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Nitrate and nitrite in Drinking-water

Background document for development of
WHO *Guidelines for Drinking-water Qua**lity***

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Preface

One of the primary goals of WHO and its member states is that “all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water.” A major WHO function to achieve such goals is the responsibility “to propose regulations, and to make recommendations with respect to international health matters ....”

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 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 public drinking-water quality is accordingly prepared/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 examined in drinking-water.

For each chemical contaminant or substance considered, a lead institution prepared a health criteria document evaluating the risks for human health from exposure to the particular chemical in drinking-water. Institutions from Canada, Denmark, Finland, France, Germany, Italy, Japan, Netherlands, Norway, Poland, Sweden, United Kingdom and United States of America prepared the requested health criteria documents.

Under the responsibility of the coordinators for a group of chemicals considered in the guidelines, the draft health criteria documents were submitted to a number of scientific institutions and selected experts for peer review. Comments were taken into consideration by the coordinators and authors before the documents were submitted for final evaluation by the experts meetings. A “final task force” meeting reviewed the health risk assessments and public and peer review comments and, where appropriate, decided upon guideline values. During preparation of the third edition of the GDWQ, it was decided to include a public review via the world wide web in the process of development of the health criteria documents.

During the preparation of health criteria documents and at experts meetings, 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 FAO/WHO Meetings 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 internet site and in the current edition of the GDWQ.

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The work of the following coordinators was crucial in the development of this document and others in the Addendum:

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GENERAL DESCRIPTION

Identity

Nitrate and nitrite are naturally occurring ions that are part of the nitrogen cycle. The nitrate ion (NO_3^-) is the stable form of combined nitrogen for oxygenated systems. Although chemically unreactive, it can be reduced by microbial action. The nitrite ion (NO_2^-) contains nitrogen in a relatively unstable oxidation state. Chemical and biological processes can further reduce nitrite to various compounds or oxidize it to nitrate (ICAIR Life Systems, Inc., 1987).

Physicochemical properties *(ICAIR Life Systems, Inc., 1987)* [Conversion to nitrogen: 1 mg NO_3^-/litre = 0.226 mg NO_3^--N/litre; 1 mg NO_2^-/litre = 0.304 mg NO_2^--N/litre]

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

Major uses

Nitrate is used mainly in inorganic fertilizers. It is also used as an oxidizing agent and in the production of explosives, and purified potassium nitrate is used for glass making. Sodium nitrite is used as a food preservative, especially in cured meats. Nitrate is sometimes also added to food to serve as a reservoir for nitrite.

Environmental fate

In soil, fertilizers containing inorganic nitrogen and wastes containing organic nitrogen are first decomposed to give ammonia, which is then oxidized to nitrite and nitrate. 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 (US EPA, 1987; van Duijvenboden & Matthijsen, 1989).

Under aerobic conditions, nitrate percolates in large quantities into the aquifer because of the small extent to which degradation or denitrification occurs. 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 other physicochemical properties are also important in determining the fate of nitrate in soil (van Duijvenboden & Loch, 1983). In surface water, nitrification and denitrification may also occur, depending on the temperature and pH. The uptake of nitrate by plants, however, is responsible for most of the nitrate reduction in surface water.

Nitrogen compounds are formed in the air by lightning or discharged into it from industrial processes, motor vehicles, and intensive agriculture. Nitrate is present in air primarily as nitric acid and inorganic aerosols, as well as nitrate radicals and organic gases or aerosols. These are removed by wet and dry deposition.

ANALYTICAL METHODS

Spectrometric techniques are used for the determination of nitrate in water. Detection limits range from 0.01 to 1 mg/litre (ISO, 1986, 1988). A molecular absorption spectrometric method is available for the determination of nitrite in potable water, raw water, and wastewater. The limit of detection lies within the range of 0.005–0.01 mg/litre (ISO, 1984). A continuous-flow spectrometric method for the determination of nitrite, nitrate, or the sum of both in various types of water is suitable at concentrations ranging from 0.05 to 5 mg/litre for nitrite and from 1 to 100 mg/litre for nitrite/nitrate, both in the undiluted sample (ISO, 1996).

Nitrate and nitrite can also be determined in water by liquid chromatography, down to a level of 0.1 mg/litre for nitrate and 0.05 mg/litre for nitrite (ISO, 1992).

ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

Air

Atmospheric nitrate concentrations ranging from 0.1 to 0.4 $\mu g/m^3$ have been reported, the lowest concentrations being found in the South Pacific (Prospero & Savoie, 1989). Higher concentrations ranging from 1 to 40 $\mu g/m^3$ have also been reported, with annual means of 1–8 $\mu g/m^3$. Mean monthly nitrate concentrations in air in the Netherlands range from 1 to 14 $\mu g/m^3$ (Janssen et al., 1989). Indoor nitrate aerosol concentrations of 1.1–5.6 $\mu g/m^3$ were found to be related to outdoor concentrations (Yocom, 1982).

Water

Concentrations of nitrate in rainwater of up to 5 mg/litre have been observed in industrial areas (van Duijvenboden & Matthijsen, 1989). In rural areas, concentrations are somewhat lower.

The nitrate concentration in surface water is normally low (0–18 mg/litre) 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 have gradually increased in many European countries in the last few decades and have sometimes doubled over the past 20 years. In the United Kingdom, for example, an average annual increase of 0.7 mg/litre has been observed in some rivers (Young & Morgan-Jones, 1980).

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. In the USA, naturally occurring levels do not exceed 4–9 mg/litre for nitrate and 0.3 mg/litre for nitrite (US EPA, 1987). As a result of agricultural activities, the nitrate concentration can easily reach several hundred milligrams per litre (WHO, 1985b). For example, concentrations of up to 1500 mg/litre were found in groundwater in an agricultural area of India (Jacks & Sharma, 1983).

In the USA, nitrates are present in most surface water and groundwater supplies at levels below 4 mg/litre, with levels exceeding 20 mg/litre in about 3% of surface waters and 6% of groundwaters. In 1986, a nitrate concentration of 44 mg/litre (10 mg of nitrate-nitrogen per litre) was exceeded in 40 surface water and 568 groundwater supplies. Nitrite levels were not surveyed but are expected to be much lower than 3.3 mg/litre (US EPA, 1987).

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. In Denmark and the

Netherlands, for example, nitrate concentrations are increasing by 0.2–1.3 mg/litre per year in some areas (WHO, 1985b). 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 most countries, nitrate levels in drinking-water derived from surface water do not exceed 10 mg/litre. 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 levels in drinking-water above 50 mg/litre ranges from 0.5 to 10% (WHO, 1985b; ECETOC, 1988); this corresponds to nearly 10 million people. Individual wells in agricultural areas throughout the world especially contribute to nitrate-related toxicity problems, and nitrate levels in the well-water often exceed 50 mg/litre.

Nitrite levels in drinking-water in the Netherlands are usually below 0.1 mg/litre. In 1993, a maximum value of 0.21 mg/litre was detected (RIVM, 1993).

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 levels, usually by 0.2–1.5 mg of nitrite per litre, but potentially by more than 3 mg of nitrite per litre (AWWARF, 1995).

Food

Vegetables and cured meat are in general the main source of nitrate and nitrite in the diet, but small amounts may be present in fish and dairy products. Meat products may contain <2.7–945 mg of nitrate per kg and <0.2–6.4 mg of nitrite per kg; dairy products may contain <3–27 mg of nitrate per kg and <0.2–1.7 mg of nitrite per kg (ECETOC, 1988). Several vegetables and fruits contain 200–2500 mg of nitrate per 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 (day)light intensity (Gangolli et al., 1994; 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 levels 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 levels of up to 400 mg/kg have been found (WHO, 1995).

Estimated total exposure and relative contribution of drinking-water

Air pollution appears to be a minor source of nitrate exposure. In general, vegetables will be the main source of nitrate intake when nitrate levels in drinking-water are below 10 mg/litre (Chilvers et al., 1984; US EPA, 1987; ECETOC, 1988).

When nitrate levels in drinking-water exceed 50 mg/litre, drinking-water will be the major source of total nitrate intake, especially for bottle-fed infants. In the Netherlands, the average population exposure is approximately 140 mg of nitrate per day (including the nitrate in drinking-water). The contribution of drinking-water to nitrate intake is usually less than 14%. For bottle-fed infants, daily intake from formula made with water containing 50 mg of nitrate per litre would average about 8.3–8.5 mg of nitrate per kg of body weight per day.

