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Nitrate and Nitrite in Drinking-water

Background document for development of
WHO *Guidelines for Drinking-water Quality*

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Preface

Access to safe drinking-water is essential to health, a basic human right and a component of effective policy for health protection. A major World Health Organization (WHO) function to support access to safe drinking-water is the responsibility "to propose ... regulations, and to make recommendations with respect to international health matters ...", including those related to drinking-water safety and management.

The first WHO document dealing specifically with public drinking-water quality was published in 1958 as *International Standards for Drinking-water*. It was subsequently revised in 1963 and in 1971 under the same title. In 1984–1985, the first edition of the WHO *Guidelines for Drinking-water Quality* (GDWQ) was published in three volumes: Volume 1, Recommendations; Volume 2, Health criteria and other supporting information; and Volume 3, Surveillance and control of community supplies. Second editions of these volumes were published in 1993, 1996 and 1997, respectively. Addenda to Volumes 1 and 2 of the second edition were published in 1998, addressing selected chemicals. An addendum on microbiological aspects reviewing selected microorganisms was published in 2002. The third edition of the GDWQ was published in 2004, the first addendum to the third edition was published in 2006 and the second addendum to the third edition was published in 2008. The fourth edition was published in 2011, and the first addendum to the fourth edition was published in 2017.

The GDWQ are subject to a rolling revision process. Through this process, microbial, chemical and radiological aspects of drinking-water are subject to periodic review, and documentation related to aspects of protection and control of drinking-water quality is accordingly prepared and updated.

Since the first edition of the GDWQ, WHO has published information on health criteria and other supporting information to the GDWQ, describing the approaches used in deriving guideline values and presenting critical reviews and evaluations of the effects on human health of the substances or contaminants of potential health concern in drinking-water. In the first and second editions, these constituted Volume 2 of the GDWQ. Since publication of the third edition, they comprise a series of free-standing monographs, including this one.

For each chemical contaminant or substance considered, a background document evaluating the risks for human health from exposure to the particular chemical in drinking-water was prepared. The draft health criteria document was submitted to a number of scientific institutions and selected experts for peer review. The draft document was also released to the public domain for comment. Comments were carefully considered and addressed as appropriate, taking into consideration the processes outlined in the *Policies and Procedures Used in Updating the WHO Guidelines for Drinking-water Quality* (http://apps.who.int/iris/bitstream/10665/70050/1/WHO_HSE_WSH_09.05_eng.pdf) and the *WHO Handbook for Guideline Development* (http://www.who.int/publications/guidelines/handbook_2nd_ed.pdf), and the revised draft was submitted for final evaluation at expert consultations.

During the preparation of background documents and at expert consultations, careful consideration was given to information available in previous risk assessments carried out by the International Programme on Chemical Safety, in its Environmental Health Criteria monographs and Concise International Chemical Assessment Documents, the International Agency for Research on Cancer, the Joint Food and Agriculture Organization of the United Nations (FAO)/WHO Meeting on Pesticide Residues and the Joint FAO/WHO Expert Committee on Food Additives (which evaluates contaminants such as lead, cadmium, nitrate and nitrite, in addition to food additives).

Further up-to-date information on the GDWQ and the process of their development is available on the WHO website and in the current edition of the GDWQ.

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The work of the following experts was crucial in the development of this document and others in the first addendum to the fourth edition:

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The draft text was discussed at the expert consultations for the first addendum to the fourth edition of the GDWQ, held on 2–5 December 2013 and 23–26 February 2015. The final version of the document takes into consideration comments from both peer reviewers and the public.

The coordinator was Ms J. De France, WHO Headquarters, with support from Mr P. Callan, Australia. Strategic direction was provided by Mr B. Gordon, WHO Headquarters. Dr A. Tritscher and Dr P. Verger, WHO Headquarters, provided liaisons with the Joint FAO/WHO Expert Committee on Food Additives and the Joint FAO/WHO Meeting on Pesticide Residues, whereas Dr R. Brown and Ms C. Vickers, WHO Headquarters, provided liaisons with the International Programme on Chemical Safety. Dr M. Perez contributed on behalf of the Radiation Programme, WHO Headquarters. Dr R. Yadav, WHO Headquarters, provided input on pesticides added to drinking-water for public health purposes.

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Many individuals from various countries contributed to the development of the GDWQ. The efforts of all who contributed to the preparation of this document and in particular those who provided peer or public domain review comments are greatly appreciated.

[1] Health Canada. *Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – Nitrate and nitrite*. Ottawa (ON): Health Canada, Healthy Environments and Consumer Safety Branch, Water and Air Quality Bureau; 2013 (http://hc-sc.gc.ca/ewh-semt/pubs/water-eau/nitrate_nitrite/index-eng.php, accessed 17 December 2014).

Abbreviations

bw	body weight
DNA	deoxyribonucleic acid
FAO	Food and Agriculture Organization of the United Nations
GDWQ	*Guidelines for Drinking-water Quality*
HPT	hypothalamic–pituitary–thyroid
LD_{50}	median lethal dose (dose estimated to be lethal to half of the animals)
LOAEL	lowest-observed-adverse-effect level
MDL	method detection limit
NADH	nicotinamide adenine dinucleotide (reduced)
NDMA	*N*-nitrosodimethylamine
NIS	sodium–iodide symporter
NOAEL	no-observed-adverse-effect level
SM	standard method
T_3	triiodothyronine
T_4	thyroxine
TSH	thyroid stimulating hormone
USA	United States of America
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

Contents

1. GENERAL DESCRIPTION

1.1 Identity

Nitrate (NO_3^-) and nitrite (NO_2^-) are naturally occurring ions that are ubiquitous in the environment. Both are products of the oxidation of nitrogen, as part of the cycle required by all living systems for the production of complex organic molecules, such as enzymes and other proteins (Environment Canada, 2003; IARC, 2010). Nitrate is the more stable form of oxidized nitrogen. However, under anaerobic conditions and in the presence of a carbon source, nitrate can be reduced by microbial action to nitrite, which is relatively unstable and moderately reactive. Under low oxygen conditions, the denitrification process further reduces nitrite to nitrogen gas (Appelo & Postma, 1996).

Nitrification is a two-step process during which ammonia is oxidized to nitrite by ammonia-oxidizing bacteria and nitrite is further oxidized to nitrate by nitrite-oxidizing bacteria (USEPA, 2002a; IARC, 2010). In addition to bacterial nitrification, organic nitrogen sources, such as organic matter in soil, manure and urea-based fertilizers, can be transformed to nitrate by mineralization and hydrolysis (Ward et al., 2005; Cartes et al., 2009).

1.2 Physicochemical properties

The Chemical Abstracts Service numbers for nitrate and nitrite are 14797-55-8 and 14797-68-0, respectively. Their molecular weights are 62.00 and 46.01, respectively. Further properties are shown in Table 1 (ICAIR Life Systems, Inc., 1987).

Table 1. Physicochemical properties of nitrate and nitrite

Property	Nitrate	Nitrite
Acid dissociation constant (pK_a)	Conjugate base of strong acid HNO_3; $pK_a = -1.3$	Conjugate base of weak acid HNO_2; $pK_a = 3.4$
Solubility in water	Very soluble in water	Very soluble in water
Reactivity	Unreactive	Reactive; oxidizes antioxidants, Fe^{2+} of haemoglobin to Fe^{3+}, and primary amines; nitrosates several amines and amides

Nitrate and nitrite are chemically expressed in terms of the concentration of the ions (i.e. mg/L as nitrate or nitrite) or as the element nitrogen (N) (i.e. mg/L as nitrate-nitrogen or nitrite-nitrogen).[1]

1.3 Major uses and sources in drinking-water

The most common sources of both nitrate and nitrite in water include agricultural activities (inorganic fertilizers and manure), wastewater treatment, nitrogenous waste products from humans and discharges from industrial processes and motor vehicles (Kirmeyer et al., 1995; Environment Canada, 2003; USEPA, 2006; Keeney & Hatfield, 2008). 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 and the process is not sufficiently well controlled.

[1] 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. To obtain equivalent ion concentrations, the given concentration is multiplied by the following conversion factors (Pfander, Brown & Garner, 1993): sodium nitrate ($NaNO_3$), 0.729; potassium nitrate (KNO_3), 0.614; sodium nitrite ($NaNO_2$), 0.667; and potassium nitrite (KNO_2), 0.541.

Nitrate and nitrite salts are also used as oxidizing agents, in the production of explosives, for glass making and to preserve food, especially in cured meats (IARC, 2010). Naturally, nitrate and nitrite are products of the oxidation of nitrogen (which comprises approximately 78% of Earth's atmosphere) by microorganisms in plants, soil and water and, to a lesser extent, by lightning (IARC, 2010).

1.4 Environmental fate

In soil, fertilizers containing inorganic nitrogen and wastes containing organic nitrogen are first decomposed to give ammonia, which is then oxidized to nitrate and nitrite. The nitrate is taken up by plants during their growth and used in the synthesis of organic nitrogenous compounds. Surplus nitrate readily moves with the groundwater (USEPA, 1987; Van Duijvenboden & Matthijsen, 1989). Generally, it is assumed that nitrate will not adsorb to soil particles and will have a high potential for mobility (Environment Canada, 2003).

Using an isotopically labelled nitrogen fertilizer, Sebilo et al. (2013) found that 61–65% of the applied fertilizer's nitrogen was taken up by plants, whereas 12–15% was still residing in the soil organic matter more than 25 years after tracer application. Between 8% and 12% of the applied fertilizer had leaked towards the hydrosphere during the 30-year observation period. The authors predicted that additional exports of nitrogen-labelled nitrate from the tracer to the hydrosphere would continue for at least another 50 years.

