Science for Communities

NITRATE IN FOOD AND WATER

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EXECUTIVE SUMMARY

Nitrate is mainly sourced through the diet via the consumption of fruit and vegetables. Nitrate is also present in varying concentrations in drinking-water, often due to animal waste, nitrogen-based fertilisers, animal feeds and septic systems. A recent epidemiological study reported an association between nitrates in drinking-water, but not through the diet, and adverse health outcomes, particularly colorectal cancer. Other studies that have investigated associations between drinking-water nitrate and colorectal cancer did not consider exposure to nitrate from the diet. Therefore, the uptake, use and excretion of nitrates were reviewed to investigate possible differences between nitrate in food and nitrate in drinking-water. Exposure data from New Zealand was then analysed to elucidate patterns of exposure to nitrates in food and drinking-water. This evidence was used to determine if there is plausible rationale for analysing different sources of nitrates independently.

The role of nitrate and the other nitrogen oxides in the human body is complex, with potential involvement in both beneficial and adverse physiological processes. Nitrates are transformed into nitrites, nitric oxide and nitrosamines within the oral and gut lumens through the action of bacteria. Nitrites have been shown to be bactericidal, preventing oral disease and pathogenic bacterial growth in the gut. At least a part of this action is due to the formation of nitric oxide. Nitric oxide is a also potent vasodilator and there is increasing evidence that nitrites and nitrates act as a storage mechanism for nitric oxide production within the body. Nitrite and nitrate storage can provide a nitrogen source for nitric acid production and therefore local vasodilation during anaerobic metabolic conditions.

Nitrite and nitrosamine production from nitrate is dependent on pH and the presence of antioxidants such as vitamin C and plant polyphenols. Nitrosamines are a large group of compounds that have been proposed as a mechanistic link between nitrate exposure and cancer. Within the body nitrosamines are metabolised by the CYP450 enzyme superfamily into mutagenic products that can cause DNA changes and initiate cancer. The concentration and conditions for this reaction are variable and there is no evidence that this would be a process limited only to nitrates from drinking-water.

Diet and consumption patterns show that New Zealanders dietary exposure to nitrates is very similar to most other countries. Exposures are within acceptable daily intakes, suggesting there is little cause for public health concern. From this analysis, less than 10% of nitrate exposure in New Zealand was from drinking-water, with little difference between adults and children. Approximately half of the total water-based exposure is through water alone, the remainder was consumed via tea and coffee (adults), or water-based fruit drinks (children).

For children, drinking-water as a beverage is generally consumed close to a meal time, with 83% of servings consumed within an hour of eating. For adults, this is reduced to 51% of servings consumed within an hour of a meal. This is reflective of all drinks, including tea, coffee and juice. Only 2.6% of nitrate exposure for adults and 0.7% of nitrate exposure for children is from drinking-water consumed on its own and not in close temporal association to food consumption.

The combination of the biology, chemistry and exposure assessment suggest that it is highly unlikely that nitrates in drinking-water or the diet present an increased risk of cancer. This conclusion is consistent with the finding of several international food safety assessments of

nitrates. The exposure analysis shows that a low overall percentage of nitrates come from drinking-water consumption and a very small proportion is from consumption of drinking-water as a beverage. Therefore, the complete assessment suggests that there is little reason to differentiate between drinking-water and food nitrate exposure.

1. INTRODUCTION

Nitrate is a naturally occurring compound that is part of the nitrogen cycle, as well as an approved food additive (EFSA, 2008). It plays an important role in the nutrition and function of plants. Higher concentrations of nitrate tend to be found in leaves whereas lower levels occur in seeds or tubers and leafy crops, such as lettuce and spinach, usually contain higher nitrate concentrations (>1000 mg/kg fresh weight). Human exposure to nitrate is mainly exogenous through the consumption of vegetables, and to a lesser extent drinking-water and other foods. Nitrate is also formed endogenously. In contrast exposure to its metabolite nitrite is mainly from endogenous nitrate conversion.

Nitrate is relatively non-toxic, but its metabolites and reaction products, such as nitrite, nitric oxide and *N*-nitroso compounds, have raised concern because of implications for adverse health effects such as methaemoglobinaemia and carcinogenesis (EFSA, 2008). However, research has also indicated that these compounds may have beneficial roles, with nitrite participating in host defence against microbial pathogens (Khatri *et al.*, 2017; McKnight *et al.*, 1999) and nitric oxide having an important role in physiological processes such as vasoregulation (Rocha *et al.*, 2011).

Despite being a major source of nitrate, increased consumption of vegetables is widely recommended because of their generally agreed beneficial effects for health (Slavin and Lloyd, 2012).

1.1 NITRATE AND COLORECTAL CANCER

Several studies have examined associations between the concentration of nitrate in drinking-water and incidence of colorectal cancer (De Roos *et al.*, 2003b; Espejo-Herrera *et al.*, 2016; Fathmawati *et al.*, 2017; Jones *et al.*, 2019; McElroy *et al.*, 2008; Schullehner *et al.*, 2018; Weyer *et al.*, 2001). These studies were inconsistent in their findings, with some reporting a positive association between exposure to nitrate from drinking-water and colorectal cancer risk (Espejo-Herrera *et al.*, 2016; Schullehner *et al.*, 2018), while others reported no association or even an inverse association (De Roos *et al.*, 2003b; Jones *et al.*, 2019; McElroy *et al.*, 2008; Weyer *et al.*, 2001). The results of studies that found positive associations between drinking-water nitrate and colorectal cancer have been used to estimate the proportion of colorectal cancer in New Zealand that may be due to drinking-water nitrate (0.6-5.6%) (Richards, 2020).

Evaluations of available human and animal data have concluded that nitrate is neither genotoxic nor carcinogenic (EFSA, 2008; IARC, 2010; JECFA, 2002a). While evidence on the carcinogenicity of nitrite is more equivocal but it has generally been concluded that, of itself, nitrite is not genotoxic or carcinogenic (IARC, 2010; JECFA, 2002b)

A potential mechanism for associations between nitrite consumption, and nitrate as a precursor of nitrite, and gastrointestinal cancers has been hypothesed, based on findings that ingested nitrites may react with secondary amines or *N*-alkylamides to generate carcinogenic *N*-nitroso compounds (NOCs) (Hord *et al.*, 2009). Although NOCs have been shown in animal models to be carcinogenic, evidence in humans in scarce. The *N*-nitrosamides and *N*-nitrosoureas have been shown to be direct mutagens, whereas *N*-nitrosoamines do not act as direct mutagens but generally require activation by microsomal enzymes within the body, perhaps by cytochrome P450 enzymes (Hord *et al.*, 2009).

Of particular interest is the study of Espejo-Herrera *et al*. (2016), in which both dietary and drinking-water exposure to nitrate were considered. This study reported associations between drinking-water nitrate and cancer of the colon and rectum at exposure levels >10 mg/day, but not for dietary exposures >133 mg/day. The authors of this study and others

have hypothesised that the lack of association for dietary nitrate exposure might be explained by the fact that vegetables, usually the main source of nitrate intake, are also a source of vitamin C and other antioxidants that inhibit endogenous formation of NOCs (Espejo-Herrera *et al.*, 2016; Grosse *et al.*, 2006). This hypothesis suggests that nitrate from drinking-water is present in the gastrointestinal tract completely separate to dietary nitrate.

1.2 CURRENT STUDY

The current study has three main objectives:

- To review of the toxicokinetics of ingested nitrate, with particular reference to the likely time-course of NOC formation following nitrate ingestion. The toxicokinetics were considered with respect to possible mechanisms of carcinogenesis and nitrate/nitrites involvement in methaemoglinaemia was not considered.
- To estimate dietary and drinking-water exposure to nitrate for the New Zealand population.
- To determine patterns of drinking-water ingestion by New Zealanders, specifically the proportion of drinking-water that is ingested purely as such and the proximity of ingestion events to food consumption events. This information can subsequently be used to determine whether it is relevant to separate food and water exposure in evaluating cancer risks associated with nitrate compounds.

2. NITRATE – TOXICOKINETICS

2.1 NITROGEN AND NITRATES

Nitrate forms a part of the wider nitrogen cycle which involves the transfer of nitrogen and nitrogen-based compounds between the atmosphere, soil and living organisms (Figure 1). Bacteria play a critical role in this cycle, with many containing unique abilities to transform nitrogen-containing compounds from one form to another (Du *et al.*, 2007). Within the air, nitrogen makes up approximately 80% of the gaseous atmosphere as its pure form (N_2). Bacteria in soil can capture this nitrogen source making it available to plants in the form of nitrates. Plants absorb these nitrates and use them as critical building blocks for amino acid production.

Animals are able to access nitrate through eating plants, with green plants such as spinach and lettuce as well as beets being particularly high in nitrates (Wolff and Wasserman, 1972). Nitrogen can also be gained through absorption of dietary amino acids, although in this form it is more likely to be utilised directly in protein production rather than released into other nitrogen compounds (Tessari, 2019). Nitrogen is used throughout the body, primarily for amino acid (protein) production but also contained within molecules such as DNA and RNA and many metabolic co-factors (Bryan and Ivy, 2015). Excess nitrogen is excreted from animals as urea (mammals and amphibians), uric acid (birds and reptiles) or ammonia (fish) via the kidneys. This nitrogen normally returns to the soil where bacteria can catalyse reactions to convert ammonium and ammonia back to nitrate which can be recycled into plants or released back into the atmosphere as N_2 (Fowler *et al.*, 2013).

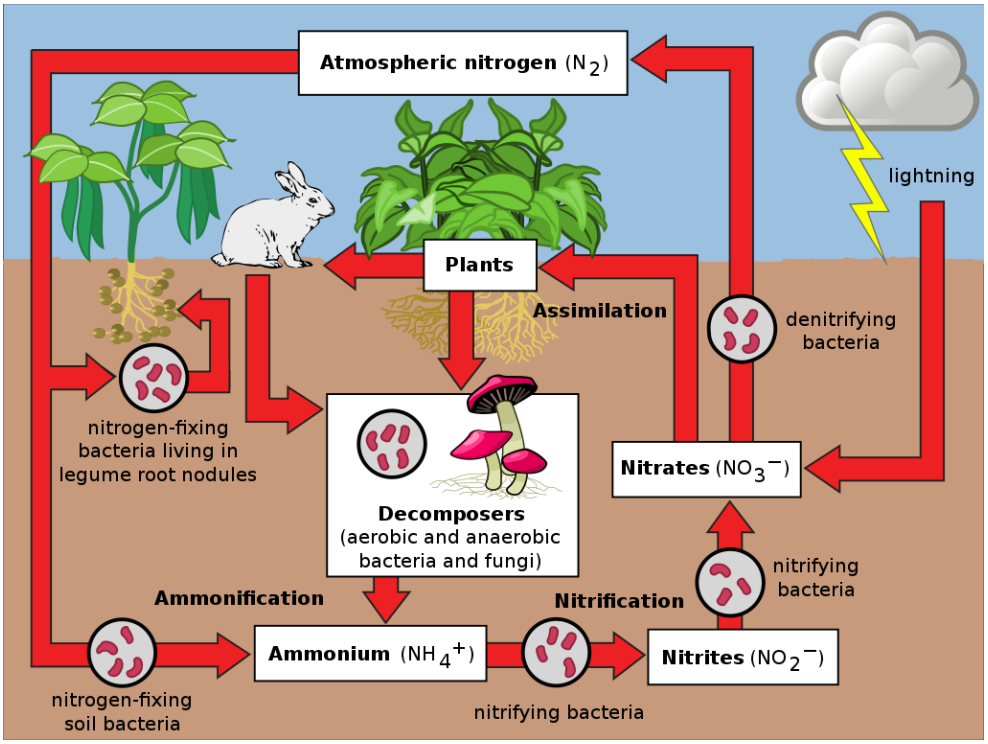

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Figure 1: The Nitrogen Cycle

Throughout this cycle nitrogen takes many forms, including multiple complexes with oxygen or hydrogen (Figure 2). Key oxygen-containing compounds include nitrite (nitrogen with two oxygens) and nitrate (nitrogen with three oxygens). Key hydrogen containing compounds are ammonia (nitrogen with three hydrogen), ammonium (nitrogen with four hydrogens) and the more complex urea (two nitrogen each with two hydrogen and an oxygen joined to a carbon). Bacteria are key to many steps in the interconversion of nitrogen-containing chemicals and there is growing awareness of the importance of bacteria in facilitating the uptake of nitrogen within the human gut (Nnate and Achi, 2016).

