, to advance our understanding of prostate cancer etiology. Prostate cancer is a solid tumor with a minimum latency of 10-15 years.[6] Our use of average contaminant concentrations at the

**Table 6.** Adjusted hazard ratios (95% CIs) for the associations of prostate cancer with a doubling and quartiles of water nitrate[a] in the Agricultural Health Study (AHS), stratified by tertile of vitamin C intake (milligrams per 1000 kilocalories per day).

| | | All prostate cancers | | Aggressive (Gleason ≥7) | |
|---|---|---|---|---|---|
| | No. | No. of cases | HR (95% CI)[b] | No. of cases | HR (95% CI)[b] |
| Per log2 nitrate-N | | | | | |
| Vitamin C tertile 1 (3.08-46.83) | 5015 | 391 | 1.03 (0.98 to 1.09) | 252 | 1.03 (0.96 to 1.10) |
| Vitamin C tertile 2 (46.85-84.87) | 5014 | 439 | 1.04 (0.99 to 1.10) | 284 | 1.04 (0.97 to 1.11) |
| Vitamin C tertile 3 (84.89-2323.7) | 5166 | 496 | 1.05 (1.00 to 1.11) | 300 | 1.08 (1.01 to 1.15) |
| *P* for interaction[c] | | | .750 | | .526 |
| Per quartile nitrate-N | Tertile 1 vitamin C (3.1-46.8 mg 1000 kilocalories per day$^{-1}$) | | | | |
| <25% | 1258 | 86 | 1.0 (reference) | 57 | 1.0 (reference) |
| 25-<50% | 1261 | 110 | 1.21 (0.91 to 1.61) | 74 | 1.27 (0.90 to 1.80) |
| 50-<75% | 1191 | 83 | 0.96 (0.70 to 1.30) | 49 | 0.90 (0.61 to 1.33) |
| ≥75% | 1305 | 112 | 1.26 (0.95 to 1.68) | 72 | 1.24 (0.87 to 1.77) |
| *P* for trend[d] | | | .196 | | .427 |
| Per quartile nitrate-N | Tertile 2 vitamin C (46.9-84.9 mg 1000 kilocalories per day$^{-1}$) | | | | |
| <25% | 1254 | 110 | 1.0 (reference) | 72 | 1.0 (reference) |
| 25-<50% | 1254 | 87 | 0.77 (0.58 to 1.02) | 55 | 0.75 (0.53 to 1.07) |
| 50-<75% | 1171 | 119 | 1.18 (0.90 to 1.54) | 74 | 1.20 (0.86 to 1.67) |
| ≥75% | 1335 | 123 | 1.03 (0.79 to 1.33) | 83 | 1.02 (0.74 to 1.41) |
| *P* for trend[d] | | | .321 | | .407 |
| Per quartile nitrate-N | Tertile 3 vitamin C (84.9-2324 mg 1000 kilocalories per day$^{-1}$) | | | | |
| <25% | 1291 | 99 | 1.0 (reference) | 53 | 1.0 (reference) |
| 25-<50% | 1391 | 145 | 1.33 (1.03 to 1.72) | 93 | 1.60 (1.14 to 2.25) |
| 50-<75% | 1154 | 106 | 1.14 (0.86 to 1.51) | 59 | 1.20 (0.82 to 1.75) |
| ≥75% | 1330 | 146 | 1.38 (1.07 to 1.79) | 95 | 1.67 (1.19 to 2.35) |
| *P* for trend[d] | | | .071 | | .034 |
| *P* for interaction[c] | | | .017 | | .013 |

[a] Water exposures were linked by address at enrollment and were assigned by the self-reported water source. Nitrate-N concentrations were modeled for private well users and represent 1990-2000 average concentrations for public water supply (PWS) users. Analyses were conducted among participants in the dietary subpopulation, with follow-up beginning at phase 2. Total person-years = 259 241.

[b] Model adjusts for age at enrollment, age at enrollment$^2$, natural log transformed total kilocalories per day, strata for state, smoking status (never, former, current, missing), education (less than high school, high school or equivalent, vocational education or some college, college or above, missing), body mass index (<25 kg m$^{-2}$, 25-<30 kg m$^{-2}$, ≥30 kg m$^{-2}$, missing).

[c] *P* value for statistical interaction was calculated using the likelihood ratio test, $\chi^2$ test statistic.

