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Nitrate in drinking water and colorectal cancer risk: A nationwide population-based cohort study

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Nitrate in drinking water may increase risk of colorectal cancer due to endogenous transformation into carcinogenic *N*-nitroso compounds. Epidemiological studies are few and often challenged by their limited ability of estimating long-term exposure on a detailed individual level. We exploited population-based health register data, linked in time and space with longitudinal drinking water quality data, on an individual level to study the association between long-term drinking water nitrate exposure and colorectal cancer (CRC) risk. Individual nitrate exposure was calculated for 2.7 million adults based on drinking water quality analyses at public waterworks and private wells between 1978 and 2011. For the main analyses, 1.7 million individuals with highest exposure assessment quality were included. Follow-up started at age 35. We identified 5,944 incident CRC cases during 23 million person-years at risk. We used Cox proportional hazards models to estimate hazard ratios (HRs) of nitrate exposure on the risk of CRC, colon and rectal cancer. Persons exposed to the highest level of drinking water nitrate had an HR of 1.16 (95% CI: 1.08–1.25) for CRC compared with persons exposed to the lowest level. We found statistically significant increased risks at drinking water levels above 3.87 mg/L, well below the current drinking water standard of 50 mg/L. Our results add to the existing evidence suggesting increased CRC risk at drinking water nitrate concentrations below the current drinking water standard. A discussion on the adequacy of the drinking water standard in regards to chronic effects is warranted.

Cancer Epidemiology

Nitrate is leached to the aquatic environment, originating mainly from human activities, especially the use of fertilizers in intensive agriculture, and is a frequent drinking water pollutant.[1–3] Denmark is among the countries with the most intensive agriculture with two-thirds of its area under cultivation, resulting in pronounced nitrate pollution of groundwater.[4] The Danish drinking water structure is decentralized and based exclusively on groundwater.[5] The drinking water standard of 50 mg/L as nitrate ion was established to protect infants from the acute condition methemoglobinemia.[1] This standard is almost equivalent to the United States Environmental Protection Agency's maximum contaminant level of 10 mg/L as nitrogen.

However, physiological pathways of possible chronic effects have been suggested, due to endogenous transformation of nitrate into genotoxic *N*-nitroso compounds.[6] Most *N*-nitroso compounds are animal carcinogens,[7] and nitrate has been classified as probably carcinogenic to humans under conditions that favor endogenous nitrosation.[8] Colorectal cancer (CRC) is the third most frequent cancer worldwide,[9] with an age-standardized incidence rate of 43.6 (males) and 33.8 (females) per 100,000 persons per year in Denmark.[10]

Previous epidemiological studies on the association between nitrate in drinking water and CRC are few and yielded inconsistent results.[6] An ecologic study in Slovakia found a positive association between nitrate levels in drinking water and cancers in all digestive organs and CRC in particular.[11] A case–control study in Iowa showed an increased colon cancer risk at elevated nitrate levels in drinking water among susceptible subgroups with elevated endogenous nitrosation, that is, low vitamin C and high red meat intake.[12] A prospective cohort study of women carried out in the same area with a similar exposure assessment found no significant association between colon cancer and the quartile exposed to

Key words: nitrate, drinking water, colorectal cancer, Denmark, cohort studies

Abbreviations: CRC: colorectal cancer; CI: confidence interval; HR: hazard ratio

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What's new?

Nitrate is considered a probable carcinogen in humans owing to its potential for endogenous transformation into genotoxic *N*-nitroso compounds. Cancer risk related to nitrate pollution in drinking water, as a consequence of intensive agriculture using fertilizers, is of particular concern. Here, analyses of water quality data and health registry data with a high spatiotemporal resolution for 2.7 million people in Denmark reveal an increased risk of colorectal cancer (CRC) in association with nitrate exposure. CRC risk was elevated at nitrate concentrations below the current drinking water standard.

the highest concentrations, while the second and third quartiles showed increased risks, and an inverse association was observed for rectal cancer.[13] A case–control study of women in Wisconsin found no overall association with colon or rectal cancer, but an increased risk of proximal colon cancer at nitrate levels around the drinking water standard.[14] A recent case–control study from Spain and Italy showed the higher the intake of nitrate from drinking water, the higher the risk of colon and rectal cancer, also at levels well below the drinking water standard.[15]

A common limitation of previous studies is the limited ability to access historical nitrate exposure for study subjects. To identify potentially small chronic effects, long-term follow-up of a large population is necessary. Large studies with well-characterized long-term exposures and inclusion of private well users were called for[6] assessing populations with large exposure contrast, even if concentrations are below the drinking water standard.[16]

We addressed these limitations by using the rich population-based Danish registers including longitudinal health and residential information,[17] linked in time and space with the likewise longitudinal information on drinking water quality with high spatial and temporal resolution, covering the entire country from 1978 onward.[5,18] The link of these unique nationwide and longitudinal data sources enabled us to study the association between nitrate in drinking water and CRC on an individual level.