Nitrogen for animals is primarily available as nitrate, sourced from plants. However, drinking-water and cured meats can also be important exposure routes for humans. Nitrate enters drinking-water through the release of nitrates from the breakdown of animal waste, nitrogen-based fertilisers, animal feeds and septic systems (Self and Waskom, 1992). Nitrates and nitrites are both used in meat curing, nitrates help keep the colour of the meat whilst nitrites are potent antimicrobial agents that prevent poisoning from bacteria such as *Clostridium botulinum* (Wolff and Wasserman, 1972). Both nitrite and nitrate are biologically active within the human body. A key function of these compounds is in the production of nitric oxide (NO), a potent vasodilator and consequently the amount of nitrite, nitrate and NO is generally tightly regulated from absorption through to excretion (Nnate and Achi, 2016). A summary of the key processes in this cycle are outlined in Figure 2.

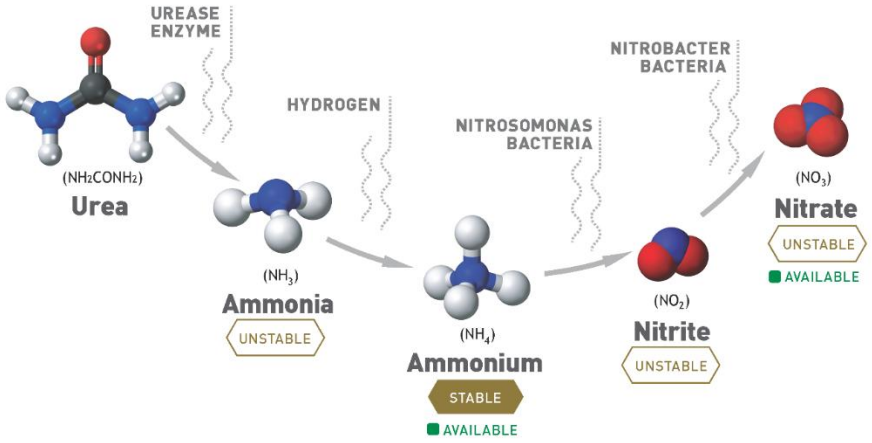

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Figure 2: Structure and conversion of nitrate compounds

2.2 NITRATE AND ITS DERIVATIVES IN THE HUMAN BODY

The interplay of nitrate, nitrite and nitric oxide is complex involving multiple sites within the body, multiple enzymes and a range of symbiotic bacteria. This initial section will focus on nitrate, tracking the absorption, distribution and excretion of this molecule within the human body. Later sections will then discuss in more detail the routes by which nitrate is converted to nitrite and nitric oxide. The biological activity of nitrate, nitrites and nitric oxide within different body systems will be discussed followed by an overview of the nitrosamines which can be formed by the reaction of nitrite with amines in the body (such as those found in food).

2.2.1 Nitrate absorption and excretion

While a proportion of ingested nitrate will pass through the oral cavity unchanged, from as early as the 1960s it was reported that nitrate interacted in some way with bacteria within the oral cavity (Goaz and Biswell, 1961) and in the 1970s it was identified that nitrate could be converted to nitrite within the mouth (Tannenbaum *et al.*, 1976). Subsequent studies using anti-bacterial agents have clearly shown that oral bacteria, located mainly on the tongue, are active in converting nitrate to nitrite (Qu *et al.*, 2016). The source of nitrate available for this conversion reaction can be dietary, but nitrates are also actively excreted from the blood plasma to the mouth via the transporter sialin (Qin *et al.*, 2012; Qu *et al.*, 2016) following rapid absorption through the stomach and small intestine (Bartholomew and Hill, 1984; Eriksson *et al.*, 2018). Studies in human volunteers recorded that stomach concentrations of nitrate rose from 133 µmol/L to 3430 µmol/L within 20 minutes of consuming 50 mL of a 2 mmol potassium nitrate solution. Within one hour gastric nitrate levels had decreased by approximately a third but remained elevated above the fasting baseline concentration for the duration of the 6-hour study (McKnight *et al.*, 1997).

While nitrite is rapidly converted to nitric oxide in the stomach and removed, nitrate has been detected in the small intestinal lumen space of pigs one hour after a bolus injection of 10 mg/kg sodium nitrate (Eriksson *et al.*, 2018). This is corroborated by studies of ileostomy fluid, where samples taken from patients eating a conventional diet had nitrate concentrations ranging from 0-48.2 nmol/g. High nitrate intake did not appear to markedly alter detected levels, as patients on a high nitrate diet recorded nitrate levels between 0-53.2 nmol/g (Radcliffe *et al.*, 1989). Similar values were found in a study of six ileostomy patients (mean nitrate 42 nmol/g) following a variety of meal options (Florin *et al.*, 1990). There is little evidence that ileal levels of nitrate correlate to nitrate ingestion, this suggests that nitrates are both absorbed and excreted across the walls of the ileum via a range of active processes (Eriksson *et al.*, 2018). Different sources of nitrate (vegetable vs supplement) do not seem to differ markedly in their bioavailability but profound inter-individual difference in uptake of nitrate has been noted (James *et al.*, 2015; McIlvenna *et al.*, 2017).

The mechanism of nitrate transport across cellular membranes is not well documented but interactions with iodide, cadmium and chlorine uptake have been reported which may indicate a common transport pathway (Santacroce *et al.*, 2006; Tonacchera *et al.*, 2004). The details of this are not currently known (Jiang *et al.*, 2016; Lévesque *et al.*, 2008).

Following absorption, increased nitrate concentrations have been recorded after 30 to 120 minutes in the liver, kidney and splanchnic circulation system (Eriksson *et al.*, 2018). Maximal plasma nitrate concentrations normally occur between 1 and 3 hours after eating (James *et al.*, 2015). Very little nitrate is considered to make it to the large intestine (colon) (Florin *et al.*, 1990), except under disease conditions (Lopez *et al.*, 2015; Winter *et al.*, 2013). Plasma concentrations of nitrate are normally higher (36.6 ± 18.5 µmol/L) as compared to nitrite levels (6.4 ± 2.2 µmol/L) with significant increases in nitrate, but not nitrite, occurring after eating (nitrate 85.2 ± 15.8; nitrite 6.8 ± 1.9 µmol/L) (Mikiwa *et al.*, 2002). Immense variation in the reported plasma level occurs across individuals and across studies due to both biological and methodological variations (Moshage *et al.*, 1995).

Nitrate is widely and rapidly distributed around the body with substantial bioaccumulation in the liver and skeletal muscle (Gilliard *et al.*, 2018; IARC, 2010; Srihirun *et al.*, 2020). Within muscle tissues, nitrate appears to be retained as a storage vehicle for nitrite and subsequently nitric oxide, which is released under anaerobic conditions to stimulate vasodilation (Srihirun *et al.*, 2020; Wylie *et al.*, 2019). Both the liver and heart are associated

with high rates of nitric oxide production, with the liver in particular containing multiple enzymes involved in the cycling of nitrate, nitrite and nitric oxide (Li *et al.*, 2008). The reason for high constitutive nitric oxide generation in the liver seem to be a protective strategy to prevent liver disease however, chronic over-production is linked to inflammation and tissue damage (Hon *et al.*, 2002; Iwakiri and Kim, 2015).

Within 48 hours of uptake, approximately 60% of nitrate is excreted into the urine via the kidneys (Wagner *et al.*, 1983). Very little nitrate is normally observed in the faeces which indicates the fate of 35-40% of an absorbed dose is not readily accounted for but may be due to storage in different sites around the body (Babateen *et al.*, 2018; Florin *et al.*, 1990; Wagner *et al.*, 1983; Witter *et al.*, 1979). Urinary excretion is relatively rapid, peaking within 4 to 6 hours of eating, and increased dietary uptake is directly linked to increased urinary excretion (Babateen *et al.*, 2018; Pannala *et al.*, 2003). This rapid excretion correlates to a relatively short plasma half-life of 5-6 hours (Lundberg *et al.*, 2008). In contrast, very low levels of nitrite are excreted through the urine (urinary nitrate 1500 µmol/L vs urinary nitrite 0.15 µmol/L) (Kapil *et al.*, 2018). For this reason, nitrite levels can be used as a diagnostic test for urinary infections, detecting nitrites formed by bacteria that have infected the urinary tract (Powell *et al.*, 1987).

A final significant pathway of excretion and reabsorption is the enterosalivary recirculation of nitrate. Up to 25% of circulating nitrate is excreted into the mouth through the salivary glands (Pannala *et al.*, 2003; Qin *et al.*, 2012). This results in an approximately 10-fold increase in salivary nitrate concentrations as compared to the plasma. In human volunteers nitrate concentrations in saliva reached 2600 µmol/L within 2 hours of eating, by contrast plasma concentrations peaked at 179 µmol/L 1 hour after eating (Pannala *et al.*, 2003). The total salivary excretion of nitrate is dependent on factors such as salivary flow rate, age, nitrate concentrations in the diet and time of day (Bos *et al.*, 1988; Eisenbrand *et al.*, 1980; Granli *et al.*, 1989; Mirvish *et al.*, 2000; Xia *et al.*, 2003). It is now well established that an estimated 16% of ingested oral nitrate is rapidly converted to nitrite by bacteria in the mouth, providing health benefits in the mouth and stomach (Duncan *et al.*, 1997; Sánchez *et al.*, 2014; Xu *et al.*, 2001). More details on the biological actions of nitrate-derived compounds are outlined below.

2.2.2 Nitrate and nitrite in the body

Nitrate was long thought to be an inactive waste product with no biological function. This understanding has changed dramatically over the last 20 years and it is now believed that nitrate and nitrite are key signalling and anti-bacterial compounds (Lundberg *et al*., 2008). As potential reservoirs for nitric oxide, nitrate and nitrite may contribute to a multitude of health effects including improved muscle function and protection against heart disease, neurological disorders, diabetes, and obesity (Lundberg *et al*., 2008). Additionally, through a direct anti-microbial effect nitrite is considered an important regulator of gastrointestinal health. The effects of nitrate and nitrite on the oral and gut microbiome are complex but may be key to wider health effects such as cardiovascular health (Rocha and Laranjinha, 2020).

2.2.3 Nitrate to nitrite to nitric oxide

The understanding of nitrate metabolism changed markedly when it was discovered that nitrate, via conversion to nitrite, could produce nitric oxide (Lundberg *et al*., 2008). Nitric oxide is a potent muscle relaxant, with particular action on the endothelial linings of arteries and veins. Although it had been known since the late 1800s that nitrate containing chemicals such as nitroglycerin could prevent heart attacks it wasn't until relatively recently that nitric oxide was identified as the causal agent (Snyder and Bredt, 1992). This is in large part due to its very short half-life in the body (approximately 0.09 seconds under normoxic conditions)

which made detection difficult (Thomas *et al*., 2001). Since its discovery, the scientific understanding of the actions of nitric oxide has developed rapidly, to the point where it was designated as “molecule of the year” by Science magazine in 1992 and researchers Louis J. Ignarro and Ferid Murad were awarded the 1998 Nobel Prize in Physiology or Medicine for their discovery of nitric oxide signalling in the cardiovascular system (Koshland, 1992). It is widely understood that nitric oxide is formed through the action of the enzyme nitric oxide synthase (NOS) with two main forms, inducible (iNOS) and endogenous (eNOS), identified (Snyder and Bredt, 1992). The ultimate source of nitrogen in this pathway is the amino acid L-arginine which is converted to citrulline releasing nitric acid in the final step of the reaction (Figure 4) (Marletta, 1994). Once formed, nitric oxide exerts its actions via binding to guanylate cyclase causing a decrease in cyclic guanosine monophosphate (cGMP) levels in the cell. This has many downstream effects including dilation of blood vessels, neuronal signalling and cell death (Lowenstein *et al*., 1994). Multiple studies have linked controlled nitric oxide production to protection from a wide range of diseases including heart disease, hypertension, diabetes, thyroid disorders, metabolic disfunction, obesity and neurological conditions such as Alzheimer’s disease (Khazan and Hdayati, 2015; Korde Choudhari *et al.*, 2013; Napoli and Ignarro, 2003).

