doi: 10.1111/1753-6405.13222

Nitrate in drinking water and cancer risk: the biological mechanism, epidemiological evidence and future research

Tim Chambers,[1] Jeroen Douwes,[2] Andrea 't Mannetje,[2] Alistair Woodward,[3] Michael Baker,[1] Nick Wilson,[1] Simon Hales[1]

1. Health, Environment & Infection Research Unit, Department of Public Health, University of Otago, Wellington
2. Research Centre for Hauora and Health, Massey University Wellington
3..School of Public Health, Auckland University, Auckland

Globally, nitrate levels in source water have increased as a result of agricultural intensification, which has subsequently increased nitrate contamination in drinking water.[1] Recent epidemiological evidence suggests a link between ingested nitrate via drinking water and increased cancer risk, with the best evidence for colorectal cancer.[2] Our own meta-analysis of six recent epidemiological studies[3-8] investigating nitrate in drinking water shows a statistically significant pooled estimate for increased risk of colon and colorectal cancers but not for rectal cancers (see Figure 1).

As highlighted in recent debates about nitrate in drinking water,[9-12] not all studies have observed a link with colorectal cancer. Therefore, we agree that results should be interpreted with caution. However, we disagree with the argument that there is no logical reason for cause and effect or that it is highly unlikely nitrate could increase risk of cancer.[9,11,12] It should be noted that these comments appeared in either a trade magazine for the major fertiliser company *Ravensdown* or were commissioned using funding from *Fonterra*, New Zealand's major dairy cooperative. The lines of argument used in these pieces undermine the extensive work conducted by the World Health Organization (WHO) and International Agency for Research on Cancer (IARC).[1,13] Conclusive statements disregarding the potential risk of nitrate contamination in drinking water does a disservice to policy makers and the public attempting to understand the potential risk nitrate poses to health. A better understanding of the biology of nitrates and colorectal cancer is vital for a healthy evidence-based public debate, and may also help explain some of the observed differences in epidemiological findings.

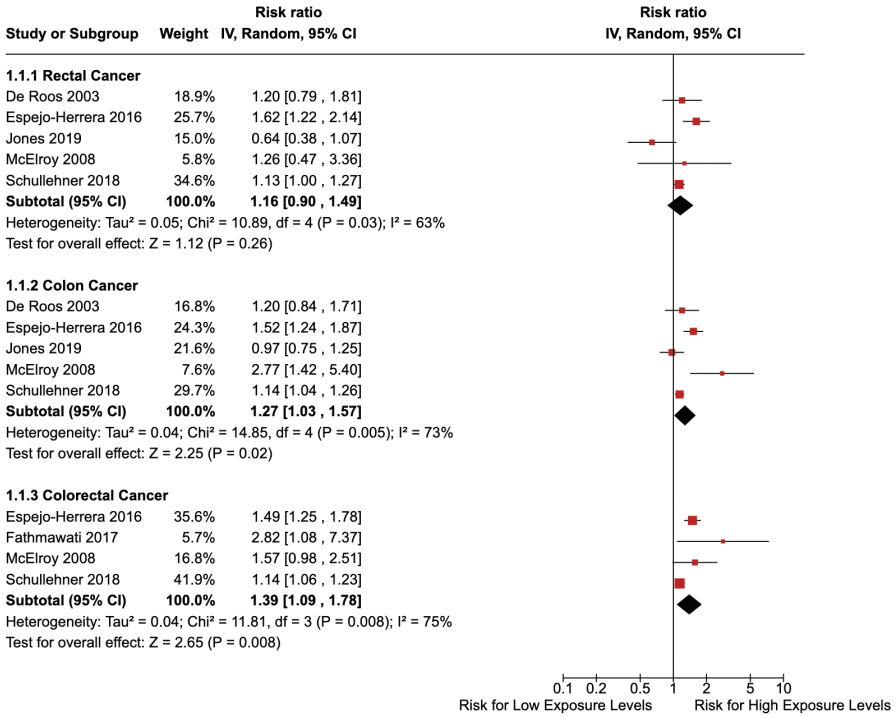
IARC states that the "combination of positive and negative results from epidemiological and animal studies is coherent with the mechanism of endogenous formation of N-nitroso compounds (NOC)."[14 p.628] N-Nitroso compounds are known carcinogens, with around 90% of all tested NOC having carcinogenic effects.[15]

