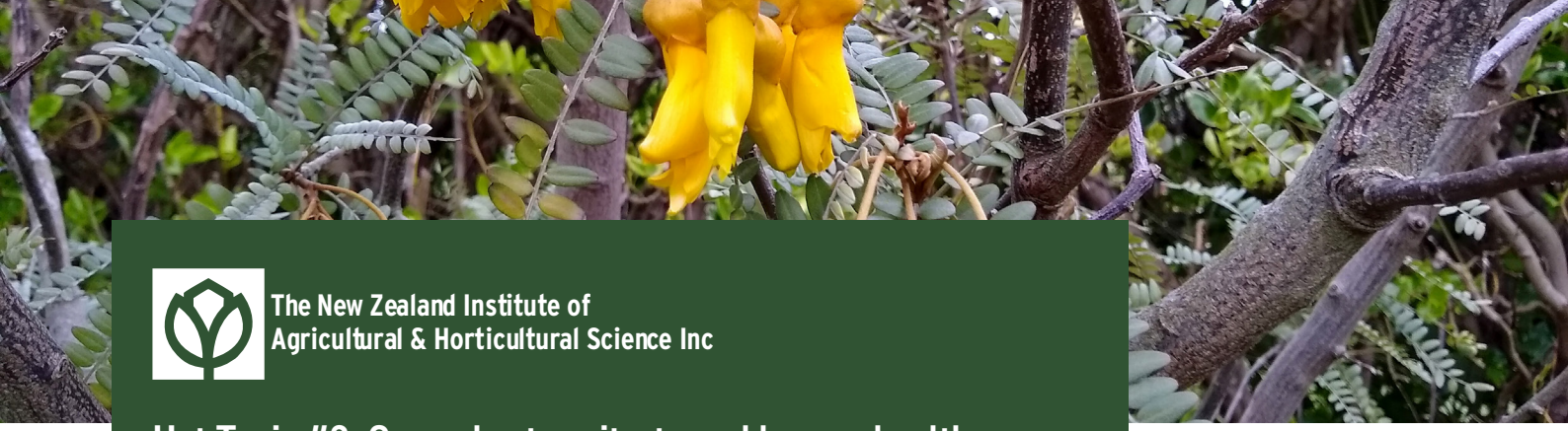

The New Zealand Institute of
Agricultural & Horticultural Science Inc

Hot Topic #2: Groundwater nitrate and human health

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Above - Kowhai (Sophora spp) along with matagouri (Discaria taumotu) are New Zealand native nitrogen fixers.

Introduction

Numerous claims circulate in the popular media about the alleged negative health impacts of nitrate (NO3-) concentrations in drinking water derived from groundwater. These claims include:

- an elevated incidence of methaemoglobinaemia (blue-baby syndrome),
- an elevated incidence of pre- and perinatal impacts, including birth defects, pre-term parturition (premature birth) and low birth weight (LBW) babies, and
- an elevated incidence of cancers of the hindgut (or colorectal cancers).

An appraisal of the peer-reviewed scientific literature around these alleged impacts, suggests that little reliance should be placed on these popularist media claims. This is explained in more detail below.

The origin of nitrate (NO3-) in groundwater

Nitrogen gas (N2) comprises ~79% of the mass of gases in the atmosphere. It is quite stable. In soil, microbial activity by nitrifying bacteria reduces the nitrogen gas to ammonia (NH3). This is called nitrogen fixation. The nitrifying bacteria convert the ammonia into nitrite (NO2-) and then into nitrate (NO3-), both of which are highly labile water-soluble ions. This process is called nitrification. Soil compounds such as nitrate, nitrite, ammonia and ammonium can then be taken up from soils by plants. This fixed inorganic nitrogen is required for the biosynthesis of organic nitrogen-containing compounds, such as amino acids and proteins, nucleoside triphosphates and nucleic acids (DNA and RNA); hence it facilitates plant growth.

Under normal conditions, the turnover of nitrogen between various soil nitrogen depots and plant tissue is such that relatively limited losses of nitrogen to groundwater occur. This turnover is also a small proportion of the total nitrogen contained in the various soil depots, and it can occur only at the rate at which relatively insoluble nitrogen-containing substances (such as decaying plant and animal tissue) break down to soluble nitrogen-rich materials that can be used as plant nutrients.

