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REVIEW

Nitrate in drinking water and pregnancy outcomes: A narrative review of epidemiological evidence and proposed biological mechanisms

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Abstract

There is emerging evidence of an association between nitrate contamination in drinking water and adverse pregnancy outcomes. Few studies have discussed the evidence in the context of plausible biological mechanisms. We performed a narrative review of the current evidence investigating associations between nitrate in drinking water and the risk of adverse pregnancy outcomes with a focus on congenital anomalies (CA) and preterm birth (PTB). We also reviewed evidence and discuss several biological mechanisms that may explain the observed associations. We reviewed cohort and case-control epidemiological studies assessing associations between maternal nitrate exposure and adverse pregnancy outcomes published until January 2023. Three plausible mechanisms may explain how maternal exposure to nitrate may impact pregnancy outcomes, which include the formation of *N*-nitroso compounds (NOC), thyroid dysfunction and oxidative stress. For CA, seven studies were included (two cohort and five case-control studies). The strongest associations observed in epidemiological studies were for neural tube defects. Inconsistent positive and negative associations were observed for anomalies of the limb, eye, ear, face and neck. Of the four PTB studies (all cohort studies), three of the largest cohort studies observed associations between PTB with nitrate levels >5 mg/L while one small cohort study did not. Emerging epidemiological evidence has observed a potential increased risk of adverse pregnancy outcomes with elevated maternal nitrate exposure in drinking water. There are also plausible biological mechanisms to support this association. However, the current evidence lacks homogeneity and additional longitudinal evidence with robust exposure asssessments is required. Given the increasing concentrations of nitrate contamination in drinking water in many countries, and the adverse effects observed at concentrations below the current

regulatory standard, a further precautionary approach should be adopted until futher evidence emerges.

Introduction

Nitrogen is essential for plant growth and a major constituent of agricultural fertilisers. The major sources of nitrate leaching from soils into groundwater, rivers and drinking water in New Zealand are fertilisers and livestock urine [1].

The World Health Organisation (WHO) has issued guidelines on safe concentrations of nitrate in drinking water that are based on the prevention of short-term health effects such as methemoglobinemia and thyroid dysfunction [2]. The current WHO guideline for the maximum acceptable value (MAV) is 50 mg/L (for total nitrate) or 11.3 mg/L as nitrate-nitrogen (the nitrogen component of the ion) [1]. To simplify communication, we have converted all nitrate concentrations in this article to nitrate-nitrogen levels. The WHO guideline has been adopted in many countries (including New Zealand and most of Europe), with the USA adopting a slightly lower value of 10 mg/L [2, 3].

Nitrate ingested through food and drinking water is absorbed by the stomach and small intestine [2, 3]. Under normal physiological condition, it is either excreted in urine or reabsorbed from blood ending up in salivary glands in the oral cavity where it is reduced to nitrite (a more reactive form of oxidised nitrogen) [2]. In humans, nitrate and nitrite metabolites can form *N*-nitroso compounds (NOC) that are carcinogenic (cancer causing) and teratogenic (causing congenital anomaly) [2, 4].

The suspected teratogenic effects of NOC have been the primary driver of research into the potential health effects of maternal exposure to nitrate in drinking water and adverse pregnancy outcomes. However, other biological mechanisms may play a role in nitrate-related adverse pregnancy outcomes including materno-fetal thyroid disruption [5–7] and oxidative stress [8, 9].

Nitrate in drinking water has also been linked to several other adverse reproductive outcomes in addition to CA and PTB, including small for gestational age [10, 11], prelabour rupture of membranes [12] and pregnancy loss [6, 13, 14]. However, the evidence for these outcomes is less consistent than for those studies investigating CA and PTB which are the focus of this article.

Four reviews assessing the impact of nitrate in drinking water on pregnancy outcomes have highlighted some limitations in the current evidence, including a reliance on ecological study designs, small sample sizes and potential confounding [2, 3, 15, 16]. Recent epidemiological studies have addressed some of these limitations but results need to be interpreted in the context of biological mechanisms underpinning the potential causal relationship ascribed to NOC exposure. In this review, we discuss the potential biological mechanisms that may explain epidemiological evidence of associations between maternal exposure to nitrate in drinking water and adverse pregnancy outcomes.

Search strategy

We performed a search on two databases (Pubmed and Embase) to identify epidemiological studies of nitrate in drinking water and congenital anomalies or preterm birth. For the literature search we used the MESH terms (and corresponding Emtree subject headings) "nitrates" (entry term: nitrate) "drinking water" (entry terms: bottled water, potable water), "congenital anomalies" (entry terms: [abnormalities, congenital], birth defects, congenital defects,

deformities, fetal anomalies, fetal malformations), "premature birth" (entry term: preterm birth), "pregnancy outcome" (entry terms: [outcome, pregnancy], [outcomes, pregnancy], pregnancy outcomes) and "maternal exposure" (entry term: maternal exposure). We also reviewed studies included in previous meta-analyses and narrative reviews on this topic.

We included cohort and case-control studies, in English, published until January 2023.

Biological mechanisms

Previous research has suggested several biological mechanisms may underly the associations observed in epidemiological studies between nitrates in drinking water and adverse pregnancy outcomes. These include the formation of NOC, thyroid dysfunction and oxidative stress. In this paper, we have focused on congenital anomalies (CA) and preterm birth (PTB) as the main adverse pregnancy outcomes.

N-nitroso compounds (NOC)

Several human studies in non-pregnant adults using NOC biomarkers in urine and saliva demonstrated that consumption of a diet high in nitrate increased the endogenous formation of NOC [17–20]. These studies examined total daily nitrate intake at levels comparable to and above the acceptable total daily intake of 0.84 mg/kg (of nitrate nitrogen) [17]. There are no epidemiological studies on CA and PTB that have used biomarkers for NOC, but an exposure-response association between exogenous exposure to NOC in drinking water during pregnancy and PTB has been reported in one study [21]. The evidence that NOC increases the risk of CA stems mainly from animal studies [22, 23] leading to difficulties when extrapolating conclusions to human populations due to the differences in fetal developmental trajectory across species. Several studies that used mothers' prescriptions of nitrosatable drugs (e.g. amoxicillin, caffeine, promethazine and pseudoephedrine) as a surrogate marker for endogenous NOC formation reported increased risks for CA [24, 25] as well as PTB [26, 27].

