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Exposure to nitrate and nitrite in drinking water and cancers (Protocol)

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Exposure to nitrate and nitrite in drinking water and cancers

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ABSTRACT

Objectives

This is a protocol for a Cochrane Review (prototype). The objectives are as follows:

To assess the association between nitrate and nitrite in drinking water and cancers in observational studies

BACKGROUND

Nitrogen is essential for plant growth and is a major constituent (typically as NO_3) of agricultural fertilisers. Both NO_3 and nitrite (NO_2 synthesised by bacteria from ammonium in fertilisers), can leach from soil into groundwater, rivers, and drinking water. Nitrate and nitrite can be ingested through food and drinking water. Nitrate levels in source water have increased as a result of agricultural intensification, which has subsequently increased nitrate contamination in drinking water (WHO 2022). The World Health Organization (WHO) issued guidelines on safe concentrations of nitrate (11.3 mg/L as nitrate-nitrogen) and nitrite (0.9 mg/L as nitrite-nitrogen) compounds in water for human use that are based on the absence of specific acute health effects (methemoglobinemia and thyroid effects (WHO 2022)).

Nitrate ingested through food and drinking water is absorbed by the stomach and small intestine. Under normal physiological conditions, most ingested nitrate is excreted in urine; the remainder is reabsorbed from the blood and ends up in salivary glands in the oral cavity, where it is reduced to nitrite (Bryan 2017). Nitrite-derived metabolites can form nitroso compounds (NOCs), including N-nitrosamines, which can be carcinogenic, in the stomach and intestine (Kobayashi 2018). These compounds are alkylating agents, and damage DNA (IARC 2010). Specific genetic somatic mutations linked to dietary factors and colorectal cancer have recently been identified (Gandarilla-Esparza 2021; Gurjao 2021; Habermeyer 2015; van Breda 2021).

In 2010, the International Agency for Research on Cancer (IARC) classified ingested nitrate and nitrite that can form NOCs as probably carcinogenic to humans, and included them in group 2A (IARC 2010). A group 2A classification is used when there is limited evidence of carcinogenicity in humans, but sufficient evidence of carcinogenicity in experimental animals, which results in endogenous nitrosation. This suggests that the agent under investigation is not nitrate or nitrite directly, but ingested nitrate or nitrite that results in endogenous nitrosation, e.g. leading to the formation of NOCs.

Why is it important to do this review?

An increasing number of observational studies have found an association between levels of nitrate in drinking water and certain forms of cancer, while a number of studies have not observed a statistically significant association (Ward 2018). The 2022 WHO drinking water guidelines concluded that "the weight of evidence does not clearly support an association between cancer and exposure to nitrate or nitrite per se", citing limitations in epidemiological studies related to exposure assessment, other risk factors, and inhibitors and precursors (WHO 2022). However, this conclusion is based on the last rolling review on nitrate and nitrite, which was conducted in 2016 (WHO 2016). There are no known plans for the next WHO revision. Likewise, the IARC's conclusion is based on evidence up to 2006, and has not been updated. Some of the larger studies that have emerged since these reviews highlight the need for up-to-date evidence synthesis.

Nitrate is one of the most common drinking water contaminants. In some countries, nitrate contamination of drinking water is widespread, e.g. USA (WHO 2016). Other countries are experiencing rapid degradation due to relatively recent land use practices, e.g. New Zealand (ME NZ 2019). There are also dramatic differences in nitrate contamination both between and within countries, which pose serious concerns for health equity (WHO 2016). In New Zealand, nitrate levels in drinking water show substantial spatial variability. Elevated nitrate levels can be found in rural centres of South Canterbury, Southland, Nelson Marlborough, Waikato, and Northland, while low nitrate levels are typically found in the urban centres of Auckland, Wellington, and Dunedin (ME NZ 2019). The populations within these areas vary greatly in ethnicity, deprivation, and health status; unequal distribution of nitrate contamination could further health inequities (Crengle 2022). Improving our understanding of these threats to human health will help policy- and decision-makers to make decisions about land use practices that could impact human health and improve health equity.

Rationale for a living systematic review

Living systematic reviews (LSR) seek to address some of the limitations of traditional systematic reviews. LSRs are continually updated, incorporating new evidence as it becomes available, which facilitates connections between evidence and practice (Cochrane 2022). LSRs are particularly appropriate when: (1) the review question is a particular priority for decision-making; (2) there is an important level of uncertainty in the existing evidence; and (3) there is likely to be emerging evidence that will impact the conclusions of the LSR (Cochrane 2019). The potential impact of nitrate or nitrite on cancer meets all three of these criteria.

Traditional systematic reviews and meta-analyses can: (1) be difficult to correct when errors occur, and (2) quickly become outdated when review conclusions are highly sensitive to just one or a few studies (Cochrane 2022).