The mean dietary intakes determined by the duplicate portion technique (WHO, 1985a) range from 43 to 131 mg of nitrate per day and from 1.2 to 3 mg of nitrite per day. 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 in the United Kingdom from 50 to 81 mg per person (Bonnell, 1995; Schuddeboom, 1995), in Denmark from 70 to 172 mg per person (Bonnell, 1995), and in Germany from 70 to 110 mg per person (Bonnell, 1995). According to the US EPA, the average nitrate intake from food is approximately 40–100 mg/day for males. The daily nitrite intake ranges 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 intake of such meat and the origin and type of cured meat consumed. Mean dietary nitrite intake from all food sources has been reported to range from <0.1 to 8.7 mg of nitrite per person per day for European diets (WHO, 1995).

KINETICS AND METABOLISMS IN LABORATORY ANIMALS AND HUMANS

Absorption, distribution, and elimination

Ingested nitrate is readily and completely absorbed from the upper small intestine. Nitrite may be absorbed directly from both the stomach and the upper small intestine. Part of the ingested nitrite reacts with gastric contents prior to absorption.

Nitrate is rapidly distributed throughout the tissues. Approximately 25% of ingested nitrate is actively secreted into saliva, where it is partly (20%) reduced to nitrite by the oral microflora; nitrate and nitrite are then swallowed and re-enter the stomach. 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, certain patients in whom hydrochloric acid secretion is slower than normal, or patients using antacids (Colbers et al., 1995). In rats, active secretion and reduction of nitrate in saliva are virtually absent (Walker, 1995). Total nitrate reduction in rats is probably less than in humans.

Absorbed nitrite is rapidly oxidized to nitrate in the blood. Nitrite in the bloodstream is involved in the oxidation of Hb to metHb: the Fe^{2+} present in the haem group is oxidized to its Fe^{3+} form, and the remaining nitrite binds firmly to this oxidized haem. The Fe^{3+} form does not allow oxygen transport, owing to the strong binding of oxygen (Jaffé, 1981; US National Research Council, 1995). Therefore, methaemoglobinaemia can lead to cyanosis.

Nitrite has been shown to cross the placenta and cause the formation of fetal methaemoglobinaemia in rats. It may react in the stomach with nitrosatable compounds (e.g. secondary and tertiary amines or amides in food) to form *N*-nitroso compounds. Such endogenous nitrosation has been shown to occur in human as well as animal gastric juice both *in vivo* and *in vitro*, mostly at higher pH values, when both nitrite and nitrosatable compounds were present simultaneously (Shephard, 1995; WHO, 1996).

The major part of the ingested nitrate is eventually excreted in urine as nitrate, ammonia, or urea, faecal excretion being negligible. Little nitrite is excreted (WHO, 1985b; ICAIR Life Systems, Inc., 1987; Speijers et al., 1989).

Endogenous synthesis of nitrate and nitrite

The excess nitrate excretion that has often been observed after low nitrate and nitrite intake originates from endogenous synthesis, which amounts, in normal healthy humans, to 1 mmol/day on average, corresponding to 62 mg of nitrate per day or 14 mg of nitrate-nitrogen per day. Gastrointestinal infections greatly increase nitrate excretion, as a result, at least in part, of increased endogenous (non-bacterial) nitrate synthesis, probably induced by

activation of the mammalian reticuloendothelial system (WHO, 1985b, 1996; Speijers et al., 1989; Wishnok et al., 1995). This endogenous synthesis of nitrate complicates the risk assessment of nitrate.

Increased endogenous synthesis of nitrate, as reported in animals with induced infections and inflammatory reactions, was also observed in humans. Infections and non-specific diarrhoea played a role in the increased endogenous synthesis of nitrate (Tannenbaum et al., 1978; Green et al., 1981; Hegesh & Shiloah, 1982; Bartholomew & Hill, 1984; Lee et al., 1986; Gangolli et al., 1994). These observations are all consistent with the induction of one or more nitric oxide synthases by inflammatory agents, analogous to the experiments described in animals and macrophages. This induction in humans has been difficult to demonstrate directly, but administration of [^{15}N]arginine to two volunteers resulted in the incorporation of ^{15}N into urinary nitrate in both individuals, confirming the arginine–nitric oxide pathway in humans (Leaf et al., 1989).