The mechanisms by which NOC may increase the risk of CA and PTB are not fully understood. The genotoxicity of NOC is likely to play a role, resulting in DNA damage and adverse fetal growth and development [21]. NOC, and in particular *N*-nitrosamines, have also been shown to induce oxidative stress, leading to impaired placental function and premature senescence in fetal membranes [21, 26]. A diet rich in antioxidants may mitigate this mechanism by reducing NOC formation and oxidative stress, while nitrosatable compounds in the diet (e.g., red meat, nitrosatable drugs) are likely to amplify it [24].

Thyroid dysfunction

Nitrate may also increase CA and PTB risk through its impact on the maternal thyroid gland. Nitrate ions (NO_3^-) compete with iodide (I^-) to bind to the sodium/iodide symporter, lowering iodine intake in the thyroid gland, which may cause reduced production of thyroid hormones and thyroid dysfunction [28]. This effect may be stronger under iodine deficient nutritional conditions [29] or for pregnant women who require more iodine to meet the increased physiological demands of pregnancy [4].

Studies examining the effect of nitrate on thyroid hormone levels in healthy non-pregnant adults have shown inconsistent results [30]. A study in people without iodine deficiency showed a dose-response association for nitrate in drinking water and thyroid volume (measured using sonography), with hypertrophy occurring at levels >11.3 mg/L [5]. An inverse association was also seen between the volume of thyroid and Thyroid Stimulating Hormone (TSH) levels suggesting the occurrence of hyperthyroidism with chronically elevated nitrate exposure [5].

Nitrate in well water >6.5 mg/L has been also associated with subclinical hypothyroidism (defined as TSH levels >4 mIU), although a clear dose-response was not observed; and no association was found in men [7]. Maternal hypothyroidism, including subclinical hypothyroidism, has been associated with PTB [31, 32].

Several mechanisms may be involved as maternal thyroid dysfunction in pregnancy has significant adverse effects on both fetal and maternal well-being (e.g. fetal hyperthyroidism, pre-eclampsia, PTB, placental abruption) [33]. Few epidemiological studies on maternal thyroid dysfunction and CA are available. The US National Birth Defects Prevention Study [34] reported an association with maternal thyroid hormone medication and four of 55 specific birth defects (anencephaly, holoprosencephaly, hydrocephaly, small intestinal atresia). A role for maternal thyroid hormone deficiencies in the development of CA is mechanistically plausible. In particular, maternal thyroid hormones orchestrate the expression of several fetal genes thereby ensuring the correct sequence of cell proliferation, differentiation and maturation in each tissue and organ [35]. In addition, during the critical time-window for CA in early gestation the embryonic thyroid gland has not yet matured [36]; consequently, the embryo still fully relies on maternal thyroid hormones [36].

Oxidative stress

Exposure to nitrate in drinking water may also increase the risk of CA and PTB through oxidative stress caused by nitrites and related reactive nitrogen and oxygen species. In both the mouth and the gastrointestinal tract, a proportion of nitrate is reduced into nitrite, which itself is an oxidising agent and can also generate other reactive oxygen and nitrogen species such as nitric oxide (NO), hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$) and superoxide anion (O_2^-) [9, 37]. This can affect the balance between pro-oxidants and antioxidants in favour of the former, resulting in oxidative stress [8]. A study of 150 Iowa non-pregnant private well users (aged 1–60 years) showed that levels of nitrate ingestion from drinking water were associated with blood methaemoglobin concentrations, a marker for oxidative stress [38]. However in another study among pregnant women in Minnesota no association between nitrate exposure and methaemoglobin levels in pregnancy was found [39]. Both studies examined effects largely below the regulatory standard of 10 mg/L of nitrate-nitrogen (e.g. the highest exposure category was <10.5 mg/L, with the second study having only 2% of study participants report nitrate levels greater than the MAV). For nitrates to induce haemoglobin to convert to methaemoglobin (which can then cause methaemoglobinaemia), high levels of nitrates need to be present in water, which was not demonstrated in either study. In addition there are a multitude of factors that influence oxidisation of haemoglobin, while methaemoglobin readily reduces back to haemoglobin making this a complex method to determine nitrate exposure [39].

A study among adult (18–45 year olds) non-pregnant Mexican women showed that water nitrate levels were associated with blood methaemoglobin levels and genotoxic damage in lymphocytes [40].

Oxidative stress has been implicated in many health complications during pregnancy including CA (specifically heart malformations, oesophageal atresia, biliary atresia, diaphragmatic hernia and autosomal dominant polycystic kidney) [41] and PTB [15]. A wide range of mechanisms may be involved as oxidative stress can induce DNA damage, telomere shortening, intrauterine infection and inflammation, premature placental aging, and it may also adversely affect the signalling pathways needed for correct embryogenesis [42]. A diet rich in antioxidants may negate the role of these mechanisms and provide some protection [42].

Epidemiological evidence

An increasing number of studies have assessed, or are currently assessing, associations between maternal nitrate exposure and adverse pregnancy outcomes. We will discuss the epidemiological evidence linking maternal nitrate exposure with CA and PTB. We have prioritised cohort and case-control studies as they provide better evidence for causal inference than ecological studies. Further, we focus on those studies with larger sample sizes and those that have appropriately controlled analyses for potential confounders or considered potential effect modifiers.

The current article is a review of existing evidence so ethical approval was not required.

Congenital anomalies

There is growing evidence for an association between nitrate levels in drinking water and the prevalence of children born with CA at concentrations well below the current WHO standard. We focus on the larger studies with robust study designs (cohort studies) with a brief summary of smaller epidemiological studies provided at the end. Descriptive characteristics of all studies investigating the association between nitrate and congenital anomalies are provided in Table 1.

Cohort studies. Stayner (2022) conducted a large retrospective cohort study (n = 1,018,914) assessing the association between nitrate concentrations in drinking water and CA [41]. The sample included Danish-born singleton births between 1991 and 2013 and identified 33,182 children with any CA. CA were defined as any diagnosis of a CA made before two years of age and were identified using the Danish National Patient Registry. Nitrate levels were taken from routine water monitoring data and linked with Danish residential addresses; from this, yearly average nitrate concentrations for each address were determined. Time-weighted exposure for each mother was calculated (taking in to account changes in residence and calendar year). Four nitrate exposure categories were defined based on the distribution of the exposure in the study population and the current EU standard. The highest exposure category of >5.65 mg/L nitrate is half the EU standard for maximal acceptable nitrate concentrations in drinking water; the lowest category <0.45 mg/L was used as the reference group.