According to Sparling *et al.* (2008), agricultural soils have an optimal gross nitrogen concentration of 0.6% by weight, hence depending on the depth to which this is measured, the soil nitrogen stock ranges from 10 - 60 tonnes of nitrogen/ha. The depth of measurement is important, because deep rooted plants like trees, can access a lot more nitrogen than shallow rooted plants, such as grasses.

The best-performed pastures harvest of the order of 600 kg of nitrogen per hectare. This pasture is available to feed livestock, but it should be noted that approximately 60% of this mass is recycled (returns by various means to the soil), with the balance removed from the pasture in animal products (e.g. protein in milk, meat and wool). Consequently, the proportion of the total soil stock of nitrogen mobilised during pasture production is no more than 3%, and as little as 0.4%.

Labile nitrogen in the soil may exist in both reduced and oxidised forms, with the latter being the more mobile, as those forms (consisting of nitrates and nitrites) are very highly dissociated into the negative ions. Conversely, reduced forms vary in mobility, with ammonium ions being largely dissociated from the cations found in the pure forms, whereas forms such as urea ((NH2)2CO from mammalian excretion, uric acid (C5H4N4O3) from avian excretion, and mucins from faecal excreta and microbial biofilms remain undissociated until soil enzyme activity breaks them down.

While the first step in this breakdown process is to release reduced nitrogen, largely as ammonium ions; such

reduced nitrogen species are useful sources of metabolic energy for many microbes, obtained by oxidising those chemical species to nitrate and/or nitrite. Thus, excreta-derived nitrogen is largely, but not entirely, converted to nitrate during its addition to the soil nitrogen bank. In this labile nitrate form it is most available for uptake for plant growth, but it is also potentially able to leach into groundwater and ultimately, possibly drinking water.

The health effects of nitrate in drinking water

The World Health Organisation (WHO) has reviewed the health impacts of the oxidised forms of nitrogen (nitrite and nitrate) in drinking water (Cotruvo *et al*., 2011), in confirming their guideline values. It is worth citing their recommendations in full:

"7. GUIDELINE VALUES

The guideline value for nitrate of 50 mg/l as nitrate is based on epidemiological evidence for methaemoglobinaemia in infants, which results from short-term exposure and is protective for bottle-fed infants and, consequently, other population groups. This outcome is complicated by the presence of microbial contamination and subsequent gastrointestinal infection, which can increase the risk for this group significantly. Authorities should therefore be all the more vigilant that water to be used for bottle-fed infants is microbiologically safe when nitrate is present at concentrations near the guideline value. It is recommended that water should not be used for bottle-fed infants when nitrate levels are above 100 mg/l, but that it may be used if medical authorities are vigilant for signs of methaemoglobinaemia when the nitrate concentration is between 50 and 100 mg/l, particularly where a high rate of gastrointestinal infection is present in infants and children in the population The latter is a minor modification of previous guidance to place greater emphasis on the role of microbiological quality."

and:

"The guideline for nitrite of 3 mg/l as nitrite is based on human data showing that doses of nitrite that cause methaemoglobinaemia in infants range from 0.4 to more than 200 mg/kg of body weight. By applying the lowest level of the range (0.4 mg/kg of body weight), a body weight of 5 kg for an infant and a drinking-water consumption of 0.75 litre, a guideline value of 3 mg (nitrite)/l (rounded figure) can be derived.

Because of the possibility of the simultaneous occurrence of nitrate and nitrite in drinking-water, the sum of the ratios of the concentration (C) of each to its guideline value (GV) should not exceed 1, i.e.

$$\frac{[\text{nitrate}]}{\text{GVnitrate}]} + \frac{[\text{nitrite}\}}{\text{GVnitrite}} < 1$$

At this time, no other values are proposed for chronic effects, in view of uncertainties regarding differences in the way in which nitrate and nitrite are handled by laboratory animals and significant uncertainties in epidemiological data, particularly for effects on the thyroid."

