

### Nitrate and nitrite<sup>1</sup>

Nitrate ( $\text{NO}_3^-$ ) is found naturally in the environment and is an important plant nutrient. It is present at varying concentrations in all plants and is a part of the nitrogen cycle. Nitrite ( $\text{NO}_2^-$ ) is not usually present in significant concentrations except in a reducing environment, because nitrate is the more stable oxidation state. It can be formed by the microbial reduction of nitrate and in vivo by reduction from ingested nitrate. Nitrite can also be formed chemically in distribution pipes by *Nitrosomonas* bacteria during stagnation of nitrate-containing and oxygen-poor drinking-water in galvanized steel pipes, or if chloramination is used to provide a residual disinfectant. An excess of free ammonia entering the distribution system can lead to nitrification and the potential increase of nitrate and nitrite in drinking-water. Nitrate can reach both surface water and groundwater as a consequence of agricultural activity (including excess application of inorganic nitrogenous fertilizers and manures), from wastewater disposal and from oxidation of nitrogenous waste products in human and other animal excreta, including septic tanks. Nitrate can also occasionally reach groundwater as a consequence of natural vegetation. Surface water nitrate concentrations can change rapidly owing to surface runoff of fertilizer, uptake by phytoplankton and denitrification by bacteria, but groundwater concentrations generally show relatively slow changes. Nitrate and nitrite can also be produced as a result of nitrification in source water or distribution systems.

In general, the most important source of human exposure to nitrate and nitrite is through vegetables (nitrate and nitrite) and through meat in the diet (nitrite is used as a preservative in many cured meats). In some circumstances, however, drinking-water can make a significant contribution to nitrate and, occasionally, nitrite intake. In the case of bottle-fed infants, drinking-water can be the major external source of exposure to nitrate and nitrite.

Guideline values <sup>2</sup>	<p><i>Nitrate</i>: 50 mg/l as nitrate ion, to be protective against methaemoglobinaemia and thyroid effects in the most sensitive subpopulation, bottle-fed infants, and, consequently, other population subgroups</p> <p><i>Nitrite</i>: 3 mg/l as nitrite ion, to be protective against methaemoglobinaemia induced by nitrite from both endogenous and exogenous sources in bottle-fed infants, the most sensitive subpopulation, and, consequently, the general population</p> <p><i>Combined nitrate plus nitrite</i>: The sum of the ratios of the concentrations of each of nitrate and nitrite to its guideline value should not exceed 1</p>
Occurrence	Nitrate levels vary significantly, but levels in well water are often higher than those in surface water and, unless heavily influenced by surface water, are less likely to fluctuate. Concentrations often approach or exceed 50 mg/l where there are significant sources of contamination. Nitrite levels are normally lower, less than a few milligrams per litre.

<sup>1</sup> As nitrate and nitrite are chemicals of significant concern in some natural waters, the chemical fact sheet on nitrate and nitrite has been expanded.

<sup>2</sup> Conversion factors: 1 mg/l as nitrate = 0.226 mg/l as nitrate-nitrogen; 1 mg/l as nitrite = 0.304 mg/l as nitrite-nitrogen.