In the primary analysis only eye anomalies were found to be significantly positively associated with increasing nitrate exposure (OR 1.09 95%CI 1.03, 1.16, per 2.2 mg/L increase in nitrate), with analyses adjusted for birth weight, birth order, maternal factors (smoking, BMI) and economic factors. There was a dose-response relationship, with exposure >5.65 mg/L being associated with the greatest increase in risk (OR 1.29 95%CI 1.00, 1.66). A statistically significant inverse exposure-response relationship was observed for diagnosis of any CA (p = 0.02) and for several specific categories (Table 1). The authors suggested that this latter finding might be explained by unobserved fetal loss. No specific analysis of effect modifiers related to any of the biological mechanisms discussed above was conducted.

Blaisdell (2019) conducted a retrospective cohort study in the state of Missouri (USA), which included 348,250 single live births for the period 2004–2008 [43]. Nitrate data from community water supplies were averaged by month and by county, and spatially linked to maternal addresses during the first trimester and the 12 months preceeding conception. The risk for limb anomaly significantly increased with increasing nitrate exposure (RR 1.26 95%CI 1.05, 1.51 per 1 mg/L increase in nitrate) adjusted for maternal age, race, ethnicity and socioeconomic status. No statisically significant associations were observed for gastroschisis, hypospadias, or anomalies of the neural tube or heart.

Both Stayner and Blaisdell's studies exhibit disparate findings. Both studies examined effects at comparable nitrate levels in drinking water, around half the current MAV or 5.65 mg/L. In both studies case numbers were low, impacting on statistical power and there was a lack of

Table 1. Description of studies included in this review on nitrate in drinking water and congenital anomalies with selected risk estimates (not all risk estimates for all outcomes reported).

Author (year published)	Location, years (design)	Participants (cases)	Exposure	Outcome	Covariates	Exposure quantiles mg/L	Outcome	Risk estimate (95% CI) crude (italicised), adjusted (not-italicised)		Findings and limitations
Arbuckle (1988)	New Brunswick, Canada 1973–1983 (case-control)	Full sample 394 (130 cases 264 controls) Final sample 171 (96 cases 75 controls)	Samples were taken directly from households and nitrate data was tested by the authors.	Canadian Congenital Anomalies Surveillance System.	Maternal age, birth order, water source, maternal birth place	<0.023 (public water)	CNS Central nervous system	1.00		Nitrate levels were largely below the MAV Maximum acceptable value. Very small participant numbers. Only 26% of households in the cohort produced a water sample.
						0.023–0.081 (public water)	CNS	**0.93**	**(0.85, 1.00)**	
						0.023–0.088 (public water)	CNS	**0.92**	**(0.84, 1.00)**	
						0.023–0.734 (public water)	CNS	0.39	(0.15, 1.05)	
						<0.023 (spring water)	CNS	1.00		
						0.023–0.081 (spring water)	CNS	0.88	(0.77, 1.01)	
						0.023–0.088 (spring water)	CNS	0.32	(0.09, 1.08)	
						0.023–0.734 (spring water)	CNS	0.00	(0.00, 2.81)	
						<0.023 (well water)	CNS	1.00		
						0.023–0.081 (well water)	CNS	1.04	(0.99, 1.09)	
						0.023–0.088 (well water)	CNS	1.22	(0.93, 1.60)	
						0.023–0.734 (well water)	CNS	2.30	(0.73, 7.29)	
Blaisdell (2019)	Missouri, USA 2004–2008 (cohort)	Final sample 348,250 (3,532)	Municipal water supply. Mean nitrate exposure 12 months prior to birth.	Missouri Birth Defects Registry. All congenital anomalies reported in the first year of life.	Maternal age, race/ethnicity, pre-pregnancy BMI, years of maternal education, maternal cigarette and alcohol use, adequacy of prenatal care, child sex and year of birth, participation in federal assistance programs.	Continuous per 1 mg/L increase	Limb anomalies	***1.23*** **1.26**	***(1.02, 1.48)*** **(1.05, 1.51)**	Statistically significant relationship with limb deficiencies. Outcomes not associated with nitrate include neural tube defects, heart defects, oral cleft defects, gastroschisis, hypospadias, Down Syndrome. Adjusted models strengthened association.
Brender (2013)	Iowa and Texas, USA 1997–2005 (case-control)	Final sample 4421 (3,300 cases 1,121 controls)	Data obtained from public water supplies routine monitoring. Groundwater modelling for private water supplies. Direct testing of a representative sample of bottled water sold in the study region	The National Birth Defect Prevention Study. Any NTD, oral cleft, limb deficiency or congenital heart defect were included.	Maternal race/ethnicity, education, study city, folic acid use, smoking, multivitamin supplementation.	<0.91	Any NTD Neural tube defect	*1.00* 1.00		Statistically significant association found for any neural tube defect, spina bifida, any oral cleft, cleft lip without cleft palate and cleft palate at highest exposure categories. No significant association found for Anencephaly, heart defects, right or left ventricular outflow tract obstruction heart defects or septal anomalies.
						0.91–4.9	Any NTD	*0.99* 1.00	*(0.68, 1.43)* (0.68, 1.45)	
						≥5.0	Any NTD	*1.39* **1.43**	*(0.99, 1.96)* **(1.01, 2.04)**	
						<0.91	Spina bifida	*1.00* 1.00		
						0.91–4.9	Spina bifida	*1.43* 1.41	*(0.87, 2.33)* (0.86, 2.32)	
						≥5.0	Spina bifida	***2.03*** **2.02**	***(1.28, 3.21)*** **(1.27, 3.22)**	
						<1.0	Any limb deficiency	*1.00* 1.00		
						1.0–5.41	Any limb deficiency	*1.27* 1.17	*(0.72, 2.24)* (0.66, 2.07)	
						≥5.42	Any limb deficiency	***1.84*** **1.79**	***(1.08, 3.11)*** **(1.05, 3.08)**	
						<1.0	Any oral cleft anomaly	*1.00* 1.00		
						1.0–5.41	Any oral cleft defect	*0.99* 0.98	*(0.74, 1.33)* (0.73, 1.32)	
						≥5.42	Any oral cleft anomaly	***1.43*** **1.45**	***(1.09, 1.88)*** **(1.10, 1.92)**	
						<1.0	Cleft lip w/o cleft palate	*1.00* 1.00		
						1.0–5.41	Cleft lip w/o cleft palate	*1.22* 1.13	*(0.70, 2.14)* (0.64, 1.99)	
						≥5.42	Cleft lip w/o cleft palate	***1.97*** **1.82**	***(1.18, 3.30)*** **(1.08, 3.07)**	
						<1.0	Cleft palate	*1.00* 1.00		
						1.0–5.41	Cleft palate	*1.12* 1.12	*(0.66, 1.88)* (0.66, 1.90)	
						≥5.42	Cleft palate	***1.88*** **1.90**	***(1.17, 3.01)*** **(1.17, 3.09)**	

