Nitrate and nitrite from drinking water and diet: summary of epidemiologic evidence

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1. Overall summary

This section summarizes epidemiologic evidence cited in the tables below and will be updated as new evidence becomes available. I would appreciate feedback on any errors or omissions. don.wigle@sympatico.ca

Health effect	Level of evidence[a]	Comments
Fetal death	Maternal prenatal expos (Inadequate)	A case-control study of late fetal deaths in Massachusetts found no association with drinking water nitrate levels in the communities of maternal prenatal residence (Aschengrau et al 1993).
Intrauterine growth retardation, preterm birth	Maternal prenatal expos (Inadequate)	A case-control study in Canada found associations between IUGR and preterm birth and average water nitrate levels in maternal prenatal residential regions (Bukowski et al 2001).
Total birth defects	Maternal prenatal expos (Inadequate)	A case-control study in Australia found a dose-response relationship between total birth defects and drinking water nitrate levels (Dorsch et al 1984). This study followed an earlier descriptive study that found elevated birth defect incidence rates in the study region which was known to have high drinking water nitrate levels. A large case-control study in Massachusetts found no association between major birth defects and nitrate in the drinking water of the maternal municipality of residence; the highest average municipal drinking water nitrate level was 0.3-4.5 mg/L (Aschengrau et al 1993).
CNS birth defects	Maternal prenatal expos Limited	A case-control study in Australia found a dose-response relationship between CNS birth defects and drinking water nitrate levels (Dorsch et al 1984). This study followed an earlier descriptive study that found elevated birth defect incidence rates in a region known to have high drinking water nitrate levels. A case-control study in Canada found a statistically non-significant association between CNS birth defects and tap water nitrate levels above 26 mg/L at the maternal residence; this association was limited to maternal residences using well water (Arbuckle et al 1988). A review concluded that there is limited evidence for an association between birth defects (especially CNS) and drinking water nitrate in humans but no evidence that nitrate is teratogenic in experimental animals (Fan and Steinberg 1996). A large case-control study in California found a statistically non-significant association between NTDs and ground water nitrate levels above 36 mg/L based on data for the water supply serving the maternal residence during the periconceptual period; this association remained after adjustment for maternal prenatal dietary nitrate intake but was still statistically non-significant (Croen et al 2001). This study found a significant association between anencephaly, a specific and severe type of NTD, and nitrate levels in the ground water supply for the maternal residence during the periconceptual period. Also, this study found no associations between NTDs and maternal periconceptual daily dietary nitrate, nitrite or N-nitroso compound intake. Given that dietary nitrate intake almost always greatly exceeds that from drinking water, these findings suggest that ingested nitrate is unlikely to cause NTDs. A case-control study in Texas found a borderline association between NTDs and drinking water nitrate levels at the maternal periconceptual residence (Brender et al 2004). This study found a strong association between NTDs and combined maternal periconceptual exposure to nitrosatable drugs and daily nitrite intakes above 10.5 mg from diet and drinking water (mostly from diet).

Cardiac birth defects	Maternal prenatal expos (Inadequate)	A case-control study in Massachusetts found no association between cardiac birth defects and detectable nitrate in the drinking water of the maternal municipality of residence (Zierler et al 1988). A Swedish cohort study found a weak association of borderline statistical significance between major cardiac birth defects and nitrate levels in the municipal water supply serving the maternal residence (Cedergren et al 2002).
Other birth defects	Maternal prenatal expos (Inadequate)	A case-control study in Australia found a dose-response relationship between musculoskeletal birth defects and drinking water nitrate levels (Dorsch et al 1984). This study followed an earlier descriptive study that found elevated birth defect incidence rates in a region known to have high drinking water nitrate levels.
Brain tumours	Maternal prenatal expos (Limited)	A multicentre case-control study in the USA and Canada found no association between childhood brain astrocytomas and maternal prenatal dietary nitrate or nitrite intake (Bunin et al 1993). Further study found an association between childhood brain astrocytomas and maternal prenatal consumption of bacon and cured meats but this only occurred among low-income and not among higher income families (Bunin et al 1994). A population-based case-control study in California and Washington State found that childhood brain tumours were associated with a prenatal maternal residence served by well water; this association was apparent in the Seattle portion but not the study as a whole (Mueller et al 2001). This study also found that childhood brain tumours were associated with nitrite but not nitrate levels in the water supply serving the prenatal maternal residence; this association was limited to children whose mothers did not drink bottled water during pregnancy. A recent report of the California/Washington State study showed a dose-response relationship between childhood brain tumours and maternal prenatal average daily dietary nitrite intake from cured meats (Pogoda and Preston-Martin 2001). A large multicentre case-control study of childhood tumours found no association with maternal periconceptual well water use or tap water nitrate levels at the maternal periconceptual residence (Mueller et al 2004). This study found a significant association between childhood astroglial brain tumours and tap water detectable nitrite levels at the maternal periconceptual residence.
Leukemia	Maternal or childhood expos (Inadequate)	A population-based case-control study in Quebec found no associations between acute lymphatic leukemia (age 0-9 years) and prenatal or postnatal average drinking water nitrate concentrations or cumulative average daily drinking water nitrate intakes (Infante-Rivard et al 2001).
Testicular cancer	Postnatal expos (Inadequate)	A cohort study of Norwegian farm families found an association between testicular cancer and use of high-nitrogen fertilizers (Kristensen et al 1996). A population-based case-control study in Denmark found an association between testicular cancer and a history of having lived in three counties with high ground water nitrate levels for most of childhood (Moller 1997). Given that the excess risk was largely limited to men who grew up in urbanized regions of the three counties with low-nitrate communal water supplies, nitrate *per se* probably was not responsible for the observed association.
General		An expert panel concluded that there is inadequate epidemiologic or toxicologic evidence for an association between cancer and drinking water nitrate intake (National Academy of Sciences 1995). An experimental study in the Netherlands exposed female volunteers age 18-46 years for one week to a test diet containing fish meal rich in amines and 220 mg nitrate per day, the current ADI for a 60 kg person (Vermeer et al 1998). The study showed that mean urinary N-nitrosodimethylamine excretion increased from 287 ng/day in the control week to 756 ng/day in the test week, demonstrating increased formation of a carcinogenic N-nitrosamine in response to ingested nitrate and dietary amines.