IARC's 2010 review of studies up to 2006 concluded that "ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (group 2A)."[13 p.325] However, there are key elements of this conclusion that are commonly misunderstood including: 1) the relationship between nitrate and nitrite; 2) the process of endogenous nitrosation; and 3) the conditions that result in endogenous nitrosation. Comprehension of these technical areas is central to understanding the potential risk nitrate in drinking water poses to human health and how to interpret the current state of evidence.

The link between nitrate and nitrite

Firstly, nitrate (NO_3^-) does not directly influence the formation of NOC. Around 5% of all ingested nitrate is broken down into nitrite (NO_2^-) by oral bacteria.[13] There are interpersonal variations in the prevalence of oral bacteria. Poor oral health is positively associated with higher concentrations of nitrate reducing bacteria, and some people can convert up to 20% of ingested nitrate to nitrite.[15] Nitrate-reducing bacteria may

Figure 1: Our meta-analysis of epidemiological studies investigating nitrate contamination and colorectal cancer [note we have only included case-control or cohort studies on colorectal cancer incidence and we have not double counted cohorts as one erroneous meta-analysis has done,[31] which included the same cohort twice, i.e.Weyer (2001)[32] is the same cohort as Jones (2019),[3] and used the rate ratio for nitrate from food from Espejo-Herrera 2016 as the rate ratio for drinking water].



also proliferate in the gastrointestinal tract (stomach, small intestine and large intestine).[16,17] Thus, nitrite, nitric oxide and other nitrosation agents are potentially bioavailable throughout the gastrointestinal tract.

The process of endogenous nitrosation

Nitrite can be metabolised further, and under acidic conditions in the stomach these breakdown products react readily with substrates such as haeme (a known nitrosatable compound).[13] Nitrosation agents can be transported to the colon attached to haeme from red meat, or be reduced from nitrate in the colon by bacteria.[17] Diet-related nitrosatable compounds include amines (from metabolised proteins – e.g. meat)[13] and haeme from red meat.[18] Levels of amines are highest in meat, fermented and cured foods, while haeme is prevalent in red but not white meat.[18] The reactions between nitrosation agents (metabolised from nitrite) and nitrosatable compounds (e.g. amines or haeme) form NOC.[13] This is the process of endogenous nitrosation.

The conditions that exacerbate or inhibit endogenous nitrosation

Many studies in which subjects consumed a combination of nitrate with amines show increases in the biomarkers of endogenous nitrosation measured as apparent total N-nitroso compounds (ATNC). One example is a 2019 feeding study where participants were provided drinking water with low nitrate-nitrogen (~1mg/L) and high nitrate-nitrogen (~11.3mg/L) with different meat-based diets (providing amines).[19] At the end of each week, participants' faecal water was measured for ATNC, markers of endogenous nitrosation. The increase in ATNC under the high drinking water nitrate condition is consistent with previous feeding studies evaluated by IARC.[13]

Previous feeding studies have also reported that exposure to high drinking water nitrate in the absence of amines did not produce significant increases in ATNC.[13] Further, that even in the presence of amines, high concentrations of antioxidants (especially vitamin C) inhibit endogenous nitrosation. The high levels of antioxidants in vegetables (the main source of dietary nitrate) may help explain why we do not see any adverse relationship between nitrate in vegetables and any cancers.[13] In fact, large meta-analyses have shown fruits and vegetables have a protective effect against colorectal cancer.[20]