(*Continued*)

Table 1. (Continued)

Author (year published)	Location, years (design)	Participants (cases)	Exposure	Outcome	Covariates	Exposure quantiles mg/L	Outcome	Risk estimate (95% CI) crude (italicised), adjusted (not-italicised)		Findings and limitations
Croen (2001)	California, USA 1989–1991 (case-control)	Final sample 1077 (538 cases 539 controls)	Diet and beverage questionnaire including medications. Nitrate levels obtained from routine monitoring data, linked to maternal addresses.	Data on birth congenital anomalies taken from State monitoring program. Neural Tube congenital anomalies only.	Maternal race/ethnicity, age, education household income, BMI, periconceptional vitamin use, tap water consumption and dietary nitrate intake	<1.13	Neural Tube defect (All water types)	*1.00* 1.00		No statistically significant association between nitrate in groundwater and mixed waters with neural tube defects. No association observed between nitrate and spina bifida. No statistically significant association between mixed water nitrate and Anencephaly. Only crude odds ratios produced for analysis between nitrate in groundwater and Anencephaly. Similar results for adjusted and non-adjusted. Dietary consumption of nitrates did not affect risk estimate. Elevated Dietary nitrate intake was protective for NTD development.
						1.13–3.49	Neural Tube defect (All water types)	*1.30* 1.30	*(0.96, 1.90)* (0.90, 2.00)	
						3.50–7.99	Neural Tube defect (All water types)	***1.40*** **1.60**	***(1.00, 2.00)*** **(1.10, 2.30)**	
						8.00–15.00	Neural Tube defect (All water types)	*1.70* 1.70	*(0.78, 3.70)* (0.76, 4.00)	
						<1.13	Anencephaly (Groundwater)	*1.00*		
						1.13–3.49	Anencephaly (Groundwater)	***2.1***	***(1.1, 4.1)***	
						3.50–7.99	Anencephaly (Groundwater)	***2.3***	***(1.1, 4.5)***	
						8.00–15.00	Anencephaly (Groundwater)	***6.9***	***(1.9, 24.9)***	
Dorsch (1984)	South Australia 1951–1979 (case-control)	Final sample 436 (218 cases 218 controls)	Government monitoring data	Hospitals registry of congenital anomalies	Maternal age, season of conception.	<1.13	Any congenital anomaly	1.00		Elevated nitrates in drinking water associated with an increase risk in birth defects in a positive dose-response relationship. Consuming groundwater during pregnancy doubled the risk of birth defects.
						1.13–3.39	Any congenital anomaly	**2.6**	**(1.6, 4.10)**	
						>3.39	Any congenital anomaly	**4.1**	**(1.3, 13.1)**	
Holtby (2014)	Nova Scotia, Canada 1988–2006 (case-control)	Final sample 2,241 (606 cases 1,635 controls)	Routine monitoring data from those on public water supplies. GIS environmental modelling used to estimate exposure in rural areas.	Province birth registry database. All congenital anomalies, central nervous system, musculoskeletal, genitourinary, cardiovascular, inguinal canal, eye, ear, nose throat and mouth.	Infant sex, season of conception, maternal age, maternal parity, pre-pregnancy weight, smoking, thyroid disease, diabetes, folate supplementation, folate fortification, water source.	<1	Any congenital anomaly	*1.00* 1.00		No statistically significant association found. Adjusted estimates are stronger. Increased risk of birth defects with maternal smoking, maternal parity of 1–2 is protective. No effect of folic acid supplementation or fortification on incidence of birth defects (study looked at all congenital anomalies).
						1–5.56	Any congenital anomaly	*1.02* 1.65	*(0.81, 1.30)* (0.83, 3.27)	
						>5.56	Any congenital anomaly	*0.97* 1.66	*(0.73, 1.29)* (0.81, 3.42)	
						Continuous per mg/L		**1.00**	**(1.001, 1.006)**	

(*Continued*)

Table 1. (Continued)

Author (year published)	Location, years (design)	Participants (cases)	Exposure	Outcome	Covariates	Exposure quantiles mg/L	Outcome	Risk estimate (95% CI) crude (italicised), adjusted (not-italicised)		Findings and limitations
Stayner (2022)	Denmark, 1991–2013 (cohort)	1,018,914 (33,182 cases)	Annual average nitrate concentrations from public waterworks samples linked with maternal addresses.	Congenital anomalies obtained from Danish National Patient Registry. All congenital anomalies diagnosed until two years of life.	Year of birth, birth order, birth weight, sex, parental age, maternal smoking, education, income and employment status.	<0.45	Any congenital anomaly	*1.00* 1.00		Only Eye birth defectsignificantly associated with nitrate. Adjusted results increase effect estimates. Outcomes not associated with nitrate include abdominal wall, male genital, female genital, heart, limbs, oro-facial clefts, respiratory and 'other' CA. Outcomes statistically significant and inversely associated with nitrate include any CA, digestive system, ear/face/neck, nervous system and urinary system CA.
						0.45 –<1.12	Any congenital anomaly	*1.01* 1.01	*(0.99, 1.04)* (0.98, 1.04)	
						1.12 –<5.65	Any congenital anomaly	***0.95*** 0.99	***(0.92, 0.99)*** (0.95, 1.02)	
						≥5.65	Any congenital anomaly	***0.91*** **0.93**	***(0.86, 0.97)*** **(0.88, 0.99)**	
						Continuous	Any congenital anomaly	***0.97*** **0.98**	***(0.96, 0.99)*** **(0.97, 1.00)**	
						<0.45	Eye anomalies	*1.00* 1.00		
						0.45 - <1.12	Eye anomalies	*1.22* 1.02	*(0.99, 1.50)* (0.91, 1.16)	
						1.12 –<5.65	Eye anomalies	*1.04* **1.23**	*(0.77, 1.39)* **(1.05, 1.44)**	
						≥5.65	Eye anomalies	*1.26* **1.29**	*(0.80, 2.00)* **(1.00, 1.66)**	
						Continuous	Eye anomalies	***1.08*** **1.09**	***(1.01, 1.15)*** **(1.03, 1.16)**	
						<0.45	Ear, face and neck anomalies	*1.00* 1.00		
						0.45 - <1.12	Ear, face and neck anomalies	*1.22* 0.96	*(0.99, 1.50)* (0.87, 1.06)	
						1.12 - <5.65	Ear, face and neck anomalies	*1.04* 1.02	*(0.77, 1.39)* (0.89, 1.16	
						≥5.65	Ear, face and neck anomalies	*1.26* **0.70**	*(0.80, 2.00)* **(0.54, 0.92)**	
						Continuous	Ear, face and neck anomalies	*1.02* 1.03	*(0.90, 1.15)* (0.91, 1.17)	
						<0.45	Digestive anomalies	*1.00* 1.00		
						0.45 - <1.12	Digestive anomalies	*0.98* 0.96	*(0.88, 1.10)* (0.87, 1.06)	
						1.12 - <5.65	Digestive anomalies	*0.93* 1.02	*(0.80, 1.08)* (0.89, 1.16)	
						≥5.65	Digestive anomalies	***0.75*** **0.70**	***(0.56, 1.00)*** **(0.54, 0.92)**	
						Continuous	Digestive anomalies	***0.90*** **0.93**	***(0.85, 0.97)*** **(0.87, 0.99)**	
						<0.45	Nervous system anomalies	*1.00* 1.00		
						0.45 - <1.12	Nervous system anomalies	*0.98* 0.98	*(0.88, 1.10)* (0.88, 1.10)	
						1.12 - <5.65	Nervous system anomalies	*0.93* 0.93	*(0.80, 1.08)* (0.80, 1.09)	
						≥5.65	Nervous system anomalies	***0.75*** **0.75**	***(0.56, 1.00)*** **(0.57, 1.00)**	
						Continuous	Nervous system anomalies	*0.94* 0.94	*(0.87, 1.01)* (0.87, 1.01)	
						<0.45	Urinary anomalies	*1.00* 1.00		
						0.45 - <1.12	Urinary anomalies	*0.97* 0.96	*(0.90, 1.05)* (0.89, 1.04)	
						1.12 - <5.65	Urinary anomalies	***0.85*** **0.88**	***(0.76, 0.95)*** **(0.79, 0.98)**	
						≥5.65	Urinary anomalies	***0.66*** **0.67**	***(0.53, 0.81)*** **(0.54, 0.83)**	
						Continuous	Urinary anomalies	***0.89*** **0.90**	***(0.84, 0.94)*** **(0.85, 0.95)**	