Nitrate excretion in excess of nitrate intake by humans was reported in 1916, but this result remained obscure until the end of the 1970s, when it was re-examined because of the potential involvement of nitrate in endogenous nitrosation. A relatively constant daily production of about 1 mmol of nitrate was confirmed. 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 oxidized to nitrate through reaction with Hb. In addition to macrophages, many cell types can form nitric oxide, generally from arginine. Under some conditions, bacteria can form nitric oxide by reduction of nitrite. These processes can lead to nitrosation of amines at neutral pH, presumably by reaction with nitrous anhydride. The question of whether the arginine–nitrate pathway can be associated with increased cancer risk via exposure to *N*-nitroso compounds remains open. Nitric oxide is mutagenic towards bacteria and human cells in culture; it causes DNA strand breaks, deamination (probably via nitrous anhydride), and oxidative damage; and it can activate cellular defence mechanisms. In virtually all of these cases, the biological response is paralleled by the final nitrate levels. Thus, while endogenously formed nitrate may itself be of relatively minor toxicological significance, the levels of this substance may potentially serve as indicators for those potentially important nitric oxide-related processes that gave rise to it (Wishnok et al., 1995).

As mentioned above, both *in vitro* and *in vivo* studies showed that nitrate can be reduced to nitrite by bacterial and mammalian metabolic pathways, via the widespread nitrate reductase (Gangolli et al., 1994). In humans, saliva is the major site for the formation of nitrite. About 5% of dietary nitrate is converted to nitrite (Spiegelhalder et al., 1976; Eisenbrand et al., 1980; Walters & Smith, 1981; Gangolli et al., 1994). A direct correlation between gastric pH, bacterial colonization, and gastric nitrite concentration has been observed in healthy people with a range of pH values from 1 to 7 (Mueller et al., 1983, 1986). In individuals with gastrointestinal disorders and achlorhydria, high levels of nitrite can be reached (6 mg/litre) (Rudell et al., 1976, 1978; Dolby et al., 1984). The situation in neonates is not clear. It is commonly accepted that infants younger than 3 months may be highly susceptible to gastric bacterial nitrate reduction, as the pH is generally higher than in 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).

EFFECTS ON LABORATORY ANIMALS AND *IN VITRO* TEST SYSTEMS

Acute exposure

The acute oral toxicity of nitrate to laboratory animals is low to moderate. LD_{50} values of 1600–9000 mg of sodium nitrate per kg of body weight have been reported in mice, rats, and rabbits. Ruminants are more sensitive to the effects of nitrate as a result of high nitrate reduction in the rumen; the LD_{50} for cows was 450 mg of sodium nitrate per kg of body weight. Nitrite is more toxic than nitrate: LD_{50} values of 85–220 mg of sodium nitrite per kg of body weight have been reported for mice and rats (Speijers et al., 1989; WHO, 1996).

Short-term exposure

In a 13-week study in which nitrite was given to rats in drinking-water, a dose-related hypertrophy of the adrenal zona glomerulosa was observed at all dose levels (100, 300, 1000, or 3000 mg of potassium nitrite per litre). Increased metHb levels were seen only in the highest dose group (Til et al., 1988). WHO (1995) concluded that the NOEL in this study was 100 mg of potassium nitrite per litre (equivalent to 5.4 mg/kg of body weight per day expressed as nitrite ion), because the hypertrophy seen at this dose was not significantly different from the controls.

An additional 13-week study in which nitrite was also given in drinking-water, including lower doses of potassium nitrite and two doses of sodium nitrite (equimolar to the low and high doses of potassium nitrite), confirmed the finding of the adrenal hypertrophy of the zona glomerulosa for potassium nitrite and also revealed hypertrophy in the animals given sodium nitrite. The NOEL for the adrenal hypertrophy of the zona glomerulosa was 50 mg of potassium nitrite per litre (equivalent to 5 mg of potassium nitrite per kg of body weight per day) (Kuper & Til, 1995). Since then, studies designed to clarify the etiology of this hypertrophy and to establish its significance for human health have been partly performed and are currently in progress. The studies already performed confirmed the adrenal hypertrophy in another rat strain. However, the effects were seen only at higher dose levels. It was also seen that the hypertrophy was still present after a 30-day recovery period but had disappeared after a 60-day recovery period. At present, the mechanism of hypertrophy induced by nitrite is not clear (Boink et al., 1995).

A variety of experimental and field studies in different mammals identified inorganic nitrate as a goitrogenic agent. It could be shown in rats by oral and parenteral application of potassium nitrate (Wyngaarden et al., 1953; Bloomfield et al., 1961; Alexander & Wolff, 1966; Wolff, 1994), of nitrate in hay (Lee et al., 1970), and of sodium nitrate (Höring et al., 1985; Seffner & Höring, 1987a,b). Antithyroid effects of nitrate were also found in sheep (Bloomfield et al., 1961) and in pigs by application of potassium nitrate (Jahreis et al., 1986, 1987). Furthermore, nitrate was goitrogenic to livestock: pigs (Körber et al., 1983), cattle (Körber et al., 1983, 1985), sheep (Körber et al., 1983), and goats (Prassad, 1983).