Considering the role of vitamin C and amines we can contextualise the epidemiological evidence. The odds ratio (OR) for the association between nitrate in public water supplies and colon cancer in a study by De Roos (2003) increased from 1.2 (95%CI: 0.9 to 1.6) to 2.0 (95%CI: 1.2 to 3.3) when accounting for vitamin C intake.[5] Likewise, Espejo-Herrera (2016) found when diets were high in vitamin C there was no statistically significant increase in risk of colon cancer from elevated nitrate levels in water (OR 1.09, 95%CI: 0.86 to 1.38).[8] In contrast, there was a statistically significant association between nitrate in drinking water and colorectal cancer when diets of cases and controls were low in vitamin C (OR=1.36, 95%CI: 1.08, 1.71).[8] Schullehner et al.[7] often referred to as the 'Danish Study', did not directly adjust for vitamin C intake but did adjust for socioeconomic status, which is strongly correlated with vitamin C intake, and reported an OR of 1.14 (95%CI: 1.04 to 1.25).[21] The remaining study, Jones et al. found a null association between nitrate and colorectal cancer (OR=0.97 95%CI: 0.75 to 1.26) in a cohort of older women in Iowa.[3] However, the authors also did not observe any association between processed meat or vitamin C with colorectal cancer [odds ratios not published], which is inconsistent with previous epidemiological studies and IARC conclusions.

N-nitroso compounds induce DNA-damaging metabolites, which can lead to a specific DNA damage signature that can be detected in cancerous lesions.[22] A recent study identified this specific DNA damage in biopsies from a cohort of 900 colorectal carcinoma cases.[23] Red meat consumption was associated with the alkylating signature in colorectal cancer sites which provided molecular evidence of the mutagenic impact of dietary nitrite via the NOC pathway.[23] The authors also found this alkylating signature was more abundant, although not specifically limited to, the distal colon. Upon reviewing the epidemiological evidence, evidence for an association between nitrate and colon cancer (OR=1.20, 95%CI: 1.02 to 1.41) is currently stronger than for nitrate and rectal cancer (OR=1.16, 95%CI: 0.90 to 1.49) which is consistent with the latest evidence on the sites in the bowel where the mutagenic impact of dietary nitrite is strongest.

The positive health effects of nitrate

The metabolised nitrate from our diets serves important physiological functions. Nitrite can be further reduced to nitric oxide (NO) and other reactive products (e.g. nitrous acid) that are toxic to pathogenic bacteria and other harmful microorganisms.[15] For example, acidified nitrite has inhibited growth of the pathogenic microbes *Salmonella, Shigella* and *Helicobacter pylori*.[24] Nitric oxide also has important roles in gastric blood flow and mucous production, regulation of blood pressure, improved cardiovascular health and cell signaling.[15] However, an oversupply of nitrate, particularly in the absence of antioxidants or the presence of amines, creates an environment in which excess nitrosating agents react to produce NOC or place cells under oxidative stress that lead to adverse health outcomes. In many parts of the world, present-day concentrations of nitrate in drinking water far exceed those that occurred before industrialisation. Moreover, modern diets may lack protective antioxidants. Therefore it is plausible that high levels of nitrate in drinking water may lead to adverse health outcomes – including cancer in some circumstances.

Comparative impact of nitrate in drinking water to other known risk factors

Colorectal cancer is a multifactorial disease with numerous risk factors including alcohol use, tobacco use, physical inactivity, red meat consumption and obesity.[18] Table 1 lists the major risk factors for colorectal cancer and their estimated population attributable fraction (PAF) using New Zealand as a case study – from Richardson et al.[25] The PAF is the estimated fraction of total cases that could be averted in the absence of the exposure (e.g. heavy alcohol consumption). The PAF accounts for the prevalence of the risk factor in the population (e.g. heavy alcohol consumption) and the increased risk of the outcome associated with that risk factor (relative risk). We have estimated that up to 800,000 New Zealanders (17%) could be consuming water with nitrate levels placing them at some risk of colorectal cancer based on international evidence.[7] Based on an exposure prevalence of 17% and a relative risk of 1.04 per mg/L from one meta-analysis[2] we estimated a colorectal cancer PAF for nitrate in drinking water of 3.3% (95%CI: 0.84 to 5.57).[26] In NZ, this

would result in 100.3 (95%CI: 25.7 to 171.3) nitrate attributable colorectal cancers per year. This is substantially lower than the PAF for obesity or alcohol consumption, but similar to the potential impact of processed meat consumption or tobacco use. In contrast to alcohol and tobacco, water is essential to human life, a basic human right and a key element of the 2030 Sustainable Development Goals.[27,28]

Table 1: Population attributable fraction of known risk factors for colorectal cancer in New Zealand - from Richardson et al. 2016[25] and with our nitrates in water estimate added.

