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IS NEW ZEALAND DRINKING WATER NITRATE STANDARD FIT FOR PURPOSE TO PROTECT FROM COLORECTAL CANCER? A CRITICAL REVIEW

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Abstract

Our drinking water standard is 50 mg nitrate (NO_3)/L, which has been set in line with the World Health Organisation (WHO) guidelines to avoid NO_3 'poisoning' (or methaemoglobinaemia) in bottle-fed infants. Ninety-seven percent of our registered water supplies have less than 10 mg NO_3/L with all supplies meeting NO_3 drinking water standard. To my knowledge, there have been no documented cases of infant death caused by methaemoglobinaemia in New Zealand.

There has been numerous reporting of drinking water NO_3 being a risk factor in colorectal cancer (CRC). A recent Danish epidemiological cohort study on drinking water NO_3 received an unprecedented attention owing to its extensive research involving the entire Danish population with ≥35-year-old age to exposure of drinking water NO_3 between 1978-2011 (Schullehner et al. 2018). The final analysis of the study used 61% of the population which claimed statistically significant relationship between CRC risk and increasing NO_3 level ≥3.8 mg/L.

The above study claimed there has been growing body of evidence along with similar studies carried out by other workers in different locations of the increased risks of CRC to exposure well below the current NO_3 drinking water standard. The study called for a discussion on reducing the current drinking water standard, which has also been echoed in New Zealand (Richards et al. 2022). Considering the above calls, this paper critically assesses the claimed CRC link to NO_3 levels well below the drinking water standard and the fit for purpose of the current drinking water NO_3 standard to protect us from CRC.

Having reviewed many well-referenced epidemiological and toxicological studies critically, as a non-epidemiologist I have been disappointed and surprised by the irrational, inconsistent, ad hoc and oversimplified way in which many studies have been researched, peer reviewed and reported with the information being critical to human health disseminated without the deserved highest possible scientific rigour. Consequently, this independent review calls on the International Agency for Research on Cancer (IARC) and WHO to set stringent protocols for toxicological and epidemiological research, data collection and analysis and reporting. Based on numerous cohort/case-control and in-vivo/in-vitro studies reviewed, I conclude there is no compelling evidence for drinking water NO_3 at or below the current standard to increase the risk of CRC, as such the standard review or reduction is not warranted.

Introduction

The current New Zealand drinking water maximum allowable value (MAV) standard for nitrate (NO_3) is 50 mg NO_3/L under the *Drinking-water Standards for New Zealand 2005 (Revised 2018)*. This standard applies concurrently with nitrite (NO_2) MAV standard of 3 mg/L where the sum of the ratio of the concentrations of NO_3 and NO_2 to each of their respective MAVs must not exceed one. Despite the above standard, the drinking water NO_2 is seldom reported or assessed probably because of substantially lower levels of NO_3 and NO_2 levels detected than that of the MAVs for most registered drinking water supplies in New Zealand.

In New Zealand, *Taumata Arowai* has been established as the Water Services Regulator under the Water Services Act 2021 which came into effect on 15 November 2021. Under Schedule 1, Part 1(2)(2) of the Water Services Act, *Taumata Arowai* must review the current Drinking-water Standards within 5 years to determine whether they are fit for purpose. As such *Taumata Arowai* is currently consulting under s53 of the Water Services Act (public submissions between 17 January 2022 and 25 March 2022) and the revised standards will be recommended by the Minister to the Governor-General to set standard by regulations by Order in Council.

In the proposed consultation, the current NO_3 (50 mg/L) and NO_2 (3 mg/L) standards are retained as 'short term' MAVs which have been established to protect against methaemoglobinaemia in bottle-fed infants. It is noteworthy that the above short-term status has only been accorded to NO_3 and NO_2 MAVs and all other chemical determinands' MAVs (e.g., lead) have been set to protect human health over 70 years consumption of 2 L/d of drinking water. The existing provisional MAV (PMAV) of 0.2 mg NO_2/L as long-term chemical determinand has been omitted in the recently proposed consultation because of the WHO's suspension of the NO_2 guideline value (GV) due to uncertainty of its accuracy.

In the meantime, considerable number of case-control (studies comparing cancer patients and healthy individuals (controls) exposed to different drinking water NO_3 levels in the same location) and cohort (studies involving large number of population for a length of study period exposed to different NO_3 levels in the same location and assessing the extent of cancer cases) epidemiological studies have associated colorectal cancer (CRC) with drinking water NO_3 levels well below the New Zealand MAV and the WHO GV.

Using an expert panel, the WHO revised its guidelines in 2017 and reinstated the current NO_3 and NO_2 GVs. Despite the above, the overseas epidemiologists from the previous studies (e.g., Schullehner et al. 2018) and more recently a New Zealand epidemiological study (Richards et al. 2022) have called for a review of the current drinking water NO_3 standard. As such, I will not be surprised if there are submissions to the *Taumata Arowai*'s drinking water standard consultation to consider more stringent and long-term NO_3 and NO_2 standard to protect from the risks of CRC.

A recent review report by Environmental and Science Research (ESR) commissioned by the New Zealand Food Safety Science Research Centre on the claimed CRC risk from drinking water concluded increased risk of cancer was unlikely from drinking water or diets and that owing to only a small proportion of NO_3 being consumed via drinking water there was little reason to differentiate the exposure from diets and drinking water (ESR 2021). In doing so, the

above report did not attempt to assess the quality of the epidemiological studies claiming the CRC and drinking water nitrate link.

This independent review which has been prepared without any financial assistance, based on critical scientific literature research by a non-epidemiologist training technical professionals in water, soil and wastewater quality in New Zealand provides new angles and insights into the claims of CRC-drinking water nitrate links.

Nitrate as a contaminant

Nitrate is a highly oxidised form of nitrogen which forms in soil, water and wastewater via microbiological processes. Nitrate has numerous sources such as farmed soils, septic tank and wastewater discharges, composts, green-wastes, landfills, plant litter and soil organic matter. It is highly mobile in soil and as such can reach groundwater. When NO_3 contaminated groundwater drains in surface water catchments it can reach surface water.

Nitrate in water is considered as a contaminant since under favourable conditions it could cause nuisance algal and macrophyte growth in surface water (1.3-1.8 mg NO_3/L) and render groundwater unsuitable for human drinking. Occasionally, NO_3 has been found to be toxic to livestock animals grazing pasture with high NO_3 resulting in animal deaths. Under favourable conditions NO_3 can accumulate in plants. Livestock ingesting high quantities of such herbage could die of 'NO_3 poisoning' which is pathologically similar to methaemoglobinaemia in human. Being one of the well-recognised and universal water contaminant, NO_3 has triggered numerous research and regulations controlling nitrate contamination globally and locally.