Under aerobic conditions, nitrate can percolate in relatively large quantities into the aquifer when there is no growing plant material to take up the nitrate and when the net movement of soil water is downward to the aquifer. Degradation or denitrification occurs to only a small extent in the soil and in the rocks forming the aquifer. Under anaerobic conditions, nitrate may be denitrified or degraded almost completely to nitrogen. The presence of high or low water tables, the amount of rainwater, the presence of other organic material and physicochemical properties are also important in determining the fate of nitrate in soil (Van Duijvenboden & Loch, 1983; Mensinga, Speijers & Meulenbelt, 2003; Fewtrell, 2004; Dubrovsky & Hamilton, 2010). In surface water, nitrification and denitrification may also occur, depending on the temperature and the pH. The uptake of nitrate by plants (e.g. phytoplankton), however, is responsible for most of the removal of nitrate from surface water.

2. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

2.1 Air

Atmospheric nitrate concentrations ranging from 0.1 to 0.4 μg/m^3 have been reported in the Pacific Island network (Prospero & Savoie, 1989). Mean monthly nitrate concentrations in air in the Netherlands ranged from 1 to 14 μg/m^3 (Janssen, Visser & Romer, 1989). In 1990, the annual average concentration of nitrate in ambient air was 0.88 μg/m^3 for 34 communities in 50 sampling locations across Canada (Environment Canada, 1992). Indoor nitrate aerosol concentrations of 1.1–5.6 μg/m^3 were found to be related to outdoor concentrations (Yocom, 1982).

2.2 Water

Concentrations of nitrate in rainwater of up to 5 mg/L have been observed in industrial areas. In rural areas, concentrations are somewhat lower (Van Duijvenboden & Matthijsen, 1989).
The nitrate concentration in surface water is normally low (0–18 mg/L; up to ~4 mg/L as nitrate-nitrogen), but can reach high levels as a result of agricultural runoff, refuse dump runoff or contamination with human or animal wastes. The concentration often fluctuates

with the season and may increase when the river is fed by nitrate-rich aquifers. Nitrate concentrations in surface water have gradually increased in many European countries in the last few decades and have in some cases doubled over the past 20 years. In the United Kingdom, for example, an average annual increase in nitrate concentration of 0.7 mg/L has been observed in some rivers (Young & Morgan-Jones, 1980).

Generally, nitrate concentrations in well water are higher than those in surface water supplies (Liebscher, Hii & McNaughton, 1992). The natural nitrate concentration in groundwater under aerobic conditions is a few milligrams per litre and depends strongly on soil type and on the geological situation. The increasing use of artificial fertilizers, the disposal of wastes (particularly from animal farming) and changes in land use are the main factors responsible for the progressive increase in nitrate levels in groundwater supplies over the last 20 years. As a result of these activities, the nitrate concentration can easily reach several hundred milligrams per litre (WHO, 1985). For example, concentrations of up to 1500 mg/L were found in groundwater in an agricultural area of India (Jacks & Sharma, 1983). In Denmark and the Netherlands, nitrate concentrations are increasing by 0.2–1.3 mg/L per year in some areas (WHO, 1985). In contrast, in the United States of America (USA), naturally occurring concentrations in groundwater do not usually exceed 4–9 mg/L as nitrate ion and 0.3 mg/L as nitrite ion (USEPA, 1987; Burkart & Stoner, 2002; DeSimone, 2009; Dubrovsky et al., 2010). Because of the delay in the response of groundwater to changes in soil, some endangered aquifers have not yet shown the increase expected from the increased use of nitrogen fertilizer or manure. Once the nitrate reaches these aquifers, the aquifers will remain contaminated for decades, even if there is a substantial reduction in the nitrate loading of the surface.

In the USA, nitrate is present in most surface water and groundwater supplies at levels below 4 mg/L, with levels exceeding 20 mg/L in about 3% of surface waters and 6% of groundwaters. In 1986, a nitrate concentration of 44 mg/L (10 mg/L as nitrate-nitrogen) was exceeded in 40 surface water and 568 groundwater supplies. Nitrite concentrations were not surveyed, but were expected to be much lower than 3.3 mg/L (USEPA, 1987). In a later survey, the median nitrite concentrations in groundwater and surface water systems in the USA were 0.07 and 0.1 mg/L (0.02 and 0.03 mg/L as nitrite-nitrogen), respectively. However, more than 635 surface water and groundwater systems reported at least one detection greater than 1 mg/L as nitrite-nitrogen (3.3 mg/L as nitrite ion), and an additional 1353 systems reported detections above 0.5 mg/L as nitrite-nitrogen (1.6 mg/L as nitrite ion) (USEPA, 2009).

In most countries, nitrate concentrations in drinking-water derived from surface water do not exceed 10 mg/L. In some areas, however, concentrations are higher as a result of runoff and the discharge of sewage effluent and certain industrial wastes. In 15 European countries, the percentage of the population exposed to nitrate concentrations in drinking-water above 50 mg/L (as nitrate ion) ranged from 0.5% to 10% (WHO, 1985; ECETOC, 1988); this corresponds to nearly 10 million people. In a national survey conducted by Health Canada in 2009 and 2010, in which 130 raw water samples and 130 treated water samples were analysed for nitrate and nitrite, nitrate was detected in 42.3% of the raw water samples at an average concentration of 3.75 mg/L (maximum of 23.9 mg/L) and in 41.5% of the treated water samples at an average concentration of 3.6 mg/L (maximum of 20.8 mg/L). Nitrite was detected in 11.5% of the raw water samples at an average concentration of 0.05 mg/L (maximum of 0.3 mg/L) and in 6.9% of the treated water samples at an average concentration of 0.05 mg/L (maximum of 0.3 mg/L) (Health Canada, 2013).

IARC (2010) reviewed available data on levels of nitrate (as nitrate-nitrogen) and nitrite (as nitrite-nitrogen) in drinking-water of various regions of the world, including Asia, Europe and North and South America. The review confirmed that agricultural activities have impacts on nitrate concentrations in both surface water and groundwater, with the most significant impact on shallow wells. The highest reported nitrate concentration was in a domestic well (>1200 mg/L as nitrate-nitrogen) in the USA. Nitrite concentrations rarely exceeded 3 mg/L; a maximum concentration of 7.9 mg/L (as nitrite-nitrogen) was reported in a private well in the USA.

Chloramination may give rise to the formation of nitrite within the distribution system, and the concentration of nitrite may increase as the water moves towards the extremities of the system. Nitrification in distribution systems can increase nitrite concentrations, usually by 0.2–1.5 mg/L, but potentially by more than 3 mg/L (AWWARF, 1995). See Section 7.3.3 for further information relating to chloramination.

2.3 Food

Vegetables and cured meat are in general the main sources of nitrate and nitrite in the diet, but small amounts may be present in fish and dairy products. Meat products may contain nitrate at concentrations ranging from <2.7 to 945 mg/kg and nitrite at concentrations ranging from <0.2 to 6.4 mg/kg; dairy products may contain nitrate at concentrations ranging from <3 to 27 mg/kg and nitrite at concentrations ranging from <0.2 to 1.7 mg/kg (ECETOC, 1988). Several vegetables and fruits contain nitrate at concentrations of 200–2500 mg/kg (Van Duijvenboden & Matthijsen, 1989). The nitrate content of vegetables can be affected by processing of the food, the use of fertilizers and growing conditions, especially the soil temperature and light intensity (Gangolli et al., 1994; FAO/WHO, 1995). Vegetables such as beetroot, lettuce, radish and spinach often contain nitrate concentrations above 2500 mg/kg, especially when they are cultivated in greenhouses. Nitrite concentrations in food are very low (generally well below 10 mg/kg) and rarely exceed 100 mg/kg. Exceptions to this are vegetables that have been damaged, poorly stored or stored for extended periods, as well as pickled or fermented vegetables. In such circumstances, nitrite concentrations up to 400 mg/kg have been measured (FAO/WHO, 1995).

2.4 Estimated total exposure and relative contribution of drinking-water

Where nitrate concentrations in drinking-water are low, the main route of exposure to nitrate for the general population will be the ingestion of food, followed by the ingestion of drinking-water. Vegetables are the main source of nitrate intake when nitrate concentrations in drinking-water are below 10 mg/L (Chilvers, Inskip & Caygill, 1984; USEPA, 1987; ECETOC, 1988).

The contribution of drinking-water to nitrate intake is usually less than 14%. When nitrate levels in drinking-water exceed 50 mg/L, drinking-water will be the major source of total nitrate intake, especially for bottle-fed infants. For bottle-fed infants, daily intake from formula made with water containing a nitrate concentration of 50 mg/L would average about 8.3–8.5 mg of nitrate per kilogram of body weight (bw) per day.

Using a multiroute exposure assessment approach (Krishnan & Carrier, 2008), it was found, on the basis of the estimated skin permeability coefficients and the air to water concentration values, that dermal and inhalation exposures to nitrate or nitrite through showering or bathing were not significant (Health Canada, 2013).