Methods

We followed all Danish residents for development of CRC considering nitrate in drinking water as the exposure of interest. Details are described in the following sections.

Study design and population

The unique personal identification number, which is assigned to all Danish residents, was used as key identifier to accurately link data from several registers. Prospectively collected and continuously updated information on date of birth, sex, residential history and vital status were retrieved from the Danish Civil Registration System.[17] The study period was January 1, 1978 to December 31, 2011, as residential history was geocoded for this period. We defined the cohort as all residents of Denmark, alive on their 35th birthday. We followed each individual from their 35th birthday until the onset of colon or rectal cancer, the end of study (31 December 2011), death, emigration or disappearance. Diagnoses of colon cancer (ICD-10 codes C18 and C19), rectal cancer (C20) and all other cancers were retrieved from the Danish Cancer Registry, which has a high validity and degree of completeness.[19]

Exposure assessment

The approach of assigning each household to its annual nitrate concentration is described in detail elsewhere.[18] In brief, we assigned annual average drinking water nitrate concentrations, registered at waterworks level, to the 2,852 public water supply areas and the 2,382,445 publicly supplied households within these. Privately supplied households (81,663) were identified and assigned nitrate concentrations of their private well. In total, 208,706 drinking water samples with precise sampling date and location were used in this study. We interpolated concentrations for years without available nitrate measurements at household level. An exposure assessment quality level based on the number of years to the closest nitrate sample was calculated for each household and year (for detailed explanation of the levels, see results from sensitivity analyses in Table 2).

We calculated each individual's average nitrate exposure between their 20th and 35th birthday by linking their residential history from 1978 onward in time and space to the longitudinal drinking water nitrate concentration data at the Danish households. To be able to calculate an individual's exposure, their exposure window had to overlap with the study period, that is, their 35th birthday had to be after the beginning of study (January 1, 1978) and before the end of study (December 31, 2011). For the main analyses, we included only individuals with a high exposure assessment quality, having lived at least 75% of the time at households with an associated nitrate sample taken within 1 year.

Covariates

Covariates were selected *a priori*. Socioeconomic status was based on the *highest attained education* of each individual from the educational registers and included in four categories: (*i*) primary school only, (*ii*) shorter education (high school and short vocational training), (*iii*) medium long education (vocational training and bachelors) and (*iv*) long education (academics).[20] We included information on any *previous cancer*