The limitations of traditional reviews can be exemplified by the epidemiological studies on nitrate in drinking water and colorectal cancer. Table 1 shows the different approaches and results from three systematic reviews (Essien 2022; Hosseini 2020; Picetti 2022). When Hosseini 2020 was first published, it had fundamental errors that led to a null finding that contradicted a previous meta-analysis (Temkin 2019), with a pooled hazard ratio (HR) of 1.04, 95% confidence interval (CI) 0.92 to 1.19. We sent a letter to the journal editor on 12 July 2021 outlining these major errors. However, the corrective action promised has yet to materialise (Chambers 2021). Likewise, in another systematic review, not included in Table 1, the authors incorrectly used an effect size of HR 0.68, 95% CI 0.66 to 0.69 for Schullehner 2018, instead of HR 1.14, 95% CI 1.06 to 1.23, which resulted in a pooled estimate from the meta-analysis of HR 1.22, 95% CI 0.74 to 1.99 (Seyyedsalehi 2023). We contacted the journal and study authors, but the review remains unchanged.

Traditional reviews can become outdated quickly, particularly when study conclusions rely on only one or two studies. For example, of the three reviews with major errors or omissions, there was a change in overall statistical significance of the association when these issues were addressed, leading to very different review conclusions (Hosseini 2020; Picetti 2022; Seyyedsalehi 2023). Review sensitivity is also compounded when study heterogeneity is high (see Table 1).

Table 1. Review characteristics and results from three systematic reviews and meta-analyses on the relationship between nitrate in drinking water and colorectal cancer

Review characteristics	Essien 2022	Hosseini 2020*	Picetti 2022**
Protocol prospectively registered	Not reported	Not reported	PROSPERO register, CRD42020186945
Databases	PubMed, Embase, the Cochrane Library databases, the Web of Science, and Google Scholar	PubMed/MEDLINE, Scopus, ISI Web of Science, Embase, and Google Scholar	MEDLINE OvidSP, PubMed OvidSP, Embase OvidSP, Global Health, Scopus, ISI Web of Science, GreenFILE, and AGRIS
Dates	Database inception to 2020	Database inception to 2020	1990 to 2021
Duplicate screening	Yes	Not reported	Partial (20%)
Duplicate full text review	Not stated	Not reported	
Duplicate data extraction	Not clear if it was in duplicate	Yes	Partial (10%)
# included papers	9	8	14
Risk of bias assessment	Newcastle–Ottawa Scale (S)	Newcastle–Ottawa Scale (S)	none
Double counting cohorts	Yes (Jones 2019; Weyer 2001)	Yes (Jones 2019; Weyer 2001)	Yes (Jones 2019; Weyer 2001)
Double counting outcomes	Yes (McElroy 2008; Morales-Suarez-Varela 1995)	Yes (De Roos 2003; Jones 2019; Weyer 2001)	Yes (De Roos 2003; Jones 2019; McElroy 2008; Weyer 2001)
Quality appraisal	None (conflated with risk of bias assessment)	None (conflated with risk of bias assessment)	Bespoke quality appraisal combining the CASP 2023 and STROBE appraisal criteria for ecological studies were adapted from Marchevsky 2000
Included study designs	Cohort, case-control, ecological	Cohort, case-control	Cohort, case-control, ecological, cross-sectional
Exposure measurement	Lowest vs highest exposure category	Lowest vs highest exposure category	Increase per 2.66 mg/L increase in nitrate-nitrogen
Outcome measurement	Incidence and mortality	Incidence	Incidence and mortality
Main finding	**Colorectal cancer =** not included **Colon cancer =** OR 1.11, 95% CI 1.04 to 1.23 **Rectum cancer =** OR 1.07, 95% CI 0.86 to 1.28	**Colorectal cancer =** Original OR 1.04, 95% CI 0.92 to 1.19; Updated OR 1.39, 95% CI 1.09 to 1.78 **Colon cancer =** not included **Rectum cancer =** not included	**Colorectal cancer =** OR 1.05, 95% CI 1.00 to 1.11 **Colon cancer =** OR 1.05, 95% CI 0.96 to 1.15 **Rectum cancer =** OR 1.09, 95% CI 0.89 to 1.33
Heterogeneity	37.3%, P = 0.072	74.1%, P = 0.009	32.9%, P = 0.02

CASP: Critical Appraisal Skills Programme; CI: confidence interval; OR: odds ratio; STROBE: STrengthening the Reporting of OBservational studies in Epidemiology checklist; vs: versus

*We presented the review characteristics post-correction.
**Includes only the results for colorectal cancer

Converting an existing review into a living review

In 2022, a systematic review and meta-analysis on nitrate and nitrite contamination in drinking water and cancer risk was published in Environmental Research (Picetti 2022). The review was led by researchers at the London School of Hygiene & Tropical Medicine. The review included 60 studies investigating the association between nitrate in drinking water and cancers. The authors observed a positive association between nitrate exposure and gastric cancer (odds ratio (OR) 1.91, 95% CI 1.09 to 3.33 per 2.66 mg/L increment in nitrate-nitrogen). They did not observe a statistically significant association for colorectal cancer (OR 1.052, 95% CI 0.995 to 1.111), but they did not include Espejo-Herrera 2016, which was the largest case-control study, with a positive association between nitrate and colorectal cancer (OR 1.47, 95% CI 1.24 to 1.79).