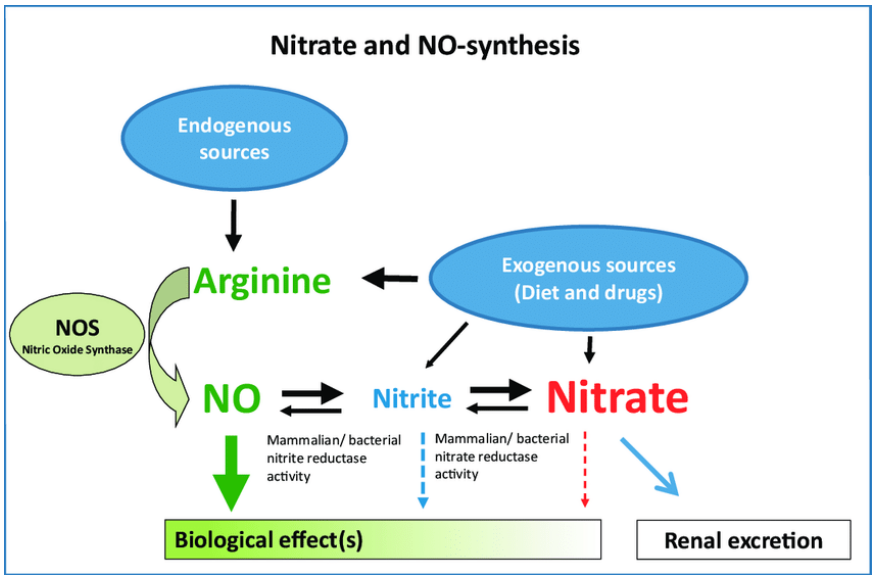


Image from Maas *et al.* (2017)

Figure 3: Interconversion of nitrate compounds in the body

Nitric oxide rapidly decays to nitrite (Lowenstein *et al*., 1994). Therefore, for many years, nitrite was seen purely as a waste product of nitric oxide signalling. Only recently has it become obvious that nitrite can also serve as a source of nitric oxide in a NOS-independent pathway. This alternate pathway has a physiological role in hypoxia and is now regarded as an important feedback system for increasing local blood flow when oxygen tension drops (Kim-Shapiro *et al*., 2006; Lundberg *et al*., 2008). The formation of nitric oxide from nitrite can involve any one of a number of other factors including the enzyme xanthine oxidoreductase, ascorbate, polyphenols or a direct reaction with protons (Lundberg *et al*., 2008). Additionally, haemoglobin and myoglobin have multiple roles. In the deoxy-state they can act as a catalyst for nitric oxide formation – causing vasodilation – but containing a haem-group the molecule also contains a potential binding site for nitric oxide. During the

haemoglobin-based formation of nitric oxide, oxidation of the Fe^{2+} occurs within the haem group forming Fe^{3+} (methaemoglobin). Methaemoglobin has a poor binding affinity for oxygen and must be reduced through the action of methaemoglobin reductase in order to carry oxygen (Bradberry, 2016). This sets up a delicate balance between the formation of nitric oxide to promote vasodilation and over-production causing reduction of haemoglobin to methaemoglobin. Several factors including pH and the conformation of haemoglobin influence this equilibrium and there is much still to be learned about the role of nitrite and nitric oxide in localised hypoxia (Bradberry, 2016; Lundberg *et al*., 2008; Maia and Moura, 2014).

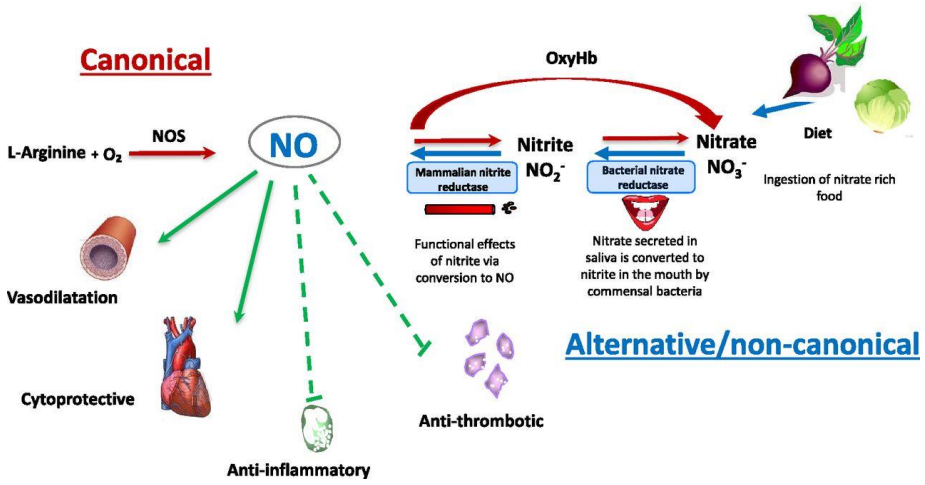


Image from Kapil *et al.* (2020)

Figure 4: Nitric oxide synthase vs haemoglobin based generation of nitric oxide

Within this scheme, both nitrate and nitrite can be viewed as storage forms of nitric oxide, with nitrite having a semi-stabile half-life and nitrate having a longer half-life for longer term retention (Maia and Moura, 2014). This fits well with the developing understanding of nitrate and nitrite storage in muscle tissues (Wylie *et al*., 2019). Athletes have long been aware that high protein dietary supplements improve performance but nitrate supplementation (e.g., through gels or beet concentrates) has been identified as providing benefits equal to protein formulas (Jones *et al*., 2018; McMahon *et al.*, 2017). Recent studies have revealed the presence of sialin nitrate transporters and xanthine oxidoreductase enzymes in muscle suggesting that there is active movement of nitrate and local nitric oxide production is possible (Wylie *et al*., 2019). Mammalian enzymes capable of converting nitrate to nitrite have been identified (Jansson *et al*., 2008; Lundberg and Govoni, 2004), although nitrite is more commonly formed from nitrate through the action of bacterial enzymes within bacteria living symbiotically within the mouth or gut (DeMartino *et al.*, 2019). Intense exercise leading to anaeorbic respiration in muscles causes significant decreases in muscle nitrate concentrations that can be restored following nitrate supplementation (Wylie et al., 2019). The discovery of mammalian enzymes that can convert nitrate to nitrite along with evidence that nitrate and nitrite levels fluctuate with exercise support the concept that these compounds form an important part of a regulatory pathway for hypoxia response (Jansson *et al*., 2008; Kim-Shapiro *et al.*, 2006). Currently work is focused on exercise and skeletal muscle but researchers have proposed that this system may also be relevant to diseases where hypoxia-ischaemia activates damaging cellular pathways (e.g. stroke, complicated neonatal deliveries). This suggests that the biological role of nitrates and nitrites may be

relevant to cellular protection and recovery and may provide a future avenue for novel therapies (Gonzalez *et al*., 2008; Premont *et al*., 2020)

2.2.4 Nitrite in the gastrointestinal system

An estimated 16% of nitrate within the mouth, from food or enterosalivary circulation, is rapidly converted to nitrite. Within the oral cavity bacteria such as *Veillonella* spp., *Staphylococcus aureus*, *Staphylococcus epidermis*, *Nocardia* spp., and *Corynebacterium pseudodiphtheriticum* contain nitrate reductase enzymes capable of catalysing the conversion of nitrate to nitrite (Bryan and Ivy, 2015; Rocha and Laranjinha, 2020; Sato-Suzuki *et al*., 2020). The rate of this conversion is highly variable and depends on individual microbiomes, mouth pH, presence of vitamin C, sex, dietary nitrate intake, and somewhat unusually, outdoor air temperature, with low nitrite production correlating to lower outdoor temperatures (Arnold *et al*., 2021; Duncan *et al.*, 1995; Kapil *et al*., 2018; Xu *et al*., 2001). Infants lack the oral microflora to promote conversion of nitrate to nitrite, this is compensated for by the composition of early breast milk, which contains nitrite in higher concentrations than detected in later milk (16.8 ± 4.5 µmol/L nitrite in colostrum vs 0.3 ± 0.1 µmol/L in mature milk) (Hord *et al.*, 2011; Kobayashi, 2020).

Nitrite is a potent oxidising agent with anti-bacterial properties (Sindelar and Milkowski, 2012). This provides protection within the mouth cavity against oral decay and likely supports the growth of beneficial bacterial species (Rosier et al., 2020). Studies in children show that higher salivary nitric oxide levels, formed from nitrite breakdown, correlate to lower incidence of dental caries (Senthil Eagappan *et al*., 2016). In oral disease, nitrite levels are often increased, which is likely to be a response to higher bacterial loads (Sánchez *et al*., 2014). Effects may be wider than just the oral cavity as changes in the oral microbiome are associated with changes in blood pressure and an increase cardiovascular disease (Briskey *et al*., 2016; Vanhatalo *et al*., 2018; Velmurugan *et al*., 2016). The full mechanism underlying these effects is still the subject of intense research but there is clear evidence that nitrates and nitrites may have more beneficial effects than has been historically recognised.

Once swallowed, there is a synergistic action between the nitrite produced in the oral cavity and the acidic environment in the stomach, which rapidly and efficiently kills a range of potentially pathogenic micro-organisms (Duncan *et al*., 1997). Under acidic conditions nitrite will degrade (both spontaneously and through the action of the NOS enzyme) to nitric oxide, a powerful oxidising agent that inactivates bacteria (McKnight *et al*., 1997). Nitric oxide also, through guanylate cyclase binding, stimulates mucous production which protects the stomach lining and increases gastric blood flow (Brown *et al.*, 1992; Li *et al.*, 2008; Pique *et al.*, 1989). The acidic conditions, particularly the co-presence of ascorbic acid (vitamin C), seem to inhibit production of N-nitroso compounds even when high levels of nitrite are present (Duncan *et al*., 1997; Kobayashi, 2018).

Nitrate is known to reach the ileum, which contains high levels of bacteria (Florin *et al.*, 1990; Radcliffe *et al*., 1989). The scientific understanding of the gut microbiome, and its effects on disease, are advancing rapidly. The term “microbiome” joined common usage in 2001 following its use by Nobel Laurate Joshua Lederberg. In the ensuing 20 years the number of publications in the field has increased dramatically (12,900 between 2013 and 2017) (Cani, 2018). It has been proposed that one of the key ways that a diet rich in fruit and vegetables promotes not just good gut health but good cardiovascular and general health may be through encouraging the growth of nitrate-reducing bacteria (Rocha and Laranjinha, 2020). While nitric oxide within the stomach is seen as largely bactericidal, nitric oxide in the higher pH environments of the intestinal, and possibly colonic, lumen can provide a signalling pathway and nutrient source for bacterial growth (Nnate and Achi, 2016; Rocha and

Laranjinha, 2020). High nitrate levels may promote preferential growth of sulphur-reducing bacteria, which are relatively insensitive to the antibacterial effects of nitrite compounds (He *et al.*, 2010; Marietou, 2016). Additionally, bacterial species such as *Escherichia coli* readily oxidise nitric oxide to nitrate under anaerobic conditions, protecting them from the antibacterial effects of this chemical (Gardner and Gardner, 2002; Vine and Cole, 2011). These findings suggest that nitrate levels could change the composition of the gut microbiome through imposing selective pressure within the growth environment (Nnate and Achi, 2016; Rocha and Laranjinha, 2020). As it is currently unclear exactly how the microbiome affects disease states and cardiovascular health the impacts of ongoing selection pressures causing changes in bacterial species over time cannot be determined.