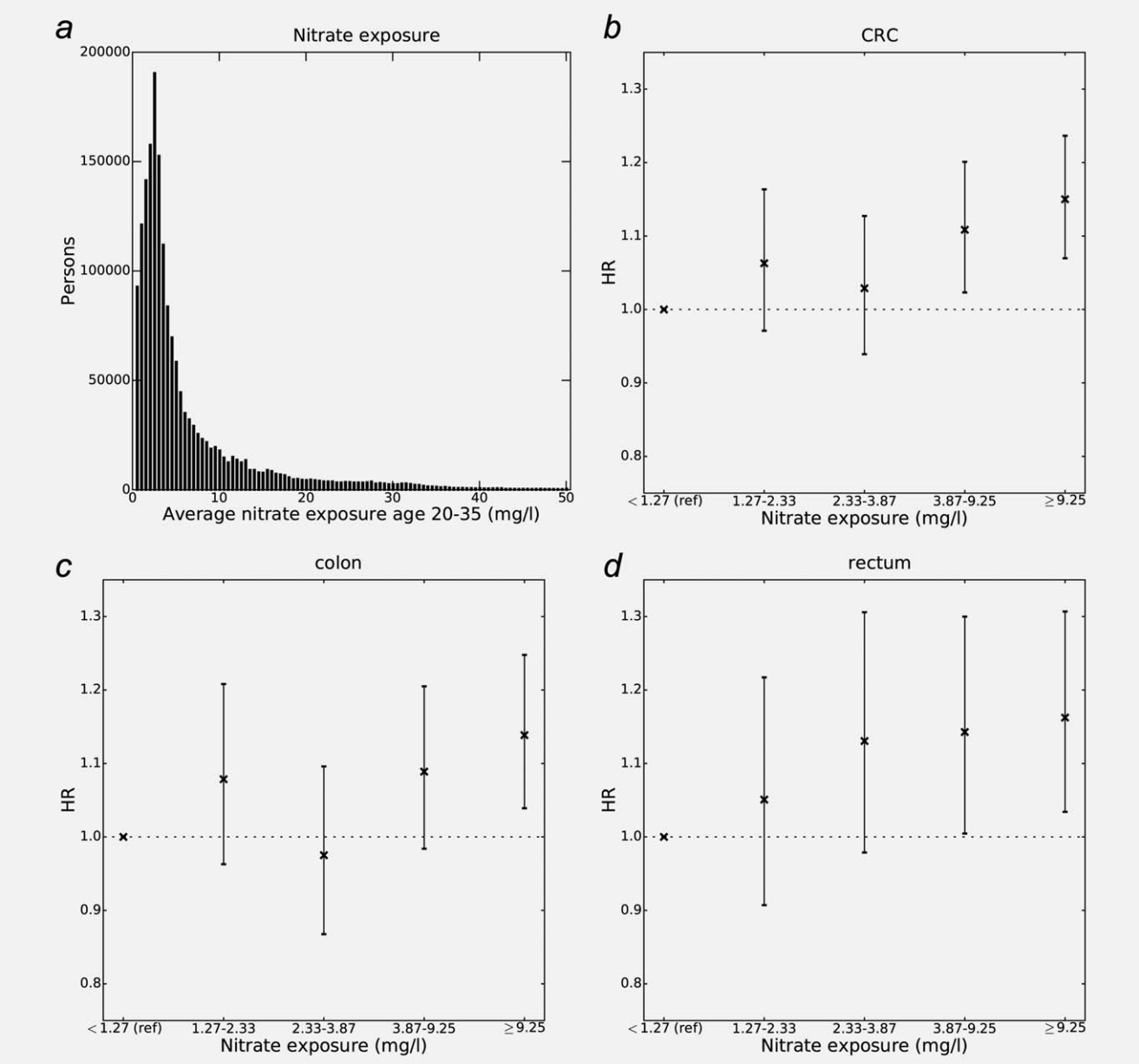

Figure 1. (*a*) Average drinking water nitrate exposure between age 20 and 35 of the study population (subjects exposed to > 50 mg/l [0.58%] not shown here). Hazard ratios (HR) and 95% CIs of nitrate exposure quintiles for (*b*) colorectal, (*c*) colon and (*d*) rectal cancer. Base adjustment.

diagnosis other than the outcome of interest, and *year of birth* in two-year bands to address birth cohort effects.

Statistical analyses

We assessed the association between drinking water nitrate and colon and rectal cancer as separate outcomes, and the combined outcome CRC. We used Cox proportional hazards models to estimate hazard ratios (HRs) using age as the underlying time scale while stratifying the baseline by sex. We included nitrate exposure as quintiles according to the distribution of nitrate exposure in the total population. The base adjustment controlled for age, sex, year of birth and previous cancer diagnosis. Additionally, we adjusted for highest attained education (2nd adjustment). We calculated a summary trend estimate, measuring the effect on a person exposed to the highest decile of nitrate concentrations in drinking water (≥16.75 mg/L) compared with a person exposed to the lowest decile of nitrate concentrations in drinking water (<0.69 mg/L), utilizing data from the in-between deciles (nominal scoring of deciles). Results are reported with 95% confidence intervals (CIs). We checked the validity of the proportional hazards assumption by assessing the null hypothesis of a zero slope of the Schoenfeld residuals on time. Analyses were done in STATA 13.1.