Risk factor	Relative risk	Prevalence of risk factor	Population attributable fraction
Obesity	1.33 (1.25–1.42)	29.9 (28.9–30.9)	9.0 (6.7–11.2)
Alcohol	1.44 (1.25–1.65)	16.1 (15.2–17.0)	6.6 (3.6–9.6)
Red meat	1.35 (1.21–1.51)	14.4 (12.7–16.1)	4.8 (2.6–7.0)
Physical inactivity	1.32 (1.23–1.39)	14.3 (13.3–15.5)	4.4 (3.1–5.6)
Nitrate[a]	1.04 (1.01-1.07)	17.3 (17.3-17.4)	3.3 (0.84-5.57)
Processed meat	1.31 (1.13–1.51)	8.6 (7.3–9.8)	2.6 (0 .9–4.3)
Smoking	1.15 (1.00–1.32)	17.2 (16.4–18.1)	2.5 (0.0–5.2)

Note:

a: We used the relative risk from Temkin et al2 which was 1.04 per mg/L increase in nitrate so is not directly comparable to other relative risks in table 1.

Policy and future research

The presence of a plausible biological pathway does not by itself justify a chronic limit for nitrate in drinking water. The current WHO guideline value for nitrate in drinking water is 11.3mg/L based on the absence of adverse health effects caused by methaemoglobinaemia.[29] Currently, there is no chronic [long term] exposure guideline for nitrate. In the latest 2017 guidelines, the WHO concluded "the weight of evidence does not clearly support an association between cancer and exposure to nitrate or nitrite per se"[1 p.13] citing limitations in epidemiological studies related to exposure assessment, other risk factors and nitrosation inhibitors and precursors. Similarly, one quantitative risk assessment conducted by Health Canada concluded that until there is improved information on the dietary concentrations of amino compounds (amine or amide substrates), dietary concentrations of amine or amide precursors and the rate of inhibition of the endogenous nitrosation it will remain extremely difficult to assess the importance of the gastric formation of N-nitroso compounds in the etiology of cancer.[30] The limitations in epidemiological and experimental research highlighted by WHO, IARC and Health Canada provide a roadmap for future research. However, importantly, no major health body or emerging evidence has challenged IARC's conclusion that there is a plausible biological pathway for ingested nitrate increasing cancer risk.

Conclusion

There is a likely biological pathway (albeit complicated) for ingested nitrate in drinking water increasing the risk of colorectal cancer. The effect of ingested nitrate is likely modified by the prevalence of nitrate-reducing bacteria, the pH of stomach acid, and the quantity of amines and antioxidants in the diet, as demonstrated in laboratory studies and epidemiological research. Therefore, greater weight should be placed on studies that have robust study designs, discriminating exposure measurements and appropriate accounting for confounders (especially, markers of endogenous nitrosation) and effect modifiers (e.g. vitamin C intake). It is important not to over-interpret epidemiological findings, but it is equally important not to dismiss them when there is a plausible biological mechanism.