The mean dietary intake of nitrate determined by the duplicate portion technique ranges from 43 to 131 mg/day (WHO, 1985). Estimates of the total nitrate intake based on the proportion

of nitrate excreted in the urine (Bartholomew et al., 1979) range from 39 to 268 mg/day, the higher values applying to vegetarian and nitrate-rich diets (ECETOC, 1988). The estimated total daily intake of nitrate ranged from 50 to 81 mg/person in the United Kingdom (Bonnell, 1995; Schuddeboom, 1995), from 70 to 172 mg/person in Denmark (Bonnell, 1995) and from 70 to 110 mg/person in Germany (Bonnell, 1995). In the Netherlands, total nitrate intake was estimated to be 160–240 mg/day, of which <20–100 mg/day was from drinking-water (Schuddeboom, 1995). EFSA (2008) indicated that average adult consumption of nitrate from all dietary sources, including water for the United Kingdom and France, was 91 and 141 mg/person, respectively. In the USA, the average adult daily intake of nitrate from food has been estimated to be 40–100 mg (OEHHA, 1997). Average daily intakes of nitrate from food in Canada have been estimated to be 44.3 mg, based on a survey of dietary habits (Choi, 1985). Other reported estimates of daily intake of nitrate from many different countries are between 53 and 350 mg (Pennington, 1998). For some individuals and communities where vegetables with particularly high nitrate levels are consumed or where well water contains elevated concentrations of nitrate, consumption may be significantly higher.

Average daily intake of nitrite from food in Canada has been estimated to be 0.50 mg, based on a survey of dietary habits (Choi, 1985). In the USA, the average adult daily intake of nitrite from food has been estimated to be 0.3–2.6 mg (OEHHA, 1997). The mean dietary intake of nitrite determined by the duplicate portion technique ranges from 1.2 to 3 mg/day (WHO, 1985). Mean dietary nitrite intake from all food sources has been reported to range from <0.1 to 8.7 mg/person per day from European diets (FAO/WHO, 1995). Other reported estimates of daily intake of nitrite from many different countries are between 0 and 20 mg (Pennington, 1998). The daily nitrite intake has been estimated to range from 0.3 to 2.6 mg/day, primarily from cured meat (NAS, 1981). Nitrite present in cured meat has been reported to account for up to 70% of total dietary intake of this substance, depending on the amount, origin and type of cured meat consumed.

The German Federal Institute of Risk Assessment (2009) issued a statement in 2009 in which it was estimated that adults in Germany take up nitrate from all sources at a median rate of 159.8 mg/day. The median intake from water and other beverages alone was estimated to be 28.3 mg/day.

Approximately 5–8% of ingested nitrate is reduced by oral bacteria to nitrite (as reviewed in Walker, 1996; Mensinga, Speijers & Meulenbelt, 2003). This nitrite represents approximately 80% of the total exposure to nitrite, the remainder coming directly from exogenous sources.

3. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

3.1 Absorption, distribution and elimination

Ingested nitrate and nitrite are rapidly and almost completely absorbed in the small intestine of humans and transferred to blood (bioavailability at least 92%); less than 2% of dietary nitrate intake reaches the terminal ileum (Mensinga, Speijers & Meulenbelt, 2003). Nitrite may also be absorbed directly by the stomach, but part of the ingested nitrite reacts with gastric contents prior to absorption. In quantitative terms, absorption through the oral route is of greater importance than that through the inhalation or dermal route (Lundberg et al., 2004).

Nitrate is rapidly distributed throughout the tissues, in breast milk and across the placenta. In humans and most laboratory animals, except the rat, approximately 25% of plasma nitrate is selectively and dose-dependently secreted by the salivary gland via an active transport mechanism shared with iodide and thiocyanate, increasing nitrate concentrations up to 10 times that in plasma (Spiegelhalder, Eisenbrand & Preussmann, 1976; Walker, 1996;

Lundberg et al., 2004). Plasma nitrite concentrations are normally lower than nitrate concentrations due to lower exposure and rapid reoxidation of nitrite to nitrate by oxygenated haemoglobin in the blood (Parks et al., 1981; Walker, 1996; Lundberg et al., 2004). In rats and dogs, nitrite is almost absent, except in saliva (Fritsch, de Saint Blanquat & Klein, 1985; Cortas & Wakid, 1991). Women who consume water with a nitrate concentration of 100 mg/L or less do not produce milk with elevated nitrate concentrations (Dusdieker et al., 1996).

The majority of ingested nitrate is eventually excreted in urine as nitrate, ammonia or urea, faecal excretion being negligible. Little nitrite is excreted because of the endogenous formation of nitrate or its reduction to ammonia by nitrate reductase (Wagner et al., 1983; WHO, 1985; ICAIR Life Systems, Inc., 1987; Speijers et al., 1989; Walker, 1999; Lundberg et al., 2004; Lundberg, Weitzberg & Gladwin, 2008). Excretion follows first-order kinetics, and the elimination half-life is approximately 5 hours (Green, Tannenbaum & Fox, 1982). Mean nitrate clearance is estimated to be 25.8 mL/minute (Cortas & Wakid, 1991). The average plasma half-life of nitrite is 30 minutes in humans and less than an hour in most species; consequently, nitrite is not normally detected in body tissues and fluids after oral administration (Kortboyer et al., 1997). Elimination of nitrite from the stomach occurs through two competing pathways: absorption and reaction with amines, resulting in the formation of nitrosamines.

3.2 Endogenous formation of nitrate, nitrite and N-nitroso compounds

In normal healthy humans, an average of 1 mmol of nitrate is synthesized endogenously per day, corresponding to 62 mg/day as nitrate or 14 mg/day as nitrate-nitrogen (Mensinga, Speijers & Meulenbelt, 2003). A major pathway for endogenous nitrate production is conversion of arginine by macrophages to nitric oxide and citrulline, followed by oxidation of the nitric oxide to nitrous anhydride and then reaction of nitrous anhydride with water to yield nitrite. Nitrite is rapidly coupled with oxyhaemoglobin, producing nitrate and methaemoglobin. Nitrite appears to be in a dynamic equilibrium with nitrate, with nitrate being the normal state (Walker, 1999; Lundberg et al., 2004). Thus, when nitrate intake is low and there are no additional exogenous sources, such as during gastrointestinal infections, endogenous production is more important than exogenous sources (Mensinga, Speijers & Meulenbelt, 2003).

In humans, saliva is the major site for the formation of nitrite. Of the approximately 25% of exogenous nitrate actively recirculated by the salivary ducts, about 20% (representing 5–8% of ingested nitrate exposure) of it is reduced by oral bacteria to nitrite (Spiegelhalder, Eisenbrand & Preussmann, 1976; Eisenbrand, Spiegelhalder & Preussmann, 1980; Walters & Smith, 1981; Gangolli et al., 1994; Walker, 1996; Mensinga, Speijers & Meulenbelt, 2003). Bacterial reduction of nitrate may also take place in other parts of the human gastrointestinal tract, but not normally in the stomach; exceptions are reported in humans with low gastric acidity, such as artificially fed infants, and certain patients in whom hydrochloric acid secretion is slower than normal or patients using antacids (Colbers et al., 1996). Endogenous microbial conversion of nitrate to nitrite is influenced by situations that alter stomach pH, such as bacterial infection, nutritional status and age (Eisenbrand, Spiegelhalder & Preussmann, 1980; Forman, Al-Dabbagh & Doll, 1985). In rats, active secretion and reduction of nitrate in saliva are virtually absent (Walker, 1995).

The situation in neonates is not clear. It is commonly accepted that infants aged under 6 months may be highly susceptible to gastric bacterial nitrate reduction to nitrite, as their gastric pH is generally higher than that of adults (Speijers et al., 1989). However, the

presence of acid-producing lactobacilli in the stomach may be important, as these organisms do not reduce nitrate and may maintain a pH low enough to inhibit colonization by nitrate-reducing bacteria (Bartholomew et al., 1980). As mentioned above, nitrite may also be produced via the arginine–nitric oxide pathway, but would be undetectable because of the rapid oxidation to nitrate. One possible example of nitrite production by this route, however, is the methaemoglobinaemia observed in infants suffering from diarrhoea (Gangolli et al., 1994).

Under certain conditions, nitrite can react with amino compounds, synthesized in the body or from food sources, to form *N*-nitroso compounds endogenously. Endogenous nitrosation may occur through several mechanisms, including acid-catalysed (particularly in the acidic stomach) and cell-mediated (bacterial cells and immune cells; at neutral stomach pH) mechanisms (Ohshima & Bartsch, 1994; Mirvish, 1995; IARC, 2010). However, many dietary substances, including vitamins C and E and polyphenols, inhibit nitrosation (Bartsch, Ohshima & Pignatelli, 1988; Crespi & Ramazzotti, 1991). Although the relative contribution of endogenous nitrosation to total exposure to *N*-nitroso compounds is still not clear, endogenous synthesis is likely the largest source of *N*-nitroso compound exposure for the general population (Shephard, Schlatter & Lutz, 1987; Bartsch, Ohshima & Pignatelli, 1988; Crespi & Ramazzotti, 1991; NRC, 1995; Fristachi & Rice, 2007).

4. EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO TEST SYSTEMS

4.1 Acute exposure

The acute oral toxicity of nitrate in experimental animals is generally low, with median lethal dose (LD_{50}) values of 1600–9000 mg of sodium nitrate per kilogram of body weight reported in mice, rats and rabbits. Nitrite is more toxic, with LD_{50} values of 85–220 mg of sodium nitrate per kilogram of body weight reported for mice and rats (Speijers et al., 1989; WHO, 1996; Boink et al., 1999).