Table 1. Adjusted hazard ratios (95% CIs) associated with high levels of nitrate exposure compared with low levels (trend estimate). Incident cases and study population size (*N*)

Cancer site	*N*[1]	Cases[1]	Base adjustment[2]	Second adjustment[3]
Colorectal	1,742,093	5,944	1.16 (1.08–1.25)	1.14 (1.06–1.23)
Colon	1,742,156	3,700	1.15 (1.05–1.26)	1.14 (1.04–1.26)
Rectum	1,742,255	2,308	1.17 (1.04–1.32)	1.13 (1.00–1.27)

[1]Incident cases for colon and rectal cancer are not mutually exclusive.
[2]Age, sex, year of birth and previous cancer diagnosis.
[3]Base and highest attained education.

Sensitivity analyses

Using the trend estimate, we assessed the robustness of our results considering potential bias due to private well users and quality, length and period of each individual's exposure assessment. We excluded persons with a previous cancer diagnosis other than the outcome of interest and residents of the Capital Region.

Ethical considerations

In keeping with Danish legislation, the Danish Data Protection Agency, the Danish Health Data Authority and Statistics Denmark approved this study.

Results

Of the 2,833,825 Danish residents whose exposure window concurred with the study period, 1,742,321 (61%) met the high exposure assessment quality criterion and were included in the main analyses. Persons who had a diagnosis before initiation of follow-up were excluded (CRC: 228; colon: 165; rectum: 66). The distribution of the average nitrate exposure between age 20 and 35 for this study population is shown in Figure 1a. During the 23 million person-years of follow-up, 5,944 persons were diagnosed with CRC, 3,700 with colon cancer and 2,308 with rectal cancer (Table 1 and Supporting Information, Table 1).

Figure 1 shows the HRs of the nitrate concentration exposure quintiles for (b) CRC, (c) colon and (d) rectal cancer. For both CRC and rectal cancer alone, the two highest exposure quintiles (>3.87 mg/L) showed statistically significant increased HRs. For colon cancer alone, only the highest exposure quintile (≥9.25 mg/L) was associated with a statistically significant increased HR. In the following, we focus on the trend estimate.

Individuals exposed to the highest level of drinking water nitrate (≥16.75 mg/L) had an increased risk of CRC [HR: 1.16 (95% CI: 1.08–1.25)] compared with individuals exposed to the lowest exposure level (<0.69 mg/L; see Table 1, base adjustment). Additional adjustment for education had only limited influence. Similar results were obtained when considering colon and rectal cancer as separate outcomes. Effect modification by sex was not observed (CRC: $p = 0.49$; colon: $p = 0.44$; rectum: $p = 0.99$; second adjustment). Stepwise reincluding individuals with a lower exposure assessment quality increased the study population to ultimately 2,692,508 individuals, followed for ∼44 million person-years (Table 2). As exposure assessment quality decreased, the observed effect sizes decreased as well.

Additional sensitivity analyses yielded robust results (Table 3). The proportional hazards assumption was not violated in any of the presented models. A previous cancer diagnosis other than the outcome of interest was associated with increased HRs for all outcomes, and a protective effect of increasing levels of education was observed (results not shown).

Discussion

This is the first nationwide population-based study using a historical longitudinal assessment of long-term drinking water nitrate exposure to assess the associated risk of CRC. Our results showed the higher the level of nitrate in drinking water, the higher the risk of CRC. Considering colon and rectal cancer as separate outcomes, we found similar results. Results for CRC combined and rectal cancer alone showed statistically significant increased HRs in the two highest quintiles of exposure (>3.87 mg/L). For colon cancer, this was only seen in the highest quintile (≥9.25 mg/L), still at concentrations substantially below the current drinking water standard of 50 mg/L. This suggests a need of lowering the drinking water standard to adequately protect the public against chronic adverse health effects of nitrate in drinking water.

From Figure 1, a dose–response relationship is suggested, which is supported by the results for the trend estimate of 1.14 (95% CI: 1.06–1.23) for CRC, 1.14 (1.04–1.26) for colon cancer alone and 1.13 (1.00–1.27) for rectal cancer alone in the full adjustment. Hazard ratios were similar in all adjustments, indicating little influence of the included covariates and sensitivity analyses showed stable and robust results. Interestingly, the higher the exposure assessment quality, the higher effect sizes were observed (Table 2). Lower exposure assessment quality levels were due to interpolation of nitrate concentrations for years with no sample taken at the respective waterworks. Consequently, effect sizes were expected to attenuate with increasing levels of misclassification.[21]