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information on individual water consumption habits and dietary nitrate intake impacting on exposure assessments.

Case-control studies. Brender (2013) conducted a case-control study of 3,300 case mothers (mothers who delivered infants with a neural tube defect, oral cleft, limb anomaly or congenital heart anomaly from live births, stillbirths and elective terminations) and 1,121 controls in Iowa and Texas (USA) [44]. The authors studied the association of nitrate from diet, nitrosatable medication and drinking water during pregnancy and the risk of deliverying an infant with CA. Control infants were randomly selected from birth certificates and hospital delivery records from the same area as case infants at the time of delivery. Nitrate exposure was estimated from data obtained from public water supplies, groundwater modelling for private supplies and adjusted for bottled water consumption through participant questionnaires.

In the primary analysis, CA were examined against daily nitrate intake from drinking water. Statistically significant associations were observed for the highest nitrate exposure tertile >5 mg per day (equivalent to ~3.5 mg/L) with any neural tube defect (OR 1.43 95%CI 1.01, 2.04), spina bifida (OR 2.02 95%CI 1.27, 3.22), any limb deficiency (OR 1.79 95%CI 1.05, 3.08), any oral cleft defect (OR 1.45 95%CI 1.10, 1.92), cleft lip without cleft palate (OR 1.82 95%CI 1.08, 3.07) and cleft palate alone (OR 1.90 95%CI 1.17, 3.09). Models were adjusted for maternal ethnicity, education, multivitamin supplementation, smoking, and US state. No associations were detected for anomalies of the heart or anencephaly. There were no statistically significant interactions with nitrosatable drug use.

Holtby (2014) conducted a case control study of 606 cases and 1,635 controls in Nova Scotia, Canada [45]. Cases were described as any infants diagnosed with any congenital anomaly (including those diagnosed in utero and pregnancies that underwent second trimester terminations) as registered in the population-based national birth registry. Controls were selected from the same birth registry, with matching conducted on infant's sex and date of conception (within 30 days). Participants' maternal residential address was spatially linked to the water supply boundaries of the public water supplies, while rural participants' nitrate was derived from spatial modelling based on the nitrate levels of municipal supplies. Nitrate exposure at 1–5.65 mg/L and >5.65 mg/L compared to <1 mg/L (reference) showed odds ratios of 1.65 (95%CI 0.83, 3.27) and 1.66 (95%CI 0.81, 3.42) for any CA, respectively, adjusted for sex, maternal age, smoking, diabetes, maternal thyroid disease and parity. Where Stayner et al acknowledge the potential risk of live birth bias within their study (they did not include births that were terminated prior to delivery), Holtby and colleagues, by including second-trimester termination pregnancies, were able to provide a broader CA data set, and did show a statistically signficant association between nitrate levels in drinking water 1–5.56 mg/L (OR 2.44 95% CI 1.05, 5.66) and CA when early terminations were included in the study population.

Croen (2001) examined the association between nitrate from all sources and neural tube defects in a case-control study with 538 cases and 539 matched controls in California, USA [46]. Controls were healthy infants, randomly selected from each area birth hospital and proportionally to the hospital's estimated contribution to the total number of live births between 1989 and 1991. Drinking water, diet and nitrosatable drug consumption were included to calculate total daily nitrate consumption. Drinking water nitrate intake was calculated by linking participants' geocoded address to the spatial water supply boundaries of public water services, adjusted for water consumption. Dietary nitrate, nitrite and NOC were calculated through a food frequency questionnaire. Exposure to nitrosatable medications was determined by classifying each medication used during pregnancy for its potential nitrosatability. Nitrate exposure categories were <1.1 (reference); 1.1–3.3; 3.4–7.7 and 7.8–15 mg/L.

The exposure level of 3.4–7.7 mg/L was associated with an increased risk of neutral tube defect (OR 1.6 95%CI 1.1, 2.3) but associations were not statistically significant for the 1.1–3.3

mg/L (OR 1.3 95%CI 0.9, 2.0) or 7.8–15 mg/L (OR 1.7 95%CI 0.8, 4.0) exposure levels. However there is a trend of increasing risk estimates with increasing nitrate exposure. Total nitrate, nitrite or NOC intake via diet were not associated with increased risk of neutral tube defects. The authors also assessed spina bifida and anencephaly risk separately. Statistically significant results were found for anencephaly with nitrate in groundwater 1.1–3.3 mg/L OR 2.1 (95% CI 1.1, 4.1) 3.4–7.7 mg/L OR 2.3 (95% CI 1.1, 4.5), 7.8–15 mg/L OR 6.9 (95% CI 1.9, 24.9). There were no statistically significant results for spina bifida alone for any water type.