[d] *P* for trend was evaluated using the median of each quartile parametrized as a continuous variable.

enrollment address does not account for drinking water exposures earlier in life that may be relevant to prostate cancer risk, though the latency period for prostate cancer following drinking water contaminant exposure is unclear.

In conclusion, our findings indicate that exposure to nitrate in drinking water, at average levels greater than 10 mg L$^{-1}$, may be a risk factor for the development of prostate cancer. Prostate cancer is the most common cancer in men in the United States after skin cancer, and it is the second-leading cause of cancer death among men after lung cancer. Thus, even small increases in the risk of prostate cancer associated with nitrate exposure can have important public health implications. Future research is needed to confirm these findings.

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## Supplementary material

Supplementary material is available at *JNCI: Journal of the National Cancer Institute* online.

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## Conflicts of interest

The authors declare no conflict of interest. R.R.J., who is a JNCI Editorial Board Member and co-author on this paper, was not involved in the editorial review or decision to publish the manuscript.

## Data availability

The analyses were conducted using AHS data releases P1REL202210.00, P2REL202210.00, P3REL202210.00, and AHSREL202405.00. The data underlying this investigation will be provided upon request as described on the AHS website: https://aghealth.nih.gov/collaboration/. For more information on the process of requesting access to these data, including any administrative and human subjects requirements, please contact the AHS Executive Committee and Coordinating Center (https://aghealth.nih.gov/about/ec.cc.html).

## References

1. National Cancer Institute. Cancer Stat Facts: Prostate Cancer. Accessed May 14, 2025. https://seer.cancer.gov/statfacts/html/prost.html.
2. Centers for Disease Control and Prevention. Prostate cancer incidence by stage at diagnosis, United States—2001–2019. Accessed July 6, 2023. https://www.cdc.gov/cancer/uscs/about/data-briefs/no34-prostate-cancer-incidence-2001-2019.htm#:~:text=In%202019%2C%20a%20total%20of,cancer%20among%20men%20in%202019.
3. Gandaglia G, Leni R, Bray F, et al. Epidemiology and prevention of prostate cancer. *Eur Urol Oncol*. 2021;4:877-892.
4. Perdana NR, Mochtar CA, Umbas R, Hamid ARA. The risk factors of prostate cancer and its prevention: a literature review. *Acta Med Indones*. 2016;48:228-238.
5. Putnam SD, Cerhan JR, Parker AS, et al. Lifestyle and anthropometric risk factors for prostate cancer in a cohort of Iowa men. *Ann Epidemiol*. 2000;10:361-369.
6. Tangen CM, Neuhouser ML, Stanford JL. *Prostate Cancer*. Oxford Academic; 2017.
7. World Cancer Research Fund/American Institute for Cancer Research. Continuous Update Project Expert Report 2018. Diet, nutrition, physical activity, and prostate cancer. 2018. Accessed May 14, 2025. https://www.wcrf.org/wp-content/uploads/2024/10/prostate-cancer-report.pdf.
8. Mahal BA, Gerke T, Awasthi S, et al. Prostate cancer racial disparities: a systematic review by the Prostate Cancer Foundation panel. *Eur Urol Oncol*. 2022;5:18-29.
9. Coughlin SS. A review of social determinants of prostate cancer risk, stage, and survival. *Prostate Int*. 2020;8:49-54.