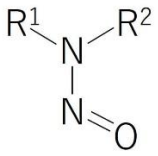
Interestingly, one study has demonstrated that disturbances in the oral bacterial species could affect colon health through changes in nitric oxide signalling (Gangula *et al*., 2015), while multiple studies have identified a dual role of nitric oxide in colon cancer depending on its concentration (pro-carcinogenic or anti-carcinogenic) (Monteiro *et al.*, 2019; Oláh *et al.*, 2018; Thomas and Wink, 2017; Voss *et al.*, 2019). Most authors suggest that nitric oxide acts as a promoter of cancer growth through its action as a vasodilator, when produced by cancerous cells it increases blood flow to tumours, thereby stimulating growth (Voss *et al*., 2019). Furthermore, many colon cancers are associated with chronic inflammation and higher nitrite and nitric acid production and other contaminants such as polycyclic aromatic hydrocarbons (Diggs *et al.*, 2011), heterocyclic amines (Helmus *et al.*, 2013) and alcohol (Rossi *et al.*, 2018). The source of the nitric oxide detected may be from plasma nitrite through the action of neutrophils rather than purely dietary (Grisham *et al*., 1992). In addition to changes in the microbiome, this may provide a second mechanism whereby nitrate concentration could affect gut health. The relative importance of the microbiome and vasodilation in maintaining a healthy gut and the wider effects of gut health on cardiovascular disease is a rapidly developing field, therefore, the role of nitrate and nitrite within this cannot be conclusively determined.

2.3 NITROSAMINES

A full discussion of all the nitrosamine compounds and their carcinogenic potential is well beyond the scope of this review. Therefore, this section will focus largely on the absorption, metabolism, distribution and elimination of the nitrosamine class of chemicals. As this class contains hundreds of possible chemicals, with over 20 commonly identified in food, this review will focus on the collective evidence as to how nitrosamines act in the body.

Nitrosamines are a varied class of compounds that all contain the chemical moiety N-N=O with various additional groups bound to the first nitrogen (Figure 5). They are present in foods that have been treated with nitrites and can also be formed in the body through the reaction of nitrites in saliva with amines, amides and carbamates (National Toxicology Program, 2016). Nitrosamines are also found in high concentrations in cigarette smoke, agricultural chemicals, drugs, solvents, plastics and in the general environment (National Toxicology Program, 2016; Robles, 2014). Animal studies have identified that *N*-nitrosodimethylamine administered in the diet causes liver tumours in rats (dose 50 ppm (=50 mg/L) up to 42 weeks) but not rabbits (escalating dose 20 ppm to 50 ppm over 24 weeks) (Magee and Barnes, 1956). Subsequently, it has been shown that several nitrosamine compounds are mutagenic, binding to DNA and causing sequence changes, via a pathway involving the metabolic dealkylation of the parent compound forming reactive products (IARC, 2000; Yang and Smith, 1996). This mechanism causes remarkable tissue-specific variations across the various nitrosamine compounds as each is preferentially metabolised by specific enzymes within a particular organ or tissue (Wiestler *et al.*, 1987;

Yang and Smith, 1996). The discovery of these compounds and their carcinogenic potential lead to research on nitrosamine-related carcinogenesis and general advice to limit consumption of nitrite-preserved meats (Sindelar and Milkowski, 2012).



Created by Eurofins (2021)

Figure 5: General structure of the nitrosamines

Both exogenous and endogenous formation of nitrosamines are important in terms of potential cancer outcomes. Endogenous formation is linked to nitrite, but not nitrate, levels and is strongly inhibited by other components in food including ascorbic acid (vitamin C) and polyphenol antioxidants (Lee *et al.*, 2006; Tannenbaum *et al.*, 1991). Within the gastric juice, various nitrosamines have been detected including the well-documented carcinogen *N*-nitrosodimethylamine (NDMA). In a study of gastric fluid samples from 71 by-pass surgery patients, mean NDMA levels were approximately 3.1 nmol/L, with total nitrosamine concentrations averaging 4.84 nmol/L but with significant inter-individual variability (Dallinga *et al.*, 1998). The concentration differences between individuals was shown to correlate to gastric pH but not salivary nitrate or nitrite concentrations (Dallinga *et al.*, 1998). There were no sex-dependent effects noted, in contrast to evidence that nitrite production in saliva varies between males and females (Xu *et al.*, 2001). Similar correlations, whereby pH is the strongest predictive factor for nitrosamine formation and no observed relationship to sex, have also been noted in a range of other studies (Lintas *et al.*, 1982; Matsuda *et al.*, 1990; Reed *et al.*, 1981; Xu and Reed, 1993). Exogenous nitrosamines present in food due to preservation methods are variable but have been detected at levels of approximately 10µg/kg in bacon and up to 70µg/L in some German beers (Lijinsky, 1999). There is debate as to whether the combination of exogenous and endogenous exposure provides sufficient exposure to pose a significant health risk in humans (EFSA, 2017; Johnson *et al.*, 2021).

Research studies in dogs suggest that nitrosamines can be absorbed directly from the stomach whereas rat studies tend to show uptake from the small intestine (Gomez *et al.*, 1977; Lintas *et al.*, 1982; Richter *et al.*, 1986). In humans, evidence favours absorption from the small intestine (Pegg, 1980). In all species, some degree of first pass metabolism occurs but this is highly variable depending on the exact chemical agent. NDMA has been shown to undergo metabolism in the liver prior to systemic absorption but studies on *N*-nitrosodiethylamine (NDEA) in rats showed very low first pass clearance (Graves and Swann, 1993; Pegg, 1980). These differences are consistent with the observation that metabolism of the nitrosamines can vary and can lead to tissue specific sensitivity to carcinogenic metabolites. The high first pass metabolism of NDMA and the development of liver tumours following administration is entirely consistent with the described mechanism of carcinogenesis. Ethanol (alcohol) is widely accepted to interact with nitrosamines and in many cases accentuates any negative effects of the nitrosamine compounds, particularly NDMA (Papp-Peka *et al.*, 2017; Schwarz *et al.*, 1983; Swann *et al.*, 1984). There is evidence that this is due to changes in the first pass metabolism kinetics, with ethanol possibly inhibiting the enzymes that are key to nitrosamine removal in the liver or possibly inducing the enzymes involved in NDMA activation (Mori *et al.*, 2002; Swann *et al.*, 1984). Timing of

ethanol ingestion is likely to be a critical factor, as the enzyme CYP2E1 has been widely shown to be involved in the metabolism of NDMA to a carcinogenic metabolite (Mori *et al.*, 2002; Pegg, 1980). Ethanol has a strong affinity for the CYP2E1 active site meaning it will readily compete with most other substrates if present concurrently. Ethanol, like many CYP2E1 substrates, will induce the action of this enzyme over time and therefore increased metabolism may occur following, but not during, ethanol consumption (Cederbaum, 2006).

Once within the body, nitrosamines are distributed widely and, depending on the exact compound, may be metabolised in a range of tissues. Most of these activation reactions are catalysed by members of the cytochrome P450 (CYP450) enzyme superfamily with CYP2E1 having a prominent role, particularly in tissues such as the liver where it is present at high concentrations (Yang and Smith, 1996). Urinary studies have been able to detect significant quantities of nitrosamine parent compound however there is no obvious direct link between dietary nitrate or nitrosamine intake and nitrosamine excretion (Levallois *et al.*, 2000; Vermeer *et al.*, 1998; Zhao *et al.*, 2019; Zhao *et al.*, 2020).

Given that nitrosamines are thought to be well absorbed through the small intestine, it seems unlikely that they could be present in high concentration within the colon. However, nitrosamines have been detected in the faeces which is likely to be due to bacterial formation of nitrosamines within the colon (De Roos *et al.*, 2003a; Hughes *et al.*, 2001; Rowland *et al.*, 1991). Given the presence of bacteria with capacity to carry out this reaction, sufficient nitrogen-based substrates and a possible lack of inhibiting substances such as ascorbic acid, it is possible that the colon is a major site of endogenous nitrosamine production, however quantification remains challenging. This is further complicated by evidence that neutrophils may actively produce nitrosamine compounds (Grisham *et al.*, 1992; Vermeer *et al.*, 2004). While studies have shown that increased nitrosamine concentrations are associated with colon cancer, the changes in both gut microbiome and inflammation during this disease state may confound the data making the actual causes and effects of the cancer difficult to elucidate (Rastogi *et al.*, 2020; Terzić *et al.*, 2010).

2.4 DIFFERENCES BETWEEN FOOD AND WATER, MECHANISTIC PERSPECTIVES

From the above summary it is clear that there are additional factors, beyond the consumed concentration of nitrate, that can affect the metabolism and uptake of these compounds. The most obvious is the presence of ascorbic acid which affects the rate of nitrate to nitrite production in the mouth and the formation of nitrosamine compounds. Factors such as gastric pH and plant polyphenols also lead to significant variation in these parameters resulting in inter-individual, inter-species, and temporal variations in nitrite and nitrosamine production (Xu *et al.*, 2001). It is possible that nitrite and nitrosamine concentrations in the gastrointestinal tract could be greater after exposure to nitrate from drinking-water, rather than food, as antioxidant compounds are less likely to be present. This effect would only be expected with consumption of drinking-water as a beverage (on its own), not water within fruit drinks or coffee/tea which contain a range of polyphenols and antioxidants (Burkholder-Cooley *et al.*, 2016). Additionally, exposure to nitrate in drinking-water is likely to be constant, while exposure to nitrate from food is likely be more variable, depending on dietary preferences and season (Brkić *et al.*, 2017).

Furthermore, as more is learned about the inter-relationship of nitrite and nitric acid, it is plausible that high nitrite and nitrate from food and water could result in chronic elevations of nitric oxide in the gut lining. Nitric oxide may facilitate cancer growth through vasodilation and increased blood supply, but its antiproliferative and cytotoxic activity may also inhibit tumour progression, depending on the local nitric oxide concentration (Monteiro *et al.*, 2019).

Whether this occurs only in established cancers mediated through elevated iNOS enzyme activity or if external nitric oxide could promote cancer growth is not clear. However, it is a theoretical possibility that chronic elevated nitrate in the gut could support more aggressive cancers even if the initial causal factor was not nitrate-derived.

Considering that food has higher antioxidant potential, causes lower pH conditions in the stomach, and releases intermittent nitrates, a hypothesis could be proposed whereby high-nitrate drinking-water creates more favourable conditions for high nitrite and nitrosamine production and potential cancer initiation and growth. However, this hypothesis depends on exposure to nitrates in food and drinking-water being considered as physiological separate. The plausibility of this separation will be explored in the second section of this report.

2.5 SUMMARY

Far from being just waste products, nitrate and nitrite are biologically active molecules with a range of physiological effects. Nitrate is rapidly absorbed from the body, mobilised, and excreted but also actively stored within tissues such as skeletal muscles. The conversion of nitrate to nitrite produces a potent bactericidal agent that assists in maintaining good oral and gut health predominantly through the formation of nitric oxide. Through this nitric oxide pathway, nitrite may also facilitate vasodilation not just in the gut but also around the body. There is emerging evidence that this facilitates recovery from exercise and possibly prevents damage from reperfusion following hypoxic-ischemic injury. Additionally, nitrate and nitrite may markedly alter the gut environment and thereby impact on the composition of the oral and gut microbiome, the relevance of this is yet to be determined.

It is clear from this work that nitrates are part of multiple dynamic and active systems. As such it is expected that absorption and excretion will be closely regulated however, this is currently largely unknown which means it is difficult to decipher how much perturbation the system can withstand. As with all chemicals, homeostasis will require a balance between too little and too much but the extent of the window between these extremes is still to be discovered.