4.2 Short-term exposure

Female Wistar rats that received sodium nitrate at 0, 50, 100, 250 or 500 mg/L in tap water for 30 weeks had increased thyroid gland weights as well as decreased iodine uptake, altered serum thyroid hormone levels and histopathological changes at 250 and 500 mg/L (Eskiocak et al., 2005). Similarly, male Wistar rats that received potassium nitrate at 0, 100, 150 or 500 mg/L in tap water for 5 months had increased thyroid weights as well as altered thyroid hormone levels and histopathological changes at 150 and 500 mg/L (Zaki et al., 2004). Rats fed a diet containing 3% potassium nitrate for 4–6 weeks also appeared to experience altered thyroid gland function (Jahreis et al., 1991; Mukhopadhyay et al., 2005). However, no significant differences in thyroid function (measured by triiodothyronine [T_3] and thyroxine [T_4] levels) were observed in any adult Beagle dogs that received 0, 300, 600 or 1000 mg sodium nitrate per litre of drinking-water for 1 year or in any puppies from the dams receiving sodium nitrate at the above concentrations (Kelley, Oehme & Hoffman, 1974). Despite some deficiencies in these studies (e.g. poor histology, lack of accounting for iodide intake and other iodide uptake inhibitors in drinking-water), they provide support for the role of nitrate in altering thyroid gland hormones and their potential functions (see Section 5.2). The no-observed-adverse-effect level (NOAEL) for thyroid effects was 100 mg potassium nitrate per litre (equivalent to 61.4 mg/L as nitrate ion). Nitrite, unlike nitrate, does not inhibit thyroidal iodide uptake (Eskandari et al., 1997) and is therefore not relevant for thyroid toxicity.

Til, Kuper & Falke (1997) reported significantly elevated methaemoglobin concentrations in weanling Wistar rats (10 of each sex per dose) given 100 or 3000 mg potassium nitrite per litre (equivalent to nitrite concentrations of 54 and 1623 mg/L, respectively) in drinking-water for 13 weeks, but not in rats given 0, 12.5, 25 or 50 mg potassium nitrite per litre (equivalent to nitrite concentrations of 0, 6.8, 13.5 and 27 mg/L, respectively). Other studies confirm nitrite's elevation of methaemoglobin levels in rats, but at higher nitrite concentrations of 667 mg/L in drinking-water for 24 months (Shuval & Gruener, 1972), 1623 mg/L for 13 weeks (Til et al., 1988) and 250 mg/L for 14 weeks (NTP, 2001). Clinical findings included brown discoloration in eyes and cyanosis of mouth, tongue and ears at a nitrite concentration of 1000 mg/L or higher in rats but not mice, possibly due to higher erythrocyte methaemoglobin reductase activity in mice compared with rats (NTP, 2001).

Both sexes of weanling Wistar rats (10 of each sex per dose) exposed to potassium nitrite in drinking-water at 1, 100, 300, 1000 or 3000 mg/L for 13 weeks experienced hypertrophy of the adrenal zona glomerulosa at all concentrations (lowest-observed-adverse-effect level [LOAEL] = 1 mg/L as potassium nitrite or 0.5 mg/L as nitrite ion). The incidence and severity of hypertrophy of the adrenal zona glomerulosa increased as concentrations of nitrite in drinking-water increased (Til et al., 1988). Additional studies support the induction of adrenal hypertrophy in rodents by the administration of sodium nitrite or potassium nitrite in drinking-water for 13 weeks (NOAEL = 50 mg/L; Kuper & Til, 1995; Til, Kuper & Falke, 1997). Mild hypertrophy is suggested to be a physiological adaptation to nitrite-induced vasodilatation rather than a harmful lesion (Boink et al., 1999). Indeed, vasodilatation, which lowers blood pressure and stimulates the renin–angiotensin–aldosterone axis, is a well-known and potentially beneficial effect of nitrite (Til et al., 1988; Gangolli et al., 1994; Vleeming et al., 1997; Lundberg et al., 2004; Lundberg, Weitzberg & Gladwin, 2008).

4.3 Reproductive and developmental toxicity

Decreased sperm count and sperm motility in mice exposed to potassium nitrate at 225–270 mg/kg bw per day in drinking-water (equivalent to nitrate doses of 138–165.8 mg/kg bw per day) for 35 days have been reported, with no effects on testis, epididymal or accessory organ weight at 175–210 mg/kg bw per day (equivalent to nitrate doses of 107.45–128.9 mg/kg bw per day) (Pant & Srivastava, 2002). Sperm count, sperm motility and the activity of enzymatic markers of spermatogenesis were significantly decreased after male Swiss albino rats were exposed orally to sodium nitrate at 50, 100 or 200 mg/kg bw per day in drinking-water (equivalent to nitrate doses of 36.45, 72.9 and 145.8 mg/kg bw per day, respectively) for 60 days, compared with controls; decreased testicular weight and histopathological changes were significant only at the two highest doses (Aly et al., 2010). In rabbits, nitrate concentrations of 250 or 500 mg/L administered in drinking-water for 22 weeks had no detrimental effects on reproductive performance after successive gestations. In sheep and cattle, no abortions were observed at nitrate dose levels causing severe methaemoglobinaemia (Speijers et al., 1989; WHO, 1996).

No nitrite-related effects on fertility parameters were observed in pair-based mouse or rat studies following oral exposures (diet and drinking-water) ranging from 5 to 425 mg sodium nitrite per kilogram of body weight per day (equivalent to nitrite doses of 3–283 mg/kg bw per day), although none of the studies was conducted under a standard multigenerational reproductive study protocol (Olsen et al., 1984; Vorhees et al., 1984; Anderson et al., 1985; NTP, 1990). Parameters examined in these studies included mean number of litters per pair, days to deliver litter, mean litter size, pup viability, post-delivery estrous cycle, gestation length, sex ratio of offspring, external malformations, timing of vaginal opening in female offspring, mean pup weight and pup survival. At high levels of sodium nitrite exposure

(1500–3000 mg/L drinking-water for 14 weeks; equivalent to nitrite concentrations of approximately 1100–2200 mg/L), estrous cycles of female mice were significantly longer (NOAEL = 750 mg sodium nitrite per litre; equivalent to a nitrite concentration of 500 mg/L), and testicular degeneration and reduced sperm motility were reported in male rats and mice; no notable histopathological changes in reproductive organs after 2 years of exposure were reported in either species or sex (NTP, 2001).

In utero exposure of mice to sodium nitrite from oral maternal exposures ranging from 20 to 243 mg/kg bw per day (equivalent to nitrite doses of 13.3–162 mg/kg bw per day) did not provide clear or consistent evidence of adverse effects on measures of fetal viability, weight, sex ratio or frequency of external or internal malformations (Globus & Samuel, 1978; Shimada, 1989). Similarly, no adverse effects on pup weight, increase in morphological malformations or increased mortality was observed in rats fed 0, 6, 47 or 580 mg sodium nitrite (equivalent to 0, 4, 31 and 387 mg nitrite) per kilogram of meat in diet (Olsen et al., 1984). In addition, no external malformations or post-weaning pup mortality was observed after rats were fed sodium nitrite (0%, 0.0125%, 0.025% or 0.05% by weight) 14 days prior to mating through to lactation; however, treatment was associated with increases in pre-weaning pup mortality and decreases in open-field locomotor activity at the highest dose (Vorhees et al., 1984). Conversely, in utero exposure of guinea pigs to sodium nitrite at 45, 50, 60 or 70 mg/kg bw (equivalent to nitrite doses of 30, 33.3, 40 and 46.7 mg/kg bw) by subcutaneous injection resulted in spontaneous abortion of litters; co-administration of methylene blue, a methaemoglobin antagonist, had a protective effect on fetuses. No gross abnormalities were noted in any living or aborted fetuses (Kociba & Sleight, 1970; Sinha & Sleight, 1971). Prenatal exposure to 300–10 000 mg potassium nitrite per litre (equivalent to nitrite concentrations of 162–5410 mg/L) in maternal drinking-water resulted in 3–100% fetal loss at all doses; fetal loss increased with increasing dose, compared with controls (Sleight & Atallah, 1968). High prenatal sodium nitrite exposure through maternal drinking-water led to reports of adverse effects on postnatal pup growth, increased mortality, decreases in haematological parameters (500–3000 mg sodium nitrite per litre; Roth et al., 1987; Roth & Smith, 1988) and effects on neurobehavioural parameters (2000 mg/L; Nyakas et al., 1990, 1994a,b).

4.4 Genotoxicity and related end-points

The mutagenicity and genotoxicity of nitrate and nitrite have been extensively reviewed by IARC (2010). In general, results are mixed for nitrate, but there is evidence for the genotoxicity of nitrite and nitrite concurrently administered with nitrosatable compounds.

Nitrate was not genotoxic in bacterial or mammalian cells in vitro (Ishidate et al., 1984; Görsdorf et al., 1990). Examination of embryonic cells from Syrian Golden hamsters for micronucleus formation, chromosomal aberrations, morphological/malignant cell transformation and drug-resistant mutation did not reveal any abnormalities (Tsuda, Inui & Takayama, 1976). Sodium nitrate given to mice by gastric intubation yielded negative results for unscheduled deoxyribonucleic acid (DNA) synthesis in early to mid spermatids and did not appear to produce any sperm abnormalities (Alavantić et al., 1988). When rats were treated intragastrically with sodium nitrate, increases in the frequency of chromosomal aberrations in bone marrow were reported (Luca et al., 1985).