Long-term exposure

The only observed effect of nitrate in rats after 2 years of oral administration was growth inhibition; this was seen at dietary concentrations of 5% sodium nitrate and higher. The NOEL in this study was 1%, which corresponds to 370 mg of nitrate per kg of body weight per day (Speijers et al., 1989; WHO, 1996). A more recent long-term study was solely a carcinogenicity study, in which the highest dose levels of 1820 mg of nitrate per kg of body weight per day did not show carcinogenic effects. However, this level could not be considered as a NOEL, because complete histopathological examinations were not performed (WHO, 1996).

One of the long-term effects of nitrite reported in a variety of animal species is vitamin A deficiency; this is probably caused by the direct reaction of nitrite with the vitamin. The most important effects reported in long-term animal studies were an increase in metHb level and histopathological changes in the lungs and heart in rats receiving nitrite in drinking-water for 2 years. The LOAEL, which gave a metHb level of 5%, was 1000 mg of sodium nitrite per litre; the NOEL was 100 mg of sodium nitrite per litre, equivalent to 10 mg of sodium nitrite per kg of body weight per day (or 6.7 mg/kg of body weight per day expressed as nitrite ion) (Speijers et al., 1989).

Reproductive and developmental toxicity

The reproductive behaviour of guinea-pigs was impaired only at very high nitrate concentrations (30 000 mg of potassium nitrate per litre); the NOEL was 10 000 mg/litre (Speijers et al., 1989; WHO, 1996). In rabbits, dose levels of 250 or 500 mg of nitrate per litre administered during 22 weeks revealed no detrimental effects on reproductive performance after successive gestations. In sheep and cattle, no abortions were observed at dose levels causing severe methaemoglobinaemia (Speijers et al., 1989; WHO, 1996).

Nitrite appeared to cause fetotoxicity in rats at drinking-water concentrations equivalent to 200 and 300 mg of sodium nitrite per kg of body weight per day, causing increased maternal metHb levels. However, after similar doses in feed in other studies, no embryotoxic effects were observed in rats. In a reproductive toxicity study in guinea-pigs at dose levels of 0, 50, or 60 mg of sodium nitrite per kg of body weight per day given by subcutaneous injection, fetal death followed by abortion occurred at the highest dose level. Teratogenic effects were not observed in reported studies in mice and rats (Speijers et al., 1989; WHO, 1996).

Mutagenicity and related end-points

Nitrate is not mutagenic in bacteria and mammalian cells *in vitro*. Chromosomal aberrations were observed in the bone marrow of rats after oral nitrite uptake, but this could have been due to exogenous *N*-nitroso compound formation. Nitrite is mutagenic. It causes morphological transformations in *in vitro* systems; mutagenic activity was also found in a combined *in vivo–in vitro* experiment with Syrian hamsters. The results of *in vivo* experiments were controversial (Speijers et al., 1989; WHO, 1996).

Carcinogenicity

Nitrate is not carcinogenic in laboratory animals. Some studies in which nitrite was given to mice or rats in the diet showed slightly increased tumour incidence; however, the possibility of exogenous *N*-nitroso compound formation in these studies could not be excluded. In studies in which high levels of nitrite and simultaneously high levels of nitrosatable precursors were administered, increased tumour incidence was seen (Speijers et al., 1989; WHO, 1996). These types of tumours could be characteristic of the presumed corresponding *N*-nitroso compound endogenously formed. However, this increase in tumour incidence was seen only at extremely high nitrite levels, in the order of 1000 mg/litre of drinking-water. At lower nitrite levels, tumour incidence resembled those of control groups treated with the nitrosatable compound only. On the basis of adequately performed and reported studies, it may be concluded that nitrite itself is not carcinogenic to animals (Speijers et al., 1989; WHO, 1996).