Together with researchers at the University of Otago, we propose to convert the existing review into an LSR, and update the review to adhere to current Cochrane methodology. The major updates will include: duplicate abstract screening, full-text review, data extraction, risk of bias in non-randomised studies - of exposure (ROBINS-E) and GRADE assessment; inclusion of criteria for establishing a living review. Other updates will include: removing the double counting of cohorts; including studies that report mg/d nitrate exposures (e.g. Espejo-Herrera 2016); separately assessing outcomes (colorectal cancer, colon cancer, rectal cancer). This protocol outlines the updated methodology of the LSR on nitrate and nitrite in drinking water and cancer.

OBJECTIVES

To assess the association between nitrate and nitrite in drinking water and cancers in observational studies

METHODS

Criteria for considering studies for this review

The populations, exposure, comparison, and outcomes (PECO) were determined a priori by the research team, with input from the Cochrane Public Health Group (Morgan 2018). Our PECO-defined question is:

Among the general population (P), what is the effect of the highest concentrations of nitrate or nitrite in drinking water (E) compared to the lowest concentrations of nitrate or nitrite in drinking water (C) on cancer incidence and cancer mortality (O)?

Types of studies

We will include prospective or retrospective longitudinal observational studies, in which the exposure concentrations were assessed at baseline; and case-control studies, in which outcome rates were compared between matched groups. Ethical considerations of purposefully exposing participants to a carcinogen precluded randomised controlled trials of any design for this research question. Nested case-control studies are eligible.

We will exclude ecological and cross-sectional study designs, as they provide limited evidence of causal inference.

Our definition of an ecological study design is one in which both the exposure and outcome are aggregated. However, we will still include case-control and cohort studies that use a semi-ecological exposure assessment (e.g. nitrate levels for an entire city water supply).

While we will not directly assess animal studies, they will be discussed in the final review.

Types of participants

We will include studies in the general population of any age, from any setting, in any country, including participants with predisposing factors or medication use related to the outcome of interest.

We will exclude studies that only use a subset of the eligible participants.

Types of exposure

We will include any study reporting the drinking water concentration of nitrate (NO_3) or nitrite (NO_2) from the water supplies of participants' residential address. We will not apply any restrictions on the exposure assessment, meaning that exposure assessments could use spot or repeated measures from quantitative laboratory testing results or estimates from environmental modelling.

Nitrate and nitrite are measured as either the concentrations of the ions nitrate (NO_3) or nitrite (NO_2), or as the element nitrogen (N) nitrate-nitrogen (NO_3-N) or nitrite-nitrogen (NO_2-N). For consistency, all concentrations of nitrate or nitrite will be converted and presented as the element nitrogen, e.g. nitrate-nitrogen mg/L and nitrite-nitrogen mg/L. We will use the following conversion formulae: nitrate-N = nitrate x 0.226; and nitrite-N = nitrite x 0.304.

Types of outcome measures

Included studies must report at least one cancer outcome. Our primary outcomes of interest will be all-cancer incidence and all-cancer mortality.

Secondary outcomes will be any site-specific cancers (e.g. colon and rectal cancers separately). We will accept self-reports and International Classification of Diseases (ICD)-10 code definitions for cancer outcomes (WHO 1992). Eligible studies will report either risk ratios (RR), hazard ratios (HR), odds ratios (OR), or outcome incidence data.

As outlined in Table 1, there were problems with double counting cohorts in previous reviews, when multiple outcomes were included in the same meta-analysis (e.g. results for colon and rectal cancers were included in a meta-analysis of colorectal cancer as separate effect sizes).

When multiple outcomes are presented (e.g. rectal cancer only, colon cancer only, colorectal cancer) we will sort studies into those groups. When a study does not provide an effect estimate for colorectal cancer, but presents results for colon and rectal cancer separately (or distal/proximal, as long as they are the only options describing the location within the colon), we will combine these effect estimates in a fixed-effect meta-analyses first, then combine results with other studies in a random-effects meta-analysis.

Search methods for identification of studies

Electronic searches

We will identify eligible peer-reviewed studies through online searches of MEDLINE Ovid, Embase, Global Health, Scopus, ISI Web of Science, GreenFILE, and AGRIS databases. We will apply no language restrictions, and will translate articles published in languages other than English. We will apply no publication date restrictions, and search from inception of the database. We will submit identified conference presentations to further online searches, if a related peer-review publication is not initially identified.