Benefits of nitrate

Whilst plants can consume dissolved organic-N, ammoniacal-N and NO_3-N from soil and foliage, since readily available in soil and accessible by plant roots, NO_3 is a valuable source of nutrient. Nitrate plays a significant and positive role in the ecosystem, owing to its highly oxidised status. When dissolved oxygen is less accessible in water, soil and wastewater microbes can use NO_3 as a source of oxygen and electron acceptor. Wastewater maturation ponds undergoing anaerobic processes are treated with the addition of NO_3 salts to aid oxidation process and to mitigate the impacts of nuisance odour. Feeding NO_3 with canola oil to cattle has been shown to reduce methanogenesis process in rumen, however, NO_3 toxicity must be avoided owing to large variation in animal NO_3 metabolism (Villar 2019).

Nitrate also has important role in human metabolism by producing NO_2 which is considered as regulating harmful bacteria in the guts and mouth cavity and NO (nitric oxide) in enhancing good blood circulation via vasodilation. High NO_3 levels found in beetroot has made it a popular supplement to treat hypertension and the past scientific trials have established positive correlation between the reduction of blood pressure and consumption of beetroot juice (Bahadoran et al. 2017).

Overall NO_3 in adult human has numerous well documented health benefits than poorly documented adverse effects. In addition to lowering of blood pressure, NO_3 intake has been shown to improve endothelial function (cell lining within heart and blood vessels which control vascular movement and enzymes regulating blood clotting, immune function and platelet adhesion), reduce platelet aggregation (which could reduce blood clotting), and reduce arterial

stiffness, all of which could mitigate atherosclerosis (thickening of arteries), coronary artery disease, and heart attack. A meta-analysis by Jackson et al. (2018) which acknowledges all the above benefits call for assessment on long-term role of NO_3 given vegetables are found to be an excellent source to deal with cardiovascular disease at large scale.

Nitrate metabolism in human

Nitrate can be ingested directly from diets, drugs, drinking water and beverages which are referred to as *exogenous sources*. Nitrate can also form within the body from amino acids such as L-arginine, which is referred to as *endogenous source*. The ESR report (2021) on *Nitrate in food and water* has an excellent compilation of the human nitrate metabolism, as such I have only outlined the key processes here.

The extent and the NO_3 sources of the overall ingested NO_3 can vary between countries, but in general, 80-90% by food and 10-20% from drinking water. Our vegetables, particularly spinach, silver beet, lettuce celery, beet can contribute 80-90% of the dietary sources with meat 10-15%. The above sources are excluding pharmaceutical drugs containing NO_3.

The New Zealand mean dietary NO_3 intake of >15-year-olds has been estimated as 0.82 mg/kg BW (body weight) (2.58 mg/L being 95th percentile of single day intake) which is well within the globally acceptable dietary intake (ADI) of 0-3.7 mg/kg BW (ESR 2021). The mean NO_3 level in public water supplies has been estimated as 4.8 mg/L (with 95th percentile being 21.8 mg/L) by Thomoson et al. (2007). Based on the ESR (2021) estimate of mean consumption of 1.44 L/d of water and beverages by >15-year-olds, daily consumption of NO_3 is likely to be 7 mg/d. Given a 65 kg adult is likely to consume 53.3 mg NO_3/d through diets, the estimated proportion of NO_3 from drinking water is only 13%.

Much of the human metabolism work has been based on dietary NO_3 or oral administration of NO_3-based salts. Ingested NO_3 is absorbed into blood within several hours which elevates plasma-NO_3 levels with some being stored in the liver and skeletal muscle tissues. The muscle tissue stored NO_3 is released as NO during high oxygen demand activities such as high physical activities (e.g., sports) which in turn promotes vasodilation.

Much of the ingested NO_3 (around 60%) is excreted via urine within 4-6 hours which indicates plasma-NO_3 half-life is also short. About 25% of the ingested NO_3 is circulated in the mouth via saliva, as such NO_3 levels found in saliva are many folds greater than that found in plasma. Saliva-NO_3 (approximately 16% of the ingested NO_3) is rapidly converted into NO_2 form by microbes residing within the mouth cavity. Such conversion is considered as beneficial to oral hygiene since NO_2 is known to kill harmful bacteria.

Nitrite in human can also be converted to NO by NO_2-reductase and numerous health benefits have been attributed to NO which reacts with guanylate cyclase and causes reduction in guanosine monophosphate which in turn is associated with health benefits such as protecting from heart disease, hypertension, diabetes, thyroid disorders, metabolic disfunction, obesity and neurological conditions such as Alzheimer's disease.

Amino acid like L-arginine has been a well-known endogenous source of NO. L-arginine upon reacting with nitric oxide synthase (NOS) releases NO. A high proportion (56%) of the daily

NO is derived from arginine stored in plasma. Whilst serum elevation of NO_2 + NO_3 (NO_x) was observed following dietary intake of L-arginine (Mirmiran et al. 2016), the overall dietary arginine contribution to NO has been estimated as very low (2-5%) . Dietary L-arginine production of NO_x in serum may be affected by sex, age, body mass index and hypertension status (Mirmiran et al. 2016). One of the negative metabolic processes of NO or NO_3 is both could trigger methaemoglobin production.

In human or livestock, methaemoglobin ($HbFe^{3+}$) is produced in blood by NO_3 being reduced to NO_2 which in turn reacts with Fe^{2+} in haemoglobin. Methaemoglobin can also be produced from NO generated from NO_2. Consequently, oxygen transfer by haemoglobin is affected by the combined effects of oxygen saturation in $HbFe^{2+}$ with lack of oxygen release and $HbFe^{3+}$ unable to carry oxygen (Nnate and Achi 2016). Fortunately, in human adults the tolerance to high intake of NO_3 is high on a bodyweight basis and $HbFe^{3+}$ is reversed readily to oxyhaemoglobin form by reducing enzyme systems such as NADH methaemoglobin reductase. However, in the case of bottle-fed infants <5-6-month-old, until they fully develop adult haemoglobin (HbA) by fully replacing *foetal haemoglobin* (HbF) they are susceptible to methaemoglobinaemia (blue-baby syndrome) when ingesting high level of NO_3.

Foetal haemoglobin (HbF) is produced in the foetus after 10-12 weeks of pregnancy and in the first 6 months after birth. Compared to HbA, HbF has high affinity to oxygen to aid maternal circulation to foetal circulation (Kaufman et al. 2021). It is this high oxygen affinity which enables heavy binding of NO_2 to HbF which accentuates methaemoglobinaemia. To protect bottle-fed babies from methaemoglobinaemia, WHO has set safe drinking water NO_3 at 50 mg/L and 3 mg NO_2/L which are also the New Zealand standards.

