Questions arising from Centre webinar on Nitrate

Q: How did the Danish study by Schullehner et al 2018 get to be published, and then so widely promoted in New Zealand by epidemiolgists and media outlets, essentially ignoring the raw data published in a supplementary dataset which showed a U shape relationship between colon and rectal cancer incidence and nitrate exposure, with little difference in incidence between the lowest and highest groups of drinking water nitrate?

A: The Schullehner study has strengths and weaknesses, but none of these would be sufficient to prevent publication. Once a study is in the public domain it can be viewed as a piece of evidence and different people will weight that evidence in different ways. There is ongoing discussion in the science arena concerning the plausibility of U-shaped dose-response curves, but the proposed mechanism for the association between nitrate exposure and colorectal cancer would be expected to conform to a monotonic dose-response relationship.

Q: What is the source or how did you establish the pie graph at 'where does our nitrate come from'?

Perhaps I'll rewrite my question,

"What was the process for establishing the pie graph, "where does our nitrate come from"?"

A: Our analysis involved estimating exposure to nitrate for each respondent in the Adult National Nutrition Survey (n = 4721) and the National Children's Nutrition Survey (n = 3275). For each individual, exposure will be made up of the amount of nitrate in the various foods and beverages consumed and the amount of those. The pie charts are the averages across the 4721 adult respondents and the 3275 child respondents, respectively. This provides a picture of what is typical or expected for the New Zealand situation based on this dataset.

Q: New topic here: Would Peter like to comment on the IARC's conclusion that "Overall evaluation Ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A)."?

A: It is important to be aware that the IARC evaluations are hazard-based, not risk-based. The final categorisation is an indication of whether a chemical or circumstance can cause cancer, not whether it will cause cancer under a particular set of circumstances. In this case it is also important to consider the IARC evaluation in full. IARC concluded that:

- There is inadequate evidence in humans for the carcinogenicity of nitrate in food.
- There is inadequate evidence in humans for the carcinogenicity of nitrate in drinking-water.
- There is limited evidence in humans for the carcinogenicity of nitrite in food. Nitrite in food is associated with an increased incidence of stomach cancer.
- There is inadequate evidence in experimental animals for the carcinogenicity of nitrate.
- There is sufficient evidence in experimental animals for the carcinogenicity of nitrite in combination with amines or amides.
- There is limited evidence in experimental animals for the carcinogenicity of nitrite per se.

The conclusion regarding nitrite in combination with amines or amides refers to the potential for these circumstances to result in formation of nitrosamines or nitrosamides and the overall evaluation states:

"Ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A). There is an active endogenous nitrogen cycle in humans that involves nitrate and nitrite, which are interconvertible in vivo. Nitrosating agents that arise from nitrite under acidic gastric conditions react readily with nitrosatable compounds, especially secondary

amines and amides, to generate N-nitroso compounds. These nitrosating conditions are enhanced following ingestion of additional nitrate, nitrite or nitrosatable compounds. Some of the N-nitroso compounds that could be formed in humans under these conditions are known carcinogens."

There is clear evidence that nitrosamines are carcinogenic, however the degree to which nitrosamines are formed and absorbed after nitrate intake is not currently fully understood (e.g. haemoglobins in red meat affect this process). IARC evaluated animal and *in vitro* data which shows that nitrosamines form from nitrate under the right conditions. The IARC working group was also clear in concluding that the concurrent intake of Vitamin C (an antioxidant) inhibits the formation of nitrosamines and therefore the carcinogenicity of nitrates, they use this as a reason to separate the risks from water and food sources. This reinforces the need for more information on consumption of water and food such as conducted in this report, if nitrates are carcinogenic under certain conditions, we need to be clear about what these conditions are and what the risks might be.

Q: Given Richards 2020 suggest up to 5.6% of CRC are due to nitrate from drinking water, what does this tell us in light of your study that says we should not differentiate between food and water sources? - is this suggesting drinking water sources might change the balance/ratio between total consumption of nitrate and total antioxidants consumed creating an increased risk in nitrosamine creation?

A: The Richards thesis started from an assumption that the relationships presented in the studies of Espejo-Herrera et al., Schullehner et al. and Temkin et al. were valid. These studies all considered nitrate from water to be distinct from nitrate in food. We believe our work casts sufficient doubt on this distinction that the relationships reported should be viewed with a high level of caution and as the potential upper estimate of the possible contribution to the CRC rates in NZ. We don't think current knowledge allows us to define the perfect balance between nitrate and antioxidants, but our study suggests that a good proportion of drinking-water nitrate will be ingested in the presence of antioxidants (and that a healthy diet with lots of vegetables is beneficial).