EFFECTS ON HUMANS

Methaemoglobinaemia

The toxicity of nitrate to humans is mainly attributable to its reduction to nitrite. The major biological effect of nitrite in humans is its involvement in the oxidation of normal Hb to metHb, which is unable to transport oxygen to the tissues. The reduced oxygen transport becomes clinically manifest when metHb concentrations reach 10% of normal Hb concentrations and above; the condition, called methaemoglobinaemia, causes cyanosis and, at higher concentrations, asphyxia. The normal metHb level in humans is less than 2%; in infants under 3 months of age, it is less than 3%.

The Hb of young infants is more susceptible to metHb formation than that of older children and adults. This higher susceptibility is believed to be the result of the large proportion of fetal Hb still present in the blood of these infants. This fetal Hb is more easily oxidized to metHb. In addition, there is a deficiency in the metHb reductase responsible for the reduction of metHb back to Hb. The net result is that a dose of nitrite causes a higher metHb formation in these infants than in adults. With respect to exposure to nitrate, these young infants are also more at risk because of a relatively high intake of nitrate and, under certain conditions, a higher reduction of nitrate to nitrite by gastric bacteria due to the low production of gastric acid (Speijers et al., 1989; WHO, 1996). The higher reduction of nitrate to nitrite in the young infants is not quantified very well, and it appears that gastrointestinal infections increase the risk of higher yield of nitrite and thus a higher metHb formation (ECETOC, 1988; Speijers et al., 1989; Möller, 1995; Schuddeboom, 1995; WHO, 1996).

Other groups especially susceptible to metHb formation include pregnant women and people deficient in glucose-6-phosphate dehydrogenase or metHb reductase (Speijers et al., 1989).

Adults and children above the age of 3 months

Cases of methaemoglobinaemia have been reported in adults consuming high doses of nitrate by accident or as a medical treatment. Fatalities were reported after single intakes of 4–50 g of nitrate (equivalent to 67–833 mg of nitrate per kg of body weight) (Speijers et al., 1989; WHO, 1996), many of which occurred among special risk groups in whose members gastric acidity was reduced. Toxic doses — with metHb formation as a criterion for toxicity — ranged from 2 to 9 g (equivalent to 33–150 mg of nitrate per kg of body weight) (WHO, 1996). In a controlled study, an oral dose of 7–10.5 g of ammonium nitrate and an intravenous dose of 9.5 g of sodium nitrate did not cause increased metHb levels in adults, although vomiting and diarrhoea occurred (Speijers et al., 1989; WHO, 1996).

Accidental human intoxications have been reported as a result of the presence of nitrite in food. The oral lethal dose for humans was estimated to range from 33 to 250 mg of nitrite per kg of body weight, the lower doses applying to children and elderly people. Toxic doses giving rise to methaemoglobinaemia ranged from 0.4 to 200 mg/kg of body weight (WHO, 1996).

Another source of information with respect to nitrite toxicity in humans is the use of sodium nitrite as medication for vasodilation or as an antidote in cyanide poisoning. Doses of 30–300 mg per person (equivalent to 0.5–5 mg/kg of body weight) were reported not to cause toxic effects (WHO, 1996).

Few cases of methaemoglobinaemia have been reported in older children. A correlation study among children aged 1–8 years in the USA showed that there was no difference in metHb levels between 64 children consuming high-nitrate well-water (22–111 mg of nitrate-nitrogen per litre) and 38 children consuming low-nitrate water (<10 mg of nitrate-nitrogen per litre).

These concentrations correspond to 100–500 and <44 mg of nitrate per litre, respectively. All the metHb levels were within the normal range, suggesting that older children are relatively insensitive to the effects of nitrate (Craun et al., 1981).