Childhood diabetes	Postnatal expos (Limited)	Ecologic studies in Colorado (Kostraba et al 1992), England (Parslow et al 1997) and Austria (Schober et al 2003) found associations between childhood diabetes incidence rates and regional water nitrate levels. No associations were found in ecologic studies in the Netherlands (van Maanen et al 2000), Sardinia (Casu et al 2000), England (Zhao et al 2001) and Finland (Moltchanova et al 2004). A case-control study in Finland found associations between childhood diabetes and both maternal and childhood average daily dietary nitrite but not nitrate intake (Virtanen et al 1994).
Infantile methemoglobinemia	Postnatal expos ? inadequate or limited (controversial)	An expert panel concluded that infantile methemoglobinemia is the only proven adverse health effect of nitrate exposure in humans (National Academy of Sciences 1995). A review of toxicologic and epidemiologic studies on methemoglobinemia concluded that case reports of infantile methemoglobinemia in the USA have been limited to drinking water nitrate levels above 45 mg/L (Fan and Steinberg 1996). Another literature review noted that infants under age 6 months are susceptible to methemoglobinemia because they have low levels of NADH-cytochrome b5 reductase, the enzyme that converts methemoglobin back to hemoglobin (Avery 1999). This review concluded that diarrheal illness can cause methemoglobinemia in young infants without high drinking water or dietary nitrate intake. Nitric oxide is produced during the inflammatory response to infection and is metabolized to nitrite that can react with hemoglobin to form methemoglobin. A cross-sectional study of young children in India found no association between drinking water nitrate levels (in five towns with average levels ranging from 26 to 459 mg/L) and percent of hemoglobin as methemoglobin (Gupta et al 2000). This study did find an association between a history of early childhood recurrent respiratory tract infections and methemoglobin level. Two case reports of infantile methemoglobinemia in Wisconsin revealed histories of consuming formula made with water from wells with nitrate concentrations of 23-27 mg/L; neither infant had evidence of infection and both improved rapidly on formula made with bottled water (Knobeloch et al 2000). A case-control study of methemoglobinemia in Romania found associations between early childhood methemoglobinemia and dietary nitrate intake and a history of diarrheal disease (Zeman et al 2002). A literature review conducted for WHO concluded that there is inadequate evidence for an association between methemoglobinemia and drinking water nitrate levels (Fewtrell et al 2004). This review also concluded that there is limited evidence for an association between methemoglobinemia and gastrointestinal illness.

[a] Sufficient evidence = based on peer-reviewed reports of expert groups or authoritative reviews that concluded that a causal relationship existed; limited evidence = relationships for which several epidemiologic studies, including at least one case-control or cohort study, showed fairly consistent associations and evidence of exposure-risk relationships after control for potential confounders; inadequate evidence = relationships for which epidemiologic studies were limited in number and quality (e.g., small studies, ecologic studies, limited control of potential confounders), had inconsistent results, or showed little or no evidence of exposure-risk relationships. Levels in parentheses are the author's interpretation of available evidence; other levels are based on expert group reviews.

2. Fetal death

Reference, location	Design	Exposure	Results	Association	Covariates
(Aschengrau et al. 1993), Boston	Case-control study nested within cohort of 14,130 women enrolled at delivery with gestation at least 20 wk, 1977-1980; included 1,039 birth defects, 77 stillbirths, 1,177 controls	Assessed drinking water quality in 155 communities where women lived during pregnancy	Stillbirths not associated with drinking water nitrate levels (crude odds ratios, nitrate 0.2 and 0.3-4.5 vs <0.2 mg/L)	1.0, 0.8	
(Fan and Steinberg 1996),	Review of literature on role of nitrate and nitrite in drinking water and methemoglobinemia and developmental toxicity	Animal studies have shown reproductive toxicity but no birth defects at high exposure levels to nitrate or nitrite	Recent epidemiologic data have suggested an association between birth defects and drinking water nitrate levels but a definite conclusion on the cause and effect relationship cannot be drawn		

Fetal death: summary

A case-control study of late fetal deaths in Massachusetts found no association with drinking water nitrate levels in the communities of maternal prenatal residence (Aschengrau et al 1993).