Basis of guideline value derivation	<p><i>Nitrate (bottle-fed infants):</i> In epidemiological studies, no adverse health effects (methaemoglobinaemia or thyroid effects) were reported in infants in areas where drinking-water consistently contained nitrate at concentrations below 50 mg/l</p>
	<p><i>Nitrite (bottle-fed infants):</i> Based on: 1) no incidence of methaemoglobinaemia at nitrate concentrations below 50 mg/l (as nitrate ion) in drinking-water for bottle-fed infants less than 6 months of age (assuming body weight of 2 kg); 2) converting 50 mg/l as nitrate to corresponding molar concentration for nitrite; 3) multiplying by a factor of 0.1 to account for the estimated conversion rate of nitrate to nitrite in infants where nitrite is formed endogenously from nitrate at a rate of 5–10%; and 4) multiplying by a source allocation factor for drinking-water of 100% or 1, as a bottle-fed infant's primary exposure to nitrite is through consumption of formula reconstituted with drinking-water that contains nitrate or nitrite. As the guideline value is based on the most sensitive subgroup of the population (bottle-fed infants less than 6 months of age), application of an uncertainty factor is not deemed necessary.</p>
	<p><i>Combined nitrate plus nitrite:</i> To account for the possibility of the simultaneous occurrence of nitrate and nitrite in drinking-water</p>
Limit of detection	<p>MDLs of 0.009 mg/l as nitrate ion and 0.013 mg/l as nitrite ion by IC; MDL of 0.04–4.4 mg/l as nitrate ion by automated cadmium reduction with colorimetry (recommended for the analysis of nitrate at concentrations below 0.4 mg/l)</p>
Treatment performance	<p><i>Nitrate:</i> Effective central treatment technologies involve the physical/chemical and biological removal of nitrate and include ion exchange, reverse osmosis, biological denitrification and electrodialysis, which are capable of removing over 80% of nitrate from water to achieve effluent nitrate concentrations as low as 13 mg/l; conventional treatment processes (coagulation, sedimentation, filtration and chlorination) are not effective</p>
	<p><i>Nitrite:</i> Treatment usually focuses on nitrate, because nitrite is readily converted to nitrate by many disinfectants</p>
Additional comments	<p>The guideline values for both nitrate and nitrite are based on short-term effects; however, they are also considered protective for any possible long-term effects.</p>
	<p>Methaemoglobinaemia is complicated by the presence of microbial contamination and subsequent gastrointestinal infection, which can increase the risk for bottle-fed infants significantly. Authorities should therefore be all the more vigilant that water to be used for bottle-fed infants is microbiologically safe when nitrate is present at concentrations near or above the guideline value. It is particularly important to ensure that these infants are not currently exhibiting symptoms of gastrointestinal infection (diarrhoea). Also, as excessive boiling of water to ensure microbiological safety can concentrate levels of nitrate in the water, care should be taken to ensure that water is heated only until it reaches a rolling boil. In extreme situations, alternative sources of water (e.g. bottled water) can be used.</p>
	<p>Nitrite is relatively unstable and can be rapidly oxidized to nitrate. Nitrite can occur in the distribution system at higher concentrations when chloramination is used, but the occurrence is almost invariably intermittent. Methaemoglobinaemia is therefore the most important consideration, and the guideline value derived for protection against methaemoglobinaemia would be the most appropriate under these circumstances, allowing for any nitrate that may also be present.</p>

All water systems that practise chloramination should closely and regularly monitor their systems to verify disinfectant levels, microbiological quality and nitrite levels. If nitrification is detected (e.g. reduced disinfectant residuals and increased nitrite levels), steps can be taken to modify the treatment train or water chemistry in order to minimize nitrite formation. Effective disinfection must never be compromised. Excessively high levels may occur in small supplies; where this is suspected from the risk assessment, testing may be appropriate.

Assessment date	2016
Principal references	Health Canada (2013). <i>Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – Nitrate and nitrite</i> WHO (2016). <i>Nitrate and nitrite in drinking-water</i>

Absorption of nitrate ingested from vegetables, meat or water is rapid and in excess of 90%; final excretion is in the urine. In humans, about 25% of ingested nitrate is recirculated in saliva, of which about 20% is converted to nitrite by the action of bacteria in the mouth. There is also endogenous formation of nitrate from nitric oxide and protein breakdown as part of normal metabolism. In normal healthy adults, this endogenous synthesis leads to the excretion of about 62 mg of nitrate ion per day in the urine. Endogenous formation of nitrate or nitrite can be significantly increased in the presence of infections, particularly gastrointestinal infections. When nitrate intake is low, endogenous formation may be the major source of nitrate in the body. Nitrate metabolism is different in humans and rats, as rats may not actively secrete nitrate in their saliva.