The search strategy was adapted from Picetti 2022. The adaption includes the introduction of an outcome concept for cancer to reduce the number of abstracts. All included studies from Picetti 2022 were identified in a MEDLINE search with the outcome restriction. The MEDLINE search strategy is shown in Appendix 1, and will be adapted to other databases. In brief, the search strategy includes three main concepts: (1) nitrate/nitrite exposure; (2) drinking water; and (3) cancer. Nitrate exposure terms include nitrate, nitrite, nitrogen, and nitroso. Drinking water search terms include drinking water, groundwater, and water supply. Cancer search terms include cancer, tumour/tumors.

Searching other resources

Online searches will be supplemented with routine screening of bibliographies and references of the included studies. At least two review authors will independently screen bibliographies and references.

Data collection and analysis

Selection of studies

We will complete all abstract and full-text screening in Covidence (Covidence). Covidence is a web-based software platform that streamlines the production of systematic and other literature reviews. After de-duplication of search records, two review authors will independently screen the titles and abstracts to identify those reports requiring full-text review. A full-text review will identify all studies eligible for inclusion. Records for which we can not obtain the full text will be classified as 'studies awaiting classification'. We will resolve any disagreements between review authors at any stage of the eligibility assessment process through discussion and consultation with a third review author.

Data extraction and management

Two review authors will independently extract data and enter them onto forms designed and piloted for this review. We will resolve disagreements through discussion and consultation with a third review author. If there are multiple publications from the same cohort, we will use data for the longest follow-up period. We will extract data on the following:

- Study details, including author details, conflict of interest declaration, funding source (if not reported, this will be requested), setting
- Methods, including design, dates, sample size, participant characteristics (sex, age, ethnicity, rurality, and deprivation), eligibility criteria, method of recruitment, participant flow details, health status, and matching parameters in case-control studies
- Exposures, including description of assessment method, sampling frequency, and water body
- Comparators,including levels of exposure per quantile, duration of follow-up in prospective observational studies, and variables controlled for
- Outcomes,including source of outcome data (i.e. death certificate, medical record, or data linkage), numeric data relevant to all primary and secondary outcomes, such as case numbers, person years, quantile variables, and any variables used to calculate comparable statistics, such as risk ratio (RR). We will preferentially extract the most adjusted RR, odds ratio (OR), hazard ratio (HR), and quantile data, when provided. We expect the most adjusted model to include adjustment for age and sex, but we will not exclude studies that do not adjust for these covariates.

Assessment of risk of bias in included studies

We will use the risk of bias instrument for non-randomised studies of exposures (ROBINS-E), consistent with Cochrane methodology, to assess the risk of bias in observational exposure studies (Higgins 2023a; Reeves 2023). ROBINS-E is modelled on the ROBINS of interventions (ROBINS-I) tool. The ROBINS-E tool covers seven domains including: (1) bias due to confounding, (2) bias in selection of participants into the study, (3) bias in classification of exposures, (4) bias due to departures from intended exposures, (5) bias due to missing data, (6) bias in measurement of outcomes, and (7) bias in selection of reported results. We will also collect information on sources of funding and conflicts of interest in each study. Each risk of bias domain is graded as low, moderate, serious, or critical. Each study is also graded as having low, moderate, serious, or critical risk of bias. The study-level risk of bias assessment will form part of the GRADE assessment outlined below, as recommended by Morgan 2019. For the risk of bias assessment using ROBINS-E, we will identify age and sex as critical confounders for adjustment in the analysis. While body mass index, smoking status, and alcohol consumption are identified as important risk factors for a range of cancers, there is no consistent evidence that these risk factors are also associated with nitrate concentrations in drinking water (exposure). Two review authors will independently assess the risk of bias. A third review author will resolve any disagreements, to reach consensus.

Measures of the effect

All outcomes of interest will be dichotomous, allowing us to present summary RR with 95% confidence intervals (CIs). When event rates are reported per person-years, followed in separate groups, we will calculate incident rate ratios with 95% CIs, so that these studies can be included in meta-analysis with studies reporting rate ratios for the same outcomes. We will calculate rate ratios by dividing the rate in the exposed group by the rate in the control group. We will calculate the 95% CI of these rate ratios by taking the antilogarithm of the natural log of the rate ratio (log(IRR)), plus or minus 1.96 times the standard error of the log(IRR). We will calculate the standard error as the square root of the sum of the inverse of events in the exposed and less exposed groups. We will impute the number of cases per quantile from the RR value, when necessary.

Dealing with missing data

We will contact study authors to request any missing or unreported data (maximum of three attempts), such as data on covariates, details of attrition, or details of the type of analysis conducted.