Infants under 3 months of age

Cases of methaemoglobinaemia related to low nitrate appear to be restricted to infants. In infants under the age of 3 months, the conversion of nitrate to nitrite and metHb formation are high, as discussed above. Gastrointestinal disturbances play a crucial role, the reduction of nitrate to nitrite in the stomach being enhanced by bacterial growth at the high pH in the stomach of these infants. Toxic effects can therefore be induced at a much lower dose of nitrate than in adults. According to Corré & Breimer (1979), assuming an 80% reduction of nitrate to nitrite in these young infants, the toxic dose ranged from 1.5 to 2.7 mg of nitrate per kg of body weight, using 10% formation of metHb as a toxicity criterion. However, in reported cases of methaemoglobinaemia, the amounts of nitrate ingested were higher: 37.1–108.6 mg/kg of body weight, with an average of 56.7 mg of nitrate per kg of body weight (WHO, 1996). In studies in which a possible association between clinical cases of infantile methaemoglobinaemia or subclinically increased metHb levels and nitrate concentrations in drinking-water was investigated, a significant relationship was usually found, most clinical cases (97.7%) occurring at nitrate levels of 44.3–88.6 mg/litre or higher (Walton, 1951; WHO, 1996), and almost exclusively in infants under 3 months of age (Walton, 1951). Some cases of infant methaemoglobinaemia have indeed been described in which increased endogenous nitrate (nitrite) synthesis as a result of gastrointestinal infection appeared to be the only causative factor (WHO, 1996). As most cases of infantile methaemoglobinaemia reported in the literature have been associated with the consumption of private and often bacterially contaminated well-water, the involvement of infections is highly probable. Most of these studies may be therefore less suitable from the point of view of the quantitative assessment of the risk of nitrate intake for healthy infants. On the other hand, bottle-fed infants under 3 months of age have a high probability of developing gastrointestinal infections because of their low gastric acidity, which is another important reason to consider these infants as a risk group.

Carcinogenicity

Nitrite was shown to react with nitrosatable compounds in the human stomach to form *N*-nitroso compounds. Many of these *N*-nitroso compounds have been found to be carcinogenic in all the animal species tested, although some of the most readily formed compounds, such as *N*-nitrosoproline, are not carcinogenic in humans. The *N*-nitroso compounds carcinogenic in animal species are probably also carcinogenic in humans. However, the data from a number of epidemiological studies are at most only suggestive. The endogenous formation of *N*-nitroso compounds is also observed in several animal species, if relatively high doses of both nitrite and nitrosatable compounds are administered simultaneously. Thus, 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).

Several reviews of epidemiological studies have been published; most of these studies are geographical correlation studies relating estimated nitrate intake to gastric cancer risk. The US National Research Council found some suggestion of an association between high nitrate intake and gastric and/or oesophageal cancer (NAS, 1981). However, individual exposure data were lacking, and several other plausible causes of gastric cancer were present. In a later WHO review (WHO, 1985b), some of the earlier associations appeared to be weakened following the introduction of individual exposure data or after adjustment for socioeconomic factors. No convincing evidence was found of an association between gastric cancer and the consumption of drinking-water in which nitrate concentrations of up to 45 mg/litre were present. No firm evidence was found at higher levels either, but an association could not be

excluded because of the inadequacy of the data available. More recent geographical correlation and occupational exposure studies also failed to demonstrate a clear relationship between nitrate intake and gastric cancer risk, although these studies were well designed. A case–control study in Canada, in which dietary exposure to nitrate and nitrite was estimated in detail, showed that exogenous nitrite intake, largely from preserved meat, was significantly associated with the risk of developing gastric cancer (ECETOC, 1988). On the other hand, case–control studies based on food frequency questionnaires tend to show a protective effect of the estimated nitrate intake on gastric cancer risk. Most likely this is due to the known strong protective effect of vegetables and fruits on the risk of gastric cancer (Möller, 1995; WHO, 1996). Studies that have assessed the effect of nitrate from sources other than vegetables, such as the concentration in drinking-water or occupational exposure to nitrate dusts, have not shown a protective effect against gastric cancer risk. For other types of cancer, there are no adequate data with which to establish any association with nitrite or nitrate intake (Gangolli et al., 1994; Möller, 1995; WHO, 1996).

It has been established that the intake of certain dietary components present in vegetables, such as vitamins C and E, decreases the risk of gastric cancer. This is generally assumed to be at least partly due to the resulting decrease in the conversion of nitrate to nitrite and in the formation of *N*-nitroso compounds. It is possible that any effect of a high nitrate intake *per se* is masked in correlation studies by the antagonizing effects of simultaneously consumed dietary protective components. However, the absence of any link with cancer in occupational exposure studies is not in agreement with this theory.

The known increased risk of gastric cancer under conditions of low gastric acidity could be associated with the endogenous formation of *N*-nitroso compounds. High mean levels of *N*-nitroso compounds, as well as high nitrate levels, were found in the gastric juice of achlorhydric patients, who must therefore be considered as a special risk group for gastric cancer from the point of view of nitrate and nitrite (NAS, 1981; WHO, 1985b, 1996; ECETOC, 1988; Speijers et al., 1989).

Other effects

Congenital malformations have been related to high nitrate levels in drinking-water in Australia; however, these observations were not confirmed. Other studies also failed to demonstrate a relationship between congenital malformations and nitrate intake (WHO, 1985b; ECETOC, 1988).