Q: In your experimental measurements of nitrate uptake from water and food, how can you distinguish one effect from the other in that anybody drinking water is also eating food . Is there any cooperative effect between these two sources?

A: This was the intent of our research – to demonstrate that nitrate from food and nitrate from drinking-water cannot be considered as distinct. In terms of 'cooperative potential', the presence of food and water components in the gut at the same time means that any nitrate present is more likely to be in the presence of antioxidants.

Q: Do the findings of your research also apply to the research around premature births and birth weight and drinking water nitrates?

A: Absolutely. In fact, the findings potentially apply to issues other than nitrate. Any association between an effect and a chemical that is present in both food and water should consider the aggregate exposure, rather than just considering exposure from one or the other. This concept was recognised (in a different context) in the early 1990s by the US National Research Council. They concluded that when looking at children's exposure to pesticides all potential routes of exposure (food, water, air, dust, etc.) should be considered, to determine the total exposure to pesticides.

Q: What does the relatively high level of dietary nitrates for vegetarians/vegans and their relatively low rate of CRC tell us about this issue?

A: I think this provides another useful piece of evidence to inform the current debate. This suggests that high exposure to nitrates *per se* is not a major determinant of CRC. However, my personal opinion is that this observation is completely consistent with the repeated observation that a diet rich in fruit and vegetables is protective against CRC.

Q: Is there a possibility that this could be construed as a reassurance that it's better for children to drink sweetened (even fruit-sweetened) drinks rather than plain water? (Presumably this would horrify dentists.) And not to drink plain water if it's not near meal-time?

A: I hope the result of this work will be the complete opposite to what you suggest. The studies on relationships between nitrate in drinking-water and colorectal cancer seem to have fuelled a fear of drinking-water in some quarters. We believe that our research casts considerable doubt on these studies and supports a view that the vast majority of people should have confidence in the safety of their drinking-water supply.

Q: Should nitrates/nitrates as an additive in foods be banned?

A: I think there are two strands to this. Firstly we need to remember why nitrites were added to cured meats in the first place, which was mainly to control the growth of *Clostridium botulinum* and prevent botulism. However, there is continual research into preservatives and preservation methods with a general guideline that preservation should be by the most effective means with the most benevolent toxicological profile. So, for this strand, I would say that use of nitrate/nitrite should only be discontinued if something better is available; more or as effective and with fewer toxicological concerns. The second strand is that the WHO guidelines are to limit consumption of processed meats, not to cease consumption. For populations with limited access to fresh meat or refrigeration, preserved meat are still a valuable protein source and this benefit needs to be balanced against any associated risks. In some situations antioxidants (vitamin C derivatives) are added in conjunction with nitrate/nitrites to lower any potential nitrosamine-based risks. Dietary exposure to nitrite is generally considered to be minor compared to endogenous production.

Q: The management of nitrates in drinking water and nitrates in food is different. Surely breaking them down into discrete studies is important for management purposes even though they both end up in the body?

A: In most parts of the world nitrates are not actively managed in foods, with the exception of nitrates used as food additives. As far as management of chemicals in food and water goes, the starting point is establishing a credible connection between the chemical and an adverse human health effect. The relative contributions of different exposure routes are then estimated, and that estimate used to drive standard setting. For example, the maximum acceptable values (MAVs) for chemicals in New Zealand drinking-water include in their derivation as a proportion of exposure that is expected to be due to drinking-water. Depending on the chemical, this can range from 10% to 100%.

Q: What level of antioxidants is required to inhibit endogenous nitrosation?

A: At this stage, this has not been defined. Most studies to date have been conducted *in vitro* but we feel this does not adequately reflect the complexity of either the gut or food exposures. Studies with tobacco which contain high levels of nitrosamines showed that a ratio of between 1:1 and 3:1 antioxidant to nitrosamine concentration was protective but the effect was highly dependent on the

exact nitrosamine and the exact antioxidant. We suspect our level of knowledge will need to advance substantially before we can fully define the relationship between antioxidants and nitrosamine cancer risks.

Q: this study uses nitrate/rectal cancer as the case study but peter indicated the wider issue is exposure - are there other low quality correlative studies that make the news but which need objective assessment?

A: Yes. Indeed any correlative study should be subjected to critical appraisal. With respect to nitrates, there have been recent studies associating nitrate in drinking-water to birth outcomes. The same criticisms that can be applied to studies of drinking-water and colorectal cancer can be applied to these studies. In general, I would encourage interested parties to become familiar with the work of Sir Austin Bradford Hill (see here: https://www.edwardtufte.com/tufte/hill). Hill was a major figure in epidemiology and his paper on associations and causality has been extremely influential in this area.