Colorectal cancer (CRC)

Factors

The colorectal cancer (CRC), also known as bowel cancer is cancer in colon and/or in rectum. Colorectal cancer has been considered a Western world disease. It has been the third most commonly occurring in men and second in women. Increasingly high CRC cases are being identified in Eastern nations (e.g., South Korea with second highest CRC) owing to the increasing influence of Western diets and sedentary lifestyle. Globally, the CRC incidence has been increasing rapidly (200,000 cases per year between 1990 and 2012) with 700,000 deaths/year (Mármol et al. 2017). In New Zealand annually more than 1100 people die of CRC.

The main *modifiable* risk factors identified to date have been smoking, obesity, lack of physical activity, high red & processed meat and alcohol consumption (Richardson et al. 2016). The *non-modifiable* risk factors have been identified as personal history of inflammatory bowel disease (IBD), age, hereditary (history of colorectal polyps or *Lynch syndrome*), type-2 diabetes, racial/ethnic background (Simon 2016). High intake of fibre, leafy vegetables, fruits, folate, polyphenols, vitamins C, D & E and calcium have been identified as beneficial factors in reducing cancer risks.

Process

Whilst technically complex, understanding the initiation, promotion and progression of CRC is critical to link with the risk factors. The theory is, when the cells are ‘normal’, they function normally. However, when the cell DNA is damaged (also referred to as gene mutation) by

hereditary or by environmental factors, polyps (tumours) form, as such, polyp formation within colon and rectum is considered as the beginning of the cancer process. This could occur in young adults (30-40-year-olds) with familial history such as *Lynch syndrome,* also known as hereditary non-polyposis colorectal cancer (HNPCC).

Depending on the factors, polyps can remain benign (non-cancerous) for a long time and could become malignant (cancerous) in 5-15 years. All polyps do not develop cancer, but all CRCs form from polyps, as such when detected polyps are removed during colonoscopy even when benign. Polyp cell growths follow typical rapid mutated or cancer cell growth pattern with mutated cells outperforming normal cells and not following normal cell damage repairs or cell deaths- normal → hyperplasia (rapid cell growth) → dysplasia (abnormal cell growth) → neoplasia (cancer cells begin) → carcinoma in situ (adenocarcinoma) → microinvasive (metastasis which is terminal cancer where cancer cells migrate to other organs). Malignant polyps could bleed with typical symptoms of malignant polyps are being rectal bleeding (non-haemorrhoidal), constipation, diarrhoea, abdominal pain, anaemia (which causes fatigue) and black stool.

Understanding the science behind DNA damage/mutation and CRC link

Since the main onset of cancer is damage to the DNA or DNA mutation, understanding the science is critical to linking causes. Depending on the origin of the DNA mutation CRC cases can be classified as *sporadic, familial* and *hereditary* (Mármol et al. 2017). The term 'sporadic' CRC is used when normal adults are diagnosed with CRC, whilst 'familial' cases are where family members are associated with some form of cancer but without any medically recognised defective genes. In sporadic cases the DNA mutation is called 'acquired gene mutation'.

In the 'inherited' cases, defective gene (e.g., Lynch syndrome or Familial Adenomatous Polyposis (FAP)) could be identified as the main cause of the cancer, as such, hereditary related CRC is relatively easy to detect and manage. Unlike normal DNAs, inherited defective DNA is unable to repair itself when the defective genes are being copied or by carrying dysfunctional tumour suppressor genes, the DNA remains defective. For example, in FAP, tumour suppressor genes are affected whilst in Lynch syndrome DNA repair pathways are affected.

In normal adult, DNA is susceptible to 'sporadic' chemical modification by endogenous (e.g., replication errors, spontaneous base deamination, oxidative damage and methylation) and exogenous (e.g., chemical agents such as alkylators, aromatic amines, polyaromatic hydrocarbons, reactive electrophiles, toxins, environmental stresses such as extreme cold, heat and oxidation) factors despite robust DNA repair pathways (Chatterjee and Walker 2017). Unlike defective genes, when a normal DNA is damaged there is natural DNA damage response (DDR) via wide-ranging repair pathways.

Understanding DNA repair pathways is becoming critical in managing the risks of CRC and managing CRC itself. For example, 85% of the CRC cases are caused by *chromosomal instability* (CIN) pathway. The remaining 15% of cases are caused by *microsatellite instability* (MSI) which is a condition where DNA mismatch repair (MMR) is impaired. Of the 15% MSI related CRC, only 3% of the MSI is identified as associated with hereditary (Lynch syndrome) and 12% are caused by sporadic DNA mutation (Boland and Goel 2010). Since the genes affected by MSI are known (i.e., oncogenes such as MLH_1, MSH_2, PMS_2 and MSH_6), using

MSI as marker, early and targeted treatment of the CRC is possible thus reducing CRC related deaths. As such once identified, increasingly MSI cases have been treated successfully by metastatic immunotherapy than chemotherapy.

As can be seen, the nub of the issue is in the familial and sporadic CRC cases, any environmental factors identified as causing cancer (e.g., alcohol, obesity, smoking) must be directly or indirectly linked to the DNA damage beyond repair or factors accentuating the damage (e.g., inflammatory bowel disease) and the factors preventing/mitigating such damage (e.g., fibre and vitamin D & E intake). As I have articulated in this section, polyp formation and cancer development are complex processes which can be affected by multiple factors, as such, it is difficult to isolate individual factors even under well planned and executed case-controlled studies.

Any causal link established with CRC must be proven scientifically with appropriate and robust statistical tools, sound hypotheses, minimal and sensible assumptions, and access to quality data/information. There must be scientifically acceptable hypotheses based on past documented information. For example, the hypothesis for alcohol being a *direct* carcinogen are, increased mucosal cell proliferation, the activation of intestinal procarcinogens, and the role of unabsorbed carcinogens and for being *indirect* carcinogens are immunodepression, activation of liver procarcinogens, changes in bile composition, alcohol nitrosamine content and increased tissue nitrosamine levels. (Kune and Vitetta 1992).

Even if we consider a single factor such as consumption of alcoholic beverages, which has already been classified as *carcinogenic* to human by the International Agency for Research on Cancer (IARC), sifting through the relevant cohort and case-control studies is not easy. Meta-analyses are good tools, but the quality is often compromised with most workers ignoring significant inconsistencies between the studies including the methodology of individual studies. Kune and Vitetta (1992) assessed 52 case-control and cohort studies held over a 35-year period and found many studies failing to link alcohol consumption with CRC. However, where there were significant links established with alcohol consumption, more often with rectal cancer, with drinking beer being riskier than spirits and wines and with several hypotheses stated above fitting beer drinking.