Nitrate probably has a role in protecting the gastrointestinal tract against a variety of gastrointestinal pathogens, as nitrous oxide and acidified nitrite have antibacterial properties. It may have other beneficial physiological roles. Hence, there may be a benefit from exogenous nitrate uptake, and there remains a need to balance the potential risks with the potential benefits.

Significant bacterial reduction of nitrate to nitrite does not normally take place in the stomach, except in individuals with low gastric acidity or with gastrointestinal infections. These may include individuals using antacids, particularly those that block acid secretion. In humans, methaemoglobinaemia is a consequence of the reaction of nitrite with haemoglobin in the red blood cells to form methaemoglobin, which binds oxygen tightly and does not release it, thus blocking oxygen transport. Although most absorbed nitrite is oxidized to nitrate in the blood, residual nitrite can react with haemoglobin. High levels of methaemoglobin (>10%) formation in infants can give rise to cyanosis, referred to as blue-baby syndrome. Although clinically significant methaemoglobinaemia can occur as a result of extremely high nitrate intake in adults and children, the most familiar situation is its occurrence in bottle-fed infants. This was considered to be primarily a consequence of high levels of nitrate in water, although there have been cases of methaemoglobinaemia in weaned infants, associated with high nitrate intake from vegetables. Bottle-fed infants are considered to be at greater risk because the intake of water in relation to body weight is high and, in infants, the development of repair enzymes is limited. In clinical epidemiological studies of

methaemoglobinaemia and subclinical increases in methaemoglobin levels associated with drinking-water nitrate, 97% of cases occurred at concentrations in excess of 44.3 mg/l, with clinical symptoms associated with the higher concentrations. The affected individuals were almost exclusively under 3 months of age.

Although drinking-water nitrate may be an important risk factor for methaemoglobinaemia in bottle-fed infants, there is compelling evidence that the risk of methaemoglobinaemia is primarily increased in the presence of simultaneous gastrointestinal infections, which increase endogenous nitrite formation, may increase reduction of nitrate to nitrite and may also increase the intake of water in combatting dehydration. Cases have been described in which gastrointestinal infection seems to have been the primary cause of methaemoglobinaemia. Most cases of methaemoglobinaemia reported in the literature are associated with contaminated private wells (predominantly when the drinking-water is anaerobic) that also have a high probability of microbial contamination, which should not occur if it is properly disinfected.

Although numerous epidemiological studies have investigated the relationship between exposure to nitrate or nitrite in drinking-water and cancer occurrence, the weight of evidence does not support an association between cancer and exposure to nitrate or nitrite per se. Nitrite can react with nitrosatable compounds, primarily secondary amines, in the body to form *N*-nitroso compounds. A number of these are considered to be carcinogenic to humans, whereas others, such as *N*-nitrosoproline, are not. Several studies have been carried out on the formation of *N*-nitroso compounds in relation to nitrate intake in humans, but there is large variation in the intake of nitrosatable compounds and in gastric physiology. Higher mean levels of *N*-nitroso compounds, along with high nitrate levels, have been found in the gastric juice of individuals who are achlorhydric (i.e. have very low levels of hydrochloric acid in the stomach). However, other studies have been largely inconclusive, and there appears to be no clear relationship with drinking-water nitrate compared with overall nitrate intake in relation to formation of *N*-nitroso compounds. Moderate consumption of a number of dietary antioxidant components, such as ascorbic acid and green tea, appears to reduce endogenous *N*-nitrosamine formation.

A significant number of epidemiological studies have been carried out on the association of nitrate intake with primarily gastric cancers. Although the epidemiological data are considered to be inadequate to allow definitive conclusions to be drawn regarding all cancers, there is no convincing evidence of a causal association with any cancer site. The weight of evidence indicates that there is unlikely to be a causal association between gastric cancer and nitrate in drinking-water. This is consistent with the conclusion by IARC that ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A), but not nitrate alone.