Note the phrase 'significant uncertainties in epidemiological data'. Further, in 2017 the World Health Organisation stated:

"Overall, these studies found no clear association between nitrate or nitrite in drinking-water and risk of cancer of the gastrointestinal tract, non-Hodgkin lymphoma, tumours of the central nervous system, urinary tract tumours, thyroid cancer, breast cancer or pancreatic cancer (Aschebrook-Kilfoy *et al*., 2012; Wu *et al*., 2013; see also numerous references cited in Health Canada, 2013)."

One can conclude that the World Health Organisation is being cautious, albeit not in the least bit alarmist in its summary of likely human health impacts of oxidised nitrogen (nitrate and nitrite) in drinking water. This doesn't mean there is any absolute guarantee of safety, albeit it also does not suggest there is a large or hidden safety issue.

Methaemoglobinaemia

Methaemoglobinaemia is a condition in which the iron in haemoglobin, the oxygen carrying compound in blood, is converted from the normal ferrous (Fe2+) state to the ferric (Fe3+) state. It is then unable to bind oxygen reversibly.

There are multiple causes of methaemoglobinaemia, but the conversion of ferrous to ferric iron may come about because of an elevated concentration of blood nitrite, which can occur when dietary nitrate intake is markedly elevated. In adults the iron in methaemoglobin is readily converted back to the ferrous (reduced) state (Anon., 2018; Chan, 2011; Duncan *et al*., 1995; Mcknight *et al*., 2021), but this is not the case in infants, meaning that they require protection from elevated dietary nitrate. This point is illustrated by the

World Health Organisation guideline values above. This is not to say that dietary nitrate is unsafe for everyone.

For example, nitrate is present in large quantities in green leafy vegetables and beetroot, and has attracted considerable attention in recent years as a potential 'health-promoting' dietary compound. Numerous studies have reported beneficial health effects of nitrate consumption on blood pressure, endothelial function, cerebrovascular blood flow, cognitive function, and exercise performance.

In understanding this, a digestive process called the entero-salivary circulation of nitrate needs to be considered. Following consumption, nitrate is absorbed in the upper gastrointestinal tract, increasing blood plasma nitrate concentration. In the blood, this exogenous nitrate mixes with endogenous (naturally body-derived) nitrate produced by the oxidation of nitric oxide. This nitric oxide is produced by nearly every type of cell in the human body and is one of the most important molecules for blood vessel health. Most (~ 60%) of the ingested nitrate is excreted in the urine, but approximately 25% is actively taken up by the salivary glands and secreted into the mouth with the saliva. In the mouth it is reduced to nitrite by anaerobic bacteria that reside on the surface of the tongue. The salivary nitrite is then swallowed and a portion is converted into nitric oxide and other nitrogen oxides in the acidic environment of the stomach. Nitric oxide (NO) is known to have a protective effect on the gastrointestinal tract. It is perhaps then not surprising that people take nitric oxide supplements as a health supplement.

It is also notable that salivary nitrate can concentrate to nine times that of blood plasma, with levels exceeding 62 mg/L, which is of course in excess of the WHO recommended maxima of 50 mg/L in drinking water. This suggests that relatively high salivary nitrate content is a consequence of acquisition of an adaptive function: that is, it benefits the animal.

Breast milk has very low content of nitrate, even when the maternal drinking water supply is >100 mg/L with respect to nitrate content (Cotruvo *et al.*, 2011). Of some concern though, is the presence of elevated nitrate in drinking water used to make up infant formula. It is notable that methaemoglobinaemia related to high nitrate levels in drinking water used to make infant formula was first reported in 1945 and that the USA EPA limit for nitrate in drinking water was set at about one-half the level at which there were no observed cases of methaemoglobinaemia. It is unclear from the literature though what the maximum 'safe' drinking water nitrate concentration should be for preparing infant formula. A recent publication of the Minnesota Department of Health (2018) proposes 10 mg/L, but it is unclear whether this refers to nitrate per se or nitrate N, as is now the normal practice. When a laboratory reports directly the concentration of nitrate, it is referring specifically to the nitrate ion (NO3-). Some testing laboratories report the amount of nitrogen that is in the nitrate ion and call it nitrate nitrogen (nitrate N) and report it chemically as NO3-N. This makes a major difference, as the nitrate concentration of a solution that is 10 mg/L of nitrate N, is almost 50 mg nitrate/L.