3. NITRATE EXPOSURE - METHODS

For dietary exposure to chemicals, exposure can be defined as:

$$E_i = \Sigma \frac{Q_{i,k} \times C_{i,k}}{bw_i}$$

Where E_i is the exposure of individual i to some chemical at some specified point in time, $Q_{i,k}$ is the amount of food k consumed by individual i, $C_{i,k}$ is the concentration of the chemical of interest in food k consumed by individual i and bw_i is the body weight of individual i. For deterministic (point) estimates of exposure these parameters (concentration, food consumption and body weight) are represented by population averages or selected percentiles. For dietary modelling, food consumption and body weight will be represented by actual reported values for an individual on one particular day or on several days, depending on the structure of the dietary survey.

3.1 CONCENTRATION DATA – NITRATE IN FOOD

While acute nitrate exposure has been proposed as a risk factor for methaemoglobinaemia, the current study is concerned with the plausibility of nitrate exposure as a risk factor for colorectal cancer. In this context, it is the ongoing, long-term (chronic) exposure to nitrate that is of interest, rather than instances of particularly high exposure. For chronic dietary exposure to chemicals the concentration metric of interest is a measure of central tendency, such as the mean or median concentration (FAO/WHO, 2009). For the current exercise mean concentrations were used.

A survey of nitrates in selected New Zealand foods was carried out in 2003-2004 (Thomson *et al.*, 2007). A summary of the survey results are included in Table 1.

Table 1. Nitrate in selected New Zealand foods

Food	Number of samples	%LC	Nitrate concentration (mg NO_3/kg)	
			Mean[a]	Range
Processed foods				
Bacon	10	10	26.8	<4-59
Ham	10	10	12.3	<4-23
Saveloys	10	0	20.8	5.8-46
Luncheon	10	0	22.5	16-39
Salami	10	20	18.5	<4-41
Beef sausages	10	90	3.0	<4-13
Pizza	10	20	4.7	<4-9.5
Corned silverside	10	0	13.2	5.1-26
Hamburger	4	0	57.6	4.4-154

Food	Number of samples	%LC	Nitrate concentration (mg NO_3/kg)	
			Mean[a]	Range
Beef mince	4	75	4.1	<4-11
Cottage cheese	4	75	3.0	<4-6.6
Dip, creamed cheese based	4	100	1.8	all <4
Cheddar cheese	4	100	1.8	all<4
Green vegetables				
Cabbage	8	0	241	88-500
Lettuce	18	0	1160	61-2500
Silverbeet	8	0	540	140-1230
Watercress	6	0	1196	630-2040
Celery	8	0	1174	640-1690
Broccoli	8	0	97	37-200
Spinach	8	0	722	73-1140
Beetroot, canned	8	0	557	190-1620
Other vegetables				
Potato	8	0	94	35-175
Carrot	8	25	43	<4-210
Pumpkin	8	50	48	<4-255

%LC: percent left-censored, the percentage of the analysed samples with results below the limit of detection (4 mg/kg)

[a] When not all analytical results were above the limit of detection (LOD), the mean was calculated by substituting a value of LOD/2 for results below the LOD

While it is likely that the New Zealand survey included the major contributors to dietary nitrate exposure, it did not include all contributors. For example, a study conducted by Food Standards Australia New Zealand (FSANZ) included 52 foods found that fruits contributed up to 30% of dietary nitrate exposure for 2-5 year olds (FSANZ, 2011). The nitrate content of plant materials can be quite variable and it is important that any external resources used to 'fill in the gaps' for a New Zealand dietary exposure assessment are consistent with the available information concerning nitrate content of New Zealand foods.

A summary of nitrate concentration data from all studies considered is given in Appendix 1. In order to identify studies with similar concentration profiles to New Zealand, a subset of foods for which nitrate concentrations data were frequently available were selected (cabbage, carrot, celery, lettuce, potato, silverbeet, spinach and watercress). Studies were compared with the New Zealand study in terms of the correlation coefficient between concentrations and the different between values, as assessed by the root mean square (RMS) of the differences in concentrations. This analysis identified three studies with

correlation coefficients greater than 0.8 and RMS less than 200 (Larsson *et al.*, 2011; Menard *et al.*, 2008; Ysart *et al.*, 1999).

The following rules were used to provide mean nitrate concentration data for the current study:

- If New Zealand data were available for the food, those data were used preferentially
- If no New Zealand data were available for the food, but data were available from one of the correlated studies then that concentration was used for the current study
- If no New Zealand data were available for the food, but data was available from more than one of the correlated studies the mean concentration across the studies was used for the current study

This approach identified concentration data for an additional 26 food items, as shown in Table 2.

Table 2. Nitrate concentration data used for the current study for plant commodities

Commodity	Mean nitrate concentration (mg/kg)				
	New Zealand (Thomson *et al.*, 2007)	Sweden (Larsson *et al.*, 2011)	France (Menard *et al.*, 2008)	UK (Ysart *et al.*, 1999)	Study value
Apple			23		23
Artichoke			16		16
Asparagus			112		112
Avocado			1256		1256
Beetroot	557	486	644	1211	557
Broccoli	97	301	442		97
Brussels sprout			24	59	42
Cabbage	241	379	498	338	241
Carrot	43	165	121	97	43
Cauliflower		139	214	86	146
Celeriac			613		613
Celery	1174		1241		1174
Chicory			135		135
Chinese cabbage		899			899
Cucumber		179	192		186
Eggplant			284		284
Endive			534		534
Fennel			1043		1043
Figs			72		72
Garlic			121		121
Green beans			449		449
Green pea			21		21
Leek		535	410		473
Lettuce	1160	1271	1974	1051	1160
Mushrooms			117		117

Commodity	Mean nitrate concentration (mg/kg)				
	New Zealand	Sweden	France	UK	
	(Thomson *et al.*, 2007)	(Larsson *et al.*, 2011)	(Menard *et al.*, 2008)	(Ysart *et al.*, 1999)	Study value
Onions			65	48	57
Parsley			1980		1980
Pepper (capsicum)			159		159
Potato	94	47	192	155	94
Pumpkin	48				48
Radish			1861		1861
Silverbeet/Chard	540		1682		540
Sorrel			3		3
Spinach, fresh	722	661		1631	722
Strawberry			78		78
Swede				118	118
Sweet potato			717		717
Tomato		4	18	17	13
Turnip			657		657
Watercress	1196		1877		1196
Zucchini			559		559

3.2 NITRATE IN WATER

Exposure to nitrate from food will only be moderately influenced by geographical location, with foods originating from various countries and various areas of New Zealand. In contrast, the nitrate content of drinking-water has a strong geographical component. However, the food consumption information used for the current study does not identify the geographical location of respondents. To assign a realistic nitrate concentration to water consumption, a plausible distribution of nitrate concentrations for New Zealand drinking-water supplies was established and sampled randomly to assign a nitrate concentration to each nitrate exposure scenario.

In association with the earlier New Zealand study, a national set of drinking-water nitrate concentrations was extracted from the national drinking-water database (Water Information New Zealand) (Thomson *et al.*, 2007). The data set included 1021 nitrate measurements covering 571 public water supplies. The mean, median and 95th percentile nitrate concentrations were 4.8, 1.1 and 21.6 mg NO_3/L, respectively. It was noted that these results are likely to be conservative, as they represent drinking-water sources for which there was an expectation that nitrate concentrations would be elevated. While these data are now moderately old (15 years), a longitudinal analysis of drinking-water nitrate levels carried out by the Taranaki Regional Council did not suggest changes over a 10-year monitoring period (TRC, 2014).

A more recent survey determined nitrate content of 135 source waters and 75 distribution zones in Canterbury, Hawkes Bay, Southland and Waikato (Nokes and Ritchie, 2018). The mean, median and 95th percentile nitrate concentrations were 5.0, 2.3 and 17.3 mg NO_3/L. Again, this survey focussed on areas and water types (groundwater) where impacts from agriculture were suspected and these summary statistics are likely to represent a conservative situation.

Combining these two data sets gives summary statistics of 4.8, 1.3 and 20.2 mg/L for the mean, median and 95th percentile, respectively.

It should be noted that these two data sets include data from registered water supplies and will not include information unregistered water supplies such as private wells and roof water. It has been estimated that up to 14% of New Zealanders may receive their drinking-water from unregistered supplies (Cressey and Horn, 2016). While there is no systematic source of information on these unregistered supplies, an unpublished study carried out in the lower North Island found that 59% of unregistered supplies were roof water, 37% were groundwater and the balance were surface water (Dr Stan Abbot, Massey University, unpublished). Roof water will contain negligible amounts of nitrate.

Another New Zealand study consolidated information on nitrate in drinking-water from a number of sources, including district council registered supplies, private registered supplies, investigator's own testing in Southland and three unregistered supplies (Richards, 2020). Although overall summary statistics were not provided, the study estimated approximately 60% of New Zealanders were receiving drinking-water from supplies containing 2.0 mg NO_3/L or less, while approximately three-quarters of the population were receiving water containing 5.0 mg NO_3/L or less. For the data set used in the current study 73.1% of nitrate values contained 5.0 mg NO_3/L or less.

In order to put the current analysis in the context of epidemiological studies that have been carried out on associations between drinking-water nitrate and colorectal cancer, the similarity of New Zealand drinking-water nitrate levels to epidemiological study levels was investigated. Table 3 provides available summary information from the epidemiological studies. For comparison, the combined New Zealand dataset described above has quartile break points of 0.31, 1.3 and 5.8 mg/L and quintile break points of 0.11, 0.84, 2.2 and 7.6 mg/L. It should be noted that the New Zealand data set is not strictly comparable to the data sets from the epidemiological studies, as it considers drinking-water supply nitrate content as opposed to individuals' drinking-water intake nitrate content.

Table 3. Nitrate levels in drinking-water in literature epidemiological studies

Country	Year	Nitrate (mg NO_3/L)	Derived summary statistics (mg NO_3/L)	Reference
Denmark	1978-2011	Quintiles: <1.27 1.27-2.33 2.33-3.87 3.87-9.25 >9.25	Median in range 2.33-3.87	(Schullehner *et al.*, 2018)
Netherlands	1986	Quintile means[a]: 0.013 1.1 2.6 4.6 11 Overall mean = 3.9	Mean 3.9	(van Loon *et al.*, 1998)
Spain/Italy	2008-2013	Categories (% of cases in category)[a]: <3.3 (42) 3.3-6.7 (24) >6.7 (34) Alternative categories (% of cases in category)[a]: ≤2.9 (38) >2.9 (62)	Median in range 3.3-6.7	(Espejo-Herrera *et al.*, 2016)

Country	Year	Nitrate (mg NO_3/L)	Derived summary statistics (mg NO_3/L)	Reference
USA (Iowa)	1986	Quartiles: <1.6 (mean = 0.9) 1.6-4.4 (mean = 3.1) 4.5-10.9 (mean = 8.5) >10.9 (mean = 24.8)	Median 4.4, mean 9.3	(Weyer *et al.*, 2001)
USA (Wisconsin)	1994	Categories (% of cases in category): <2.2 (31) 2.2-8.4 (22) 8.9-26.1 (29) 26.6-43.8 (12) >44.3 (7)	Median in range 2.2-8.4	(McElroy *et al.*, 2008)
USA (Iowa)	1986-1989	Categories (% of cases in category): ≤4.4 (46) >4.4-≤13.3 (30) >13.3-≤22.1 (8) >22.1 (16)	Median in range 4.4-13.3	(De Roos *et al.*, 2003b)
USA (Iowa)	1986-2010	Quintiles: <1.7 1.7-3.5 3.6-6.0 6.0-15.5 >15.5	Median in range 3.6-6.0	(Jones *et al.*, 2019)

[a] Quintile means/categories are expressed as 'mg/day'. These were converted to water concentrations assuming a mean daily water consumption of 1.5 L.

3.3 FOOD AND WATER CONSUMPTION DATA

3.3.1 National Nutrition Survey (NNS) records

Periodic national nutrition surveys (NNSs) are carried out in New Zealand. The most recent are the 2008-2009 Adult Nutrition Survey (2009ANS) covering adult New Zealanders, aged 15 years and over (University of Otago and Ministry of Health, 2011) and the 2002 National Children's Nutrition Survey (2002CNS) covering New Zealand children aged 5-14 years (Ministry of Health, 2003). No nationally-representative survey of the food consumption patterns of children less than 5 years of age has been conducted in New Zealand.