Is the science behind CRC-NO_3 or CRC-nitroso compounds sound to establish a link?

The theory behind CRC and ingested-NO_3 link has been based on harmful nitroso compounds forming following the ingestion of NO_3 or NO_2 which in turn causing cancer. Nitroso compounds can be ingested exogenously or can form endogenously within the human guts. The processes behind the formation of endogenous nitroso compounds from ingested NO_3 is not well understood. Endogenous formation relies on *nitrosating* agent NO_2 and any one or combination of the precursors alkylamines, aromatic amines, amino acids, amides, peptides, ureas and guanidines and as such the process is referred to as *nitrosation*.

$$NO_2 + \text{precursors (e.g., amines and amides)} \text{----nitrosation}\rightarrow \text{nitroso compounds}$$

Often high red meat consumption is associated with haem and precursor (e.g., amines or amino acids) formation which could also enhance nitrosation whilst the presence of vitamin-C & E and polyphenols appears to inhibit the process (Ward et al. 2018). Nitroso compounds are

processed and excreted regularly by human via urine and faeces which can contain both exogenous and endogenous nitroso compounds. The extent of endogenous nitrosation is often measured by monitoring the excreted nitroso compounds (Breda et al 2019). An unvalidated crude estimate showed that 45-75% of the human exposure could be from endogenous nitroso compounds (Tricker, 1997). Whilst the CRC research focus has been on endogenous nitroso compounds, the exogenous sources such as diets, chlorinated drinking water, smoking, occupational exposures, cosmetics and pharmaceuticals are confounding factors and as such must be integral part of the study to provide meaningful results.

Before testing any hypothesis on causative link between ingested drinking water NO_3 and CRC, the questions must be whether there has been evidence on (a) endogenous nitroso compounds formation from *drinking water* NO_3 and (b) the formed compounds from drinking water causing human cancer. If drinking water is consumed without diet, technically there is no opportunity for endogenous nitrosation from drinking water NO_3 because of the absence or lack of the precursors such as amino acids. However, if consumed with diets, drinking water NO_3 endogenous nitrosation must be inhibited by dietary intake of inhibitory agents such as vitamin-C and polyphenols. The fact that there has been no conclusive evidence on *any* nitroso compounds causing human cancer, let alone endogenously formed compounds will demand compelling evidence for any causative link.

Regardless of the origin, proving carcinogenicity of nitroso compounds or any other cancer agent is not easy. There have been numerous in-vitro trials (simulated in laboratory conditions outside the target living organisms) assessing chemical analyses, alkylating potential, mutagenicity, carcinogenicity and assays comparison. Most studies have been performed with unrealistically high NO_3, NO_2 and precursor levels to simulate nitrosation. Using the above processes, considerable scientific data and information have been accumulated on 'stable' and high concentration nitroso compounds. However, little was known about the formation of chemically unstable nitroso compounds using low levels of nitrite and their genotoxicity in target cells (Shephard and Lutz 1989).

On the other hand, in-vivo trials (experiments held within human or animals) have been difficult to perform because of the trial results are considered as complex function of (a) amount of precursor (e.g., amines) and nitrite ingested (b) the rates of in-vivo nitrosation and (c) the carcinogenic potential of the resulting N-nitroso compound (Shephard and Lutz 1989). Despite the above complexity, even useful studies seldom performed have oversimplified with unrealistically high NO_3 in drinking water rendering the results redundant (e.g., study by Breda et al. 2019 used >120 mg NO_3/L water based on 3.7 mg NO_3/body weight use).

It must be emphasised that not all nitroso compounds are endogenously formed from NO_3 and if formed not all endogenously formed nitroso compounds are considered as carcinogenic. If carcinogenic they may not be relevant to CRC. International Agency for Research on Cancer (IARC) has been tasked to classify cancer causing agents. In 1987, it classified N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) and in 2010, ingested NO_3 and NO_2 under conditions that result in endogenous nitrosation as *probably* carcinogenic to human under Group 2A. Under Group 2A the evidence for human cancer may be *limited/inadequate*, cancer in experimental animals *sufficient/less than sufficient* and mechanistic evidence may be *strong, limited or inadequate*.

The IARC classification of NO_3 and NO_2 as Group 2A based on endogenous nitrosation has been misinterpreted by researchers attempting to link drinking water NO_3 with cancer. High consumption of red meat has been shown as the main source of the endogenous nitroso compounds such as NDMA and NDEA (Ward et al. 2018) and there has been no compelling evidence of the above two nitroso compounds endogenously formed from the ingested drinking water NO_3. Despite decades of research many remaining nitroso compounds are still held in Group 2B (possibly carcinogenic) and 3 (not classifiable). As such any hypothesis on CRC link to generic endogenous nitroso compounds from drinking water NO_3 is baseless.

In the context of the agents which are classified as *human carcinogens* (e.g., alcohol beverages, smoking, solar radiation), they are not considered to cause cancer in everyone exposed to well above the thresholds, as such when the epidemiological studies are performed only a small proportion of the population has been found to be affected by the proven human carcinogens. Such a technical conundrum rendering studies attempting to link CRC with *probable* carcinogens are likely to generate numerous unproven hypotheses and more questions than answers.

One such hypothesis is that endogenous nitrosation is not inhibited when NO_3 is ingested with drinking water but dietary NO_3 is inhibited by simultaneous intake of substances such as folates, vitamins and polyphenols. Notwithstanding endogenous nitrosation is not possible without precursors such as amides or amino acids, given the longer half-life of most ingested polyphenols is 1.3-19.9 h (Manach et al. 2005) and vitamin-C is 16 h (Hellman and Burns 1958) and the short half-life of 5-6 h for ingested NO_3, there has been no cancer related metabolic study assessing the effect of plasma residing nitrosation/cancer inhibiting agents such vitamin-C or polyphenols.

Despite IARC's regular reviews of the potentially carcinogenic agents, most such studies argue some nitroso compounds are animal carcinogens and as such there is 'potential' for such substances to cause cancer in human. Unfortunately, many studies demonstrating animal cancer are conducted with unrealistically high levels of nitroso compounds or combination of high levels of nitrosation precursor (amines/amino acids) and nitrite concentrations. For example, one such study reviewed by Shephard and Lutz (1989) stated cancer tumour development in experimental rats were observed 18 months after regularly feeding nitrosated fish extract obtained under drastic conditions (70 nM nitrite (calculated as 3220 mg/L), pH 3, 3 hours at 25^{o}C) for 6 months. I hope in future, IRAC would vet such studies under stringent conditions to accept the studies which correctly simulate human nitrosation metabolism.