10. Williams PA, Zaidi SK, Ramian H, Sengupta R. AACR cancer disparities progress report 2024: achieving the bold vision of health equity. *Cancer Epidemiol Biomarkers Prev*. 2024;33:870-873.
11. Togawa K, Leon ME, Lebailly P, et al. Cancer incidence in agricultural workers: findings from an international consortium of agricultural cohort studies (AGRICOH). *Environ Int*. 2021;157:106825.
12. Koutros S, Beane Freeman LE, Lubin JH, et al. Risk of total and aggressive prostate cancer and pesticide use in the Agricultural Health Study. *Am J Epidemiol*. 2013;177:59-74.
13. Donat-Vargas C, Kogevinas M, Castaño-Vinyals G, et al. Long-term exposure to nitrate and trihalomethanes in drinking water and prostate cancer: a multicase–control study in Spain (MCC-Spain). *Environ Health Perspect*. 2023;131:37004.
14. Morales-Suarez-Varela MM, Llopis-Gonzalez A, Tejerizo-Perez ML. Impact of nitrates in drinking water on cancer mortality in Valencia, Spain. *Eur J Epidemiol*. 1995;11:15-21.
15. Ward MH, Jones RR, Brender JD, et al. Drinking water nitrate and human health: an updated review. *Int J Environ Res Public Health*. 2018;15:1557.
16. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Ingested nitrate and nitrite, and cyanobacterial peptide toxins. In: *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*. International Agency for Research on Cancer; 2010.
17. Nolan BT, Ruddy BC. *Nitrate in the Ground Waters of the United States—Assessing the Risk*. U.S. Geological Survey Fact Sheet 092-96. National Water-Quality Assessment (NAWQA) Program; 1996.
18. Ransom KM, Nolan BT, Stackelberg PE, Belitz K, Fram MS. Machine learning predictions of nitrate in groundwater used for drinking supply in the conterminous United States. *Sci Total Environ*. 2022;807:151065.
19. Levin R, Villanueva CM, Beene D, et al. US drinking water quality: exposure risk profiles for seven legacy and emerging contaminants. *J Expo Sci Environ Epidemiol* 2024;34:3-22.
20. United States Environmental Protection Agency. National Primary Drinking Water Regulations. Accessed July 7, 2023. https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations.
21. Inoue-Choi M, Virk-Baker MK, Aschebrook-Kilfoy B, et al. Development and calibration of a dietary nitrate and nitrite database in the NIH–AARP Diet and Health Study. *Public Health Nutr*. 2016;19:1934-1943.
22. United States Environmental Protection Agency. Stage 1 and Stage 2 disinfectants and disinfection byproducts rules. Accessed July 7, 2023. https://www.epa.gov/dwreginfo/stage-1-and-stage-2-disinfectants-and-disinfection-byproducts-rules.
23. Rahman MB, Driscoll T, Cowie C, Armstrong BK. Disinfection by-products in drinking water and colorectal cancer: a meta-analysis. *Int J Epidemiol*. 2010;39:733-745.
24. Xie B, Chen J, Kai J, Li J. Association between drinking water disinfection byproducts exposure and human bladder cancer: a time-updated meta-analysis of trihalomethanes. *J Hazard Mater*. 2025;490:137833.
25. Zhang M, Deng YL, Liu C, Lu WQ, Zeng Q. Impacts of disinfection byproduct exposures on male reproductive health: current evidence, possible mechanisms and future needs. *Chemosphere*. 2023;331:138808.
26. Plewa MJ, Muellner MG, Richardson SD, et al. Occurrence, synthesis, and mammalian cell cytotoxicity and genotoxicity of haloacetamides: an emerging class of nitrogenous drinking water disinfection byproducts. *Environ Sci Technol*. 2008;42:955-961.
27. Richardson SD, Plewa MJ, Wagner ED, Schoeny R, DeMarini DM. Occurrence, genotoxicity, and carcinogenicity of regulated and