Nitrite is genotoxic in numerous bacterial and mammalian cells (Kodama, Umeda & Tsutsui, 1976; Tsuda, Inui & Takayama, 1976; Tsuda & Kato, 1977; Ishidate et al., 1984; Budayová, 1985; Brams et al., 1987; Luca et al., 1987; Nakamura et al., 1987; Tsuda & Hasegawa, 1990; Prival, Simmon & Mortelmans, 1991; Zeiger et al., 1992; Balimandawa, de Meester &

Léonard, 1994). Morphological transformation of hamster embryonic cells in utero was reported following exposure to nitrite (Inui et al., 1979). Negative results were obtained for rats orally exposed to sodium nitrite when the pyloric mucosa was examined for single-strand breaks or unscheduled DNA synthesis. However, it should be noted that sperm head abnormality was detected after treatment (Alavantić et al., 1988; Ohshima et al., 1989). Positive results were reported for chromosomal aberrations in mouse, rat and chinchilla bone marrow cells following nitrite exposure (El Nahas, Globus & Vethamany-Globus, 1984; Luca et al., 1987; Alavantić et al., 1988; Ohshima et al., 1989) and in liver cells from embryos after exposure of pregnant rats (El Nahas, Globus & Vethamany-Globus, 1984). In contrast, negative results were reported for chromosomal aberrations following in utero exposure in the hamster (Inui et al., 1979).

Concurrently administered nitrite and nitrosatable compounds were shown to be genotoxic, giving positive results in in vitro genotoxicity assays and inducing genetic modifications either by reduction of DNA synthesis or by methylation of nucleic acids in vivo (numerous studies reported in Health Canada, 2013).

4.5 Carcinogenicity

There is inadequate evidence in experimental animals for the carcinogenicity of nitrate (IARC, 2010; Health Canada, 2013). No increase in tumour incidence was observed in mice ingesting a nitrate dose of approximately 18 or 182 mg/kg bw per day in drinking-water for 18 months (Mascher & Marth, 1993) or in rats ingesting a nitrate dose of 910 or 1820 mg/kg bw per day in feed for 2 years (Maekawa et al., 1982). Other studies conducted in mice (Greenblatt & Mirvish, 1973; Sugiyama, Tanaka & Mori, 1979) and rats (Lijinsky, Greenblatt & Kommineni, 1973) demonstrated that nitrate has no carcinogenic activity.

There is no definitive evidence of the direct carcinogenicity of nitrite per se in experimental animals by different routes of exposure (IARC, 2010; Health Canada, 2013). In most of the studies in which mice or rats were exposed to sodium nitrite by gavage, in diet (250–5000 mg/kg diet for 1–2 years) or in drinking-water (750–5000 mg/L for 2 years), the incidences of tumours were not significantly higher than those in controls. The few studies that reported an increase in liver neoplasms or forestomach squamous papillomas did so at high sodium nitrite concentrations (>2 g/L or 2 g/kg) or could not exclude the possibility of exogenous *N*-nitroso compound formation (Health Canada, 2013). High sodium nitrite concentrations have also been reported to promote forestomach carcinogenesis in rats initiated with various carcinogens (Hirose et al., 1993; Yoshida et al., 1994).

There is sufficient evidence in experimental animals for the carcinogenicity of nitrite in combination with amines or amides (IARC, 2010). Current science suggests an association between cancer (numerous target organs) and exposure to high concentrations of nitrite in drinking-water (>1500 mg/L) co-administered with nitrosatable compounds when conditions result in endogenous nitrosation (Health Canada, 2013). This was not the case for nitrite or nitrosatable compounds alone or for the co-administration of nitrate and nitrosatable compounds to rodents (Health Canada, 2013). Thus, current evidence indicates that nitrite alone may not act directly as a carcinogen in animals.

5. EFFECTS ON HUMANS

The oral lethal doses for humans range from 67 to 833 mg/kg bw for nitrate and from 33 to 250 mg/kg bw for nitrite (Boink et al., 1999), the lower doses applying to children, the elderly and people with a deficiency in nicotinamide adenine dinucleotide (NADH)–dependent methaemoglobin reductase, also known as cytochrome b_5 reductase, which

converts methaemoglobin to haemoglobin. Methaemoglobinaemia is the main acute effect, whereas effects on the thyroid have been reported after longer-term exposure.

5.1 Methaemoglobinaemia

Scientific studies published since the 1950s consistently show methaemoglobinaemia in infants as the end-point of concern for nitrate or nitrite exposure in humans. Based on its mode of action, nitrite is the toxic moiety of concern. Nitrite, either directly ingested from drinking-water or formed endogenously from nitrate, binds haemoglobin and disrupts its transport of oxygen to the tissues, causing methaemoglobinaemia (>2% methaemoglobin in blood). Hence, studies of nitrate exposure are important for assessing nitrite-induced methaemoglobinaemia. The original study by Walton (1951) found acute cases of clinical infantile methaemoglobinaemia associated with the ingestion of nitrate in drinking-water at nitrate concentrations exceeding 45 mg/L. A review of the literature found no incidences of methaemoglobinaemia at nitrate concentrations below 45 mg/L in drinking-water for bottle-fed infants aged under 6 months (Fan & Steinberg, 1996). The majority of studies that were published since Walton (1951) looking at associations between infantile methaemoglobinaemia and the ingestion of nitrate in drinking-water report associations at concentrations of nitrate exceeding 100 mg/L (Shuval & Gruener, 1972; Fan & Steinberg, 1996; Zeman, Kross & Vlad, 2002).

However, most studies of methaemoglobinaemia failed to account for bacterial contamination of the drinking-water, which may cause intestinal inflammation in infants and increase the endogenous conversion of nitrate to nitrite and subsequently methaemoglobinaemia (Avery, 1999). Based on the above human data and on the mode of action for nitrite's toxicity (see Section 6), infants aged under 6 months are the most sensitive subpopulation. Infants are more susceptible to methaemoglobinaemia because 1) their stomach pH is less acidic, promoting the growth of bacteria that convert nitrate to nitrite, which binds to haemoglobin to cause methaemoglobinaemia, and 2) the amount and activity of the enzyme NADH-dependent methaemoglobin reductase, which reduces methaemoglobin, are deficient in infants until approximately 6 months of age (Avery, 1999; Gupta et al., 1999; Knobeloch et al., 2000; Sanchez-Echaniz, Benito-Fernandez & Mintegui-Raso, 2001). Other groups especially susceptible to methaemoglobinaemia include the fetus and individuals genetically deficient in NADH-dependent methaemoglobin reductase.

Young children do not appear to be as sensitive as infants. In the USA, methaemoglobin levels were not significantly different in 64 children aged 1–8 years who consumed well water containing nitrate at concentrations of 22–111 mg/L as nitrate-nitrogen (97–491 mg/L as nitrate), when compared with 38 children consuming well water containing less than 10 mg/L as nitrate-nitrogen (44.3 mg/L as nitrate ion) (Craun, Greathouse & Gunderson, 1981). Methaemoglobinaemia has also been reported in a home dialysis patient (with anaemia) using private well water in the dialysate with a nitrate-nitrogen concentration of 94 mg/L (415 mg/L as nitrate ion) (Carlson & Shapiro, 1970).[1]

5.2 Thyroid effects

Current evidence 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

[1] The WHO *Guidelines for Drinking-water Quality* (WHO, 2017) note that water of higher quality than drinking-water is required for dialysis.

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 (Van Maanen et al., 1994; Gatseva & Dimitrov, 1997; Gatseva, Vladeva & Pavlov, 1998; Tajtáková et al., 2006; Hunault et al., 2007; Below et al., 2008; Gatseva & Argirova, 2008a,b; Rádiková et al., 2008).

Other factors may confound thyroid hormone function, including iodine insufficiency, age and pregnancy. Iodine insufficiency in the population due to lack of iodine in the diet or from other dietary exposures (e.g. thiocyanates in tobacco or brassica vegetables) (Vanderpas, 2006) or pollutant goitrogens (e.g. perchlorate) (Blount et al., 2006) may increase susceptibility to the effects of increased nitrate exposure. In addition, the effects on thyroid hormone synthesis can be more profound during pregnancy and for newborns.

Mode of action data (see Section 6) 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 due to increased thyroid hormone turnover and low intrathyroidal stores in fetal and early life. However, the findings from the only study that examined the effects of drinking-water nitrate on thyroid function in pregnant women were inconclusive (Gatseva & Argirova, 2008b). Decreased thyroid function has been observed only in school-aged children exposed to nitrate in drinking-water at concentrations of 50–264 mg/L in studies conducted in Bulgaria and Slovakia (Gatseva & Dimitrov, 1997; Gatseva, Vladeva & Pavlov, 1998; Tajtáková et al., 2006; Gatseva & Argirova, 2008a; Rádiková et al., 2008). No study has examined nitrate's effect on infant thyroid function. Although infants' thyroidal iodine turnover is lower than that of school-aged children, their average drinking-water consumption is lower. The lack of appropriate scientific data does not allow for the calculation of a conversion factor from school-aged children to infants. However, levels protective for school-aged children are expected to be similarly protective for infants.

5.3 Reproductive and developmental toxicity

Evidence of an association between nitrate and fetal mortality, growth restriction or birth defects is weak. However, there are critical data gaps in individual exposure assessment, co-exposure to other contaminants and exposure to nitrate from food sources, which is likely more relevant than exposure from drinking-water.