Q: What concentration of nitrate in drinking water was used to calculate ~10% contribution to total nitrate intake? And what % contribution would drinking water have if it was at the Maximum Acceptable Value for nitrate (50 mg/L)?

A: We established a distribution of drink-water nitrate concentrations from surveys carried out in New Zealand. These surveys were slanted towards areas that were expected might have high nitrate concentration. We assigned a random value from this distribution to each of the approximately 8000 respondents in the national nutrition surveys and calculated the resultant exposure to nitrate from the food and beverages they consumed. The dataset we used had a mean nitrate concentration of 4.8 mg/L, but contained values up to 130 mg/L, expressed as nitrate. Using a 'back of the envelope' calculation, if water with an average concentration of 4.8 mg/L accounts for 9.1% of an average exposure (adult) of 0.82 mg/kg body weight per day (0.075 from water), then an average drinking-water concentration of 50 mg/L would equate to exposure of 0.78 mg/kg bw per day from water and total exposure from food and water of 1.53 mg/kg bw per day, with the split between food and water being about 50:50. The internationally-accepted acceptable daily intake for nitrate is 3.7 mg/kg bw per day.

Q: Thanks for a very interesting presentation. Would you recommend to increase intake of antioxidant and multivitamins? If so how much?

A: Our general advice on diets, particularly after completing this report, would be to eat a varied diet containing a high proportion of fruits and vegetables. We don't believe that the science is sufficiently developed to say that additional synthetic antioxidants would be of value.

Q: Do you have a comment to make on the choice of 'level of education' as a adjusment criteria for determining risk of colo-rectal cancer in the Schellehner study 2018?

A: A really interesting question. Schullehner et al. state that "we adjusted our analyses for highest attained education, an especially appropriate proxy for lifestyle, smoking and diet in the Danish population". This statement is referenced to a Danish study, that is unfortunately only available in Danish. Unless the associations between education and known colorectal risk factors are very strong in Denmark, I feel that this is a less than ideal means of accounting for known confounders.

Q: Final question, is there a reason that Fonterra's financial support for this study wasn't mentioned in acknowledgements, given that their conflict of interest in this issue is so great?

A: The NZFSSRC and ESR have openly acknowledged in news articles that Fonterra have co-funded this research. Acknowledgement of their funding on the presentation was a simple oversight. Fonterra have not been involved in the undertaking of the exposure assessment, the writing of the report or the formation of conclusions.

Nitrate research by Peter Cressey and Dr Belinda Cridge, ESR, commissioned by the NZ Food Safety Science & Research Centre, with co-funding from the Ministry of Business, Innovation and Employment, and Fonterra

Q: What are the key conclusions of this research?

A: How did the researchers reach the conclusion that, based on the current evidence, it is highly unlikely that nitrates in drinking water present an increased risk of bowel cancer?

While some studies have reported associations between drinking water nitrate and bowel cancer, studies of nitrate in food have not reported associations or have reported negative associations (lower cancer rates with higher nitrate).

Drinking water accounts for less than 10% of the nitrates NZers are ingesting.

Most drinking water is consumed either in a 'food-like' form (tea, coffee, porridge, etc.) or drunk together with a meal, or within an hour of eating, in which case the nitrates are likely to be cancelled out by antioxidants in food.

Nitrate in drinking-water cannot sensibly be considered separately to nitrate in foods.

Q: What is the difference between bowel cancer and colorectal cancer?

A: These are different terms for the same thing. Both refer to cancers of the large intestine (the colon), the rectum and the anus.

Q: What are the rates of bowel cancer in New Zealand compared to other countries? Has our incidence improved or got worse?

A: According to the Global Cancer Observatory (Globocan) New Zealand's rate of new cases of colorectal cancer in 2020 was 70.6 cases per 100,000 population. Some countries reported rates greater than 100 cases per 100,000 (Japan, Portugal and Hungary), while many developing countries have rates less than 10 cases per 100,000, with the lowest reported being Guinea, with 1.5 cases per 100,000.

The Ministry of Health's data collation *Cancer: Historical summary 1948-2017* shows that New Zealand's rate of colorectal cancer peaked in about 1994-1996 and has been decreasing steadily since that time. New Zealand's age-standardised rate of colorectal cancer decreased by 30% between 1994 and 2017.

Q: Why is the cancer rate in New Zealand so high?

A: There are no clear reasons why the rates of colorectal cancer are higher in NZ than elsewhere. Diet, alcohol consumption, smoking, age, exercise and genetic factors can all affect the rates of CRC. New

Zealand has recently started a bowel cancer (colorectal cancer) screening programme which should help detect bowel cancer early when it still responds to treatment.