Dorsch (1984) performed a small case-control study (n = 218 case-controls pairs) matched on hospital, birth date and maternal age of all deliveries in the period 1951–1979 based in South Australia [47]. Cases were defined as structural defects at birth, identified via the delivery registeries of three hospitals in South Australia. Maternal addresses were linked to government monitoring data on levels of nitrate at each water source. Nitrate exposure was divided into 3 categories; <5 mg/L, 5–15 mg/L and >15 mg/L. There was a statistically significant dose-response association between increasing nitrate levels and risk of CA: <5 mg/L, reference; 5–15 mg/L, RR 2.6 (95%CI 1.6, 4.1); and >15 mg/L, RR 4.1 (95%CI 1.3, 13.1). Models adjusted for maternal and infant sex, maternal marital status and occupation.

Arbuckle (1988) assessed the association between maternal exposure to nitrates in drinking water and risk of central nervous system anomalies in New Brunswick, Canada [48]. Cases (n = 130) were identified via government surveillance systems and classified as per the International Classification of Disease (ICD) codes. Two controls per case were matched by date of birth and county of maternal residence for the period 1973–1983. Nitrate data was obtained either by directly sampling maternal households (26%) or average levels obtained from sampling the corresponding municipal distribution system. A positive but statistically non-significant association was observed (OR 2.3 95%CI 0.73, 7.29).

Preterm birth

Preterm birth (birth prior to 37 weeks gestation [49, 50]) is a leading cause of mortality in infants and children under 5 years [51]. Compared to those born at full term, survivors of PTB have higher rates of chronic health conditions including neurological and developmental disabilities, mental health, emotional, cardio-metabolic and respiratory problems [52, 53]. The lower the gestational age at birth, the greater the immediate and longer term costs to health and wellbeing [52]. The legacy of preterm birth continues well beyond the newborn period and has been described by the WHO as a 'silent emergency' [54] that requires a coordinated and research-informed response to both reduce the number of children born too early and mitigate the life-course impact on survivors and their families.

To our knowledge, only four cohort studies investigating the association between nitrate in drinking water and preterm birth have been conducted to date, while no case-control studies were identified. Studies investigating the association between nitrate and preterm birth are summarized in Table 2.

Sherris (2021) published the largest of the studies, a retrospective cohort study in California from 2000–2011 [49]. Residential addresses of mothers during pregnancy were geocoded to spatially-linked water supply boundaries of the public water utilities. Nitrate levels were retrieved from the routinely (quarterly or yearly) collected monitoring data. The final analytic sample of singleton births included 4.1 million births from 3.2 million mothers.

The authors observed an increased risk of early PTB (20–31 weeks) for mothers exposed to nitrate levels of 5–10 mg/L (OR 1.49 95%CI 1.42, 1.56) and >10 mg/L (OR 1.34 95%CI 1.12, 1.60) [49]. Associations with near term PTB (32–36 weeks) were weaker for mothers exposed to nitrate levels of 5–10 mg/L (OR 1.12 95%CI 1.09, 1.14) and >10 mg/L (OR 1.07 95%CI 0.97,

Table 2. Description of studies included in this review on nitrate in drinking water and preterm birth.

Author (year published)	Location, years (design)	Participants (cases)	Exposure	Outcome	Covariates	Exposure quantiles mg/L	Outcome	Risk estimate (95%CI) crude (italicised), adjusted (not-italicised)		Findings and limitations
Albouy-Llaty (2016)	Deux-Sèvres, 2005–2010 France (cohort)	Full sample 13,654 mother/ neonate pairs (586) Final sample 4,697 (~200)	Nitrate in community water systems were linked to maternal place of residence on the date of birth.	Birth records of the district office of maternal and childhood protection via the mandatory health certificates. Preterm birth was defined as birth before 37 weeks of amenorrhoea	Maternal age, mother's occupation, smoking, single parent family, history of preterm birth, primiparity, neighbourhood deprivation.	<3.64	Preterm (<37 weeks)	1.00		No statistically significant association detected. Direction of associations reversed by adjustment for confounding.
						3.64–6.14	Preterm (<37 weeks)	1.126	(0.920, 1.378)	
						>6.14	Preterm (<37 weeks)	1.009	(0.820, 1.241)	
Coffman (2022)	Denmark, 1991–2015 (cohort)	Final sample 1,009,189 births (Total 51,747 PTB; Extremely preterm 2,117; very preterm 5,094; moderately preterm 44,536)	Unique personal identification number assigned to each liveborn resident in Denmark linked to household level of nitrate in drinking water from the Danish national monitoring geodatabase Jupiter	Danish Medical Birth Registry. Extremely early preterm birth (20–27 weeks); very early preterm birth (28–31 weeks); moderate preterm birth (32–36 weeks)	Sex, year of birth, gravidity, urbanicity, maternal age, smoking, maternal income, maternal education, employment status.	≤0.45	All preterm	1.00		No association for extremely preterm births (20–27 weeks) or very early preterm (28–31 weeks). Association between nitrate in drinking water and moderate preterm birth and all preterm births.
						>0.45–1.13	All preterm	**1.03**	**(1.01, 1.06)**	
						>1.13–5.64	All preterm	**1.04**	**(1.01, 1.07)**	
						>5.64	All preterm	**1.05**	**(1.00, 1.10)**	
						Continuous per 2.26mg/ L	All preterm	**1.01**	**(1.00, 1.03)**	
						≤0.45	Moderate preterm	1.00		
						>0.45–1.13	Moderate preterm	**1.04**	**(1.01, 1.06)**	
						>1.13–5.64	Moderate preterm	**1.04**	**(1.01, 1.07)**	
						>5.64	Moderate preterm	**1.06**	**(1.01, 1.12)**	
						Continuous per 2.26mg/ L	Moderate preterm	**1.02**	**(1.00, 1.03)**	
Huang (2018)	USA, California, 2009–2012 (cohort)	1,812,145 individual births with a final analytic sample of 1,448,600. A total of 99,519 preterm births	Census tract codes from birth records were matched to aggregated nitrate values for census areas from the CalEnviroScreen 3.0 dataset.	A total of 99,519 preterm births defined as <37 weeks. The preterm prevalence in the final analytic sample was 6.87%.	Maternal race/ ethnicity, maternal age, maternal education and payment of delivery costs.	Continuous per 2.1 mg/ L	All preterm	**1.02**	**(1.01, 1.03)**	Statistically significant association between preterm birth outcomes and nitrate in drinking water. Exposure assessment may have led to several areas of misclassification bias.