These two surveys include 24-hour dietary recall records (24HDR). These include a complete listing of all foods and beverages consumed by an individual during one 24-hour period. Days of the week and time of year are randomised across the survey to avoid bias due to these factors. The 2009ANS contains 24HDR records for 4,721 respondents and the 2002CNS contains 24HDR records for 3,275 respondents. The surveys also carried out a second 24HDR for a proportion of the respondents.

3.3.2 Mapping of NNS records to nitrate-containing foods and beverages

The NNSs contain almost 11,000 unique food and beverage descriptors. In order to estimate the nitrate concentration of each of these items it is necessary to map the items for which nitrate concentrations are available to the list of unique NNS food and beverage descriptors. Three situations arise:

- The food or beverage for which nitrate concentration information is available is sufficiently similar to the NNS food descriptor to allow direct application of the determined nitrate concentration;
- The NNS food or beverage is unrelated to any food or beverage for which nitrate concentration information is available and is unlikely to contain appreciable nitrate. Such foods and beverages are assumed to have a nitrate concentration of zero; or
- The NNS food or beverage is similar to or contains (as part of a recipe) one of the foods or beverages for which nitrate concentration information is available. Given the focus of the current study it was particularly important to identify foods or beverages that, when consumed, would contain an appreciable proportion of tap water

3.4 CHRONIC DIETARY EXPOSURE ASSESSMENT

The food and beverage mapping was used to assign a mean nitrate concentration to all instances of consumption of relevant foods and beverages reported in the 24HDR components of the 2002CNS and 2009ANS. Concentration values were multiplied by the food consumption amount and summed for each NNS respondent to give 3275 (2002CNS) and 4721 (2009ANS) individual estimates of daily dietary nitrate exposure Dietary exposure estimates were divided by the respondent's body weight. Where body weight information was not available for a respondent (missing data) the mean body weight for the age and gender from the remainder of the data set was substituted. All calculations were carried out using Microsoft Excel.

Dietary risk assessments should consider vulnerable populations (FAO/WHO, 2009). While no specific at risk populations have been identified for the association of nitrate exposure to colorectal cancer, consumers of large amounts of nitrate-containing foods and beverages will have greater exposure (high consumers). For the current study, a high consumer was defined as an individual with dietary exposure to nitrates at the 95th percentile, based on 24-hour dietary recall food consumption information.

3.5 ESTIMATION OF USUAL DIETARY EXPOSURE TO NITRATE

While the 24HDR records provide a very good record of the food and beverage intake and resultant dietary exposure to nitrate by an individual on a particular day, this is not the same as the individual's habitual long-term (usual) food intake and may include consumption of foods rarely eaten by the individual or exclude foods commonly eaten by the individual. This will mean that any dietary exposure estimate based on 24HDR records may not be a true representation of habitual exposure for an individual. While the mean of dietary exposures derived from single day 24HDR are likely to be good estimates of the true mean, it is expected that the variability in dietary exposure derived from 24HDR records will be greater than the true population habitual dietary exposure variability, as it will include both between person variability (inter-person) and within person variability (intra-person) (Dodd *et al.*, 2006; Hoffmann *et al.*, 2002; Nusser *et al.*, 1996). Between-person variability is the parameter of interest for risk assessment associated with chronic exposure, as is the case for nitrate.

For the 2009ANS and 2002CNS, 24HDR dietary information was collected on a second day for approximately 15% of respondents. These duplicate days can be used to estimate intra-person variability and correct the overall estimate of exposure variability to only represent inter-person variability (Dodd *et al.*, 2006; Hoffmann *et al.*, 2002; Nusser *et al.*, 1996).

3.6 DETAILED ANALYSIS OF WATER CONSUMPTION

Two further analyses were carried out on drinking-water servings within the food consumption databases. Servings were analysed to determine whether they related to drinking-water as a beverage or drinking-water as an ingredient in a beverage (tea, coffee, etc.) or food (stews, casseroles, etc.). Servings that were determined to represent consumption of drinking-water as a beverage were further analysed to determine their temporal proximity to non-water foods. The 2009ANS data set does not include the time of each food consumption event and for this analysis an older 1997 NNS data set was used (Russell *et al.*, 1999). It was assumed that general patterns of food and beverage consumption would not have changed appreciably between the two studies.

Microsoft Excel functions were used to carry out these analyses.

3.7 RISK CHARACTERISATION

Safety evaluations have been carried out for nitrate in food (EFSA, 2008; JECFA, 2002a) and drinking-water (WHO, 2011). JECFA concluded that the pivotal observed toxic effects of nitrate are consequent on its conversion to nitrite *in vivo.* JECFA retained the acceptable daily intake (ADI) of 0–5 mg/kg bw expressed as sodium nitrate, or 0–3.7 mg/kg bw, expressed as nitrate ion, established at its forty-fourth meeting in 1995. JECFA derived the ADI from the no observed adverse effect level (NOAEL) for nitrate in a long-term rat study (370 mg/kg bw per day) and application of a 100-fold uncertainty factor.

JECFA also derived an ADI based on the proportion of nitrate converted to nitrite in humans was, taken as 5% for the average individual and 20% for those with a high level of conversion, and the NOAEL for nitrite (6 mg/kg bw per day, expressed as nitrite ion) to calculate a 'transposed' NOAEL for nitrate, expressed as nitrate ion. The resulting NOAELs were estimated to be 160 and 40 mg/kg bw per day for average and high responders, respectively. As these figures were derived in part from data on human pharmacokinetics, rather than animals, use of an uncertainty factor of less than 100 was considered to be justified. Using the conversion rate for a normal person and an uncertainty factor of 50 an ADI of 0-3.2 mg/kg bw per day could be derived. As the two approaches resulted in very similar ADIs, JECFA retained the previously established ADI.

In their 2008 evaluation, EFSA noted that no new data had been identified that would require a revision of the ADI (EFSA, 2008).

The assessment of nitrate in drinking-water carried out by WHO also focussed on the effects of nitrite formed from reduction of nitrate and the ability of nitrite to oxidise haemoglobin (Hb) to methaemoglobin (metHb) (WHO, 2011). Methaemoglobin is unable to transport oxygen to the tissues and if metHb concentrations reach 10% of Hb levels a condition called methaemoglobinaemia may occur, characterised by cyanosis and, at higher metHb concentrations, asphyxia.

WHO derived a guideline value for nitrate in drinking-water of 50 mg/L, based on epidemiological evidence for occurrence of methaemoglobinaemia in infants. Based on an infant body weight of 5 kg and drinking-water ingestion of 0.75 L/day this equates to a NOAEL of 7.5 mg/kg bw per day or about twice the ADI.

A conservative approach was taken, with the ADI of 0-3.7 mg/kg bw per day used for risk characterisation in the current study.

4. NITRATE EXPOSURE - RESULTS

4.1 DIETARY EXPOSURE

Table 4 gives summary statistics for the estimates of nitrate exposure from consumption of foods and beverages.

Table 4. Oral exposure to nitrates from food and beverages, New Zealand adults (15+ years) and children (5-14 years)

Parameter	Adults (15+ years)	Children (5-14 years)
ADI (mg/kg bw per day)	0-3.7	
Number of respondents	4721	3275
Mean nitrate exposure (mg/kg bw per day), single day, weighted by survey weights	0.82	0.86
Median nitrate exposure, single day (mg/kg bw per day)	0.45	0.54
95th percentile nitrate exposure, single day (mg/kg bw per day)	2.58	2.58
Mean nitrate exposure (mg/kg bw per day), usual intake, weighted by survey weights	0.82	0.88
Median nitrate exposure, usual intake (mg/kg bw per day)	0.74	0.82
95th percentile nitrate exposure, usual intake (mg/kg bw per day)	1.64	1.63

Table 4 shows little difference in dietary exposure to nitrate between adults and children. This is somewhat unusual, as children generally have higher exposure to chemicals in foods, due their greater food consumption, on a body weight basis. However, other studies of dietary exposure to nitrates have noted similar minor differences between estimated dietary exposures for adults and children. A French study derived lower-upper bound mean estimates of nitrate exposure for adults (>15 years) and children (3-14 years) of 1.5-1.5 and 1.9-2.0 mg/kg bw per day, respectively (Menard *et al.*, 2008).[1] An Italian study estimated dietary exposure to nitrate from consumption of leafy vegetables only for adults and 4-6 years girls (De Martin and Restani, 2003). Estimates of dietary exposure were 1.1 and 0.73 mg/kg bw per day for adults and young girls, respectively. A Spanish study estimated lower-upper bound dietary exposure to nitrate for adults and young people (6-15 years) of 0.27-

[1] Lower bound estimates of dietary exposure are derived by assigning a value of zero to analytical results below the limit of detection (LOD). Upper bound estimates are derived by assigning a value equal to the LOD to analytical results below the LOD. If few analytical results are below the LOD then the lower and upper bound dietary exposure estimates will be very similar.

0.28 and 0.36-0.38 mg/kg bw per day (Quijano *et al.*, 2017). It is likely that the modest difference between dietary nitrate exposure for adults and children is at least partially due to lower consumption of vegetables by children.

While the current study does not include all potential sources of nitrate in the diet, if is expected to include all major sources of nitrate. The dietary exposure assessment carried out by FSANZ employed a total diet approach and included a diverse array of commonly consumed foods (FSANZ, 2011). Foods not included in the current study were only minor contributors to dietary nitrate exposure.

Table 5 provides a comparison of estimated nitrate exposures from the current study to other estimates available in the scientific literature.

Table 5. Comparison of international estimates of nitrate exposure

Country	Cohort	Includes drinking-water	Mean (high percentile)[a, b] exposure, mg/kg bw per day	Main contributing food	Reference
New Zealand	Adults (15+ years) Children (5-14 years)	Yes	0.82 (1.64) 0.88 (1.63)	Potato, lettuce	Current study
New Zealand	Adults (15+ years)	Yes	0.52	Green vegetables, other vegetables	(Thomson *et al.*, 2007)
Australia	9 months 2-5 years 6-12 years 13-16 years 17+ years 16-44 years female	Yes	1.1 (2.2)[c] 1.5 (2.6) 1.0 (1.9) 0.73 (1.4) 0.73 (1.5) 0.73 (1.5)	Lettuce, stalk and stem vegetables, root vegetables	(FSANZ, 2011)
China (north)	General population	Only vegetables considered	7.0[d]	Celery, Chinese cabbage	(Zhong *et al.*, 2002)
Cyprus	Adolescents	Only vegetables considered	1.12 (3.42)	Lettuce and other leafy vegetables	(Stavroulakis *et al.*, 2018)
Denmark	General population	Yes	1.0 (1.9)[d]	Potato, lettuce	(Petersen and Stoltze, 1999)
Estonia	Adults 4-6 years 1-3 years Infants	Only vegetables and vegetable products considered	0.97 1.5 1.7 0.84	Potato, cabbage, beetroot	(Tamme *et al.*, 2006)
France	Adults (>15 years) Children (3-14 years)	Yes	1.5 (3.3)[e] 2.0 (4.9)	Vegetables, excluding potatoes	(Menard *et al.*, 2008)
Hong Kong SAR	Adults	Only vegetables considered	4.4 (13)	NS	(Chen *et al.*, 2011)
Ireland	Adults (18+ years) Children (5-12 years)	Yes	0.46-0.86 (1.62-2.20)[e] 0.46-0.91 (1.30-1.82)	Vegetables	(FSAI, 2016)
Italy	Adults Girls (4-6 years)	Only leafy vegetables considered	1.1 0.73	Lettuce	(De Martin and Restani, 2003)
Spain (Valencia)	Adults (16-95 years) Young people (6-15 years)	Only vegetables considered	0.28 (1.17) 0.38 (1.53)	Lettuce, potato, chard, spinach	(Quijano *et al.*, 2017)
Sweden	4 years 8-9 years 11-12 years	Yes	0.84 (1.62) 0.68 (1.24) 0.45 (0.92)	Vegetables, drinking-water	(Larsson *et al.*, 2011)
UK	General population	No	0.95 (1.8)[d, e]	Potato, green vegetables	(Ysart *et al.*, 1999)
UK	General population	Yes	0.90[d]	Potato, green vegetables	(Meah *et al.*, 1994)

Country	Cohort	Includes drinking-water	Mean (high percentile)[a, b] exposure, mg/kg bw per day	Main contributing food	Reference
USA	Males Females	NS	1.1[d] 1.2	Lettuce, green leafy vegetables	(Inoue-Choi *et al.*, 2016)

[a] High percentiles are 95th percentiles unless otherwise stated

[b] Exposure estimates expressed as two hyphenated numbers are lower and upper bound estimates of exposure. These are usually determined by assuming that analytical results below the limit of detection (LOD) are equal to zero (lower bound) or equal to the LOD (upper bound)

[c] 90th percentile

[d] Results were reported as 'mg/day' and have been converted using a body weight of 60 kg

[e] 97.5th percentile

The information in Table 5 suggests that internationally dietary nitrate exposure estimates fall within quite a narrow range, with the exception of studies conducted in China and Hong Kong (Chen *et al.*, 2011; Zhong *et al.*, 2002), with mean estimates in the range 0.3 to 2.0 mg/kg bw per day. In this context, New Zealand dietary exposure to nitrate including nitrate from drinking-water is unremarkable.