Q: Has any other work been done in New Zealand in respect of nitrates in water and bowel cancer?

A: A Masters student from Loughborough University in the UK used the relationships between drinking-water nitrate and colorectal cancer reported in some studies, including the Danish study, and information on nitrate in New Zealand water supplies, to estimate the number of colorectal cancer cases potentially attributable to nitrate in water. It was estimated that 0.6-5.6% of colorectal cancers in New Zealand could be due to nitrate in drinking-water.

Q: How might the nitrosamines actually cause cancer?

A: Nitrosamines are formed from the reaction of nitrite with amines. Nitrites are formed both from nitrates in the body, and they are present in some food and cigarette smoke. Some nitrosamines become mutagenic when the right enzymes are present – this means that they can then react with DNA, causing changes in that DNA which can lead to cancer. Some food components (vitamin C, polyphenols) have been shown to prevent the formation of nitrosamines in the body.

Q: What are amines?

A: Amines are chemical compounds containing nitrogen and are usually found in the body due to digestion of proteins, which are made up of amino acids.

Q: Has anyone measured nitrates or their derivatives in urine?

A: Yes, at least 60% of nitrate that we take in goes out again in urine. Very little is excreted in faeces. It is thought that the remainder is stored in the body, particularly the muscles, and is used to produce nitrite and nitric oxide. Nitrite acts as an antimicrobial in the mouth and stomach, while nitric oxide regulates vasodilation, increasing blood flow.

Q: What is the official advice from MoH and other NZ health agencies about how to prevent bowel (colorectal) cancer?

A: The MoH advises that you can reduce your risk of bowel cancer (and other diseases) by:

- Maintaining a healthy body weight.
- Being physically active for at least 30 minutes on most days of the week. It is even better if you increase the 30 minutes to 45–60 minutes and make some of the activity vigorous.
- Eating plenty of vegetables and fruit.
- Choosing wholemeal and wholegrain breads, cereals or grain products.
- Choosing foods low in salt, sugar and fat, particularly animal (saturated) fat.
- If drinking alcohol, doing so in moderation.
- Being smoke free

Q: What is the current dietary advice about red meat, cured meats?
A: Consumption of red meat and particularly consumption of processed meat has been linked to an increased risk of colorectal cancer, but not in all studies. Current health advice is that consumption of processed meats should be limited.

Q: What is the association between obesity and bowel cancer?

A: A higher body mass index (BMI) is associated with increased risk of colorectal cancer in both men and women, but the increases are higher in men than women.

Q: What percentage of CRC incidence is attributed to genetic inheritance?

A: About 75% of patients with colorectal cancer have sporadic disease with no apparent evidence of having inherited the disorder. The remaining 10% to 30% of patients have a family history of colorectal cancer that suggests a hereditary contribution, common exposures or shared risk factors among family members, or a combination of both. However, it seems likely that genetics can create a predisposition to the effects of environmental triggers.

Q: How can I get my water tested for nitrate content?

A: Many laboratories that carry out water testing will be able to analyse the nitrate content of drinking water. Information on laboratories accredited (IANZ) for testing drinking water can be found on the Ministry of Health website.

Q: Can I put anything in my water to counteract nitrates?

A: Water treatment systems, such as reverse osmosis, distillation, and ion exchange, can remove nitrates from water. However, before buying a home filtration system do your research to ensure that the system you are considering is appropriate.

Q: Does boiling affect nitrate content?

A: Boiling water is not sufficient to remove nitrates. In fact, nitrates may accumulate in the boiled water due to evaporation.

Q: Are reticulated (registered) water supplies tested for nitrate content?

A: The Priority 2 (P2) Chemical Determinands Identification Programme operated from 1995 to 2004. It identified chemicals in drinking-water supplies that were at a level that required ongoing surveillance. Nitrate was identified as a P2 determinand for four drinking-water treatment plants. These drinking water sources are in Canterbury (Rolleston, Darfield and Ashburton) and the lower Waitaki and supply water to about 42,000 people. Occasional surveys of the nitrate content of a wider range of drinking-water supplies has been carried out.

Q: Will nitrates in our water get worse before they get better?

A: At this stage it is impossible to tell. Nitrates come from a variety of sources including human and animal waste, nitrogen-based fertilisers, decaying plant matter, refuse dumps and some commercial processes. A number of measures have already been put in place to reduce nitrate release into waterways and a range of other measures are in the development phase. The translation of these measures into changes in water nitrates is likely to vary from region to region.