Globally, the incidence of methaemoglobinaemia has fallen markedly since the 1950's. It is believed that this is due to a number of factors, including better recognition of symptoms at the primary care level (leading to effective intervention), greater emphasis on breastfeeding into the second six months of life, and the addition of the antioxidant vitamin C to infant formula, which slows the rate of reduction of nitrate to nitrite.

In summary, occasional global instances of methaemoglobinaemia in neonates are largely secondary to other causes of ill-heath, and they are normally prevented by the levels of antioxidants in infant formula that are required for food safety.

Reproductive impacts

Large studies of the relationship between drinking water nitrate concentration and various markers for intrauterine growth have been unsuccessful in demonstrating any important effects. For instance, a study of outcomes among nearly one million live births in Denmark (Coffman *et al.*, 2021) highlighted a statistically significant reduction in birthweight for a water nitrate concentration of 25 mg/L, but the effect was a miniscule nine gram reduction in birthweight relative to birthweights at 0 mg/L nitrate. This would suggest that nitrate levels are not that important for neonatal health, especially as other putative effects were negligible.

In other smaller studies in the USA, it proved almost impossible to assign a realistic value for maternal drinking water nitrate intake limits, when reviewing retrospective information for possible linkages between intake and different measures of pre-term birth effects. In those studies, no real effect was observed, even when observations were controlled for herbicide exposure.

In the Netherlands, said to have much higher drinking

water nitrate exposure than in New Zealand, the incidence of pre-term birth is lower than the mean value in this country, and it fell over the period from 2010 to 2015. Within New Zealand, rates of pre-term birth are higher in Hawke's Bay than in Waikato, despite the latter having a greater number of drinking water sources with elevated nitrate, than the former.

It might be concluded that there is no substantive evidence for an impact of elevated drinking water nitrate levels on measures of human reproductive performance.

Colorectal cancers

Lobbyists in New Zealand have of late placed a huge weighting on a study by Schuellehner *et al*. (2018) that suggests that elevated nitrate levels in drinking water are causing colorectal cancer. This paper postulated a significant relationship between groundwater nitrate content in Danish non-urban well-water supplies and colorectal cancer. This postulated link has been used in popular media to suggest that drinking water nitrate concentration limits should be 'dramatically reduced' in New Zealand, which has led to the expenditure of enormous quantities of ink on both sides of this argument.

The problem with using a single study to justify any argument, is that it is a potentially flawed approach to ascertaining what the weight of evidence suggests about any given complex matter, and whether all factors have been considered in that study. Sadly, the answer to this is 'no' for the Schuellehner *et al*. *(op. cit.)* paper.

To explain; any study of a complex situation that claims a relationship between two things, has to attempt to understand the various factors that affect that relationship. In the case of any putative relationship between nitrate levels in drinking water and cancer, one needs to account for all the other factors that might potentially confound the result, such as cigarette smoking, alcohol consumption, red meat consumption, processed meat consumption, other food and water contaminants, etcetera. The failure to account for these things weakens a study, and while it doesn't necessarily say the relationship doesn't exist, it also doesn't prove beyond doubt that it does.

By their own admission Schuellehner *et al*. *(op. cit.)* could not account for all the potential factors that may have led to their conclusion. In their words: "Given our study design, we were limited to include only covariates available in nationwide registers. We could for example not control for individual-level information on lifestyle and diet", and later in their paper "As diet (e.g., red meat), alcohol intake, smoking and lifestyle factors such as physical inactivity are established CRC" (i.e. colorectal cancer) "risk factors that we could not include in our analyses, the possibility of confounding our results needs to be considered."

The pundits who cite papers like Schuellehner *et al*. *(op. cit.)*, rarely mention phrases like those above in their commentary. It would of course weaken their simplistic arguments, although it is the very fact that they do not mention these things, which should make everyone wary of any simple argument they mount. Science is seldom simple!