Reviews of the reproductive and developmental effects of exposure to nitrate or nitrite in drinking-water are provided by Manassaram, Backer & Moll (2006) and a publication from a symposium sponsored by the International Society for Environmental Epidemiology (Ward et al., 2005). Manassaram, Backer & Moll (2006) concluded that the current literature does not provide sufficient evidence of a causal relationship between exposure to nitrate in drinking-water and adverse reproductive and developmental effects; epidemiological evidence is sparse and suggestive at best. However, findings of excess birth defects in some of the studies reviewed suggest the need for further studies. Ward et al. (2005) concluded that the results of a few published studies regarding water nitrate and reproductive outcomes have been inconsistent, but elevated risks for neural tube defects have been observed after intake of nitrate. The Manassaram, Backer & Moll (2006) and Ward et al. (2005) conclusions were based on reviews of studies on fetal mortality, growth restriction and birth defects. From these reviews, no significant increased risk of fetal mortality (spontaneous abortions and stillbirths) was associated with drinking-water nitrate concentrations of ≤55 and 43–123 mg/L (Gelperin, Moses & Bridger, 1975; Super et al., 1981; Aschengrau, Zierler & Cohen, 1989, 1993). Growth restriction (prematurity, intrauterine growth restriction and

decreased birth weight) was associated with nitrate concentrations of ≥3.1 and 8–54 mg/L (Tabacova, Balabaeva & Little, 1997; Tabacova, Baird & Balabaeva, 1998; Bukowski, Somers & Bryanton, 2001), but not with concentrations above 20 mg/L (Super et al., 1981). Birth defects (central nervous system and cardiac) were not significantly associated with drinking-water nitrate concentrations of 0.2–4.5, >2, >3.5, 5, 26 and >45 mg/L (Arbuckle et al., 1988; Ericson, Kallen & Lofkvist, 1988; Aschengrau, Zierler & Cohen, 1993; Croen, Todoroff & Shaw, 2001; Cedergren et al., 2002; Brender et al., 2004). However, an increased risk of anencephaly was associated with nitrate concentrations above 45 mg/L (Croen, Todoroff & Shaw, 2001).

Recent studies do not provide strong evidence for a causal association between nitrate in drinking-water and birth defects or growth restriction (Brender et al., 2013; Huber et al., 2013). However, more research is needed, especially examining mixtures of contaminants in drinking-water, to determine the role of nitrate in the formation of birth defects.

5.4 Carcinogenicity

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 clearly support an association between cancer and exposure to nitrate or nitrite per se. Overall, these studies found no clear association between nitrate or nitrite in drinking-water and risk of cancer of the gastrointestinal tract, non-Hodgkin lymphoma, tumours of the central nervous system, urinary tract tumours, thyroid cancer, breast cancer or pancreatic cancer (Aschebrook-Kilfoy et al., 2012; Wu et al., 2013; see also numerous references cited in Health Canada, 2013). Many of these studies lacked individual exposure data, information on cancer risk factors and information on nitrosation inhibitors and precursors. This conclusion is consistent with the conclusions by IARC (2010) that 1) there is inadequate evidence in humans for the carcinogenicity of nitrate per se from exposure in food or in drinking-water, 2) there is limited evidence in humans for the carcinogenicity of nitrite in food and 3) nitrite in food is associated with an increased incidence of stomach cancer.

A link between cancer risk and endogenous nitrosation as a result of high intake of nitrate and/or nitrite and nitrosatable compounds is possible (Speijers et al., 1989; WHO, 1996, 2003a,b). This is consistent with the conclusion by IARC (2010) that ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A).

5.5 Other effects

Together, the data suggest some association between intake of nitrogen-containing compounds and risk of insulin-dependent type 1 diabetes mellitus. However, the data are limited and inconsistent; more accurate estimation of the total intake of nitrate, nitrite or nitrosamines at an individual level may be necessary for a conclusive assessment of their relationship with insulin-dependent type 1 diabetes mellitus (Health Canada, 2013).

In addition to the endogenous production of nitrate and its role in the nitric oxide pathway, there is increasing evidence for the beneficial role of this pathway in human health. There is evidence for its importance in protecting against oral and gastrointestinal diseases (Duncan et al., 1997) and also for its role in vascular fitness and exerting antihypertensive effects (Bryan & Loscalzo, 2011; Carlström et al., 2011; Lansley et al., 2011; Montenegro et al., 2011; Tang, Jiang & Bryan, 2011; Zhu et al., 2011).

6. MODE OF ACTION FOR CRITICAL EFFECTS

6.1 Methaemoglobinaemia

The key events in the mode of action by which nitrate and nitrite are reported to cause methaemoglobinaemia in humans and experimental animals are summarized from Health Canada (2013) as follows:

1. *Reduction of nitrate to nitrite*: As described in Section 3, microorganisms in saliva and the gastrointestinal tract reduce exogenous nitrate to nitrite in humans and in most laboratory animals, except in rats, where this process is deficient. In addition, changes to a more neutral intestinal pH promote the growth of microorganisms and hence the reduction of nitrate to nitrite. In infants, the variable stomach pH (2–5) may permit the growth of nitrate-reducing bacteria (Zeman, Kross & Vlad, 2002) and therefore increase the infants' risk of forming methaemoglobin.
2. *Oxidation of haemoglobin to methaemoglobin*: In the presence of nitrite, the ferrous ion (Fe^{2+}) of haemoglobin is oxidized to the ferric ion (Fe^{3+}) to form methaemoglobin (Gupta et al., 1999). Methaemoglobin formation was evident in both humans (>100 mg/L as nitrate ion; see Section 5.1) and experimental animals (250 mg/L as nitrite ion; see Section 4.2). Fetal haemoglobin has the same oxidation/reduction potential and auto-oxidation rate as adult haemoglobin, and thus differences in oxidation/reduction potential and auto-oxidation rate are not likely to contribute to increased infant susceptibility (Avery, 1999).
3. *Deficient methaemoglobin reduction*: Methaemoglobin can be reduced to haemoglobin by NADH-dependent methaemoglobin reductase (Gupta et al., 1999). NADH-dependent methaemoglobin reductase activity is 10 times higher in blood from rat fetuses than in pregnant rat or human cord blood; the activity was 1.5 times higher in blood from pregnant women than in human cord blood (NAS, 1981). Importantly, the development of the infant NADH-dependent methaemoglobin reductase system is incomplete; infants begin making adult levels of this enzyme at about 6 months of age (Avery, 1999; Gupta et al., 1999; Knobeloch et al., 2000; Sanchez-Echaniz, Benito-Fernandez & Mintegui-Raso, 2001). Thus, relatively lower amounts and activities of NADH-dependent methaemoglobin reductase in human neonates likely contribute to their susceptibility to methaemoglobinaemia.
4. *Increased percentage of haemoglobin as methaemoglobin in blood*: Under normal conditions, less than 2% of haemoglobin circulates in the blood as methaemoglobin (Fan, Willhite & Book, 1987). As methaemoglobin cannot bind oxygen, symptoms of methaemoglobinaemia appear in both humans and experimental animals as the percentage of methaemoglobin in the blood increases. Clinical methaemoglobinaemia is defined as greater than 2% methaemoglobin in blood. However, clinical symptoms are generally not present until the methaemoglobin level in blood reaches 3–15% of total haemoglobin (Avery, 1999; Zeman, Kross & Vlad, 2002).

As an alternative mode of action, some studies report that endogenous formation of nitrite resulting from overproduction of nitric oxide by tissues inflamed as a result of bacterial infection may be a significant cause of infant methaemoglobinaemia, greater than or instead of that caused by ingested nitrate (Hegesh & Shiloah, 1982; Avery, 1999). Infants suffering from diarrhoea and methaemoglobinaemia (without exposure to nitrate-contaminated water) excrete up to 10 times more nitrate daily than they ingest through food and water (Hegesh & Shiloah, 1982; Avery, 1999; Health Canada, 2013).

6.2 Thyroid effects

Disruption of thyroid hormones can lead to numerous adverse outcomes, including thyroid tumours and birth defects. However, humans do not get thyroid carcinomas as a result of decreased T_3 and T_4 levels, because they are less susceptible than rodents to the effects of thyroid stimulating hormone (TSH) on thyroid cell proliferation (Crofton, 2008). Thus, the mode of action by which nitrate and nitrite are reported to cause thyroid effects and subsequently birth defects and not tumours in humans and experimental animals as summarized from Health Canada (2013) is as follows:

1. *Inhibition of iodide uptake to thyroid*: Ingested nitrate inhibits thyroid uptake of iodide circulating in the blood by competitively binding to the sodium–iodide symporter (NIS) on the surface of thyroid follicular cells (Greer et al., 2002; Tonacchera et al., 2004). If sufficient inhibition of iodide uptake occurs, formation of thyroid hormones is depressed. The kinetics for the inhibition of iodide uptake by nitrate in humans and experimental animals has not been reported. Hunault et al. (2007) reported no significant effects on thyroidal iodide uptake in 10 human volunteers receiving sodium nitrate at a dose of 15 mg/kg bw (equivalent to a nitrate dose of 10.9 mg/kg bw) in 200 mL of drinking-water for 28 days. Other drinking-water contaminants are also iodide uptake inhibitors. The relative potency of perchlorate to inhibit radioactive iodide uptake at the NIS in humans was found to be 15, 30 and 240 times that of thiocyanate, iodide and nitrate, respectively, on a molar concentration basis (Tonacchera et al., 2004). Nitrite is not transported by the NIS (Eskandari et al., 1997) and is therefore not relevant to the mode of action for thyroid toxicity.
2. *Serum T_3 and T_4 changes*: Depression of thyroid hormone formation, secondary to the inhibition of thyroidal iodide uptake, results in decreased thyroid hormone secretion into the circulation. Lower concentrations of thyroid hormones in the serum can activate the feedback mechanism to the hypothalamic–pituitary–thyroid (HPT) axis, resulting in increased TSH secretion, which in turn leads to signalling the thyroid to produce more thyroid hormones. However, with inhibition of iodide uptake, the production of thyroid hormones may be insufficient. It is not known to what levels thyroid hormone synthesis must be reduced before serum thyroid hormone levels are affected to the extent that adverse effects occur in either humans or experimental animals. What is known is that, given the same dose of the antithyroid compound propylthiouracil, rats exhibit a significant reduction in circulating thyroid hormone levels sooner than humans; the serum half-life of T_4 is 7–10 days in humans (Vulsma, Gons & de Vijlder, 1989; Greer et al., 2002), but only 1 day in rats (Zoeller & Crofton, 2005). In addition, the adult human thyroid stores a large supply – maybe several months' worth – of thyroid hormones (Greer et al., 2002). The human neonate has a serum half-life of T_4 of approximately 3 days (Vulsma, Gons & de Vijlder, 1989), and intrathyroidal stores of T_4 are estimated to be less than 1 day's worth (reported in Zoeller & Crofton, 2005). The shorter T_4 half-life in neonates and rats implies that they must produce much more T_4, thus requiring more iodide uptake. Therefore, neonates and rats are more sensitive than adult humans to uptake inhibitors. The most commonly used biomarker of effect for exposure to thyroid-disrupting chemicals is serum total T_4 concentration (DeVito et al., 1999; Zoeller, Tyl & Tan, 2007). Thyroid hormones are evolutionarily conserved molecules present in all vertebrates (Heyland & Moroz, 2005). However, species differences in serum total T_4 levels and consequent adverse effects have not been reported.