Our results showed a statistically significant positive association between nitrate in drinking water and CRC at levels well below the current drinking water standard, which is in agreement with the findings of a recent case–control study.[15] Espejo-Herrera *et al.* found an increased risk for colon cancer from 5 mg/d waterborne nitrate intake (corresponding to drinking water concentrations of ∼4.3 mg/L), and for CRC and rectal cancer from ∼8.6 mg/L. Espejo-Herrera *et al.* had individual-level data on endogenous nitrosation factors, diet, lifestyle and water consumption, allowing controlling for established CRC risk factors and additional covariates. They observed higher effect sizes in groups with high red meat intake, in agreement with a previous study.[12]

Table 2. Stepwise reinclusion of individuals with at least 75% of their exposure window at given, or higher, exposure assessment quality level. Trend estimate: hazard ratios (95% CIs), study population size (*N*) and number of cases for colon and rectal cancer. Second adjustment (age, sex, year of birth, previous cancer diagnosis and highest attained education)

Exposure assessment quality	Explanation	*N*[1]	Colon	Rectum
High (main analyses)	At least one nitrate sample taken within 1 year at waterworks supplying the residence[2]	1,742,156	1.14 (1.04–1.26)	1.13 (1.00–1.27)
		Cases	3,700	2,308
Medium high	At least one nitrate sample taken within 5 years at waterworks supplying the residence[2]	2,139,124	1.11 (1.03–1.19)	1.10 (1.01–1.21)
		Cases	6,025	3,764
Medium	At least one nitrate sample taken within 10 years at waterworks supplying the residence[2]	2,299,309	1.08 (1.01–1.16)	1.10 (1.01–1.19)
		Cases	6,966	4,384
Medium low	At least one nitrate sample taken outside time window of 10 years at waterworks supplying the residence[2]	2,615,138	1.09 (1.02–1.16)	1.08 (0.99–1.16)
		Cases	8,652	5,495
Low	No nitrate sample taken at waterworks supplying the residence[2]	2,692,508	1.09 (1.03–1.15)	1.07 (0.99–1.15)
		Cases	8,844	5,618

[1]Study population *N* for colon cancer analyses.
[2]Residence: longitudinal data refers to exposure assessment quality of each individual's residence at any point in time during the exposure window.

Table 3. Sensitivity analyses: hazard ratios (95% CIs) of trend estimate and study size *N*. Full adjustment: age, sex, year of birth, previous cancer diagnosis and education

Scenario	*N*[1]	Colon	Rectum
Main analysis (Table 1)	1,742,156	1.14 (1.04–1.26)	1.13 (1.00–1.27)
Excluding private well users	1,684,944	1.14 (1.04–1.25)	1.13 (1.00–1.27)
At least 5 years of exposure data	1,562,072	1.15 (1.01–1.31)	1.07 (0.90–1.26)
At least 10 years of exposure data	1,351,232	1.18 (0.98–1.41)	1.06 (0.84–1.33)
Only individuals with colon/rectum cancer as first cancer diagnosis	1,681,694	1.13 (1.02–1.25)	1.11 (0.98–1.26)
New exposure window: age 30–40	1,798,350	1.13 (1.05–1.21)	1.07 (0.98–1.18)
Excluding capital region	1,195,094	1.18 (1.06–1.31)	1.09 (0.95–1.25)

[1]Study population *N* for colon cancer analyses.

While we could not include individual-level data on diet and lifestyle, the strength of our study lies in its large population size and the comprehensive long-term exposure assessment. By including the entire population (up to 2.7 million persons followed for up to 34 years), we avoided selection bias. Register data used in this study are deemed to be of very high validity and completeness.[17,19] All administrative, health and drinking water quality data were prospectively collected, thereby eliminating bias due to differential recall and loss to follow-up.

In contrast to previous studies, our exposure assessment was based on exhaustive longitudinal drinking water quality data, registered in one nationwide database. We did not need to model historical nitrate concentrations at the waterworks, but could rely on the actual measurements of nitrate concentrations in drinking water samples taken and analyzed by certified laboratories.[22] We used the physical drinking water supply areas to assign nitrate concentrations to each household and knew the precise residential history of all study participants. Here, our exposure assessment is superior to earlier studies that needed to model historical exposure both spatially and temporally, or estimated exposure by nitrate concentrations at a given location at a single point in time.