Data synthesis

We will pool the reported RR from more than one study with a DerSimonian and Laird random-effects model to compare the highest exposure levels with the lowest. When individual studies report results separately by sex, we will combine these effect size estimates with a fixed-effect model, before including them in the broader pooled estimate. Studies reporting incidence or mortality will be analysed separately. When data are reported in a suitable format, we will consider dose-response relationships with the Greenland and Longnecker method, assuming linearity with a two-stage, dose-response, random-effects analysis. The Greenland and Longnecker method considers exposure concentrations from the lowest level reported in a publication to the highest level of exposure concentration reported in a publication across the included studies, considering every value between these values (Greenland 1992). As a result, this method accounts for differences in exposure concentrations between studies, which has been applied by review authors in previous reviews (Reynolds 2019). We will use the average or mid-point of each defined quantile for the dose amount. If the quantile dose range is open-ended, we will use half the range of the adjacent quantile to establish the average exposure level of that quantile. When presenting the dose response values, we will use 1 mg/L to represent one viable increase in exposure concentration. We will assess non-linear dose-response by restricted cubic splines with three knots at 10%, 50%, and 90% distribution, combined with multivariate meta-analyses. We will show linear and spline (with 95% CI) models together, with each data point overlaid as circles. Circle size will indicate the weighting of each data point, with bigger circles indicating greater influence. We will not use duplicate data.

The PECO formulation will account for the fact that the exposure is a continuous variable. The impact of exposure 'x' on incidence 'I' will be modelled as multiplicative I(x + 1) = beta I(x), where beta = RR per unit exposure (mg/L); and then as additive I(x + 1) = I(x) + lambda, where lambda = risk difference per unit exposure. These models differ in implication in the meta-analysis because background cancer rates and exposure levels differ among the contributing studies. Both models are appropriate, because a priori, there is evidence that nitrate may act on cancer in an additive and multiplicative way. We will also construct a combined multiplicative-additive model. The data synthesis involves fitting these basic models to these inputs, and then allowing more complicated dose-response relationships, as with the Greenland and Longnecker method, where the data permit.

Meta-regression analysis and investigation of heterogeneity

We will perform meta-regression analyses when data allow, to identify driving differences between studies and potential sources of heterogeneity, with these subgroups.

- Exposure assessment – laboratory results or modelled
- Geographic region
- Exposure period – latency period in years and age of exposure
- Adjustment for dietary nitrate and nitrite
- Health equity: sex, ethnicity, deprivation, and rurality

Assessment of heterogeneity

For each meta-analysis, we will use the I^2 statistic to estimate the level of heterogeneity among the studies. We will define substantial heterogeneity as $I^2 > 50\%$. When substantial heterogeneity is identified, we will explore reasons for this incongruency by subgrouping differences in the studies, such as risk of bias, meta-regression, and influence analyses, as explained in Meta-regression analysis and investigation of heterogeneity. We will use caution in the interpretation of results with high levels of unexplained heterogeneity, and will GRADE the evidence accordingly.

Assessment of reporting biases

For each meta-analysis, we will assess the possibility of small-study effects with an Egger's test, and in the case of asymmetry ($P < 0.05$), conduct a trim and fill analysis to consider the pooled effect size with potential missing data extrapolated.

Sensitivity analysis

We will conduct sensitivity analyses for all outcomes, assessing the impact of:

- Each individual study, by removing them one at a time and considering their effect on the pooled result with an influence analysis.
- Risk of bias, by only including results with a low or moderate risk of bias.
- Removing studies with a modelled exposure assessment.
- Removing studies in which outcomes have been combined (e.g. colon and rectal outcomes into colorectal cancer).
- Removing studies with a funder with a clear competing interest.

Certainty of evidence - GRADE

We will use GRADE protocols to judge the certainty of evidence (CoE) as either high, moderate, low, or very low for each outcome. The *Cochrane Handbook for Systematic Reviews of Interventions* states that authors conducting GRADE assessments on observational studies generally start the assessment with a low grading (Higgins 2023). However, this has been challenged in the field of environmental health, in which observational studies provide a substantial contribution to evidence-based decision-making, and overcome the challenges of implementing randomised control trials (e.g. because it would be unethical to randomise people to a condition that is hypothesised to cause

adverse health impacts (Woodruff 2014)). For example, the US Institute of Medicine panel concluded that observational studies were generally the most appropriate for answering questions related to incidence, prevalence, and etiology (Sox 2008). As a result, we will start the GRADE rating at moderate.

GRADE considers eight domains that can impact the CoE for each outcome, which include: risk of bias, inconsistency, indirectness, imprecision, publication bias, magnitude of effect, dose-response gradient, and opposing residual confounding. The first five of these domains can lead to downgrading the CoE, while the last three domains can lead to upgrading the CoE (Higgins 2023). We will use the study-level assessments of risk of bias to inform our GRADE assessment (Morgan 2019).

We will downgrade the CoE by one level for each serious risk, by two levels for each very serious risk, and by three levels for each critical risk of the following criteria: risk of bias, inconsistency of effect, indirectness, imprecision, or other considerations (e.g. conflicts of interest). We will describe the decisions and reasons for downgrading or upgrading the certainty of evidence in the footnotes of the summary of findings tables.

Summary of findings tables

We will present the meta-regression analysis results and GRADE assessment for all-cancer incidence, all-cancer mortality and site-specific cancer in our summary of findings table.