3. *Tissue T_3 changes*: Peripheral tissues contain deiodinases, which convert T_4 to T_3. The biological actions of thyroid hormones are driven by T_3 binding to nuclear thyroid receptors, which then act as signal transducers and transcription factors to exert their diverse biological effects. Thyroid hormones regulate the transcription of many proteins and control neuronal migration, differentiation and apoptotic modelling (Kirk, 2006). The mechanisms by which thyroid hormones function through nuclear receptors to alter gene expression are highly conserved across species (studies reported in Miller et al., 2009). Chronic stimulation of the thyroid gland by TSH can lead to proliferative changes in follicular cells, ultimately leading to hypertrophy, hyperplasia and hypothyroidism (Capen, 1997; Tonacchera et al., 2004; De Groef et al., 2006; Vanderpas, 2006). Adult experimental animals and humans are relatively resistant to adverse outcomes of impaired thyroid hormone production, as the HPT axis can compensate to a considerable extent for reduced thyroid hormone production. If nitrate exposure is sufficiently high to overcome this compensation, persists for long enough to exhaust thyroid gland stores of thyroid hormones or is combined with exposure to other thyroid-disrupting chemicals or with dietary iodide deficiencies, hypothyroidism or enlargement of the thyroid will likely occur. In addition, pregnancy causes increased demand on the thyroid gland, and hypothyroidism is twice as common during pregnancy (Aoki et al., 2007). In humans, exposure to nitrate in drinking-water at concentrations at and above 50 mg/L resulted in increased thyroid volume and thyroperoxidase levels as well as increased incidence of goitre (see Section 5.2). In rats, exposure to sodium nitrate concentrations of 50 mg/L (equivalent to 36.45 mg/L as nitrate ion) and above for 30 weeks increased the weight of the thyroid (see Section 4.2).
4. *Altered development and birth defects*: Moderate or even transient thyroid hormone insufficiency can cause specific developmental defects in rodents and humans. For example, small differences in point estimates of maternal T_4 levels during the early fetal period are associated with adverse outcomes (e.g. reduced intelligence quotient scores), even though these deficits do not constitute clinical hypothyroidism (see references cited in Miller et al., 2009). Thyroid hormones are essential to neurological development, skeletal growth, regulation of metabolism and serum lipids, and the normal function of the pulmonary, cardiovascular and renal systems (Kirk, 2006; De Escobar et al., 2008; Woodruff et al., 2008; Miller et al., 2009). In addition to the degree of thyroid hormone insufficiency, the developmental timing of thyroid hormone insufficiency and the duration of the perturbation are important (Kirk, 2006; Miller et al., 2009). Formal dose–response studies have not been conducted to determine to what extent plasma thyroid hormone levels must decrease before altered development and birth defects occur in either experimental animals or humans. Evidence of developmental and birth defects has been reported in humans for nitrate concentrations above 45 mg/L in drinking-water (see Section 5.3); however, further studies of the role of thyroid hormones and the validity of these end-points are needed before strong conclusions can be made.

7. PRACTICAL CONSIDERATIONS

7.1 Analytical methods and achievability

The United States Environmental Protection Agency (USEPA) currently has three approved methods for the analysis of nitrate and nitrite in drinking-water. Method 300.0 revision 2.1 and Method 300.1 revision 1.0 are based on ion chromatography and have method detection

limits (MDLs) of 0.002 mg/L as nitrate-nitrogen (equivalent to 0.009 mg/L as nitrate ion) and 0.004 mg/L as nitrite-nitrogen (equivalent to 0.013 mg/L as nitrite ion). Method 353.2 revision 2.1 uses an automated cadmium reduction with colorimetry for the analysis of nitrite singly or nitrate and nitrite combined in drinking-water. No MDLs are reported for this method (USEPA, 1993). The range reported for an equivalent method (SM 4500-NO_3-F) is 0.01–1.0 mg/L as nitrate-nitrogen (equivalent to 0.04–4.4 mg/L as nitrate ion). This method is recommended particularly for concentrations of nitrate below 0.1 mg/L as nitrate-nitrogen (equivalent to 0.4 mg/L as nitrate ion), where other methods might lack adequate sensitivity (APHA et al., 2005).

Two manual cadmium reduction methods are also available for nitrate and nitrite analysis: SM 4500-NO3-E (APHA et al., 2005) and American Society for Testing and Materials method D3867-99B (ASTM, 1999). In these methods, nitrate is reduced to nitrite in the presence of cadmium by manually adding a sample to a reduction column and measured using colorimetry after addition of a colour reagent. No detection limits are reported for these methods.

Additional methods have been developed for the analysis of either nitrate or nitrite, including ion selective electrode methods for the analysis of nitrate and spectrophotometric methods for the analysis of nitrite (APHA et al., 2005).

Low-cost test kits that use colorimetry are also available from several companies. They can be found online by searching "nitrate test kits". The performance ranges are typically from 1 to 50 mg/L as nitrate ion.

7.2 Treatment methods and performance

A key requirement for treating drinking-water to reduce the risk of methaemoglobinaemia is disinfection to eliminate pathogens and to convert nitrite to nitrate. Therefore, drinking-water treatment methods focus on the treatment of nitrate, because nitrite is readily converted to nitrate by many disinfectants. However, nitrite can also be formed during chloramination in some water systems with a long retention time if nitrification occurs.

Conventional municipal water treatment processes (coagulation, sedimentation, filtration and chlorination) are not effective for nitrate removal, because nitrate is a stable and highly soluble ion with low potential for co-precipitation and adsorption. Effective central treatment technologies involve the physical/chemical and biological removal of nitrate and include ion exchange, reverse osmosis, biological denitrification and electrodialysis; these are capable of removing over 80% of nitrate from water (Beszedits & Walker, 1998) to achieve effluent concentrations as low as 3 mg/L as nitrate-nitrogen (equivalent to 13 mg/L as nitrate ion). Treatment processes capable of nitrate removal at the household level include reverse osmosis, distillation and ion exchange. All of these technologies are, however, more costly than conventional water treatment technologies and not easily applied in many locations.

7.2.1 Central treatment

Detailed information on the effectiveness and operational considerations of the various treatment technologies for nitrate removal are available (Dahab, 1991; Clifford & Liu, 1995; Kapoor & Viraraghavan, 1997; Meyer et al., 2010; Seidel et al., 2011). Selection of an appropriate treatment process will depend on many factors, including the characteristics of the raw water supply, the source and concentration of nitrate and nitrite, the operational conditions of the specific treatment method and the utility's treatment goals.

Anion exchange is the most common nitrate removal process for municipal-scale treatment (Wachinski, 2006; Ruppenthal, 2007; Wang et al., 2007). Strong base anion exchange resins and nitrate-selective resins are typically used. However, as strong base anion exchange resins have a greater preference for sulfate ions than for nitrate ions, the effectiveness and capacity of this type of resin will be limited when the sulfate concentration in the source water is high. Disposal of the resin regenerant is a major consideration for ion exchange treatment plants; disposal options include discharge to wastewater systems, volume reduction using drying beds, off-site approved land disposal and deep well injection (Seidel et al., 2011). Additional considerations include mineral imbalances in the water caused by replacement of nitrate and other anions with chloride ions during anion exchange, which could increase the corrosive nature of the treated water and require post-treatment corrosion control measures (Schock & Lytle, 2011), and the potential for the release of nitrosamines from strong base anion exchange resins as a result of the shedding of manufacturing impurities (Kemper et al., 2009).

Reverse osmosis is an effective technology for producing water with low nitrate concentrations, but it is costly. It is typically used for nitrate removal when high concentrations of other dissolved solids need to be removed. Systems must demonstrate high nitrate rejection, high water flux and a high recovery rate for reverse osmosis to be economically viable (Dahab, 1991; Duranceau, 2001; MWH, 2005). Reverse osmosis treatment systems typically require prefiltration for particle removal and often include other pretreatment steps, such as the addition of anti-scaling agents, prechlorination/dechlorination and softening. As with other treatment technologies, post-treatment typically includes pH adjustment, addition of corrosion inhibitors and disinfection (Cevaal, Suratt & Burke, 1995; Schoeman & Steyn, 2003). Well over 90% nitrate reduction is achievable. As with anion exchange, reverse osmosis treatment produces a brine that must be properly disposed of, as well as corrosive product water (Taylor & Wiesner, 1999; Schock & Lytle, 2011).