References

1. World Health Organization. *Nitrate and Nitrite in Drinking-water: Background Document for Development of WHO Guidelines for Drinking-water Quality* [internet]. Geneva (CHE): WHO; 2017[cited 2021 Aug 25]. Available from: https://www.who.int/water_sanitation_health/dwq/chemicals/nitrate-nitrite-background-jan17.pdf
2. Temkin A, Evans S, Manidis T, Campbell C, Naidenko OV. Exposure-based assessment and economic valuation of adverse birth outcomes and cancer risk due to nitrate in United States drinking water. *Environ Res.* 2019;176:108442.
3. Jones RR, DellaValle CT, Weyer PJ, Robien K, Cantor KP, Krasner S, et al. Ingested nitrate, disinfection by-products, and risk of colon and rectal cancers in the Iowa Women's Health Study cohort. *Environ Int.* 2019;126:242-51.
4. Fathmawati, Fachiroh J, Gravitiani E, Sarto, Husodo AH. Nitrate in drinking water and risk of colorectal cancer in Yogyakarta, Indonesia. *J Toxicol Environ Health A.* 2017;80(2):120-8.
5. De Roos AJ, Ward MH, Lynch CF, Cantor KP. Nitrate in public water supplies and the risk of colon and rectum cancers. *Epidemiology.* 2003;14(6):640-9.
6. McElroy JA, Trentham-Dietz A, Gangnon RE, Hampton JM, Bersch AJ, Kanarek MS, et al. Nitrogen-nitrate exposure from drinking water and colorectal cancer risk for rural women in Wisconsin, USA. *J Water Health.* 2008;6(3):399-409.
7. Schullehner J, Hansen B, Thygesen M, Pedersen CB, Sigsgaard T. Nitrate in drinking water and colorectal cancer risk: A nationwide population-based cohort study. *Int J Cancer.* 2018;143(1):73-9.
8. Espejo-Herrera N, Gràcia-Lavedan E, Boldo E, Aragonés N, Pérez-Gómez B, Pollán M, et al. Colorectal cancer risk and nitrate exposure through drinking water and diet. *Int J Cancer.* 2016;139(2):334-46.
9. Nadine P. Bowel Cancer NZ Questions links between the disease and low nitrate levels in drinking water. *Stuff* [Internet]. 2021 Feb[cited 2021 Aug 3];24;5:00am. Available from: https://www.stuff.co.nz/national/health/124330345/bowel-cancer-nz-questions-links-between-the-disease-and-low-nitrate-levels-in-drinking-water
10. Cressey P, Cridge B. *Nitrate in Food and Water* [Internet]. Wellington (NZ): Institute of Environmental Science and Research; 2021 [cited 2021 Aug 31]. Available from: https://mcusercontent.com/ac7d10ed90f765f0df9b564b7/files/0d6eaae9-6e25-328b-835e-10330f48888c/CSC21025_Nitrate_in_food_and_water_FINAL_13_August_2021.pdf.
11. Cressey P, Cridge B, McLeod C, Lewis G. Unlikely nitrates in water or food increase New Zealand cancer risk - study. *Stuff* [Internet]. 2021 Aug[cited 2021 Aug 31];30;5:00am. Available from: https://www.stuff.co.nz/science/300392822/unlikely-nitrates-in-water-or-food-increase-new-zealand-cancer-risk--study
12. Frizelle FA, Keenan J. *Hype gets in the way of cause and effect.* Ground Effect: *Ravensdown Ground Effect.* 2021[cited 2021 Sep 4]Spring;13:18-19. Available from: https://www.ravensdown.co.nz/media/5460/ravs-j004868-ground-effect_edition-13-spring-2021_web-10mb-spreads.pdf
13. International Agency for Research on Cancer. *Ingested Nitrate and Nitrite, and Cyanobacterial Peptide Toxins* [Internet]. Lyon (FRA): IARC; 2010 [cited 2021 Feb 25]. Available from: https://publications.iarc.fr/_publications/media/download/2867/c9f9c85d6dd616d774bdbbe67bae77bddeb1b4de.pdf
14. Grosse Y, Baan R, Straif K, Secretan B, El Ghissassi F, Cogliano V. Carcinogenicity of nitrate, nitrite, and cyanobacterial peptide toxins. *Lancet Oncol.* 2006;7(8):628-9.
15. Bryan NS, Loscalzo J. *Nitrite and Nitrate in Human Health and Disease.* Berlin (GER): Springer; 2017.
16. Xu G, Reed P. N-nitroso compounds in fresh gastric juice and their relation to intragastric pH and nitrite employing an improved analytical method. *Carcinogenesis.* 1993;14(12):2547-51.
17. Kobayashi J. Effect of diet and gut environment on the gastrointestinal formation of N-nitroso compounds: A review. *Nitric Oxide.* 2018;73:66-73.
18. Seiwert N, Heylmann D, Hasselwander S, Fahrer J. Mechanism of colorectal carcinogenesis triggered by heme iron from red meat. *Biochim Biophys Acta Rev Cancer.* 2020;1873(1):188334.
19. van Breda SG, Mathijs K, Sági-Kiss V, Kuhnle GG, Van der Veer B, Jones RR, 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):87.
20. Johnson CM, Wei C, Ensor JE, Smolenski DJ, Amos CI, Levin B. et al. Meta-analyses of colorectal cancer risk factors. *Cancer Causes Control.* 2013;24(6):1207-22.
21. Carr AC, Rowe S. Factors affecting vitamin C status and prevalence of deficiency: A global health perspective. *Nutrients.* 2020;12(7):1963.
22. Zhu Y, Wang PP, Zhao J, Green R, Sun Z, Roebothan B, et al. Dietary N-nitroso compounds and risk of colorectal cancer: A case-control study in Newfoundland and Labrador and Ontario, Canada. *Br J Nutr.* 2014;111(6):1109-17.
23. Gurjao C, Zhong R, Haruki K, Li YY, Spurr LF, Lee-Six H, et al. Discovery and features of an alkylating signature in colorectal cancer. *Cancer Discov.* 2021;11(10):2446-55.