Living systematic review considerations

We plan to make all data sets and analyses available as part of the living systematic review and meta-analyses on this topic. We are happy to share all the information collated and generated with future research teams to maintain an up-to-date, viable, evidence synthesis on nitrate and nitrite in drinking water on cancer outcomes. We will develop this review as a living systematic review, and the searches will be re-run every six months.

Whenever new evidence is identified that is relevant to the review, we will extract the data and assess the risk of bias. We will wait until the accumulating evidence changes the findings of one or more outcomes, e.g. a change in size or direction of an effect, before incorporating the evidence and re-publishing the review. Upon each publication update, including the original publication, we will conduct a cumulative meta-analysis, assessing the direction and strength of the effect over time.

At the time of each update, we will publish: (1) the number of new papers identified, (2) the reason for any exclusions, and an update to the PRISMA diagram, (3) the number of papers meeting the inclusion criteria but not included in an updated estimate (e.g. because it did not change effect size or direction, or alter study conclusions). Any changes in published review updates will also be included and freely accessible.

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Editorial and peer-reviewer contributions

The following people conducted the editorial process for this article:

- Sign-off Editor (final editorial decision): Lisa Bero, Cochrane Editorial Board; University of Colorado, USA
- Managing Editor (selected peer reviewers, provided editorial guidance to authors, edited the article): Liz Bickerdike, Cochrane Central Editorial Service
- Editorial Assistant (conducted editorial policy checks, collated peer-reviewer comments and supported editorial team): Leticia Rodrigues, Cochrane Central Editorial Service
- Copy Editor (copy editing and production): Victoria Pennick, Cochrane Central Production Service
- Peer-reviewers (provided comments and recommended an editorial decision): Jennifer Hilgart, Cochrane (methods), Steve McDonald, Cochrane Australia (search), Louis Leslie, University of Colorado Anschutz Medical Campus (clinical), Daniel Axelrad, Independent Consultant, Washington, DC, USA (clinical). One additional peer reviewer provided clinical peer review but chose not to be publicly acknowledged.

REFERENCES

Additional references

Bryan 2017

Bryan NS, Loscalzo J. Nitrite and Nitrate in Human Health and Disease. NJ: Springer; Humana Totowa, 2017. [DOI: 10.1007/978-1-60761-616-0]

CASP 2023

CASP Team. Critical Appraisal Skills Programme (CASP) appraisal checklists. casp-uk.net/casp-tools-checklists/ (accessed March 2024).

Chambers 2021

Chambers T, Hales S, Anglemyer A. Letter to the editor: Correction "Nitrate-nitrite exposure through drinking water and diet and risk of colorectal cancer: A systematic review and meta-analysis of observational studies". *Clinical Nutrition* 2021;**40**(11):5443-4. [DOI: 10.1016/j.clnu.2021.09.027]

Cochrane 2019

Cochrane. Guidance for the production and publication of Cochrane living systematic reviews: Cochrane Reviews in living mode. Available at community.cochrane.org/sites/default/files/uploads/inline-files/Transform/201912_LSR_Revised_Guidance.pdf 2019.

Cochrane 2022

Cochrane. Living systematic reviews. Available from community.cochrane.org/review-development/resources/living-systematic-reviews (accessed 13 March 2024).

Covidence [Computer program]

Covidence. Version (accessed 13 March 2024). Melbourne, Australia: Veritas Health Innovation. Available at covidence.org.

Crengle 2022

Crengle S, Davie G, Whitehead J, de Graaf B, Lawrenson R, Nixon G. Mortality outcomes and inequities experienced by rural Māori in Aotearoa New Zealand. *Lancet Regional Health – Western Pacific* 2022;**28**:100570. [DOI: 10.1016/j.lanwpc.2022.100570]

De Roos 2003

De Roos AJ, Ward MH, Lynch CF, Cantor KP. Nitrate in public water supplies and the risk of colon and rectum cancers. *Epidemiology* 2003;**14**(6):640-9. [DOI: 10.1097/01.ede.0000091605.01334.d3]

Espejo-Herrera 2016

Espejo-Herrera N, Gràcia-Lavedan E, Boldo E, Aragonés N, Pérez-Gómez B, Pollán M, et al. Colorectal cancer risk and nitrate exposure through drinking water and diet. *International Journal of Cancer* 2016;**139**(2):334-46. [DOI: 10.1002/ijc.30083]

Essien 2022

Essien EE, Said Abasse K, Côté A, Mohamed KS, Baig MM, Habib M, et al. Drinking-water nitrate and cancer risk: a systematic review and meta-analysis. *Archives of Environmental & Occupational Health* 2022;**77**(1):51-67. [DOI: 10.1080/19338244.2020.1842313]