4.2 RISK CHARACTERISATION

Mean exposure to nitrate, including food and beverages, for New Zealand adults and children are in the range of 22 to 24% of the ADI of 0-3.7 mg/kg bw per day (Table 4). The usual 95th percentile estimates of exposure are still less than 50% of the ADI. Based on single day estimates of nitrate exposure, 2.2% of adults and 1.6% of children exceed the ADI. However, when usual exposure estimates were compared to the ADI, the probability of either adults or children exceeding the ADI was less than 0.01%.

4.3 CONTRIBUTORS TO NITRATE EXPOSURE FROM FOOD AND BEVERAGES IN NEW ZEALAND

The contribution of the various foods and beverages included in the current study was estimated by summing contributions to dietary exposure for each food across all respondents and dividing by the total cohort exposure to nitrate. Major contributors to nitrate exposure for adults and children are listed in Table 6.

Table 6. Major contributors to dietary nitrate exposure in New Zealand

Rank	Food (percent contribution)	
	Adults	**Children**
1	Lettuce (23.5)	Potato (42.0)
2	Potato (17.8)	Lettuce (13.9)
3	Kumara (9.7)	Drinking-water (7.5)
4	Drinking-water (9.1)	Kumara (5.9)
5	Avocado (8.9)	Cabbage (5.1)
6	Cabbage (3.8)	Apple (4.9)
7	Green beans (3.0)	Avocado (2.8)
8	Silverbeet (2.8)	Watercress (2.7)
9	Spinach (2.3)	Hamburger (2.0)
10	Watercress (1.6)	Silverbeet (1.5)

The foods identified as major contributors to dietary nitrate exposure in New Zealand are consistent with those identified in other studies (Table 5).

Drinking-water from public supplies contributed less than 10% of nitrate exposure overall, although there will be individuals for which this contribution is greater. It should be noted that drinking-water includes all instances where water was an ingredient in a food or beverage, particularly beverages such as tea and coffee.

The dominance of food over drinking-water as a contributor to dietary nitrate exposure is consistent with other studies. For example, the French study of Menard *et al.* (2008) estimated that approximately 11-13% of dietary nitrate exposure was due to water consumption. The Australian assessment conducted by FSANZ (2011) concluded that drinking-water contributed less than 5% to dietary nitrate exposure for all age groups considered, except infants (19%). The Swedish study of Larsson *et al*. (2011) indicated a greater contribution of drinking-water to dietary nitrate exposure for young children, in the range 21-26%.

4.4 DETAILED ANALYSIS OF DRINKING-WATER CONSUMPTION

Epidemiological studies that have included consideration of nitrate from the diet and from drinking-water have tended to treat these as distinct sources of nitrate exposure. The reality is likely to be somewhat different with drinking-water being consumed as an ingredient of foods or beverages or in association with foods. The following sections will examine the degree to which this is the case in the New Zealand context.

4.4.1 Form in which drinking-water is consumed

Table 7 shows a breakdown of the 17743 servings of water identified in the 2009ANS and the 6750 servings identified in the 2002CNS.

Table 7. Analysis of drinking-water servings for adult and child New Zealanders

Food or beverage	Number of servings (percent of total)		Mean volume (percent of total), mL/day	
	Adults (15+ years)	Children (5-14 years)	Adults (15+ years)	Children (5-14 years)
Total	17743	6750	1440	477
Drinking-water (as a beverage)	5668 (31.9)	3823 (56.6)	704 (48.9)	279 (58.5)
Tea	5523 (31.1)	490 (7.3)	304 (21.1)	31.3 (6.6)
Coffee	4905 (27.6)	71 (1.1)	261 (18.1)	4.9 (1.0)
Other hot beverages	486 (2.7)	613 (9.1)	24.4 (1.7)	34.6 (7.3)
Water-based fruit drinks	464 (2.6)	1445 (21.4)	74.0 (5.1)	114 (23.9)
Porridge	275 (1.5)	-	17.5 (1.2)	-
Other	422 (2.3)	308 (4.6)	56.1 (3.9)	13.2 (2.8)

The analysis in Table 7 is consistent with a similar analysis conducted for the Australian population, with adult Australians consuming approximately 50% of drinking-water as a beverage, 24% as tea and 25% as coffee (EnHealth, 2012). A survey of the UK population showed a more extreme composition of drinking-water consumption, with 49.2% by volume consumed as tea, 29.1% as coffee and only 9.2% as water (MEL Research, 1996).

It has been hypothesised that the lack of associations between nitrate in the diet and colorectal cancer is due to the presence of antioxidant compounds, such as vitamin C, that inhibit nitrosamine formation in the gut. Tea and coffee are reported as rich sources of polyphenolic antioxidants, while water-based fruit drinks generally contain vitamin C as an antioxidant.

4.4.2 Timing of consumption of drinking-water

According to the analysis in the previous section, approximately half of drinking-water is consumed as water not mixed with other ingredients. However, for this water ingestion to be considered as distinct from nitrate from food it would need to be present in the gut at a time when food was not also present in the gut.

Transit times for ingesta through the entire gut, from mouth to anus, have been reported to be in excess of a day (Chaddock *et al.*, 2014; Nandhra *et al.*, 2020) and, in some cases, greater than three days (Keendjele *et al.*, 2021). Passage through the stomach (gastric emptying) can be similarly variable, but may last for several hours (Nandhra *et al.*, 2020). While this appears to provide considerable potential for components of meals and between meal ingesta to be present in the gut at the same time, there is surprisingly little information on the relative transit times of foods and liquids. A rather old study, using radiolabelled solid

and liquid foods, found that although liquids transited the stomach more rapidly than solid foods, both liquid and solid foods progressed through the small intestine at similar rates (Malagelada *et al.*, 1984). A more recent study arrived at similar conclusions (Bennink *et al.*, 1999).

Data from the NNSs were examined to determine when drinking-water as a beverage was consumption in relation to ingestion of non-water foods. It should be noted that times of food consumption were not reported in the 2009ANS and the analysis for adults comes from the earlier 1997NNS. Data are summarised in Table 8.

Table 8. Proximity of drinking-water consumption to consumption of non-water foods

Proximity (hours)	Number of servings (percent)		Mean[a] volume (percent of total), mL/day	
	Adults (15+ years)	Children (5-14 years)	Adults (15+ years)	Children (5-14 years)
Total	3892	3823	422	279
Zero (consumed with food)	1074 (27.6)	2444 (63.9)	100 (23.7)	181 (65.0)
≤0.5 hours	1650 (42.4)	2982 (78.0)	147 (34.8)	220 (78.8)
≤1 hour	2002 (51.4)	3182 (83.2)	177 (41.8)	235 (84.2)
>1 hour	1890 (48.6)	641 (16.8)	245 (58.1)	44 (15.7)

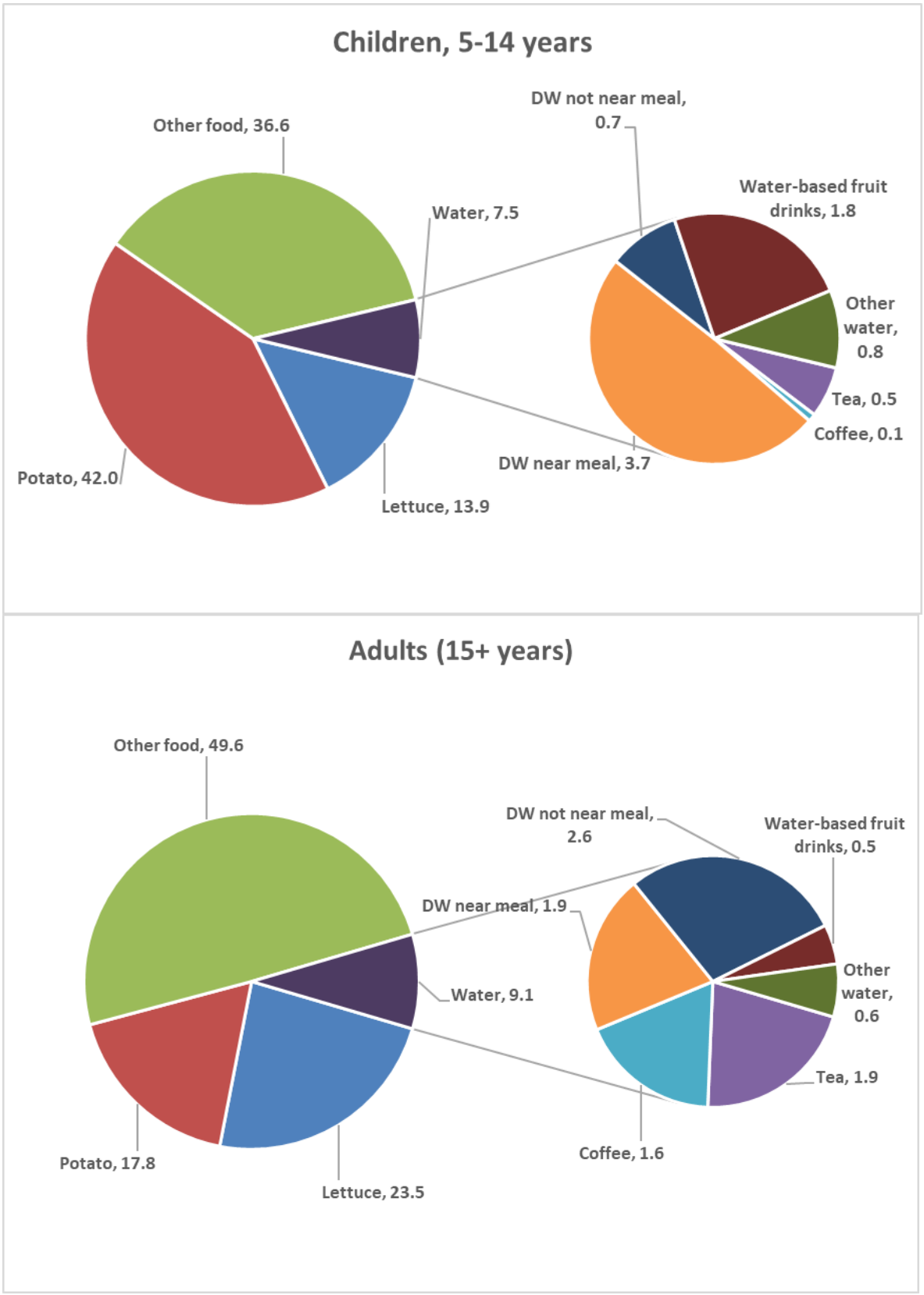
[a] Mean volumes are calculated as the mean across all survey respondents

The information in Table 8 indicates that most drinking-water consumed as a beverage is consumed with or within a short time period of food. This phenomenon is most pronounced for children, with the majority (65%) of drinking-water being consumed in association with a meal. However, for adults about half of water drinking events and almost 60% of the volume of drinking-water consumption occurs more than an hour away from a meal.