3. Low birth weight, intrauterine growth retardation, preterm birth

Reference, location	Design	Exposure	Results	Association	Covariates
(Bukowski et al. 2001), Prince Edward Island, Canada	Case-control study, 336 preterm cases, 210 IUGR cases, 4098 term live birth controls, 1991-1994	Nitrate measurements for public and private wells used to calculate average water nitrate levels in each of 46 geographic regions	IUGR associated with average water nitrate levels in maternal prenatal residential region; odds ratio, ≥3.1 vs ≤1.3 mg/L	2.4 (1.8-3.3)	3rd trimester maternal smoking, previous low birth weight or preterm birth, parity, maternal height, weight, insufficient maternal weight gain, pregnancy-induced hypertension
			Preterm birth associated with average water nitrate levels in maternal prenatal residential region; odds ratio, ≥3.1 vs ≤1.3 mg/L	1.9 (1.5-2.5)	3rd trimester maternal smoking, previous low birth weight or preterm birth, parity, maternal height, insufficient maternal weight gain, history of abortion

Intrauterine growth retardation, preterm birth: summary

A case-control study in Canada found associations between IUGR and preterm birth and average water nitrate levels in maternal prenatal residential regions (Bukowski et al 2001).

4. Birth defects

Reference, location	Design	Exposure	Results	Association	Covariates
(Dorsch et al. 1984), Australia	Case-control study, 218 birth defect cases, 218 matched controls, 1951-1979	Identified water source (rain, well); measured nitrate level in water samples from subjects' homes	Birth defects associated with drinking water nitrate levels; odds ratios, 5-15 and >15 vs <5 mg/L	2.6 (1.6-4.1) 4.1 (1.3-13)	Matched for maternal age, parity, DOB; adjusted for infant sex and maternal residence region
			CNS and musculoskeletal birth defects associated with ground water use; odds ratios, ground vs rain water	CNS 3.5 (1.1-15) Musculoskel 2.9 (1.2-8.0)	As above
(Zierler et al. 1988), Massachusetts	Case-control study, 270 cases cardiac birth defects, 665 live birth controls	Assessed maternal exposure to nitrate and 10 other chemicals in drinking water in communities where the mothers resided during pregnancy	Total cardiac birth defects not associated with drinking water nitrate level; odds ratio, detectable vs non-detectable nitrate	1.1 (0.7-1.6)	Other chemicals in drinking water, drinking water source, maternal education
(Arbuckle et al. 1988), New Brunswick, Canada	Case-control study, 130 cases CNS birth defects, 260 controls, 1973-1983	Sampled tap water in subjects' homes; for homes on well water, nitrate levels at the 37.5, 62.5 and 87.5 percentiles were 1.3, 6.3 and 26.0 mg/L	Statistically non-significant association between CNS birth defects and tap water nitrate levels above 26 mg/L among subjects using well water but not among those using public water; odds ratios, 1.3-6.2, 6.3-25.9 and 26+ vs ≤0.1 mg/L	1.0 (1.0-1.1) 1.2 (0.9-1.6) 2.3 (0.7-7.3)	Matched by DOB and county of maternal residence; adjusted for chloride level, maternal birthplace
(Aschengrau et al. 1993), Massachusetts	Case-control study nested within cohort of 14,130 women enrolled at delivery with gestation at least 20 wk, 1977-1980; included 1,039 birth defects, 77 stillbirths, 1,177 controls	Assessed drinking water quality in 155 communities where women lived during pregnancy	Major birth defects not associated with drinking water nitrate levels (crude odds ratios, nitrate 0.2 and 0.3-4.5 vs <0.2 mg/L)	1.0, 0.9	Maternal race, age, hospital insurance coverage, previous infant with birth defect, alcohol, water source
(Croen et al. 2001), California	Case-control study, 454 cases neural tube birth defects (including live born and stillborn infants and	Assessed maternal periconceptual drinking water and dietary nitrate/nitrite intake; water supply nitrate	Statistically non-significant association between NTDs and ground water nitrate levels above 36 mg/L based on data for the water supply serving the maternal	1.3 (0.7-2.3) 1.4 (0.8-2.5) 2.4 (0.6-9.9)	Maternal race/ethnicity, age, education, income, BMI, vitamin use