(*Continued*)

Table 2. (Continued)

Author (year published)	Location, years (design)	Participants (cases)	Exposure	Outcome	Covariates	Exposure quantiles mg/L	Outcome	Risk estimate (95%CI) crude (italicised), adjusted (not-italicised)		Findings and limitations
Sherris (2021)	California, USA 2000–2011 (cohort)	4,160,998 individual-level sample with a sibling sample size of 1,443,318 used in the within-mother analysis (early preterm birth 20–31 weeks, 31,090; near-term birth 32–36 weeks, 146,059, 37–40 weeks, 3,983,849)	Geocoded residences were linked to water supplies, and public monitoring records of nitrate levels were used.	Preterm birth (early preterm birth 20-31weeks, 31,090; near-term birth 32–36 weeks) from Californian birth certificate registry.	Maternal age, education, ethnicity, health care payer type for delivery, parity, interpregnancy interval, infant sex, birth year, month of conception, region and prenatal care intitiation.	<5	Early preterm (individual-level analysis)	**1.00**		Statistically significant association between preterm birth outcomes and nitrate in drinking water. Stronger associations detected in the early preterm group 20–31 weeks than in the near-term group. Stronger associations also detected in within-mother analyses
						5 - <10		**1.49**	**(1.42, 1.56)**	
						≥10		**1.34**	**(1.12, 1.60)**	
						Continuous per mg/L		**1.011**	**(1.009, 1.013)**	
						<5	Early preterm (within-mother analysis)	**1.00**		
						5 - <10		**1.47**	**(1.29, 1.67)**	
						≥10		**2.52**	**(1.49, 4.26)**	
						Continuous per mg/L		**1.015**	**(1.008, 1.021)**	
						<5	Near-term (individual-level analysis)	**1.00**		
						5–10		**1.12**	**(1.09, 1.14)**	
						>10		1.07	(0.97, 1.17)	
						Continuous per mg/L		**1.003**	**(1.002. 1.004)**	
						<5	Near-term (within-mother analysis)	**1.00**		
						5–10		**1.08**	**(1.02, 1.15)**	
						>10		1.05	(0.85, 1.31)	
						Continuous per mg/L		**1.003**	**(1.001, 1.006)**	

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1.17). The authors also conducted a within-mother analysis of exposure-discordant consecutive births (n = 1.4 million), which controlled for major potential confounders by design.

The within-mother analysis showed pregnancies exposed to 5–10 mg/L (OR 1.47 95%CI 1.29, 1.67) and >10 mg/L (OR 2.52 95%CI 1.49, 4.26) had increased odds of early PTB compared to pregnancies exposed to <5 mg/L. Again, associations with near term PTB were weaker at 5–10 mg/L (OR 1.08 95%CI 1.02, 1.15) and >10 mg/L (OR 1.05 95% 0.85, 1.31). One limitation was the lack of maternal information on diet, BMI or smoking status, however, the associations were strengthened in the within-mother analysis, which, at least partly, adjusts for these factors.

Huang (2018) published a retrospective cohort study from California which included 1.8 million births from 2009 to 2012 (this cohort overlapped with the cohort described above in Sherris [49, 55]). Nitrate data was extracted via the CalEnviroScreen 3.0 dataset from California Communities Environmental Health Screening Tool. The CalEnviroScreen 3.0 dataset contains seven exposure and five environmental effects variables and five socioeconomic variables for 56 census tracks in California. Mother's census track during pregnancy was linked to CalEnviroScreen data. Nitrate concentrations within CalEnviroScreen were the aggregated value of all water distribution zones within any given census tract area.

The final analytic sample for the analysis on nitrate was 1.4 million births. The prevalence of PTB in the sample was 6.84%. The nitrate values for census tracks ranged from 0 to 22.7 mg/L. The authors conducted logistic regression to calculate the increased risk of PTB per interquartile range (2.1 mg/L) increase in nitrate. The authors observed an increased risk of PTB (RR 1.02 95%CI 1.01, 1.03) per 2.1 mg/L increase in nitrate accounting for maternal race/ethnicity, maternal age, maternal education and payment of delivery costs. The authors acknowledged the aggregation of water distribution zones and other potential sources of misclassification bias in their exposure assessment. It is likely that this biased the results towards the null.

Coffman (2022) published a retrospective cohort of 1 million births in Denmark between 1991 and 2015 [50]. The authors linked residential addresses of mothers during pregnancy to household level data on nitrate in drinking water. PTB was divided into extremely early PTB (20–27+6 weeks), very PTB (28 - <32 weeks) and moderate PTB (32–36 weeks of gestation). Co-variates included maternal age, BMI, socioeconomic status, employment status, educational attainment, smoking status, urbanicity, gravidity and birth year. Exposure cut-offs were <0.45 mg/L, 0.45-<1.13 mg/L, 1.13 - <5.64 mg/L and >5.64 mg/L. Exposure at >5.64 mg/L compared to <0.45 mg/L was associated with a statistically significant increased risk of all PTB (OR 1.05 95%CI 1.00, 1.10) and moderate PTB (OR 1.06 95%CI 1.01, 1.12), but not extremely early PTB (OR 0.93 95%CI 0.72, 1.19) or very PTB (OR 1.03 95%CI 0.89, 1.19). The major limitation of this study is lack of data on dietary sources of nitrate and nitrite, or antioxidants which may moderate the effect of nitrate. Further, as also highlighted by the authors, the inclusion of PTB due to induction for pregnancy or birth complications, potentially unrelated to nitrate exposure (e.g. pre-eclampsia), may have resulted in bias.

Albouy-Llaty (2016) published a prospective cohort study of 13,654 births conducted between 2005–2010 in Deux-Sevres, France [56]. The final analytic cohort included 4,697 mother/neonate pairs, including 200 PTB (4.3%) defined as <37 weeks gestation. Residential addresses were linked to nitrate levels in municipal drinking water supplies. There were no statistically significant associations between PTB and nitrate levels 3.64–6.14 mg/L (OR 1.12 95% CI 0.92, 1.38) or >6.14 mg/L (OR 1.01 95%CI 0.82, 1.24) compared to <3.64 mg/L. Co-variates included maternal smoking, occupation, age, medical histories and child sex and birth weight. Some major limitations of this study included the exclusion of 81% of the original cohort primarily due to missing exposure information, conflation of early and late PTB and small number of PTB relative to other cohorts.

Discussion

To date, research examining the association between nitrates in drinking water and pregnancy outcomes (CA and PTB) is limited. For CA, the strongest and most consistent associations were observed for neural tube anomalies. Many studies, particularly those focusing on CA, considered a large number of outcomes and additional analyses and it was not always clear whether results were adjusted for multiple testing.