There have been suggestions that nitrate in drinking-water could be associated with congenital malformations, but the overall weight of evidence does not support this.

Nitrate appears to competitively inhibit iodine uptake, with the potential for an adverse effect on the thyroid. Current evidence also suggests that exposure to nitrate in drinking-water may alter human thyroid gland function by competitively inhibiting

thyroidal iodide uptake, leading to altered thyroid hormone concentrations and functions. Although studies found that exposure to nitrate concentrations above 50 mg/l are weakly associated with altered thyroid function, the evidence is limited, conflicting and based on studies with important methodological limitations. Mode of action data suggest that pregnant women and infants are the most sensitive populations, owing primarily to the importance of adequate thyroid hormones for normal neurodevelopment in the fetus and infant, but also to increased thyroid hormone turnover and low intrathyroidal stores in fetal and early life.

There have been suggestions of an association between nitrate in drinking-water and the incidence of childhood diabetes mellitus. However, subsequent studies have not found a significant relationship, and no mechanism has been identified.

In some studies on rats treated with high doses of nitrite, a dose-related hypertrophy of the zona glomerulosa of the adrenal was seen; one strain of rats appeared to be more sensitive than others. However, this minimal hyperplasia was considered to be due to physiological adaptation to small fluctuations in blood pressure in response to high nitrite doses.

Nitrate is not carcinogenic in laboratory animals. Nitrite has been frequently studied, and there have been suggestions of carcinogenic activity, but only at very high doses. The most recent long-term studies have shown only equivocal evidence of carcinogenicity in the forestomach of female mice, but not in rats or male mice. In view of the lack of evidence for genotoxicity, this led to the conclusion that sodium nitrite was not carcinogenic in mice and rats. In addition, as humans do not possess a forestomach and the doses were high, the significance of these data for humans is very doubtful.

The guideline value for nitrate of 50 mg/l, as nitrate ion, is based on an absence of health effects (methaemoglobinaemia and thyroid effects) in epidemiological studies and is protective for bottle-fed infants and, consequently, other parts of the population. Methaemoglobinaemia is complicated by the presence of microbial contamination and subsequent gastrointestinal infection, which can increase the risk for this group significantly. Authorities should therefore be all the more vigilant that water to be used for bottle-fed infants is microbiologically safe when nitrate is present at concentrations near the guideline value. It is particularly important to ensure that these infants are not currently exhibiting symptoms of significant gastrointestinal infection (diarrhoea). Also, as excessive boiling of water to ensure microbiological safety can concentrate levels of nitrate in the water, care should be taken to ensure that water is heated only until it reaches a rolling boil. In extreme situations, alternative sources of water (e.g. bottled water) can be used.

The guideline for nitrite of 3 mg/l, as nitrite ion, is based on: 1) no incidence of methaemoglobinaemia at nitrate concentrations below 50 mg/l in drinking-water for bottle-fed infants less than 6 months of age (assuming body weight of 2 kg), 2) converting 50 mg/l nitrate to the corresponding molar concentration for nitrite, 3) multiplying by a factor of 0.1 to account for the estimated conversion rate of nitrate to nitrite in infants where nitrite is formed endogenously from nitrate at a rate of 5–10% and 4) multiplying by a source allocation factor for drinking water of 100% or 1, as a bottle-fed infant's primary exposure to nitrite is through consumption of for-

formula reconstituted with nitrate- or nitrite-containing drinking-water. As the health-based value is based on the most sensitive subgroup of the population (bottle-fed infants less than 6 months of age), application of an uncertainty factor is not deemed necessary.

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

$$\frac{C_{\text{nitrate}}}{GV_{\text{nitrate}}} + \frac{C_{\text{nitrite}}}{GV_{\text{nitrite}}} \leq 1$$

The guideline values are based on short-term effects; however, they are also considered protective for long-term effects.