Lack of technical rigour in the most recent CRC-drinking water NO_3 overseas and NZ epidemiological studies

Danish study by Schullehner et al. (2018) claiming increased CRC risk of ≥3.87 mg NO_3/L (0.87 mg NO_3-N/L) in drinking water

The above Danish cohort study on drinking water NO_3 received an unprecedented attention owing to its extensive research involving the entire Danish population with 2852 public water supplies, 2,382,445 houses and 81,663 private wells along with CRC records of all people ≥35 years old between 1978 and 2011. The final analysis of the study used 61% of the population with NO_3 exposure and claimed statistically significant relationship between increasing NO_3 levels and CRC risk at ≥3.87 mg NO_3/L.

Superficially, the above finding from a peer-reviewed scientific paper with significant data assessment appears compelling as such I am not surprised by the overwhelming interest generated, including calls on our government to review and tighten our drinking water standard. However, when I reviewed the above paper thoroughly along with many other drinking water NO_3-linking cancer studies, it is apparent that the Danish paper is an *oversimplified* cohort study without considering already well-researched factors such as dietary NO_3 intakes, precursors of nitrosation and cancer inhibiting agents and the well-known confounding factors such as obesity, lack of physical activity, smoking and alcohol, red meat and processed meat consumption.

The study also failed to consider drinking water *ingested* NO_3 which is critical to such studies than the average source water quality alone which is referred to as *residential* NO_3. Since much of the data had been retrospective and gathered from the 1978-2011 Danish public health register, there was limited access to critical data. As such, the Danish workers admission of "… *any observational study of human health, including the present, cannot exclude the possibility of residual confounding by unobserved factors*…" was correct and must be considered by other similar future studies. However, it is baffling why the Danish workers proceeded with such a costly and laborious project with the full knowledge of data deficient public health register and called for discussion to reduce drinking water NO_3 level based on a highly deficient study with conflicting results.

The exclusion of the key confounding factors recognised belatedly by Schullehner et al. (2018) explain the erratic hazard ratio (HR) values obtained between drinking water NO_3 exposure quintiles and colon, rectal and colorectal cancers. The work revealed increasing colon cancer risk at 1.27-2.33 mg NO_3/L (HR>1) which reduced at 2.33-3.87 mg NO_3/L (HR>1) and colon cancer with 'beneficial effect' (HR<1) at 2.33-3.87 mg NO_3/L. Of the 3700 colon cancer cases reported approximately in two of the three cases (69%) colon cancer cases were not statistically significantly associated with increasing drinking water NO_3 levels from 1.27 to 9.23 mg NO_3/L with cancer risk only established at $\geq$9.25 mg NO_3/L.

Like many other workers, the Danish workers failed to offer any scientific explanation to the reducing risk or reaching HR value <1 with increasing drinking water NO_3 levels on CRC or colon cancer risks. Similar reduced or no risks or 'beneficial effects' with increasing drinking water NO_3 levels against CRC have also been evident in other cohort (e.g., Weyer et al. 2001) and case-control studies (De Roos et al. 2003, Yang et al. 2007, McElroy et al. 2008 and Espejo-Herrera et al. 2016).

The use of Cox hazard ratio by the Danish study and many similar epidemiological studies is also questionable. The *Cox proportional hazard regression analysis* which is a semi-parametric model (different to the conventional linear regression analysis) is a well-known and widely used statistical tool which is used to relate several risk factors/exposures together against survival time. The key assumption in the Cox model is that the hazards are proportional, which means that the relative hazard remains constant over time for individual involved with different risk factors or covariates (Kuitunen et al. 2021). Such an assumption is very strong in long-term cohort studies where it is impossible to maintain constant hazard in biological and ageing system.

The Danish study claimed that it did check for the validity of the proportional hazards assumption by assessing the null hypothesis by *Schoenfeld residuals* on time which claimed to have resulted in zero slope indicating no violation of the assumption. This was despite the study experienced increased diagnosis resulting in increased HR in all outcomes with an increase in observation in one of the two covariates such as education.

The non-violation of the assumption in such a complex multi-factor and dynamic setting may be a deliberate outcome of the long-term cohort study by retaining simple covariates such as education and cancer history and neglecting or omitting the critical and dynamic confounding factors such as BMI, physical activity, smoking and alcohol, vegetable, vitamins and red meat/processed diet consumptions. The above misuse of statistical methods has been the feature of many similar studies I have reviewed fully. Statistics experts involved in medical research have been warning numerous researchers neglecting non-proportionality which has been undermining the overall research efforts and have called for specific reporting guidelines for researchers (Kuitunen et al. 2021).

Based on the above information it is evident that the CRC-drinking water NO_3 link claim by the Danish cohort study suffered significant flaw in the methodology and as such considerably lacked in scientific rigour. Unfortunately, our own oversimplified work (Richards et al 2022) published recently which attracted considerable local media attention has been the simplest study I have reviewed to date with full disregard to New Zealand based confounding factors. I have assessed that work below.

The recent New Zealand study by Richards et al (2022)

Compared to the above Danish cohort study deficient of key confounding factors and with confusing results, the recent New Zealand desktop study by Richards et al (2022) is simplified further by using risk factor derived from an overseas data based meta-analysis from Temkin et al (2019). This New Zealand inaugural CRC-drinking water NO_3 epidemiological study used 2013 Ministry of Health CRC data to assess CRC link with 2018-2020 drinking water NO_3-N data (note, the study used NO_3-N than NO_3). Given CRC development can take 5-15 years from the gene mutation, technically, drinking water collected between 1997 and 2008 should have been used to assess causal link with 2013 CRC data.

The study assigned drinking water quality (reported as NO_3-N noting my review has reported as NO_3) of 0.49 mg NO_3-N/L (2.17 mg NO_3/L) for 84.4% of the New Zealand population and 0.84 mg NO_3-N/L (3.72 mg NO_3/L) for the entire population. It used a simple relationship between population attributable fraction (PAF) and effective risk ratio (RR) to assess PAF. The effective risk ratio was obtained by multiplying the average exposure (i.e., drinking water nitrate concentration) by the relative risk (0.04) from the meta-analysis of Temkin et al (2019).

$$\boldsymbol{PAF} = \boldsymbol{Pe}\ (\boldsymbol{RR}\text{-}1)/[\boldsymbol{Pe}\ (\boldsymbol{RR}\text{-}1)+1] \times 100\%$$

where: ***PAF*** is the population attributable fraction, ***Pe*** is the prevalence of exposure (0.84 mg NO_3-N/L), ***RR*** is the relative risk (1.04), hence PAF was derived as 3.26% as the percentage of the entire population likely to develop CRC. To assess the proportion of the newly registered CRC patients and CRC related deaths affected by drinking water nitrate, 2013 Ministry of

Health data of 3075 and 1252 respectively were used. As such drinking water NO_3 affected patients and CRC casualties in 2013 were estimated as 100 and 40 respectively.