Gandarilla-Esparza 2021

Gandarilla-Esparza DD, Calleros-Rincón EY, Macias HM, González-Delgado MF, Vargas GG, Sustaita JD, et al. FOXE1 polymorphisms and chronic exposure to nitrates in drinking water cause metabolic dysfunction, thyroid abnormalities, and genotoxic damage in women. *Genetics and Molecular Biology* 2021;**Oct 4**:44. [DOI: 10.1590/1678-4685-GMB-2021-0020]

Greenland 1992

Greenland S, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. *American Journal of Epidemiology* 1992;**135**(11):1301-9. [DOI: 10.1093/oxfordjournals.aje.a116237]

Gurjao 2021

Gurjao C, Zhong R, Haruki K, Li YY, Spurr LF, Lee-Six H, et al. Discovery and features of an alkylating signature in colorectal cancer. *Cancer Discovery* 2021;**11**(10):2446-55. [DOI: 10.1158/2159-8290.CD-20-1656]

Habermeyer 2015

Habermeyer M, Roth A, Guth S, Diel P, Engel KH, Epe B, et al. Nitrate and nitrite in the diet: how to assess their benefit and risk for human health. *Molecular Nutrition & Food Research* 2015;**59**(1):106-28. [DOI: 10.1002/mnfr.201400286]

Higgins 2023

Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 6.4 (updated August 2023). Cochrane, 2023. Available from www.training.cochrane.org/handbook.

Higgins 2023a

Higgins J, Morgan R, Rooney A, Taylor K, Thayer K, Silva R, et al, ROBINS-E Development Group. Risk Of Bias In Non-randomized Studies - of Exposure (ROBINS-E). Launch version, 20 June 2023. Available from www.riskofbias.info/welcome/robins-e-tool.

Hosseini 2020

Hosseini F, Majdi M, Naghshi S, Sheikhhossein F, Djafarian K, Shab-Bidar S. Nitrate-nitrite exposure through drinking water and diet and risk of colorectal cancer: a systematic review and meta-analysis of observational studies. *Clinical Nutrition* 2021;**40**(5):3073-81. [DOI: 10.1016/j.clnu.2020.11.010]

IARC 2010

International Agency for Research on Cancer (IARC). Ingested nitrate and nitrite, and cyanobacterial peptide toxins; IARC monographs on the evaluation of carcinogenic risks to humans Volume 94. Available at monographs.iarc.who.int/ 2010.

Jones 2019

Jones RR, DellaValle CT, Weyer PJ, Robien K, Cantor KP, Krasner S, et al. Ingested nitrate, disinfection by-products, and risk of colon and rectal cancers in the Iowa Women's Health

Study cohort. *Environment International* 2019;**126**:242-51. [DOI: 10.1016/j.envint.2019.02.010]

Kobayashi 2018

Kobayashi J. Effect of diet and gut environment on the gastrointestinal formation of N-nitroso compounds: a review. *Nitric Oxide* 2018;**73**:66-73. [DOI: 10.1016/j.niox.2017.06.001]

Marchevsky 2000

Marchevsky D. Critical appraisal of different study designs. In: Critical Appraisal of Medical Literature. Springer Science & Business Media, 2000:139-42.

McElroy 2008

McElroy JA, Trentham-Dietz A, Gangnon RE, Hampton JM, Bersch AJ, Kanarek MS, et al. Nitrogen-nitrate exposure from drinking water and colorectal cancer risk for rural women in Wisconsin, USA. *Journal of Water and Health* 2008;**6**(3):399-409. [DOI: 10.2166/wh.2008.048]

ME NZ 2019

Ministry for the Environment and Statistics New Zealand (ME NZ). New Zealand's Environmental Reporting Series: Environment Aotearoa 2019. Available from environment.govt.nz/publications/environment-aotearoa-2019/ 2019.

Morales-Suarez-Varela 1995

Morales-Suarez-Varela MM, Llopis-Gonzalez A, Tejerizo-Perez ML. Impact of nitrates in drinking water on cancer mortality in Valencia, Spain. *European Journal of Epidemiology* 1995;**11**(1):15-21. [DOI: 10.1007/BF01719941]

Morgan 2018

Morgan RL, Whaley P, Thayer KA, Schünemann HJ. Identifying the PECO: a framework for formulating good questions to explore the association of environmental and other exposures with health outcomes. *Environment International* 2018;**121**(Pt 1):1027-31. [DOI: 10.1016/j.envint.2018.07.015]

Morgan 2019

Morgan RL, Thayer KA, Santesso N, Holloway AC, Blain R, Eftim SE, et al. A risk of bias instrument for non-randomized studies of exposures: a users' guide to its application in the context of GRADE. *Environment International* 2019;**122**:168-84. [DOI: 10.1016/j.envint.2018.11.004]

Picetti 2022

Picetti R, Deeney M, Pastorino S, Miller MR, Shah A, Leon DA, et al. Nitrate and nitrite contamination in drinking water and cancer risk: a systematic review with meta-analysis. *Environmental Research* 2022;**210**:112988. [DOI: 10.1016/j.envres.2022.112988]

Reeves 2023

Reeves BC, Deeks JJ, Higgins JPT, Shea B, Tugwell P, Wells GA. Chapter 24: Including non-randomized studies on intervention effects. In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 6.4 (updated August 2023). Cochrane, 2023. Available from www.training.cochrane.org/handbook.