	therapeutic abortions), 462 population controls, 1989-1991	data available for season of conception for 72% of study addresses	residence during the periconceptual period; odds ratios, nitrate 5-15, 16-35 and 36-67 vs <5 mg/L		
			Borderline association between NTDs and ground water nitrate levels after adjustment for dietary nitrate intake; odds ratio, nitrate 36-67 vs <5 mg/L	1.9 (0.7-4.7)	
			Among NTDs, anencephaly but not spina bifida associated with ground water nitrate levels in supply for maternal residence during periconceptual period; crude odds ratios, nitrate 5-15, 16-35 and 36-67 vs <5 mg/L	Anencephaly 2.1 (1.1-4.1) 2.3 (1.1-4.5) 6.9 (1.9-25) Spina bifida 0.9, 0.9, 1.1	
			NTDs not associated with maternal periconceptual dietary nitrate, nitrite or N-nitroso compound intakes; odds ratios for upper 3 vs lowest quartiles	Dietary NO_3 0.9, 0.9, 1.0 Dietary NO_2 1.0, 0.9, 0.9 Dietary N-nitroso cpds 0.6, 0.9, 0.8	Maternal race/ethnicity, age, education, income, BMI, vitamin use, dietary folate, zinc & protein
(Cedergren et al. 2002), Sweden	Cohort study, 753 major cardiac birth defects among infants of 58,669 mothers at residences using municipal water supplies, 1982-1996	Drinking water quality data for the 80 municipal water supplies in the study region (one county)	Borderline association between major cardiac birth defects and drinking water nitrate levels (odds ratio for ≥2 vs <2 mg/L)	1.2 (1.0-1.4)	Maternal age, parity, education
(Brender et al. 2004), Texas	Case-control study, 184 NTD cases (including live births, stillbirths and spontaneous or therapeutic abortions with NTDs), 225 population controls, enrolled 1995-2000	Mother-reported food frequency questionnaire; measured drinking water nitrate (range 0-28 mg/L) and nitrite levels at periconceptual residence for 27% of all subjects	Borderline association between NTDs and drinking water nitrate levels; odds ratio, 3.5+ vs <3.5 mg/L	1.9 (0.8-4.6)	
			NTDs associated with combined maternal periconceptual exposure to nitrosatable	0.9 (0.3-3.4) 2.7 (0.8-11)	Household income, maternal BMI, folate

		drugs and relatively high nitrite intakes from diet or diet plus water; odds ratios, drugs + total nitrite intake of <7.5, 7.5-10.5 and >10.5 mg/day vs no drug intake and same nitrite intake	7.5 (1.8-45)	intake

Birth defects: summary

Total birth defects

A case-control study in Australia found a dose-response relationship between total birth defects and drinking water nitrate levels (Dorsch et al 1984). This study followed an earlier descriptive study that found elevated birth defect incidence rates in the study region which was known to have high drinking water nitrate levels. A large case-control study in Massachusetts found no association between major birth defects and nitrate in the drinking water of the maternal municipality of residence; the highest average municipal drinking water nitrate level was 0.3-4.5 mg/L (Aschengrau et al 1993).

CNS birth defects

A case-control study in Australia found a dose-response relationship between CNS birth defects and drinking water nitrate levels (Dorsch et al 1984). This study followed an earlier descriptive study that found elevated birth defect incidence rates in a region known to have high drinking water nitrate levels. A case-control study in Canada found a statistically non-significant association between CNS birth defects and tap water nitrate levels above 26 mg/L at the maternal residence; this association was limited to maternal residences using well water (Arbuckle et al 1988). A review concluded that there is limited evidence for an association between birth defects (especially CNS) and drinking water nitrate in humans but not evidence that nitrate is teratogenic in experimental animals (Fan and Steinberg 1996). A large case-control study in California found a statistically non-significant association between NTDs and ground water nitrate levels above 36 mg/L based on data for the water supply serving the maternal residence during the periconceptual period; this association remained after adjustment for maternal prenatal dietary nitrate intake but was still statistically non-significant (Croen et al 2001). This study found a significant association between anencephaly, a specific and severe type of NTD, and nitrate levels in the ground water supply for the maternal residence during the periconceptual period. Also, this study found no associations between NTDs and maternal periconceptual daily dietary nitrate, nitrite or N-nitroso compound intake. Given that dietary nitrate intake almost always greatly exceeds that from drinking water, these findings suggest that ingested nitrate is unlikely to cause NTDs. A case-control study in Texas found a borderline association between NTDs and drinking water nitrate levels at the maternal periconceptual residence (Brender et al 2004). This study found a strong association between NTDs and combined maternal periconceptual exposure to nitrosatable drugs and daily nitrite intakes above 10.5 mg from diet and drinking water (mostly from diet).

Cardiac birth defects

A case-control study in Massachusetts found no association between cardiac birth defects and detectable nitrate in the drinking water of the maternal municipality of residence (Zierler et al 1988). A Swedish cohort study found a weak association of borderline statistical significance between major cardiac birth defects and nitrate levels in the municipal water supply serving the maternal residence (Cedergren et al 2002).

Other birth defects

A case-control study in Australia found a dose-response relationship between musculoskeletal birth defects and drinking water nitrate levels (Dorsch et al 1984). This study followed an earlier descriptive study that found elevated birth defect incidence rates in a region known to have high drinking water nitrate levels.