I have assessed the quality of the meta-analysis by Temkin et al. (2019) in detail including the that of the key cohort and case-controlled studies used. Whilst the meta-analysis made considerable effort in collation and its own additional data analysis, it was evident clearly that the meta-analysis faced difficulties in finding comparable studies. The studies used by Temkin et al (2019) were a mixture of colon and CRC studies, with or without adjusting or including confounding factors and in majority of cases involving residential drinking water NO_3 than ingested which resulted in undesirably high statistical heterogeneity pooled study as identified by the workers themselves. I have collated a summary of the studies and results used by the meta-analysis by Temkin et al. (2019) in Table 1.

Table 1. Summary of the overseas studies used in the meta-analysis by Temkin et al. (2019) from which the NZ study by Richards et al. (2022) derived relative risk factor

Studies	Sample size	Country of study	Age	Sex	Cancers or diseases studied	Confounding factors considered	Ingested or Residential drinking water nitrate	Statistic used	Cancer and nitrate[1] relationship
Case control studies									
De Roos et al (2003)	Colon cancer cases 685 Rectum cancer cases 655 Control cases 2434	Iowa, US	40-85	F	Colon, rectum, brain, pancreas, bladder, kidney	Dietary nitrate/nitrite, vitamins C, A & E, alcohol, smoking, physical activity, BMI, bowel inflammation, family history, chlorinated water, education, dietary quality and quantity	Residential	Odds ratio and logistic regression model	[2]Reduced risk between >13.3 and ≤22.1 mg/L than >4.4 and ≤13.3 and increased risk ≥ 22.1 mg/L Beneficial effects from increasing dietary nitrate
Espejo-Herrera et al. (2016)	CRC cases Spain 1562 CRC cases Italy 307	Spain and Italy	≤57->72	M/F	Colon, rectum and CRC	Education, smoking, physical activity, family history, oral contraceptive and anti-inflammatory drug use, BMI, energy, fibre, Vit C, Vit E, red and processed meat and water intakes	Ingested	Odds ratio	Increasing risk between ≤3.6 mg/L, >3.6- 7.1 mg/L and ≥ 7.1 mg/L Beneficial effect on rectal cancer at >3.6-7.1 mg/L Beneficial effect from Vit E and fibre intake
Chiu et al (2010)	Colon cancer deaths 3707 Control deaths 3707	Taiwan		M/F	Colon	Magnesium in water	Residential	Odds ratio	Increasing risk between <1.7 mg/L, 1.7-2.5 mg/L and >2.7 mg/L
Yang et al (2007)	Colon cancer death 2234	Taiwan		M/F	Colon	Nil	Residential	Odds ratio	No effect between ≤0.9 mg/L, 1-2

Studies	Sample size	Country of study	Age	Sex	Cancers or diseases studied	Confounding factors considered	Ingested or Residential drinking water nitrate	Statistic used	Cancer and nitrate[1] relationship
	Control death 2234								mg/L and 2.1-12.7 mg/L
Fathmawati et al., (2017)	CRC patients 75 and controls 75	Indonesia		M/F	CRC	Protein intake, age, family history and smoking	Ingested (well)	Relative risks	>50 and <50 mg/L were compared and found increased risk with >50 mg/L
McElroy et al 2008	CRC cases of 475 and control of	Wisconsin, US	20-74	F	CRC	Family history, smoking alcohol consumption, BMI, education	Residential (randomised well water nitrate)	Odds ratios	Increased risk at 2.2-8.4 and ≥44.2 but reduced risk at 8.8-26 and 26.5-43.8 mg/L
Cohort studies									
Weyer et al (2001)	Sample size 21977 Colon case 385 and Rectal cases 129 between 1955-88	Iowa, US	55-69	F	Non-Hodgkin lymphoma, leukaemia, melanoma, and cancers of colon, rectum, breast, lung, pancreas & kidney	Confounding considered only to assess overall cancer risk not separately for CRC	Ingested based on 2L/d (municipal and well)	Relative risks	Colon risk-Increased between 1.6-10.9 mg/L and decreased >10.9 mg/L Rectal risk-Beneficial effect >1.6 mg/L with greatest benefit >10.9 mg/L
Schullehner et al (2018)	Total studied 2.83M but data analysis on 1.74M	Denmark	>35	M/F	Colon, rectal and CRC	Education,& family history	Residential (municipal and well)	Hazard ratio	CRC risk-increasing risk in all quintiles except decreasing risk 2.33-3.87 mg/L Colon risk-beneficial effect at 2.33-3.87 mg/L but increasing risk ≥9.25 mg/L

[1] Where needed, nitrate values were converted from NO_3-N by multiplying by 4.426

[2] Reduced or increased risk ratio stays ≥1.00 whilst beneficial effect risk ratio was <1.00

Given the significant differences between the methodology of the studies and countries of origin, the usefulness and the universal transferability of the estimated relative risk (RR) values to assess CRC risk must be questioned. The above concern is particularly critical in human health studies. Many such studies including the New Zealand study (Richards et al. 2022) have not only resorted to crude epidemiological assessment but extended such assessment to extrapolate social/economic costs whose applicability to public health policy development is almost nil given the lack of quality, accuracy and technical rigour.

My critical review of the frequently referred epidemiological papers linking CRC with drinking water NO_3 has found, many such studies have yielded unexpected and inexplicable results with increasing drinking water NO_3 levels yielding reduced risks (RR values >1 but with reduction

between exposures) and beneficial effect (with RR <1) of CRC between exposures (see Table 1 final column). Despite the frequent reduced risk observations of the increasing drinking water NO_3, no workers have attempted to offer any scientific explanation of such findings.

Most cohort and case-control studies were based on municipal water supplies which were of good quality obviously generating numerous data from the exposure of relatively very low drinking water NO_3 levels. Despite private/rural wells are known to have greater NO_3 levels than municipal water supply water sources, strangely exclusive epidemiological studies on private well water users have been rare and if conducted, key epidemiological studies have failed to access NO_3 data from private wells and water sources with >50 mg NO_3/L.

A study by Weyer et al. (2001) provides an insight into CRC cases from female residents using private well water without individual well NO_3 data or the extent of bottled water or rainwater use. Using the data in Table 4 in the above study I have estimated the actual disease rate per 10,000 person-years for private well and municipal water users whose disease rate trend is similar to that of the relative risk computed by Weyer et al. (2001).