Reynolds 2019

Reynolds A, Mann J, Cummings J, Winter N, Mete E, Te Morenga L. Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *Lancet* 2019;**393**(10170):434-45. [DOI: 10.1016/S0140-6736(18)31809-9]

Schullehner 2018

Schullehner J, Hansen B, Thygesen M, Pedersen C, Sigsgaard T. Nitrate in drinking water and colorectal cancer risk: a nationwide population-based cohort study. *International Journal of Cancer* 2018;**143**(1):73-9. [DOI: 10.1002/ijc.31306]

Seyyedsalehi 2023

Seyyedsalehi MS, Mohebbi E, Tourang F, Sasanfar B, Boffetta P, Zendehdel K. Association of dietary nitrate, nitrite, and n-nitroso compounds intake and gastrointestinal cancers: a systematic review and meta-analysis. *Toxics* 2023;**11**(2):190. [DOI: 10.3390/toxics11020190]

Sox 2008

Sox H, McNeil B, Wheatley B, Eden J, editor(s), Institute of Medicine. Knowing What Works in Health Care: A Roadmap for the Nation. Washington, DC: The National Academies Press, 2008. [DOI: 10.17226/12038]

Temkin 2019

Temkin A, Evans S, Manidis T, Campbell C, Naidenko OV. Exposure-based assessment and economic valuation of adverse birth outcomes and cancer risk due to nitrate in United States drinking water. *Environmental Research* 2019;**176**:108442. [DOI: 10.1016/j.envres.2019.04.009]

van Breda 2021

van Breda SG, Mathijs K, Pieters HJ, Sági-Kiss V, Kuhnle GG, Georgiadis P, et al. Replacement of nitrite in meat products by natural bioactive compounds results in reduced exposure to N-Nitroso compounds: the PHYTOME project. *Molecular Nutrition & Food Research* 2021;**65**(20):2001214. [DOI: 10.1002/mnfr.202001214]

Ward 2018

Ward MH, Jones RR, Brender JD, De Kok TM, Weyer PJ, Nolan BT, et al. Drinking water nitrate and human health: an updated review. *International Journal of Environmental Research and Public Health* 2018;**15**(7):1557. [DOI: 10.3390/ijerph15071557]

Weyer 2001

Weyer PJ, Cerhan JR, Kross BC, Hallberg GR, Kantamneni J, Breuer G, et al. Municipal drinking water nitrate level and cancer risk in older women: the Iowa Women's Health Study. *Epidemiology* 2001;**12**(3):327-38. [DOI: 10.1097/00001648-200105000-00013]

WHO 1992

World Health Organization (WHO). The ICD-10 classification of mental and behavioural disorders: clinical descriptions and diagnostic guidelines (Vol. 1). Available from www.who.int/publications/i/item/9241544228 1992.

WHO 2016

World Health Organization (WHO). Nitrate and nitrite in drinking-water; background document for development of who guidelines for drinking-water quality. WHO/FWC/WSH/16.52. Available at cdn.who.int/media/docs/default-source/wash-documents/wash-chemicals/nitrate-nitrite-background-jan17.pdf?sfvrsn=1c1e1502_4 2016.

WHO 2022

World Health Organization (WHO). Guidelines for drinking-water quality: fourth edition incorporating the first and second addenda. Available at www.who.int/publications/i/item/9789240045064 2022.

Woodruff 2014

Woodruff TJ, Sutton P. The Navigation Guide systematic review methodology: a rigorous and transparent method for translating environmental health science into better health outcomes. *Environmental Health Perspectives* 2014;**122**(10):1007-14. [DOI: 10.1289/ehp.1307175]

APPENDICES

Appendix 1. MEDLINE search strategy

MEDLINE Ovid

1	(nitrate* or nitrite* or no3* or no2* or nitroso or nitrogen).kf,tw,mp.
2	exp Nitrates/ or exp Nitrites/
3	1 or 2
4	(cancer* or tumo?r* or carcino* or neoplas* or malignan* or adenocarcinoma*).kf,tw,mp.
5	exp Neoplasms/
6	4 or 5
7	(((public or well or drinking or bottled or suppl* or consum* or tap or potable) adj5 water*) or groundwater).kf,tw,mp.
8	exp Drinking Water/
9	7 or 8
10	3 and 6 and 9
11	10 not (exp animals/ not humans.sh.)

CONTRIBUTIONS OF AUTHORS

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DECLARATIONS OF INTEREST

TC has declared that they have no conflict of interest.
RW has declared that they have no conflict of interest.

AR has declared that they have no conflict of interest.
AA has declared that they have no conflict of interest.
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