

doi: 10.1111/1753-6405.13222

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

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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 studies³⁻⁸ 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.

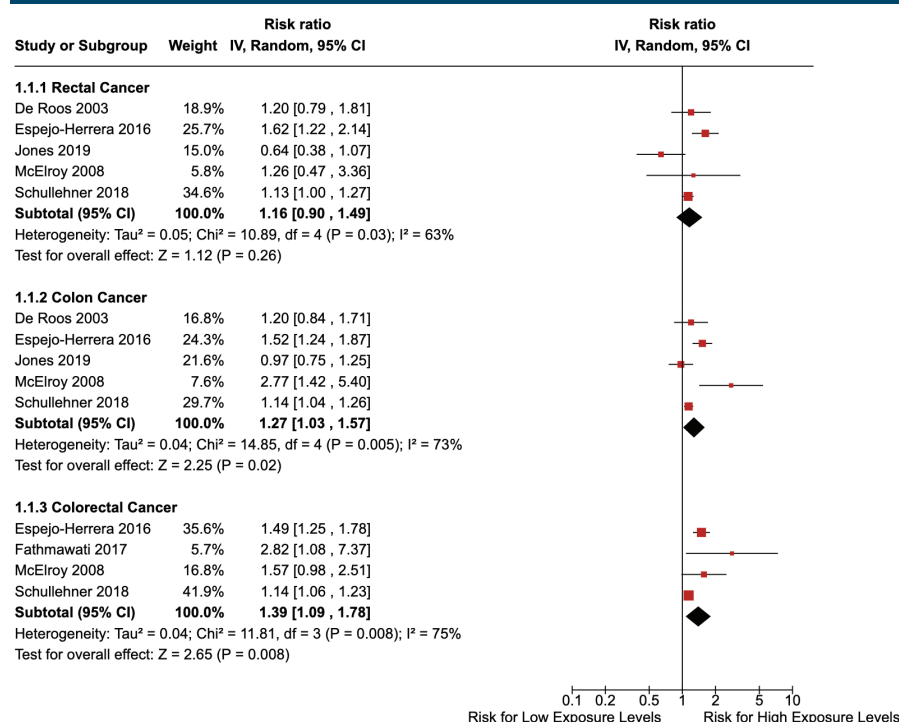
IARC states that the "combination of positive and negative results from epidemiological and animal studies is coherent with the mechanism of endogenous formation of N-nitroso compounds (NOC)."¹⁴ N-Nitroso compounds are known carcinogens, with around 90% of all tested NOC having carcinogenic effects.¹⁵

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 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 (NO₃⁻) does not directly influence the formation of NOC. Around 5% of all ingested nitrate is broken down into nitrite (NO₂⁻) 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].



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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-analysis² 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}

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”³¹ 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.

Table 1: Population attributable fraction of known risk factors for colorectal cancer in New Zealand - from Richardson et al. 2016²⁵ 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.

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.

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