Table 2. Cohort study on cancer association with drinking water NO_3 in Iowa female population with municipal water (with 1955-88 data) and private well use with cancer case assessment between 1986-1998 (sourced from Weyer et al. (2001) with cancer rate estimated in the current paper)

Cancer type	Private wells		Municipal water							
	Unknown nitrate levels 64,276 person-years		<1.6 mg NO_3/L 48,438 person-years		1.6-4.4 mg NO_3/L 48,163 person-years		4.5-10.9 mg NO_3/L 47,821 person-years		>10.9 mg NO_3/L 48,011 person-years	
	Cases	Rate per 10,000	Cases	Rate per 10,000	Cases	Rate per 10,000	Cases	Rate per 10,000	Cases	Rate per 10,000
All cancers	730	113	586	120	620	128	630	131	584	121
Colon	85	13	58	12	86	17	92	19	64	13
Rectum	23	3	33	6	25	5	32	6	16	3
Colon + Rectum	108	16	91	18	111	23	124	26	80	16
Other digestive	21	3	11	2	12	2	16	3	16	3
Breast	275	42	208	43	209	43	185	38	208	43
Bladder	10	1	7	1	14	3	8	1	18	3

As seen in Table 2, *all* cancer rates for private well water users were found to be lower than that for the municipal water users. As for CRC, the risk reduced in females drinking municipal water with >10.9 mg NO_3/L compared to that drinking water with substantially lower NO_3 levels. The overall CRC risk appeared similar in private water users and the municipal water users using drinking water with >10.9 mg NO_3/L. Assuming private well water users using well water as a primary source of drinking water and judging by the reducing risk of increasing NO_3 from municipal water studies, it could be inferred there may beneficial health effects on drinking water NO_3 or lack of quality data or lack of confounding factor considerations causing erratic results. As stated before, my review of such studies has raised more questions than drawing any meaningful answers.

In conclusion of this subsection, based on the erratic CRC risks exhibited by increasing drinking water NO_3 exposure, widely differing methodology and confounding factors between study locations in the meta-analysis by Temkin et al (2019), the oversimplified CRC risk assessment by the NZ study by Richards et al. (2022) which used a single relative risk factor derived from the above meta-analysis cannot be used to establish link between CRC in New Zealand and drinking water NO_3 because of substantial lack of technical rigour.

Recommendations for meaningful and useful drinking water nitrate toxicological and epidemiological studies

I am not surprised despite five decades of research there has been *no proof* of either drinking water NO_3 or its endogenously formed nitroso compounds causing CRC. Unfortunately, most studies have been based on inconsistent, deficient and inappropriate or oversimplified methods and unproven hypotheses, with numerous assumptions in the absence of appropriate or quality data. The above situation equally applies to numerous studies which have established no risk or beneficial link between drinking water NO_3 and cancer. Under the circumstances meta-analyses performed on inconsistent and deficient studies hold little or no value to the end user.

Clearly, epidemiological cohort or case-control studies performed in the absence of key confounding factors hold little or no value to anyone and as such must be avoided. Compelling evidence is possible only when cohort/case-control studies assess reliable, quality and long-term data on key factors causing and inhibiting cancer with appropriate statistical tools. Such studies must be objective and *country specific* owing to significant differences in environmental, health, lifestyle and dietary differences which are all co-factors in cancer studies. Judging by the high degree of methodology inconsistencies and variation between confounding factors between/within the countries in the epidemiological studies, the transfer and use of relative risk factors in another country must be avoided unless technically justified.

I hope when assessing carcinogenic agents regularly IARC does not accord any weight to the human health studies with poor methodologies and misuse of statistical methods. Unlike studies on well-documented confounding CRC factors such as smoking, studies proving agents such as NO_3 with multiple sources and with numerous well documented beneficial health effects causing irreparable gene mutation which in turn causing CRC will be challenging. As such, any studies investigating the effects of NO_3 on CRC must be of the highest possible quality and open to assessing both positive and negative effects.

Researchers must also not lose the sight of the tenuous NO_3 link to CRC or any human cancer. Since nitroso compounds such as NDMA and NDEA are considered are probably carcinogenic, NO_3 has also been considered as probably carcinogenic by the IARC on the assumption of it endogenously producing the above compounds in the human digestive system. There has been no evidence on the formation of NDMA and NDEA in human from the *ingested drinking water* NO_3 let alone the above compounds causing CRC. The main reason for the above two compounds being classified as probable carcinogens has been laboratory trials involving animals subjected to unusually sustained or high levels of the above nitroso compounds.

I call upon the IARC and WHO to play more active role in providing protocol for research associated with carcinogenic substances including drinking water contaminants. Such research can be categorised as in-vivo and in-vitro studies on human and animals and cohort and case-

control epidemiological studies. Whilst substantial research effort has been made in the past five decades, much of the data derived from many studies lacked in technical rigour to be useful for the purpose of IARC or WHO. If the correct research protocols are set by the expert panels of IARC and WHO are followed, comparable, meaningful and robust research data and information can flourish.

Fit for purpose of the current drinking water nitrate

This paper is not intended to assess the current drinking water standard's fit for purpose to protect infant death from drinking water NO_3 since the standard has been promoted as guideline by WHO and being in use in almost all OECD countries. The ongoing debate has been to reduce NO_3 level substantially from 50 mg/L below 3 mg/L to avoid the risk of CRC or any other cancer risks.

Based on the information available to date, there is no evidence for drinking water NO_3 to form cancer causing nitroso compounds in the human digestive system or such compounds causing cancer. To date, no nitroso compounds have been identified as Group 1 cancer agent by the IARC and that no cohort or case-control studies have isolated drinking water NO_3 as one of the key confounding factors causing CRC with compelling evidence.

Based on the information available to date including the beneficial or reducing effect of increasing drinking water NO_3 exposure on CRC, there is no scientific rationale to consider precautionary measures in reducing drinking water NO_3 level until compelling evidence is in hand. However, establishing causal effect of drinking water NO_3 on CRC will be extremely challenging given the well documented beneficial effects of NO_3 ingestion from multiple sources, lack of endogenous nitrosation opportunity owing to the absence of nitrosating precursors, the complex interactions of the well documented dominant confounding factors and the pathological complexities associated with CRC.

Although 97% of all registered water supplies in New Zealand have <10 mg NO_3/L, there is no technical or policy justification to reduce current drinking water standard of 50 mg NO_3/L to 10 mg NO_3/L. However, since NO_3 is a well-recognised and significant surface water contaminant with potential for algal growth >1.3-1.8 mg NO_3/L (or >0.3-0.4 mg NO_3-N/L), our focus must be on minimising or avoiding groundwater and surface water NO_3 contamination to better maintain and enhance our river, lake and estuarine ecosystems.