5. Childhood cancer

Reference, location	Design	Exposure	Results	Association	Covariates
(Bunin et al. 1993),	Case-control study, 166 cases primitive neuroectodermal brain tumors, 166 matched controls, age < 6 yr	Assessed maternal diet and alcohol consumption during pregnancy; note – diet is usually much more important than drinking water as a source of nitrate uptake	Childhood brain astrocytomas not associated with maternal prenatal dietary nitrate intake; odds ratio, highest vs lowest quartile	0.54 p>0.05	Matched by telephone area code, DOB, race
			Childhood brain astrocytomas not associated with maternal prenatal dietary nitrite intake; odds ratio, highest vs lowest quartile	1.06 p>0.05	
(Bunin et al. 1994), United States and Canada	Case-control study, 155 astrocytoma brain tumours, 155 matched population controls, age < 6 yr	Assessed maternal diet and alcohol consumption during pregnancy	Among low-income families; childhood brain astrocytomas associated with maternal prenatal consumption of bacon and cured meats; odds ratios, bacon consumed at least once/wk vs less often	Low income 2.5 (1.2-5.3) Higher income 0.7 (0.3-1.3)	Matched by telephone area code, DOB, race
(National Academy of Sciences, 1995), USA	Expert panel review of health effects of nitrate and nitrite in drinking water	For more than 99% of the US population, about 97% of nitrate and about 99% of nitrite intake comes from diet	There is inadequate epidemiologic or toxicologic evidence for an association between cancer and drinking water nitrate		
(Kristensen et al. 1996), Norway	Cohort study, linked birth registry and five agricultural and horticultural censuses, identified 166,291 male offspring of farmers and cumulated 2.9 million person-years of follow-up; 158 cases of testicular cancer among offspring of farm holders, age < 40 yr; compared testicular cancer incidence rates in cohort and general total rural population of Norway	Expenditures on pesticides, fertilizer, possession of tractor pesticide spraying equipment (based on agricultural census closest to time of birth)	Testicular cancer associated with use of fertilizers with high nitrogen:phosphorus ratio (SIR)	2.0 (1.5-2.6)	
			Association strongest for non-	4.2	YOB, calendar

			seminoma subtype of testicular cancer	(2.1-8.3)	year, paternal time/yr working on farm
(Moller 1997), Denmark	Population-based case-control study, 296 testicular cancer cases, 287 controls; self- and maternal-reported information on residence and occupational history	Ecologic index of ground water nitrate level, i.e., residence in any of 3 counties with known high ground water nitrate levels; 22% of water supplies in high nitrate region had levels exceeding 25 mg/L in 1994	Testicular cancer associated with living in counties with high ground water nitrate levels for larger part of childhood; odds ratio	1.4 (1.1-1.8)	YOB
			Excess risk largely limited to men who grew up in urbanized regions of the 3 counties; these regions used communal water supplies low in nitrate, i.e., nitrate *per se* probably was not responsible for the observed association		
(Vermeer et al. 1998), the Netherlands	Experimental study, 25 female volunteers age 18-46 yr	Test diet for 1 wk containing fish meal rich in amines and 220 mg nitrate/day (the current ADI for a 60 kg person); mean urinary nitrate excretion increased from 76 mg/d during first control week to 180 mg/d in the test week and declined to 77 mg/d during the second control week; measured urinary volatile N-nitrosamines, N-nitrosodimethylamine (NDMA) and N-nitrosopiperidine (NPIP)	Mean urinary NDMA excretion increased from 287 ng/d in the control week to 756 ng/d in the test week and declined to 383 ng/d in the second control week; this demonstrates increased formation of carcinogenic N-nitrosamines		
			Urinary NPIP was not related to nitrate intake and diet composition		
(Mueller et al. 2001), California, Washington State	Population-based case-control study, 540 brain tumours, 791 matched controls, age 0-19 yr, 1984-1991	Mother-reported prenatal dietary history, prenatal and childhood water source, prenatal bottled water consumption; current residence tap water dipstick nitrate/nitrite measurement (if in same residence as during pregnancy with index child)	Brain tumours associated with prenatal maternal residence served by well water in Seattle region but not in study as a whole (respective odds ratios, well water only vs public water only)	Seattle 2.6 (1.3-5.2) all regions 1.2 (0.8-2.2)	Matched for YOB, sex; adjusted for child's age, sex
			Brain tumours associated with nitrite but not nitrate levels in the water supply serving the prenatal	nitrite 8.8 (2.1-46)	As above

			maternal residence (odds ratios, detectable vs non-detectable levels)	nitrate 0.6 (0.3-1.1)	
			Brain tumours associated with detectable nitrite or nitrate in the water supply serving the prenatal maternal residence among children whose mothers did not drink bottled water during pregnancy but not in whole group (odds ratios, detectable nitrate or nitrite vs non-detectable levels)	subgroup 2.1 (1.0-4.4) whole group 1.1 (0.7-2.0)	As above
(Infante-Rivard et al. 2001), Quebec	Population-based case-control study, 491 acute lymphatic leukemia cases, 491 matched controls, age < 10 yr, 1980-1993	Child's residential history from conception to present, parent-reported drinking water source, water quality data for arsenic, cadmium, chromium, lead, zinc, nitrate, 1970-1993, 1995-1996 tap water survey (227 homes)	ALL not associated with prenatal or postnatal avg drinking water nitrate exposure (odds ratio, >2 vs ≤2 mg/L)	prenatal 0.7 (0.3-1.7) postnatal 0.6 (0.2-1.6)	Matched for age, sex, region; adjusted for maternal age, education
			ALL not associated with prenatal or postnatal cumulative drinking water nitrate exposure (odds ratio, >95th vs ≤95th percentile of mg.day.L^{-1})	prenatal 0.9 (0.5-1.6) postnatal 0.6 (0.3-1.1)	As above
(Pogoda and Preston-Martin 2001), California, Washington State	Multicentre population-based case-control study, 540 cases brain tumours, 801 controls, age 0-19 yr, enrolled 1984-1991	Estimated maternal prenatal dietary nitrite intake from diet history (frequency, portion sizes); nitrite-cured meats deliver much higher gastric nitrite concentrations compared to nitrite formed from dietary nitrate	Childhood brain tumours associated with maternal prenatal dietary nitrite intake from cured meats; odds ratios, <0.5, 0.5-0.9, 1.0-1.9, 2.0-2.9 and 3+ mg/day vs none	1.1 (0.8-1.5) 1.9 (1.2-2.9) 1.3 (0.8-2.3) 1.8 (0.8-4.1) 3.0 (1.2-7.9) p-trend=0.008	Geographic area, sex, age at diagnosis, YOB; results not changed by adjustment for race or SES; brain tumours not associated with maternal smoking or alcohol
(Mueller et al. 2004),	Multicentre case-control study, 1218 childhood brain tumour	Mother-reported information on drinking water source from 1month before	Childhood brain tumours not associated with water source	1.2 (0.8-1.8)	