Since the Schuellehner *et al*. *(op. cit.)* paper, another large study (Jones *et al*., 2019) has been released. This was based in Iowa in the USA. Unlike the study of Schuellehner *et al*. *(op. cit.)*, this study did account for a large number of known lifestyle and dietary factors, and they did not find a link between nitrate levels and colorectal cancer. In their words: "Ingested nitrate from drinking water at levels below the MCL" (the Maximum Contaminant Level) "was not associated with colon or rectal cancer risk in the IWHS (the Iowa Women's Health Study). They went on to suggest that ingestion of the highest average disinfection by-products (DBP) levels (i.e. the breakdown products of substances added to disinfect their drinking water), was a risk factor for rectal cancer. They also reported positive associations for individual haloacetic acid and trihalomethane levels and rectal cancer.

The take home message again, would appear to be that it is more complex than simply how much nitrate is in drinking water. This once again does not mean that Schuellehner *et al*. *(op. cit.)* are wrong, or that Jones *et al*. *(op. cit.)* are right: it is much more complex and nuanced than that.

Clearly, the World Health Organisation are unwilling to countenance the 10-fold reduction in guideline values demanded by Schuellehner and others, and this is likely because although the correlation between groundwater nitrate and colorectal cancer is (barely) statistically significant. There is no plausible cause and effect relationship. What is more, Sashegyi and Ferry (2017), suggest that little reliance should be put on hazard ratios, as were calculated in the Schuellehner *et al*. *(op. cit.)* paper, when the scale of the ratio is small, as it was.

Cressey *et al*. (2021) pointed out that while perhaps 10% of dietary nitrate is obtained from drinking water, less than 1% is consumed independently (i.e. more than 1 hour from ingestion of foods or water-containing beverages such as tea or coffee). This interval is a somewhat arbitrary period,

but is suggested as appropriate to avoid confounding water-derived nitrate with the (rather larger) amounts of food-derived nitrate consumed in meals.

The Implausibility of the suggested mechanism that causes colorectal cancer

The aetiology of colorectal cancer has long been studied (Manne *et al*., 2011), and >95% of such cancers are said to arise from adenomatous polyps of sporadic origin. Manne *et al. (op. cit.)* cite a range of predisposing factors, including exposure to secondary nitrosamines formed during cooking, but do not refer to production of such substances as a result of reactions between nitrate or nitrite and protein-derived materials capable of forming such nitrosamines. This is almost certainly because nitrate (and nitrite) from the diet is cleared rapidly from digesta in the stomach and proximal duodenum (Florin & Dunn, 2021), and therefore cannot take part in the nitrosamine-forming reactions.

Two separate lines of evidence are adduced to support this. Firstly, ileostomy patients show very low levels of nitrate in ileostomy fluid, the level of which is not correlated at all with nitrate uptake (Florin *et al*., 1990; Duncan *et al*., op. cit; Cressey & Cridge, op. cit). Consequently, the flow of nitrate in the gut that is able to participate in the formation of carcinogenic nitrosamines would be low, and not correlated to dietary intake. Secondly, in a study of healthy males, 15N labelled nitrate was evidently cleared from the digestive tract almost entirely (Wagner *et al*., 1983), with less than 0.1% of label recovered from faeces.

These two sets of findings suggest that dietary nitrate can almost certainly not cause colorectal cancer, and that some environmental factor(s) only coincidentally linked to drinking water or groundwater nitrate concentration is responsible for the putative health effects observed.

Beneficial health impacts of dietary nitrate.

As stated above, dietary nitrate is almost entirely removed from the digesta before it is transferred to the small intestine, and is quickly reduced to nitrite in the entero-salivary circulation (Duncan *et al*., op. cit.), and thence to nitrous oxide. These species are important for vascular function, and also modulate immune function. They also play an important role in managing and preventing microbial infections of the GI tract, including by species thought to be involved in causing colorectal cancer!

Other positive impacts are also reported (Shannon *et al*., 2021). Nitrate concentration in foods is sufficiently high to meet most needs, but the action of foodborne antioxidant compounds delays its health-giving actions, meaning that the amounts consumed in drinking water may have significant health **benefits**. On balance, it could be concluded that levels of drinking water nitrate up to the WHO standard, will reduce human mortality and morbidity.

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