Studies relating cardiovascular effects to nitrate levels in drinking-water gave inconsistent results (WHO, 1985b).

Possible relationships between nitrate intake and effects on the thyroid have also been studied, as it is known that nitrate competitively inhibits iodine uptake. In addition to effects of nitrate on the thyroid observed in animal studies and in livestock, epidemiological studies revealed indications for an antithyroid effect of nitrate in humans. If dietary iodine is available at an adequate range (corresponding to a daily iodine excretion of 150–300 µg/day), the effect of nitrate is weak, with a tendency to zero. The nitrate effect on thyroid function is strong if a nutritional iodine deficiency exists simultaneously (Höring et al., 1991; Höring, 1992).

Hettche (1956a,b) described an association between high nitrate concentrations in drinking-water and goitre incidence in 1955. As well, Höring & Schiller (1987), Sauerbrey & Andree (1988), Höring et al. (1991), Höring (1992), and van Maanen et al. (1994) found that inorganic nitrate in drinking-water is a manifested factor of endemic goitre. A dose–response relationship could be demonstrated by Höring et al. (1991) (nitrate in drinking-water vs incidence of goitre) as well as by van Maanen et al. (1994) (nitrate in drinking-water vs

thyroid volume). Both the experimental and epidemiological studies give the impression that nitrate in drinking-water has a stronger effect on thyroid function than nitrate in food. The differences in nitrate kinetics after ingestion through drinking-water and through food could be the cause of the difference in thyroid effects. However, no adequate studies regarding this question exist at present. Furthermore, some of the above-mentioned studies demonstrate that dietary iodine deficiency is much more effective than nitrate exposure in causing goitre.

In addition to the effect of nitrite on the adrenal zona glomerulosa in rats, a study in humans indicated that sodium nitrite (0.5 mg of sodium nitrite per kg of body weight per day, during 9 days) caused a decreased production of adrenal steroids, as reflected by the decreased concentration of 17-hydroxysteroid and 17-ketosteroids in urine (Til et al., 1988; Kuper & Til, 1995). Similar results were also found in rabbits (Violante et al., 1973). Although the mechanism is not clear, the effects of nitrite seen in rats seem relevant for the hazard assessment for humans, unless mechanistic studies prove otherwise.

GUIDELINE VALUES

With respect to chronic effects, JECFA recently re-evaluated the health effects of nitrate/nitrite, confirming the previous ADI of 0–3.7 mg/kg of body weight per day for nitrate ion and establishing an ADI of 0–0.06 mg/kg of body weight per day for nitrite ion (WHO, 1995). However, it was noted that these ADIs do not apply to infants below the age of 3 months. Bottle-fed infants below 3 months of age are most susceptible to methaemoglobinaemia following exposure to nitrate/nitrite in drinking-water.

For methaemoglobinaemia in infants (an acute effect), it was confirmed that the existing guideline value for nitrate ion of 50 mg/litre is protective. For nitrite, human data reviewed by JECFA support the current provisional guideline value of 3 mg/litre, based on induction of methaemoglobinaemia in infants. Toxic doses of nitrite responsible for methaemoglobinaemia range from 0.4 to more than 200 mg/kg of body weight. Following a conservative approach by applying the lowest level of the range (0.4 mg/kg of body weight), a body weight of 5 kg for an infant, and a drinking-water consumption of 0.75 litre, a guideline value for nitrite ion of 3 mg/litre (rounded figure) can be derived. The guideline value is no longer provisional.

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

It seems prudent to propose a guideline value for nitrite associated with chronic exposure based on JECFA's analysis of animal data showing nitrite-induced morphological changes in the adrenals, heart, and lungs. Using JECFA's ADI of 0.06 mg/kg of body weight per day, assuming a 60-kg adult ingesting 2 litres of drinking-water per day, and allocating 10% of the ADI to drinking-water, a guideline value of 0.2 mg of nitrite ion per litre (rounded figure) can be calculated. However, owing to the uncertainty surrounding the relevance of the observed adverse health effects for humans and the susceptibility of humans compared with animals, this guideline value should be considered provisional.

Because of known interspecies variation in the conversion of nitrate to nitrite, the animal model was not considered appropriate for use in human risk assessment for nitrate.

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. 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 should be taken to modify the treatment train or water chemistry in order to maintain a safe water quality. Efficient disinfection must never be compromised.

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