SEARCH study	cases, 2223 population controls, age 0-19 yr, enrolled 1976-1994	conception to child's 1st birthday; dipstick water nitrate/nitrite measurements for about 35% of all subjects	during periconceptual month; crude odds ratio, well vs public water source (calculated from data in paper)		
			Childhood brain tumours not associated with drinking water nitrate level at residence during pregnancy; odds ratios, 10-24, 25-49 and 50+ mg/L vs no detectable nitrate	0.5 (0.3-0.9) 0.5 (0.2-1.1) 1.0 (0.4-2.2)	Study centre, age, sex, year of diagnosis
			Non-statistically significant association between childhood brain tumours and drinking water nitrite level at maternal prenatal; residence; odds ratios, 1-4 and 5+ vs no detectable nitrite	1.7 (0.8-3.7) 2.1 (0.6-7.4)	As above
			Childhood brain astroglial tumours associated with drinking water nitrite level at maternal prenatal; residence; odds ratios, 1-4 and 5+ vs no detectable nitrite; astroglial tumours not associated with nitrate levels	4.3 (1.4-13) 5.7 (1.2-27)	As above
(Mensinga et al. 2003),	Review of literature on health effects of environmental nitrate/nitrite	Intense use of nitrogen fertilizers and manure in agriculture may increase nitrate levels in food and water; 25% of ingested nitrate is secreted in saliva, where some 20% is converted to nitrite by oral bacteria; nitrite so formed is swallowed and absorbed in the small intestine	Nitrate *per se* is generally regarded as nontoxic; toxicity usually results from conversion *in vivo* to nitrite		
		The Joint Expert Committee of the Food and Agriculture Organization of the United Nations/World Health Organization (JECFA) and the European Commission's Scientific Committee on Food have set an acceptable daily intakes for nitrate of 0-3.7 mg nitrate ion/kg bw;	The two major toxic effects of nitrite are methemoglobinemia (can cause tissue hypoxia and possibly death) and reaction of nitrite with secondary or N-alkyl-amides to form N-nitroso carcinogens.		

	the JECFA ADI for nitrite is 0-0.07 mg nitrite ion/kg bw; these levels appear to be safe for healthy neonates, children and adults	

Childhood cancer

Brain tumours

A multicentre case-control study in the USA and Canada found no association between childhood brain astrocytomas and maternal prenatal dietary nitrate or nitrite intake (Bunin et al 1993). Further study found an association between childhood brain astrocytomas and maternal prenatal consumption of bacon and cured meats but this only occurred among low-income and not among higher income families (Bunin et al 1994). A population-based case-control study in California and Washington State found that childhood brain tumours were associated with a prenatal maternal residence served by well water; this association was apparent in the Seattle portion but not the study as a whole (Mueller et al 2001). This study also found that childhood brain tumours were associated with nitrite but not nitrate levels in the water supply serving the prenatal maternal residence; this association was limited to children whose mothers did not drink bottled water during pregnancy. A recent report of the California/Washington State study showed a dose-response relationship between childhood brain tumours and maternal prenatal average daily dietary nitrite intake from cured meats (Pogoda and Preston-Martin 2001). A large multicentre case-control study of childhood tumours found no association with maternal periconceptual well water use or tap water nitrate levels at the maternal periconceptual residence (Mueller et al 2004). This study found a significant association between childhood astroglial brain tumours and tap water detectable nitrite levels at the maternal periconceptual residence.

Leukemia

A population-based case-control study in Quebec found no associations between acute lymphatic leukemia (age 0-9 years) and prenatal or postnatal average drinking water nitrate concentrations or cumulative average daily drinking water nitrate intakes (Infante-Rivard et al 2001).

Testicular cancer

A cohort study of Norwegian farm families found an association between testicular cancer and use of high-nitrogen fertilizers (Kristensen et al 1996). A population-based case-control study in Denmark found an association between testicular cancer and a history of having lived in three counties with high ground water nitrate levels for most of childhood (Moller 1997). Given that the excess risk was largely limited to men who grew up in urbanized regions of the three counties with low-nitrate communal water supplies, nitrate *per se* probably was not responsible for the observed association.