emerging disinfection by-products in drinking water: a review and roadmap for research. *Mutat Res*. 2007;636:178-242.
28. Komulainen H, Vaittinen SL, Vartiainen T, et al. Carcinogenicity of the drinking water mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2 (5H)-furanone in the rat.. *J Natl Cancer Inst* 1997;89:848-856.
29. Sinha R, Park Y, Graubard BI, et al. Meat and meat-related compounds and risk of prostate cancer in a large prospective cohort study in the United States. *Am J Epidemiol*. 2009;170:1165-1177.
30. Loh YH, Jakszyn P, Luben RN, Mulligan AA, Mitrou PN, Khaw KTN. Nitroso compounds and cancer incidence: the European Prospective Investigation into Cancer and Nutrition (EPIC)–Norfolk Study. *Am J Clin Nutr*. 2011;93:1053-1061.
31. Manley CK, Spaur M, Madrigal JM, et al. Drinking water sources and water quality in a prospective agricultural cohort. *Environ Epidemiol*. 2022;6:e210.
32. Giovannucci E, Liu Y, Platz EA, Stampfer MJ, Willett WC. Risk factors for prostate cancer incidence and progression in the Health Professionals Follow-Up Study. *Int J Cancer*. 2007;121:1571-1578.
33. Hurwitz LM, Agalliu I, Albanes D, et al.; Prostate Cancer Cohort Consortium (PC3) Working Group. Recommended definitions of aggressive prostate cancer for etiologic epidemiologic research. *J Natl Cancer Inst*. 2021;113:727-734.
34. Messier KP, Wheeler DC, Flory AR, et al. Modeling groundwater nitrate exposure in private wells of North Carolina for the Agricultural Health Study. *Sci Total Environ*. 2019;655:512-519.
35. Wheeler DC, Nolan BT, Flory AR, DellaValle CT, Ward MH. Modeling groundwater nitrate concentrations in private wells in Iowa. *Sci Total Environ*. 2015;536:481-488.
36. Sebastian RS, Wilkinson C, Goldman JD. *Drinking Water Intake in the US: What We Eat in America, NHANES 2005-2008*. United States Department of Agriculture Agricultural Research Service; 2011.
37. National Cancer Institute. Diet history questionnaire, version 1.0. Accessed May 14, 2025. https://epi.grants.cancer.gov/dhq/forms/.
38. Ammons SM, Manley J, Spaur CK, et al. Nitrate and disinfection by-products in drinking water and risk of ovarian cancer. *Environ Epidemiol*. 2025;9:e382.
39. R Core Team. *R: A Language and Environment for Statistical Computing* 4.3.1. R Foundation for Statistical Computing; 2025.
40. Bylsma LC, Alexander DD. A review and meta-analysis of prospective studies of red and processed meat, meat cooking methods, heme iron, heterocyclic amines and prostate cancer. *Nutr J*. 2015;14:125.
41. Wu K, Spiegelman D, Hou T, et al. Associations between unprocessed red and processed meat, poultry, seafood and egg intake and the risk of prostate cancer: a pooled analysis of 15 prospective cohort studies. *Int J Cancer*. 2016;138:2368-2382.
42. van Breda SG, Mathijs K, Sági-Kiss V, et al. Impact of high drinking water nitrate levels on the endogenous formation of apparent N-nitroso compounds in combination with meat intake in healthy volunteers. *Environ Health*. 2019;18:1-12.
43. Cross AJ, Pollock JR, Bingham SA. Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat. *Cancer Res*. 2003;63:2358-2360.
44. Koutros S, Cross AJ, Sandler DP, et al. Meat and meat mutagens and risk of prostate cancer in the Agricultural Health Study. *Cancer Epidemiol Biomarkers Prev*. 2008;17:80-87.
45. Roh T, Lynch CF, Weyer P, Wang K, Kelly KM, Ludewig G. Low-level arsenic exposure from drinking water is associated with prostate cancer in Iowa. *Environ Res*. 2017;159:338-343.
46. Bulka CM, Jones RM, Turyk ME, Stayner LT, Argos M. Arsenic in drinking water and prostate cancer in Illinois counties: an ecologic study. *Environ Res*. 2016;148:450-456.
47. Lombard MA, Bryan MS, Jones DK, et al. Machine learning models of arsenic in private wells throughout the conterminous United States as a tool for exposure assessment in human health studies. *Environ Sci Technol*. 2021;55:5012-5023.
48. Ravalli F, Yuanzhi Yu Y, Bostick BC, et al. Sociodemographic inequalities in uranium and other metals in community water systems across the US. *Lancet Planet Health*. 2006-2011;6:e320-e330.
49. Bellamri M, Turesky RJ. Dietary carcinogens and DNA adducts in prostate cancer. In: Dehm S, Tindall D, eds. *Prostate Cancer*. Springer; 2019:29-55.
50. Xie L, Mo M, Jia HX, Liang F, Yuan J, Zhu J. Association between dietary nitrate and nitrite intake and site-specific cancer risk: evidence from observational studies. *Oncotarget*. 2016;7:56915-56932.
51. Jakszyn PG, Allen NE, Lujan-Barroso L, et al. Nitrosamines and heme iron and risk of prostate cancer in the European Prospective Investigation into Cancer and Nutrition. *Cancer Epidemiol Biomarkers Prev*. 2012;21:547-551.
52. American Cancer Society. Screening tests for prostate cancer. Accessed May 14, 2025. https://www.cancer.org/cancer/types/prostate-cancer/detection-diagnosis-staging/tests.html#:~:text=you%20have%20cancer.-,Prostate%2Dspecific%20antigen%20(PSA)%20blood%20test, PSA%20tests%2C%E2%80%9D%20below).&text=Men%20with%20a%20PSA%20level, prostate%20cancer%20is%20over%2050%25.



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