**Nitrate and nitrite**

Nitrate (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 (NO$_2^-$) is not usually present in significant concentrations except in a reducing environment, as 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.

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 animal excreta, including septic tanks. 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. Some groundwaters may also have nitrate contamination as a consequence of leaching from natural vegetation.

In general, the most important source of human exposure to nitrate and nitrite is through vegetables (nitrite and nitrate) 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**

<table>
<thead>
<tr>
<th>Nitrate</th>
<th>50 mg/l as nitrate ion (or 11 mg/l as nitrate-nitrogen) to protect against methaemoglobinaemia in bottle-fed infants (short-term exposure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrite</td>
<td>3 mg/l as nitrite ion (or 0.9 mg/l as nitrite-nitrogen) to protect against methaemoglobinaemia in bottle-fed infants (short-term exposure)</td>
</tr>
<tr>
<td>Combined nitrate plus nitrite</td>
<td>The sum of the ratios of the concentrations as reported or detected in the sample of each to its guideline value should not exceed 1.</td>
</tr>
</tbody>
</table>
## 12. CHEMICAL FACT SHEETS

**Limit of detection**
- 0.005–0.01 mg/l (nitrite) by a molecular absorption spectrometric method;
- 0.01–1 mg/l (nitrate) by spectrometric techniques;
- 0.022 mg/l (nitrate) and 0.035 mg/l (nitrite) by ion chromatography;
- 0.1 mg/l (nitrate) and 0.05 mg/l (nitrite) by LC.

**Treatment performance**
- **Nitrate:** 5 mg/l or lower should be achievable using biological denitrification (surface waters) or ion exchange (groundwaters).
- **Nitrite:** 0.1 mg/l should be achievable using chlorination (to form nitrate).

**Additional comments**
- Nitrite can occur in the distribution system at higher concentrations when chloramination is used, but the occurrence is almost invariably sporadic. Methaemoglobinemia is therefore the most important consideration, and the guideline derived for protection against methaemoglobinemia would be the most appropriate under these circumstances, allowing for any nitrate that may also be present.

- Methaemoglobinemia in infants appears to be associated with simultaneous diarrhoeal disease. 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 or in the presence of endemic infantile diarrhoea. Water should not be used for bottle-fed infants if the concentration of nitrate is above 100 mg/l but can be used if the concentration is between 50 and 100 mg/l if the water is microbiologically safe and there is increased vigilance by medical authorities.

- 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. Efficient disinfection must never be compromised.

- The occurrence of nitrite in the distribution system as a consequence of chloramine use will be intermittent, and average exposures over time should not exceed about 0.2 mg/l.

**Assessment date**
- 1998, revised in 2007 and 2010

**Principal references**
- FAO/WHO (2003) Nitrite (and potential endogenous formation of N-nitroso compounds)
- Schmoll et al. (2006) *Protecting groundwater for health*
- WHO (2011) *Nitrate and nitrite in drinking-water*

Absorption of nitrate ingested from vegetables, meat or water is rapid and in excess of 90%, and 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. There may therefore 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 (greater than 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 nitrate reduction to nitrite and may also increase the intake of water in combating 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 that also have a high probability of microbial contamination and predominantly when the drinking-water is anaerobic, which should not occur if it is properly disinfected.

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.
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 (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. 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.

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; however, this would be an issue only under circumstances of high nitrate intake and simultaneous iodine deficiency, although the nitrate intakes at which this becomes significant are uncertain.

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 (or 11 mg/l if reported as nitrate-nitrogen) is based on epidemiological evidence for methaemoglobinaemia in infants, which results from short-term exposure, and is protective for bottle-fed infants and, consequently, other parts of the population. This outcome 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.
The guideline value for nitrite of 3 mg/l as nitrite (or 0.9 mg/l if reported as nitrite-nitrogen) is based on human data showing that doses of nitrite that cause methaemoglobinaemia in infants range from 0.4 mg/kg body weight to more than 200 mg/kg body weight. By applying the lowest level of the range (0.4 mg/kg body weight), a body weight of 5 kg for an infant and a drinking-water consumption of 0.75 litre, a guideline value of 3 mg/l (rounded figure) can be derived.

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:

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

For chronic exposure, JECFA proposed an ADI of 0–3.7 mg/kg body weight for nitrate and an ADI of 0–0.07 mg/kg body weight for nitrite, expressed as nitrite ion. The value for nitrate is based on a NOEL of 370 mg/kg body weight per day in laboratory animal studies; in view of the known interspecies variation in nitrate/nitrite metabolism, however, it was not considered appropriate at this time to use this in the risk assessment for humans. The JECFA ADI for nitrite was based on effects on heart and lung in a 2-year study in rats using a safety factor of 100. However, owing to the uncertainty surrounding the susceptibility of humans compared with experimental animals, this value was considered provisional and has now been suspended and is being subjected to review in light of evidence on the differences in nitrite metabolism between laboratory rodents and humans.

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, the careful siting of pit latrines and septic tanks, sewer leakage control, as well as management of fertilizer and manure application and storage of animal manures. It may also take the form of denitrification of wastewater effluents.

Methaemoglobinaemia 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 indications that microbiological quality might also be poor, a number of actions can be taken. Water should be boiled 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 (normally for groundwaters) and biological denitrification (normally for surface waters). However, there are disadvantages associated with both approaches, including the need for regeneration and disposal of spent regenerant with ion exchange, the complexities of operation and the potential for microbial and carbon feed contamination of the final water with biological denitrification.

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 where chloramines are used to control Legionella.

**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.

<table>
<thead>
<tr>
<th>Guideline value</th>
<th>0.2 mg/l (200 µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occurrence</td>
<td>Concentrations in drinking-water usually do not exceed a few micrograms per litre, although concentrations as high as 35 µg/l have been measured</td>
</tr>
<tr>
<td>TDI</td>
<td>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)</td>
</tr>
<tr>
<td>Limit of detection</td>
<td>0.2 µg/l using GC with a nitrogen-specific detector</td>
</tr>
<tr>
<td>Treatment performance</td>
<td>No information found on removal from water</td>
</tr>
<tr>
<td>Guideline value derivation</td>
<td></td>
</tr>
<tr>
<td>• allocation to water</td>
<td>50% of TDI</td>
</tr>
<tr>
<td>• weight</td>
<td>60 kg adult</td>
</tr>
<tr>
<td>• consumption</td>
<td>2 litres/day</td>
</tr>
</tbody>
</table>