General

An expert panel concluded that there is inadequate epidemiologic or toxicologic evidence for an association between cancer and drinking water nitrate intake (National Academy of Sciences 1995). An experimental study in the Netherlands exposed female volunteers age 18-46 years for one week to a test diet containing fish meal rich in amines and 220 mg nitrate per day, the current ADI for a 60 kg person (Vermeer et al 1998). The study showed that mean urinary N-nitrosodimethylamine excretion increased from 287 ng/day in the control week to 756 ng/day in the test week, demonstrating increased formation of a carcinogenic N-nitrosamine in response to ingested nitrate and dietary amines.

5. Other effects

Reference, location	Design	Exposure	Results	Association	Covariates
(Kostraba et al. 1992), Colorado, USA	Ecologic study, type I diabetes, 1280 cases, age 0-17 yr, 1978-1988, lived in 63 counties; identified cases using Colorado IDDM Registry	County-level drinking water weighted avg nitrate levels, 1984-1988	Childhood diabetes incidence rates by county associated with avg county drinking water nitrate levels; Spearman correlation coefficient	r_s= 0.26, p=0.03	
(Virtanen et al. 1994), Finland	Population-based case-control study, 684 cases childhood type I diabetes, 595 controls, age 0-14 yr, 1986-1989	Assessed exposure of children and parents to nitrate and nitrite from food and drinking water	Childhood diabetes associated with maternal and childhood dietary nitrite intake; odds ratios, childhood dietary nitrite, higher vs 1st quartiles	1.2 (0.8-1.7) 1.5 (1.1-2.1) 2.4 (1.8-3.4)	Maternal education, child's age, place of residence
			Childhood diabetes not associated with dietary nitrate intake; odds ratios, higher vs 1st quartiles	0.8 (0.6-1.1) 1.0 (0.7-1.4) 0.9 (0.7-1.3)	As above
(National Academy of Sciences, 1995), USA	Expert panel review of health effects of nitrate and nitrite in drinking water	For more than 99% of the US population, about 97% of nitrate and about 99% of nitrite intake comes from diet	Methemoglobinemia in infants is the only proven adverse health effect of nitrate exposure		
(Fan and Steinberg 1996), USA	Review of toxicologic and epidemiologic studies on methemoglobinemia and developmental toxicity of nitrate and nitrite in drinking water		Limited and inconclusive epidemiologic evidence for an association between CNS birth defects and maternal drinking water nitrate levels		
			Nitrate and nitrite at high levels in drinking water cause adverse developmental effects including fetal death, reduced fetal growth and anemia in prenatally exposed experimental animals		
			Case reports of infantile methemoglobinemia in the USA have been associated with drinking water nitrate levels above 45 mg/L		

(Parslow et al. 1997), England	Ecologic study, 1797 registered cases type I diabetes, age < 17 yr, diagnosed during 1978-1994, living in 148 water supply areas	Measured nitrate levels for 9330 samples in 148 regions, 1990-1995	Childhood diabetes associated with nitrate levels; standardized incidence ratios, 3.2-14.8 and 14.9-40 vs < 3.2 mg/L	1.1 (1.0-1.3) 1.3 (1.1-1.5)	Ethnicity, population density, SES
(Avery 1999), USA	Review of literature on infantile methemoglobinemia	Infants under age 6 mos are susceptible to methemoglobinemia because they have low levels of NADH-cytochrome b5 reductase, the enzyme that converts methemoglobin back to hemoglobin	Diarrheal illness can cause methemoglobinemia in young infants without high drinking water or dietary nitrate intake; inflammatory response to infection raises nitric oxide production in several tissues; nitric oxide is metabolized to nitrite that reacts with hemoglobin to form methemoglobin		
(Gupta et al. 2000), India	Cross-sectional study, 88 children age 0-8 yr, residents of 5 villages; measured methemoglobin levels, cytochrome b5 reductase activity, mother-reported history of acute respiratory tract infections	Avg nitrate levels in 5 towns were 26, 45, 95, 222 and 459 mg/L	Methemoglobin levels not associated with drinking water nitrate levels; % of hemoglobin as methemoglobin in towns with increasing water nitrate levels	10.4, 16.1, 19.4, 7.9 and 15.4 % of hemoglobin	
			History of recurrent respiratory tract infections associated with methemoglobin level; Pearson's correlation coefficient	r=0.73	
(van Maanen et al. 2000), The Netherlands	Ecologic study, type I diabetes, 1064 cases, age 0-14 years, 1993-1995, residents of 3932 postal code areas; cases identified from national registry	Avg drinking water nitrate levels in postal code geographic areas, 1991-1995	Childhood diabetes incidence rate not associated with avg nitrate level in regional drinking water supply; incidence rate ratio, 10-25 and >25 vs <10 mg/L	1.0 (0.9-1.2) 1.5 (0.9-2.4) p-trend=0.57	
(Casu et al. 2000), Sardinia	Ecologic study, 1975 cases of type I diabetes, age 0-29 yr, 1989-1998	5541 tap water samples from 353 municipalities; calculated median nitrate level for each municipality	Type I diabetes incidence rates not associated with median drinking water nitrate levels in municipality of residence; Pearson's correlation coefficient	age 0-19 yr r=-0.06, p>0.05 age 0-29 yr r= -0.17, p>0.05	
(Knobeloch et al. 2000),	Case report, 2 infants with methemoglobinemia		History of consuming formula made with water from wells with nitrate		