24. Dykhuizen R, Frazer R, Duncan C, Smith C, Golden M, Benjamin N, et al. Antimicrobial effect of acidified nitrite on gut pathogens: Importance of dietary nitrate in host defense. *Antimicrob Agents Chemother.* 1996;40(6):1422-5.
25. Richardson A, Hayes J, Frampton C, Potter J. Modifiable lifestyle factors that could reduce the incidence of colorectal cancer in New Zealand. *N Z Med J.* 2016;129(1447):13-20.
26. Richards J, Chambers T, Hales S, Joy MK, Radu T, Woodward A, et al. Nitrate contamination in drinking water and colorectal cancer: Exposure assessment and estimated health burden in New Zealand. *Environ Res.* 2022;204(Pt C):112322.
27. United Nations General Assembly. *Resolution 64/292: The Human Right to Water and Sanitation* [Internet]. Geneva (CHE): UN; 2010 [cited 2021 Feb 17]. Available from: https://www.un.org/ga/search/view_doc.asp?symbol=A/RES/64/292
28. United Nations General Assembly. *Transforming Our World: The 2030 Agenda for Sustainable Development* [Internet]. Geneva (CHE): UN; 2015 [cited 2021 Mar 15]. Available from: https://sdgs.un.org/sites/default/files/publications/21252030%20Agenda%20for%20Sustainable%20Development%20web.pdf
29. World Health Organization. *Guidelines for Drinking-water Quality: Fourth Edition Incorporating the First Addendum* [Internet]. Geneva (CHE); WHO; 2017 [cited 2020 Aug 11]. Available from: https://apps.who.int/iris/bitstream/handle/10665/254637/9789241549950-eng.pdf;jsessionid=653DBF3E25683F182CA7C095E5F61A04?sequence=1
30. Health Canada. *Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – Nitrate and Nitrite* [Internet]. Ontario (CAN): Health Canada Healthy Environments and Consumer Safety Branch Water and Air Quality Bureau; 2013 [cited 2021 Aug 25]. Available from: https://www.canada.ca/content/dam/canada/health-canada/migration/healthy-canadians/publications/healthy-living-vie-saine/water-nitrate-nitrite-eau/alt/water-nitrate-nitrite-eau-eng.pdf
31. Hosseini F, Majdi M, Naghshi S, Sheikhhossein F, Djafarian K, Shab-Bidar S. Nitrate-nitrite exposure through drinking water and diet and risk of colorectal cancer: A systematic review and meta-analysis of observational studies. *Clin Nutr.* 2021;40(5):3073-81.
32. Weyer PJ, Cerhan JR, Kross BC, Hallberg GR, Kantamneni J, Breuer G, et al. Municipal drinking water nitrate level and cancer risk in older women: The Iowa Women's Health Study. *Epidemiology.* 2001;12(3):327-38.

Correspondence to: Dr Tim Chambers, University of Otago, 23a Mein Street, Newtown, Wellington, New Zealand 6023;
e-mail: tim.chambers@otago.ac.nz