Biological denitrification treatment processes are based on the removal of nitrate in source water through its biological reduction to nitrogen gas (denitrification) in an anoxic environment. Biological denitrification is more frequently applied as post-secondary wastewater treatment, but there are applications in drinking-water treatment. These complex systems, which involve adding carbon sources such as acetic acid or ethanol to facilitate microbial activity to convert nitrate to nitrogen, are difficult to manage and costly. Biological denitrification systems can be designed as fixed bed reactors, fluidized bed reactors, membrane bioreactors and membrane biofilm reactors. Design and operational considerations for biological denitrification plants include electron donor and nutrient dosing, dissolved oxygen, pH and temperature control as well as biofilm management (Meyer et al., 2010). In general, biological denitrification treatment systems require post-treatment (e.g. aeration, filtration, activated carbon and disinfection) to remove biomass and biodegradable organic materials that are present in the reactor effluent (MWH, 2005; Meyer et al., 2010). A full-scale denitrification plant has been reported to achieve greater than 90% removal of nitrate with raw water containing 16–18 mg/L as nitrate-nitrogen (equivalent to 71–80 mg/L as nitrate ion) (Mateju et al., 1992).

Electrodialysis, which is a membrane process that uses an electric potential for removing charged species from water by forcing them through cation or anion exchange membranes, is less commonly reported as being used in public water treatment. Although electrodialysis treatment systems produce less reject water and have lower power consumption than other membrane processes, there are a number of considerations for systems using this technology for nitrate removal, including the operational complexity of the system, disposal of the reject water and the need for pH adjustment of the treated water (Kapoor & Viraraghaven, 1997).

7.2.2 Household treatment

Point-of-use reverse osmosis and point-of-entry anion exchange technologies are available for home or small-volume applications (McGowan & Harrison, 2000). Certified units are available, but they are costly, require good maintenance and are not readily available in many locations. Point-of-entry anion exchange utilizes strong base anion exchange resins that are regenerated with sodium chloride. Concurrent sulfate presence will significantly affect nitrate removal performance, as sulfate is preferentially retained and will displace nitrate back into the water if the system is operated beyond its nitrate capacity. Point-of-use reverse osmosis systems are potentially useful, but they operate at low line pressures, so they are much less efficient than high-pressure central desalination systems. Water reject levels can be of the order of up to 80%. Point-of-entry reverse osmosis is not used because of the corrosivity of the treated water to pipe and plumbing components (for more information, refer to the WHO website on household water treatment and safe storage at http://www.who.int/water_sanitation_health/water-quality/household/en/).

For individual households that obtain their drinking-water from private wells, residential drinking-water treatment devices are an option for reducing nitrate and nitrite concentrations in drinking-water. Before a treatment device is installed, the water should be tested to determine its general water chemistry and verify the presence and concentrations of nitrate and nitrite in the source water. As bacterial contamination of a well water supply frequently occurs in conjunction with nitrate contamination, the bacterial and chemical aspects of the water quality should be considered prior to selecting a water treatment device, and disinfection is essential.

7.3 Prevention and control

7.3.1 Prevention of nitrate contamination

The approach to reducing exposure to nitrate from drinking-water generally includes management of activities within the watershed/aquifer, treatment to decrease nitrate levels in the water supply and management of nitrification in the distribution system.

The most appropriate means of controlling nitrate concentrations, particularly in groundwater, is the prevention of source water contamination, which may take the form of appropriate management of agricultural activities (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; Schmoll et al., 2006).

Shallow wells that are located in agricultural areas are particularly susceptible to nitrate and nitrite contamination. For 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 also important that the household use of manures and fertilizers on small plots near wells is 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 indicates that there are sources of nitrate close by that could be causing contamination, steps should be taken to protect the well and ensure that sources of nitrate are removed from the vicinity of the well.

Management and control strategies for catchment areas are essential for smaller, urban areas where either large numbers of on-site sanitation facilities/leaking sewers or intensive agricultural activities can result in significant contamination of groundwater used as a source of drinking-water. More detailed information on managing nitrate/nitrite in groundwater

supplies can be found in Section IV of *Protecting Groundwater for Health – Managing the Quality of Drinking-water Sources* (Schmoll et al., 2006).

7.3.2 Reduction of methaemoglobinaemia risk

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 concentrations are particularly high, providing bottled water from safe sources or providing advice as to where such water can be obtained.

Nitrate-contaminated surface water or ground source waters should always be disinfected to eliminate pathogens and convert any nitrite to nitrate to reduce methaemoglobinaemia risks. Blending may be employed if an additional source of low-nitrate water is available, but this is unlikely. Alternatively, bottled water may need to be consumed if nitrate or nitrite concentrations are high and pregnant women or infants are exposed.

Where there are elevated concentrations of nitrate or where inspection of the well indicates 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. However, as excessive (i.e. continuous or extended) boiling of water to ensure microbiological safety can concentrate levels of nitrate in the water, care should be taken to ensure that the guidance or directions for boiling water (i.e. heat only until the water reaches a rolling boil, as articulated in the WHO *Guidelines for Drinking-water Quality* [GDWQ]) are followed. Where alternative supplies (e.g. bottled water) 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 microbiological contamination are removed from the vicinity of the well.

7.3.3 Prevention of nitrification in the distribution system

Nitrate and nitrite can be formed in the distribution system as a result of nitrification of chloramines or excess ammonia that occurs naturally in the source water and is not removed prior to disinfection or in systems that add ammonia as part of chloramination for secondary disinfection. Nitrification usually occurs in water systems with warmer water and with long retention times. Nitrification can have adverse impacts on water quality, including increasing nitrate and nitrite levels, increasing bacterial regrowth and lowering chloramine residuals, pH and dissolved oxygen (Kirmeyer et al., 1995, 2004; Odell et al., 1996; Wilczak et al., 1996; USEPA, 2002b; Zhang, Love & Edwards, 2009). Studies have also reported possible links between corrosion problems and nitrification (USEPA, 2002a; Edwards & Dudi, 2004; Zhang et al., 2009, 2010).

Many preventive and control measures can be taken to address nitrification (Kirmeyer et al., 1995; Skadsen & Cohen, 2006; Zhang, Love & Edwards, 2009). Preventive methods include control of water quality parameters (pH, free ammonia entering the distribution system, organic matter) and operating parameters (chlorine:ammonia-nitrogen weight ratio and residual chloramine), corrosion control programmes, distribution system pipe flushing, establishing booster chlorination or chloramination stations, temporary/seasonal free chlorination (breakpoint chlorination) and chlorite addition. Corrective methods are similar to the preventive methods and include distribution system pipe flushing, temporary/seasonal

free chlorination (breakpoint chlorination), reservoir cycling or cleaning and chlorite addition. However, the addition of chlorite is considered to be controversial, as its presence can lead to the formation of chlorate (Skadsen & Cohen, 2006).

The different measures used to control nitrification vary in their effectiveness and their ability to provide long-term improvements. For these reasons, comprehensive strategies aimed at the prevention of nitrification episodes are recommended over strategies aimed at controlling nitrification as it occurs.

8. GUIDELINE VALUES

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

8.1 Nitrate

The guideline value for nitrate in drinking-water is 50 mg/L as nitrate ion (equivalent to 11 mg/L as nitrate-nitrogen), to be protective of the health of the most sensitive subpopulation, bottle-fed infants. Therefore, it is protective of other population groups, such as older children and adults. This guideline value is based on the absence of adverse health effects (methaemoglobinaemia and thyroid effects) below 50 mg/L in drinking-water in epidemiological studies. 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 (i.e. continuous or extended) boiling of water to ensure microbiological safety can concentrate levels of nitrate in the water, care should be taken to ensure that the guidance or directions for boiling water (i.e. heat only until the water reaches a rolling boil, as articulated in the GDWQ) are followed. In extreme situations, alternative sources of water (e.g. bottled water) can be used.

There is no clear evidence of carcinogenicity from nitrate per se in humans. The human risk from cancer has been calculated based on the endogenous formation of a specific *N*-nitroso compound, *N*-nitrosodimethylamine (NDMA), using a number of worst-case assumptions. The estimated lifetime excess cancer risk from the endogenous formation of NDMA associated with the ingestion of drinking-water containing nitrate at the guideline value is less than two additional cases of cancer per 100 000 people drinking water containing the substance at the guideline value for 70 years. For more details on the cancer risk assessment, see Section 9.3 of the Health Canada (2013) document.

8.2 Nitrite

The guideline value for nitrite in drinking-water is 3 mg/L as nitrite ion (equivalent to 0.9 mg/L as nitrite-nitrogen). The guideline value is protective against methaemoglobinaemia induced by nitrite from both endogenous and exogenous sources in bottle-fed infants and the general population. The guideline value for nitrite-induced infantile methaemoglobinaemia is derived based on 1) no incidence of methaemoglobinaemia at nitrate concentrations below 50 mg/L in drinking-water for bottle-fed infants aged under 6 months, 2) converting 50 mg/L as nitrate (molecular weight 62 g/mol) to the corresponding molar concentration for nitrite (molecular weight 46 g/mol), 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 the 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 aged under 6 months), application of an uncertainty factor is not deemed necessary.

8.3 Nitrate plus nitrite

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. In other words,

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

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 or nitrite is present at concentrations near or above its guideline value. It is particularly important to ensure that these infants are not currently exhibiting symptoms of gastrointestinal infection (diarrhoea). Also, as excessive (i.e. continuous or extended) boiling of water to ensure microbiological safety can concentrate levels of nitrate in the water, care should be taken to ensure that the guidance for boiling water (i.e. heat only until the water reaches a rolling boil, as articulated in the GDWQ) is followed. In extreme situations, alternative sources of water (e.g. bottled water) can be used.

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