Wisconsin			concentrations of 23-27 mg/L; both infants had no evidence of infection and improved rapidly on formula made with bottled water		
(Zhao et al. 2001), England	Ecologic study, type I diabetes, 517 cases, age 0-15 yr, 1975-1996, residents of 40 water supply zones	Measured drinking water nitrate levels, 40 water supply regions, 1993-1997	Childhood diabetes standardized incidence ratios not associated with drinking water nitrate levels in region of residence; standardized incidence ratios, 1-3.6, 3.7-7.8 and >7.8 mg/L vs not detectable	1.1 (1.0-1.3) 1.0 (0.9-1.2) 0.9 (0.8-1.1)	
(Zeman et al. 2002), Transylvania region, Romania	Case-control study, 26 cases methemoglobinemia, 45 controls, age <5 yr	Nitrate/nitrite intake (mg/kg/day) based on dietary history and drinking water and blood lead measurements	Early childhood methemoglobinemia associated with dietary nitrate intake; crude odds ratio, >10 vs ≤10 mg/day (calculated from data in paper)	13.5 (3.9-52)	
			Early childhood methemoglobinemia associated with history of diarrheal disease; crude odds ratio, yes vs no (calculated from data in paper)	2.9 (1.0-8.0)	
(Schober et al. 2003), Austria	Ecologic study, 1449 cases type I diabetes, age 0-14 yr, 1989-1999; cases identified through national registry based on pediatric hospitals, clinics and diabetologists; conducted Poisson regression and Bayesian spatial conditional autoregression	Postal code of residence used to assign cases to one of 99 geographic regions; 1901 nitrate measurements available for ground and public drinking water sources; calculated avg levels for each region	Borderline association between childhood diabetes incidence and average regional drinking water nitrate levels; regression coefficient	0.0036 (-0.0004, 0.0076), p=0.07	Proportion of population age <15 yr, infant mortality rate, population density, industrial employment
			Borderline association between childhood diabetes incidence and average regional drinking water nitrate levels; conditional autoregression coefficient	0.00244 (-0.0021, 0.0069)	Proportion of population age <15 yr
(Moltchanova et al. 2004), Finland	Ecologic study, 3564 cases type I diabetes, age 0-14 yr, 1987-1996; cases identified through national hospital-based registry; conducted Bayesian spatial conditional autoregression	Geologic survey data on nitrate levels in natural springs and wells; interpolated data for cells in a 10X10 km grid	No association between childhood diabetes incidence rates and ground water nitrate levels; regression coefficient (% increased risk of diabetes per ground water nitrate increment of 1 mg/L)	0.3% (-0.9, 1.4)	Geographic regions coded as urban or rural

(Fewtrell 2004), UK	Review of literature on drinking water nitrate and methemoglobinemia		Concluded that there is inadequate evidence for an association between methemoglobinemia and drinking water nitrate levels; also concluded that there is limited evidence for an association between methemoglobinemia and gastrointestinal illness.

Other health effects: summary

Childhood diabetes

Ecologic studies in Colorado (Kostraba et al 1992), England (Parslow et al 1997) and Austria (Schober et al 2003) found associations between childhood diabetes incidence rates and regional water nitrate levels. No associations were found in ecologic studies in the Netherlands (van Maanen et al 2000), Sardinia (Casu et al 2000), England (Zhao et al 2001) and Finland (Moltchanova et al 2004). A case-control study in Finland found associations between childhood diabetes and both maternal and childhood average daily dietary nitrite but not nitrate intake (Virtanen et al 1994).

Infantile methemoglobinemia

An expert panel concluded that infantile methemoglobinemia is the only proven adverse health effect of nitrate exposure in humans (National Academy of Sciences 1995). A review of toxicologic and epidemiologic studies on methemoglobinemia concluded that case reports of infantile methemoglobinemia in the USA have been limited to drinking water nitrate levels above 45 mg/L (Fan and Steinberg 1996). Another literature review noted that infants under age 6 months are susceptible to methemoglobinemia because they have low levels of NADH-cytochrome b5 reductase, the enzyme that converts methemoglobin back to hemoglobin (Avery 1999). This review concluded that diarrheal illness can cause methemoglobinemia in young infants without high drinking water or dietary nitrate intake. Nitric oxide is produced during the inflammatory response to infection and is metabolized to nitrite that can react with hemoglobin to form methemoglobin. A cross-sectional study of young children in India found no association between drinking water nitrate levels (in five towns with average levels ranging from 26 to 459 mg/L) and percent of hemoglobin as methemoglobin (Gupta et al 2000). This study did find an association between a history of early childhood recurrent respiratory tract infections and methemoglobin level. Two case reports of infantile methemoglobinemia in Wisconsin revealed histories of consuming formula made with water from wells with nitrate concentrations of 23-27 mg/L; neither infant had evidence of infection and both improved rapidly on formula made with bottled water (Knobeloch et al 2000). A case-control study of methemoglobinemia in Romania found associations between early childhood methemoglobinemia and dietary nitrate intake and a history of diarrheal disease (Zeman et al 2002). A literature review conducted for WHO concluded that there is inadequate evidence for an association between methemoglobinemia and drinking water nitrate levels (Fewtrell et al 2004). This review also concluded that there is limited evidence for an association between methemoglobinemia and gastrointestinal illness.

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