References

Bahadoran, Z., Mirmiran, P., Kabir, A., Azizi, F., and Ghasemi, A. 2017. The Nitrate-independent blood pressure–lowering effect of beetroot juice: A systematic review and meta-analysis. *Advances in Nutrition (American Society for Nutrition)*, 8: 830-838.

Boland, C.R. and Goel, A. 2010. Microsatellite instability in colorectal cancer. *Gastroenterology*, 138(6): 2073-2087.

Breda, S.G.V., Mathijs, K., Sági-Kiss, V., Kuhnle, G.G., Veer, B.V.D., Jones, R.R., Sinha, R., Ward, M.H. and Kok, T.M.D. 2019. Impact of high drinking water nitrate levels on the endogenous formation of apparent N-nitroso compounds in combination with meat intake in healthy volunteers. *Environmental Health*, 18:87. pp 12.

Chatterjee, N. and and Walker, G.C. 2017. Mechanisms of DNA damage, repair and mutagenesis. *Environmental Molecular Mutagenesis*, 58(5): 235-263.

Chiu, H., Tsai, S., Wu, T., Yang, C., 2010. Colon cancer and content of nitrates and magnesium in drinking water. *Magnesium Research*, 23 (2), 81‑89.
De Roos, A.J., Ward, M.H., Lynch, C.F., Cantor, K.P. 2003. Nitrate in public water supplies and the risk of colon and rectum cancers. *Epidemiology* 14 (6): 640‑649.

Espejo-Herrera, N., Gracia-Lavedan, E., Boldo, E., Aragones, N., Perez-Gomez, B., Pollan, M. et al. 2016. Colorectal cancer risk and nitrate exposure through drinking water and diet. *International Journal of Cancer*, 139(2): 334-346.

ESR. 2021. Nitrate in food and water. Prepared by Peter Cressey and Belinda Cridge, ESR. Client report CSC21025. pp 63.

Fathmawati, Fachiroh J., Gravitiani, E., Sarto, Husodo, A.H., 2017. Nitrate in drinking water and risk of colorectal cancer in Yogyakarta, Indonesia. *Journal of Toxicology & Environmental Health*, Part A. 80 (2): 120‑128.

Hellman, L and Burns, J.J. 1958. Metabolism of L-ascorbic acid-1-C^{14} in man. *Journal of Biological Chemistry*, 230: 923-930.

Jackson, J.K., Patterson, A.J., MacDonald-Wicks, L.K., Oldmeadow, C., and McEvoy, M.A. 2018. The role of inorganic nitrate and nitrite in cardiovascular disease risk factors: a systematic review and meta-analysis of human evidence. *Nutrition Reviews*, 76(5): 348-371.

Kaufman D.P., Khattar J., Lappin S.L. 2021. Physiology, Fetal Hemoglobin. [Updated 2021 Mar 29]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan. https://www.ncbi.nlm.nih.gov/books/NBK500011/

Kuitunen, I., Ponkilainen, V.T., Uimonen, M.M., Eskelinen, A., and Reito, A. 2021. Testing the proportional hazards assumption in cox regression and dealing with possible non-proportionality in total joint arthroplasty research: methodological perspectives and review. *BMC Musculoskeletal Disorders*, 22(489): pp 7.

Kune, G.A. and Vitetta, L. 1992. Alcohol consumption and the etiology of colorectal cancer: a review of the scientific evidence from 1957 to 1991. Nutr Cancer 1992;18(2):97-111.

McElroy, J.A., Trentham-Dietz, A., Gangnon, R.E., Hampton, J.M., Bersch, A.J., Kanarek, M.S., Newcomb, P.A. 2008. Nitrogen-nitrate exposure from drinking water and colorectal cancer risk for rural women in Wisconsin, USA. *Journal of Water and Health* 6 (3): 399‑409.

Mirmiran, P., Bahadoran, Z., Ghasemi, A., and Azizi, F. 2016. The association of dietary L-arginine intake and serum nitric oxide metabolites in adults: a population-based study. *Nutrients*, 8(311).

Nnate, D.A. and Achi, N.K. 2016. Nitrate metabolism: A curse or blessing to humanity? *Journal of Scientific Research & Reports*, 11(4): 1-19.

Manach, C., Williamson, G., Morand, C., Scalbert, A. and Rémé, C. 2005. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *American Journal of Clinical Nutrition*, 81: 230S-242S.

Richards, J., Chambers, T., Hales, S., Joy, M., Radu, T., Woodward, A., Humphrey, A., Randal, E., and Baker, M.G. 2022. Nitrate contamination in drinking water and colorectal cancer: Exposure assessment and estimated health burden in New Zealand. *Environmental Research,* 204, 112322.

Richardson, A., Hayes, J., Frampton, C. and Potter, J. 2016. Modifiable lifestyle factors that could reduce the incidence of colorectal caner in New Zealand. *New Zealand Medical Journal*, 129 (1447).

Schullehner, J., Hansen, B., Thygesen, M., Pedersen, C.B. and Sigsgaard, T. 2018. Nitrate in drinking water and colorectal cancer risk: A nationwide population-based cohort study. *International Journal of Cancer*, 143(1): 73-79.

Shephard, S.E. and Lutz, W.K. 1989. Nitrosation of dietary precursors. *Cancer Surveys*, 8(2): 401-421.

Simon, K. 2016. Colorectal cancer development and advances in screening. *Clinical Intervention in Aging*,11: 967-976.

Temkin, A., Evans, S., Manidis, T., Campbell, C. and Naidenko, O.V. 2019. Exposure-based assessment and economic valuation of adverse birth outcomes and cancer risk due to nitrate in United States drinking water. *Environmental Research,* 176, 108442.

Thomson B.M., Nokes C.J. and Cressey P.J. 2007. Intake and risk assessment of nitrate and nitrite from New Zealand foods and drinking water. *Food Additives and Contaminants*, 24(2): 113-121.

Tricker, A.R. 1997. N-nitroso compounds in man. Sources of exposure, endogenous formation and occurrence in body fluids. *European Journal of cancer prevention*, 6:226-268.

Villar, M.L. 2019. Nitrate and nitrite metabolism in ruminant livestock. PhD thesis, School of Environmental and Rural Sciences, University of New England. pp 259.

Ward, M.H., Jones, R.R., Brender, J.D., De Kok, T.M., Weyer, P.J., Nolan, B.T., et al., 2018. Drinking water nitrate and human health: an updated review. *International Journal of Environmental Research Public Health* 15, 1557.

Yang, C., Wu, D. and Chang, C., 2007. Nitrate in drinking water and risk of death from colon cancer in Taiwan. *Environmental International,* 33(5): 649‑653.