When the results from the analyses presented here are combined only 2.6% of adult and 0.7% of child nitrate exposure is from drinking-water not consumed as an ingredient of another food and not consumed within one hour of a meal.

Figure 6 shows the breakdown of nitrate exposure for adults and children from food and beverages, including water. The contributions from water are further broken down into beverage types and proximity of consumption to a meal.

Figure 6. Estimated exposure to nitrate from consumption of food and beverages, including water



5. CONCLUSIONS

The study sought to examine evidence that nitrate from drinking-water, but not nitrate from the diet could plausibly be involved in the causation of colorectal cancer.

The role of nitrate and the other nitrogen oxides in the human body is complex, with potential involvement in both beneficial and adverse physiological processes. Nitrate is rapidly absorbed and has a short half-life within the body. Excretion is predominantly via the kidneys and urine. It is well documented that nitrate is reduced to nitrite both in the human mouth and small intestine. Nitrite has important physiological functions including anti-bacterial actions and as a pre-cursor for nitric oxide. Nitrosamines, formed from reactions between nitrite and amines and long-considered the potential link between nitrate consumption and cancer, have been detected in the ileum and colon. However, the rate of formation is variable, species-dependant and inhibited by co-consumption of vitamin C and polyphenols. Nitrosamines present in the gut are absorbed into the body and undergo metabolism in different tissues depending on the exact nitrosamine chemical present.

New Zealanders exposure to nitrate from food and beverages, including drinking-water, is very similar to most other countries that have carried out studies of nitrate exposure. Exposures are well within acceptable daily intakes, suggesting little cause for public health concern. Public drinking-water accounts for less than 10% of estimated nitrate exposure in New Zealand, with little difference in exposure patterns between adults and children. About half of the drinking-water consumed, by volume, is as a beverage in its own right. The remainder of drinking-water is consumed as an ingredient of antioxidant-rich beverages, such as tea and coffee.

For children, most of the drinking-water consumed without other ingredients (65%) is consumed with a meal, with 85% of drinking-water consumed with or within one hour of a meal. The pattern is somewhat different for adults, with about one-quarter of drinking-water consumed with a meal.

In total, only 2.6% of nitrate exposure for adults and 0.7% of nitrate exposure for children is from drinking-water consumed on its own and not in close (<1 hour) temporal association to food consumption. This indicates that most nitrate from drinking-water cannot be considered as separate to dietary nitrate. The theoretical scenario suggesting that drinking-water could cause an increase in cancer risk as compared to food assumes high exposure to nitrates from drinking-water as a beverage, not as a component of a food or meal. However, the exposure analysis shows that a low overall percentage of nitrates come from water consumption and a very small proportion is from consumption of drinking- water as a beverage. For children, while more drinking-water is consumed as a beverage it is more likely to be in conjunction with a meal, balancing the overall risk. Therefore, the complete assessment suggests that there is little reason to differentiate between drinking-water and food nitrate exposure. Evaluation of the total nitrate intake from both sources is the most appropriate strategy for risk analysis purposes.

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APPENDIX 1. CONCENTRATIONS OF NITRATE IN PLANT-BASED FOODS

International studies, countries A-I

Food	Mean nitrate concentration (mg/kg fresh weight)												
	Australia 2011	Brazil 2000	China 2002	Denmark 1999	Estonia 2006	Estonia 2010	Europe 2008	Fiji 2008	France 2002	France 2008	Hong Kong 2011	Iran 2016	Italy 2003
Almonds												285	
Apple	15.0									23		103	
Apricot												178	
Artichoke							174			16			
Arugula (rocket)													3705
Asparagus							209			112			
Avocado	14.5									1256			
Baked beans	13.0												
Banana	78.3											444	
Barley												596	
Basil							2292					2360	
Borage							1918						
Beetroot	1466	7465		1505	1446		1379			644	3000	4950	
Bread												513	
Broad beans												354	
Broccoli	164.3	565				314	279			442	420		
Brussels sprout							24			24			
Cabbage	252.7		1530	333	437	382	311	1425		498	1200	1980	
Cantaloupe												468	

Food	Mean nitrate concentration (mg/kg fresh weight)												
	Australia 2011	Brazil 2000	China 2002	Denmark 1999	Estonia 2006	Estonia 2010	Europe 2008	Fiji 2008	France 2002	France 2008	Hong Kong 2011	Iran 2016	Italy 2003
Carrot	5.1				148		296		113	121	220	503	
Cauliflower					287	314	148			214	250	1020	
Celeriac							390			613			
Celery	1114		3600		565	2056	1103	4707		1241	1700	2610	
Cherries												212	
Chickpea												322	
Chicory										135			2529
Chinese cabbage			2120		1243	1068.6					1300		
Chives							748						
Coriander							2445				3200	2370	
Corn												279	
Cowpea												106	
Cucumber	180.6		170		160	303				192	110	877	
Dill					2936		1332					1830	
Eggplant			479				314			284	350	1100	
Endive							1465			534			
Fennel							1024			1043			
Fenugreek												6560	
Figs										72		241	
Fruit juice												1250	
Garlic							69			121	18	350	
Gherkin							69						
Grapes	14.2											160	
Green beans	292.3						323		712	449		466	
Green onion													

Food	Mean nitrate concentration (mg/kg fresh weight)												
	Australia 2011	Brazil 2000	China 2002	Denmark 1999	Estonia 2006	Estonia 2010	Europe 2008	Fiji 2008	France 2002	France 2008	Hong Kong 2011	Iran 2016	Italy 2003
Green pea	5.6						30			21		882	
Kale		1832					537						
Kiwifruit												97	
Kohlrabi							987						
Leek				284			345			410		1770	
Lemon												248	
Lentil												113	
Lettuce	834.3	1419		1859	2167	2348	1324	1297	805	1974	950	3650	1473
Mango	7.7												
Melon												223	
Mint												2790	
Mushrooms	5.3						61			117	43	654	
Loives, preserved	16.0												
Onions	7.2				55		164			65	13	609	
Orange	7.9											132	
Orange juice	8.2												
Parsley	1427.7				966		958			1980		1710	
Parsnip							83						
Peach	7.9											75	
Pear												173	
Pepper (capsicum)							108			159	77		
Pineapple	6.4												
Pistachio												591	
Plum												127	
Pomegranate												97	

Food	Mean nitrate concentration (mg/kg fresh weight)												
	Australia 2011	Brazil 2000	China 2002	Denmark 1999	Estonia 2006	Estonia 2010	Europe 2008	Fiji 2008	France 2002	France 2008	Hong Kong 2011	Iran 2016	Italy 2003
Potato	72.0		164	182	94		168			192	170	376	
Potato chips	314.8											414	
Pumpkin	121.0				174		894				260		
Radish		1461			1309		967			1861	1400	6260	
Red bean												157	
Rhubarb					201		2943						
Rice												243	
Scallion			704									1010	
Silverbeet/Chard							1690			1682			
Sorrel										2.5			
Soy bean												103	
Spinach, fresh	2000	527		1783	2508	1987	1066		1591		3100	1830	1757
Spinach, preserved													
Split pea												375	
Spring onion					477								
Strawberry	125.6				55					78			
String bean							618				190		
Sultanas	15.8												
Sweet corn	8.0												
Sweet potato										717			
Tangerine												326	
Tarragon												4240	
Tomato	12.0		78		41		43		19	18	57	175	
Tomato paste												362	
Turnip		2097			307		663			657		2230	

Food	Mean nitrate concentration (mg/kg fresh weight)												
	Australia 2011	Brazil 2000	China 2002	Denmark 1999	Estonia 2006	Estonia 2010	Europe 2008	Fiji 2008	France 2002	France 2008	Hong Kong 2011	Iran 2016	Italy 2003
Water melon	8.3				95							110	
Watercress		1365					136			1877	1300	2250	
Wax gourd			635										
White bean												185	
Zucchini					421		416			559		1080	

Australia 2011: (FSANZ, 2011), Brazil 2000: (Ximenes *et al.*, 2000), China 2002: (Zhong *et al.*, 2002), Denmark 1999: (Petersen and Stoltze, 1999), Estonia 2006: (Tamme *et al.*, 2006), Estonia 2010: (Tamme *et al.*, 2010), Europe 2008: (EFSA, 2008), Fiji 2008: (Prasad and Chetty, 2008), France 2002: (Malmauret *et al.*, 2002), France 2008: (Menard *et al.*, 2008), Hong Kong 2011: (Chung *et al.*, 2011), Iran 2016: (Bahadoran *et al.*, 2016), Italy 2003: (De Martin and Restani, 2003)

International studies, countries J-U

Food	Mean nitrate concentration (mg/kg)												
	Japan 2003	Jordan 2001	Korea 2003	New Zealand 2007	Portugal 2010	Slovenia 2006	Spain 2017	Spain 2020	Sweden 2011	Turkey 2007	Turkey 2010	UK 1999	USA 2015
Adzuki bean	0.5												
Apple						3.3							
Artichoke							80						
Arugula (rocket)								3266					
Beetroot				557					486			1211	
Broccoli	1515			97					301				394
Brussels sprout												59	
Cabbage	2485	34	725	241	547	881			379		510	338	
Cantaloupe													
Carrot	1663		316	42		264	40		165	190		97	
Cauliflower	4321	1.3							139			86	418

Food	Mean nitrate concentration (mg/kg)												
	Japan 2003	Jordan 2001	Korea 2003	New Zealand 2007	Portugal 2010	Slovenia 2006	Spain 2017	Spain 2020	Sweden 2011	Turkey 2007	Turkey 2010	UK 1999	USA 2015
Celery	13226			1175									1496
Chinese cabbage	1773		1740						899				
Colza					73								
Cucumber		23	212			93			179				
Eggplant	1272												
Garlic	0.8		124										
Grapes						5.6							
Green onion	521		436										
Green pea	0.2												
Kale					472								
Leek	883								535		40		
Lettuce	1978	30	2430	1159	1156	1074	788	724	1271	860	1439	1051	851
Mushrooms	0.2												
Okra	20												
Onions	913		23									48	
Parsley	3079	26			891					1513	1070		
Peach						4							
Pear						2.8							
Pepper (capsicum)	225.7		76										
Potato	321		452	94		158	174		47			155	
Potato chips													
Pumpkin	31			48									
Radish	4397		1878								3428		
Silverbeet/Chard				540			1502	1750					

Food	Mean nitrate concentration (mg/kg)												
	Japan 2003	Jordan 2001	Korea 2003	New Zealand 2007	Portugal 2010	Slovenia 2006	Spain 2017	Spain 2020	Sweden 2011	Turkey 2007	Turkey 2010	UK 1999	USA 2015
Soy bean	0.1												
Soybean sprouts			56										
Spinach, fresh		25	4259	722	1112		1267	1494	661	1456	1132	1631	2797
Spinach, preserved							573						
Squash		41-48	639										
Strawberry						94							
String bean						298							
Swede												118	
Tomato	2.5	7.4				4.3			4	11		17	
Turnip	71				444								
Turnip sprouts					489								
Watercress				1199				791					

Japan 2003: (Himeno *et al.*, 2003), Jordan 2001: (Amr and Hadidi, 2001), Korea 2003: (Chung *et al.*, 2003), New Zealand 2007: (Thomson *et al.*, 2007), Portugal 2010: (Correia *et al.*, 2010), Slovenia 2006: (Susin *et al.*, 2006), Spain 2017: (Quijano *et al.*, 2017), Spain 2020: (Martín León and Luzardo, 2020), Sweden 2011: (Larsson *et al.*, 2011), Turkey 2007: (Ayaz *et al.*, 2007), Turkey 2010: (Mor *et al.*, 2010), United Kingdom (UK): (Ysart *et al.*, 1999), United States of America (USA): (Nunez de Gonzalez *et al.*, 2015)

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