The influence of nitrate exposure in risk of preterm birth is also limited and inconsistent. The three largest cohort studies observed that drinking water nitrate concentrations were associated with increased risk of PTB. However, one smaller study found no significant association, albeit the study had limited statistical power. A common limitation across these studies was the lack of information on dietary or other sources of maternal nitrate or exposure during pregnancy. Futhermore, given the modifying effect of antioxidants on the biological impact of nitrates, any concurrant antioxidant exposure should be explored as this may further influence pregnancy outcomes.

In contrast to the above, two studies included total nitrate consumption from drugs and diet in addition to drinking water [44, 46]. Neither found that dietary or drug sources of nitrates were strongly linked with pregnancy outcomes, either in isolation or in combination with nitrate concentrations in drinking water. Future studies need to examine the interaction of these potential effect modifiers on the association between nitrate and adverse pregnancy outcomes. Examining the role of potential effect modifiers in the proposed pathways (e.g. nitrosatable compounds, iodine, dietary antioxidants) could help elucidate which specific mechanistic pathways may be most relevant.

In addition to potential effect modifiers discussed above, there is also substantial interpersonal variation between individuals in how they breakdown and recycle nitrate that cannot be easily adjusted for in epidemiological research [2]. For example, people exposed to nitrate in drinking water, over longer periods, tend to have greater propagation of oral and gastrointestinal nitrate-reducing bacteria [2]. As a result, people with prolonged exposure (e.g. prior to becoming pregnant) to nitrate in drinking water may more readily convert nitrate into nitrite and its other metabolites at any given nitrate concentration compared to people with shorter durations of exposure.

Differences in exposure assessments between studies affects comparability. The most common exposure assessment linked an individual's address to the spatial boundary of a municipal supply and its associated nitrate data. One limitation of this is that exposure assessments often do not account for individuals on non-municipal, private supplies. These individuals are typically most at risk of nitrate contamination as they tend to draw water from shallow wells and are often located in rural areas of land use intensification and nitrate leaching. Some studies have used environmental modelling to address data gaps; these use a range of environmental datasets to predict nitrate levels based on the local conditions [2, 57]. In addition, bottled water use was routinely not taken in to account. A study by Weyer et al (2014) found that approximately 30% of the pregnant women included in their sample used bottle water as a primary source of their drinking water but that the nitrate content was found to be lower than community water supplies [58]. Finally, nearly all studies used different exposure quantiles and these were rarely above half of the current drinking water standard. Further, some studies had quite wide quantile bands (e.g. from 0.9 to 4.9 mg/L) which may mask potential affects at moderate levels of exposure.

More consistent defintions of outcome measures would facilitate aggregation of study results and enable better understanding of the current evidence. For example with respect to PTB, some studies aggregated all births less than 37 weeks into one category while others split PTB up into two or three categories. The WHO tightly prescribes the defintions of PTB with the following categories: extremely preterm (less than 28 weeks); very preterm (28 to less than 32 weeks); and moderate to late preterm (32 to less than 37 weeks) [54]. While the lower gestational age threshold at which therapeutic (rather than palliative) neonatal care is offered varies across jurisdictions, most births $<$23 weeks are regarded as non-viable due to ethical, technical and physiological constraints at these extremes of birthweight (typically $<$500 g) and organ immaturity [59]. Future studies should adhere to commonly defined categories and naming conventions for PTB to enhance comparability.

The high level of heterogeneity and relatively small numbers of studies were factors influencing the decision to conduct a narrative review as opposed to a systematic review and meta-analysis. When a limited number of studies are available, meta-analyses can be highly senstive to the results of a single or small number of studies. For example, a systematic review and meta-analysis published as a report in 2021 concluded "the evidence of a relationship between nitrate in drinking water and preterm birth is inconsistent and inconclusive" [3, p.21], based on two studies (OR 0.98 95%CI 0.93, 1.03). The report was then published in a peer-reviewed

journal with an additional study with the conclusion "increased nitrate in drinking water may be associated with an increased risk of preterm birth" [3, p.1] based on three studies (OR 1.01 95% CI 1.00, 1.01).

To partly address this problem, future reviews may want to consider conducting a living review, which is continually updated, incorporating relevant new evidence as it becomes available and which facilitates connections between evidence and practice [60]. However often the burden of maintaining these reviews lies on researchers so long-term funding is required to ensure these reviews are kept up-to-date. This may require a shift by funders to prioritise living reviews over more conventional review types.

We examined the three biological mechanisms that current literature suggests may underly the associations seen between nitrate in drinking water and adverse pregnancy outcomes. These include NOC formation, thyroid dysfunction and oxidative stress. The evidence for these mechanisms is based on animal studies, predominantly rats or mice, and is often decades old. The proposed mechanisms primarily rely on reduction of nitrate to nitrite by bacteria in human saliva, however this process is absent in rats [61]. For this reason, the FAO/WHO Joint Committee confirmed that rats are not a good surrogate species for humans in relation to NOC formation [61]. Future animal studies could aim to identify a more reliable animal surrogate with similar biological processes for nitrate reduction as observed in humans.

Several experimental human studies have investigated the impact of nitrate ingestion on the formation of NOC [17, 18, 62, 63]. However, these studies have typically been conducted on healthy, non-pregnant, volunteers. The formation of NOC is influenced by oral and gastrointestinal microflora, stomach acid pH and volume and other dietary factors [64]. Thus, it is likely the process of NOC formation is exacerbated in individuals with high levels of nitrate reducing bacteria (e.g. with poor oral health), more alkaline stomach pH (facilitating bacterial proliferation) and with a high intake of other sources of amines (red meat or certain drugs) and low intake of NOC inhibitors (polyphenols, vitamin C). Pregnant women can experience physiological changes that may make them more prone to endogenous formation of NOC [65, 66]. While it would be unethical to randomise pregnant women to conditions that could put them or their baby at risk, future studies could investigate these biological mechanisms in non-randomised observational studies of pregnant women or randomised control trials of non-pregnant volunteers.

Conclusions

Associations between nitrate concentrations in drinking water and congenital anomalies and preterm birth are apparent in the aforementioned studies, and may be causal. There are a number of plausible biological mechanisms that may explain associations between nitrate in drinking water and adverse pregnancy outcomes. Emerging epidemiological evidence has observed increased risks of adverse pregnancy outcomes at nitrate concentrations below the current maximum acceptable value allowed in drinking water.

Additional longitudinal evidence with robust exposure assessments, outcome definitions and information on important confounders and effect modifiers is required. Given the widespread contamination of drinking water with nitrate in some countries, a precautionary approach should be adopted until further evidence emerges.

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