Estimating waterborne nitrate intake from residential tap water is reasonable in the Danish context; the annual bottled water consumption is the lowest in Europe with 26 L per person.[23] Furthermore, it has been shown that nitrate levels do not change within a given distribution system and that seasonal variations in drinking water nitrate levels at public supplies are negligible in Denmark.[24] Groundwater abstracted

for drinking water production has a typical age (time since recharge) of 10–60 years.[24] We do not have adequate data to assess seasonal variability in private wells. However, seasonal variability in shallow wells has been observed in other locations.[25] Private wells are often shallower than public supply wells; therefore, we cannot exclude seasonal variability in private wells. Since Danish waterworks abstain from using chemical disinfection, confounding by disinfection by-products was not a concern in this study.[26] Water samples used in this study were taken after all treatment steps at the waterworks. The use of in-home water treatment installations to reduce nitrate concentrations is uncommon in Denmark and authorities have been restrictive in giving permission to use such installations at private wells.[27]

The possibility of including information on private wells is another strength of our work. It was earlier shown that the drinking water sampling frequency for private wells is much lower compared to public supplies.[5,18] Therefore, residing a long time at a privately supplied household decreased an individual's exposure assessment quality level. The stepwise inclusion of lower exposure assessment quality levels into the model was therefore crucial to include those who lived many years with private well supply. Even though we could only retrieve nitrate concentrations of approximately half of the 55,752 private wells that we identified,[18] we knew the location of the remaining wells and could therefore exclude their users in our sensitivity analyses.

Given our study design, we were limited to include only covariates available in nationwide registers. We could for example not control for individual-level information on lifestyle and diet. A study on the dietary intake of nitrate in the Danish population estimated an average nitrate intake of 61 mg/d for adults.[28] Therefore, at elevated levels as seen in parts of the Danish population (Figure 1a), drinking water will be a major source of nitrate exposure. As diet (e.g., red meat), alcohol intake, smoking and lifestyle factors such as physical inactivity are established CRC risk factors that we could not include in our analyses, the possibility of confounding our results needs to be considered. To address this issue, we adjusted our analyses for highest attained education, an especially appropriate proxy for lifestyle, smoking and diet in the Danish population.[29] Furthermore, studies suggest that dietary nitrate intake is not associated with CRC, or even has a protective effect, because of antioxidants and nitrosation inhibitors in nitrate-containing foods.[6] Nevertheless, any observational study of human health, including the present, cannot exclude the possibility of residual confounding by unobserved factors.

Another limitation is the omission of drinking water nitrite levels in our models. Nitrite is an intermediate in the endogenous transformation of nitrate into genotoxic *N*-nitroso compounds. Nitrite occurs in groundwater in the anoxic nitrate reducing zone but can also be formed at the waterworks due to oxidation of ammonium. Drinking water samples are historically not measured for nitrite to the same extent as nitrate. The restrictive drinking water standard of 0.01 mg/L nitrite is complied with at the large waterworks[30]; however, little is known for smaller waterworks and private wells. An earlier study showed that even though nitrite is taken up through drinking water and food, up to 77% of the total exposure to nitrite is due to the reduction of nitrate *in vivo*.[31] Furthermore, nitrate in drinking water could also be a proxy for additional agricultural pollutants, such as pesticides, which we did not consider here.

We used the average nitrate concentration an individual was exposed to between their 20th and 35th birthdays as the exposure estimate. We assumed that this exposure period was representative of the relevant relationship between exposure and outcome. As geocoded residences were available from 1978 onward, our main analyses included the early cases of CRC only, with an age at diagnosis below 69, before incidence rates peak. Shifting the exposure window to age 30–40, we could include more cases (until age 74), however, at the expense of moving the exposure closer to the time of disease onset. We observed a high agreement between the estimated nitrate exposures in the two competing exposure models. Changing the exposure window to age 30–40 did not substantially change the associated HRs.

In conclusion, our study adds to the growing body of evidence that suggests an increased risk of CRC at nitrate levels in drinking water below the current drinking water standard. Several studies carried out in different locations with different designs and each of their strengths and limitations imply this association. While our study contributed with a large study population, the resulting statistical power, and a detailed exposure assessment, other studies' strengths lay in the inclusion of a number of additional covariates. Considering all evidence, not only in the light of the precautionary principle, a discussion about a reduction of the drinking water standard is warranted.

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