#### Practical considerations

The most appropriate means of controlling nitrate concentrations, particularly in groundwater, is the prevention of contamination. This may take the form of appropriate management of agricultural practices (e.g. management of fertilizer and manure application and storage of animal manures) and sanitation practices (e.g. the careful siting of pit latrines and septic tanks, sewer leakage control).

Methaemoglobinæmia has most frequently been associated with private wells. It is particularly important to ensure that septic tanks and pit latrines are not sited near a well or where a well is to be dug and to ensure that animal manure is kept at a sufficient distance to ensure that runoff cannot enter the well or the ground near the well. It is particularly important that the household use of manures and fertilizers on small plots near wells should be managed with care to avoid potential contamination. The well should be sufficiently protected to prevent runoff from entering the well. Where there are elevated concentrations of nitrate or where inspection of the well indicated that there are sources of nitrate close by that could be causing contamination, particularly where there are also indications that microbiological quality might also be poor, a number of actions can be taken. As noted above, water should be heated only until the water reaches a rolling boil or disinfected by an appropriate means before consumption. Where alternative supplies are available for bottle-fed infants, these can be used, taking care to ensure that they are microbiologically safe. Steps should then be taken to protect the well and ensure that sources of both nitrate and microbial contamination are removed from the vicinity of the well.

In areas where household wells are common, health authorities may wish to take a number of steps to ensure that nitrate contamination is not or does not become a problem. Such steps could include targeting mothers, particularly expectant mothers, with appropriate information about water safety, assisting with visual inspection of wells to determine whether a problem may exist, providing testing facilities where a problem is suspected, providing guidance on disinfecting water or, where nitrate levels are particularly high, providing bottled water from safe sources or providing advice as to where such water can be obtained.

With regard to piped supplies, where nitrate is present, the first potential approach to treatment of drinking-water supplies, if source substitution is not feasible,

is to dilute the contaminated water with a low-nitrate source. Where blending is not feasible, a number of treatment techniques are available for drinking-water. The first is disinfection, which may serve to oxidize nitrite to the less toxic nitrate as well as minimize the pathogenic and non-pathogenic reducing bacterial population in the water. Nitrate removal methods include ion exchange, biological denitrification, reverse osmosis and electrodialysis. However, there are disadvantages associated with all of these approaches, including cost, operational complexities and the need for disposal of resin, brine or reject water. Conventional municipal water treatment processes (coagulation, sedimentation, filtration and chlorination) are not effective for nitrate removal, as nitrate is a stable and highly soluble ion with low potential for co-precipitation and adsorption.

In systems with a water source containing naturally occurring ammonia or that add ammonia for chloramination, free ammonia entering the distribution system can be one of the causative factors of nitrification and the potential increase of nitrate and nitrite in the distribution system. Care should be taken with the use of chloramination for providing a residual disinfectant in the distribution system. It is important to manage this to minimize nitrite formation, either in the main distribution system or in the distribution systems of buildings.

### ***Nitrilotriacetic acid***

Nitrilotriacetic acid, or NTA, is used primarily in laundry detergents as a replacement for phosphates and in the treatment of boiler water to prevent accumulation of mineral scale.

Guideline value	0.2 mg/l (200 µg/l)
Occurrence	Concentrations in drinking-water usually do not exceed a few micrograms per litre, although concentrations as high as 35 µg/l have been measured
TDI	10 µg/kg body weight, based on nephritis and nephrosis in a 2-year study in rats and using an uncertainty factor of 1000 (100 for interspecies and intraspecies variation and 10 for carcinogenic potential at high doses)
Limit of detection	0.2 µg/l using GC with a nitrogen-specific detector
Treatment performance	No information found on removal from water
Guideline value derivation	
• allocation to water	50% of TDI
• weight	60 kg adult
• consumption	2 litres/day