

## **INGESTED NITRATES AND NITRITES (Group 2A)**

For definition of Groups, see Preamble Evaluation.

**VOL.:** 94

### **5. Summary of Data Reported**

**[The text of these Summaries and Evaluations may be edited for language and clarity during the checking of the main text of the Monographs.]**

#### **5.1 Exposure data**

Nitrate and nitrite are naturally occurring ions that are part of the global nitrogen cycle and are ubiquitous in the environment. Since the early 1900s, nitrate and other nitrogen compounds have been used extensively as fertilizers in agriculture. Moreover, nitrate and nitrite are added to meat and fish for the purpose of preservation, as colour fixatives and as flavouring agents.

The natural background level of nitrate in groundwater is generally below 10 mg/L. Both groundwater and surface water can be contaminated by nitrate as a result of agricultural activities. Levels of nitrate contamination are usually significantly higher in groundwater, particularly in shallow wells, in which concentrations that exceed the current WHO guideline of 50 mg/L, and exceptionally exceed 500 mg/L, have been reported in intensive agricultural regions. Nitrite is not frequently detected in drinking-water; when it is present, its concentrations rarely exceed 3 mg/L.

Human exposure to nitrate is principally from exogenous sources, i.e. through the ingestion of food and water, whereas exposure to nitrite is primarily endogenous (see Section 5.4.1 on the endogenous formation of nitrite).

For nitrate, the main sources of exposure are vegetables, especially leafy vegetables. Other food sources include baked and processed cereal products and cured meat. Drinking-water is generally a minor source of nitrate, but may become a major contributor to overall nitrate intake at concentrations above 50 mg/L. For an average adult consumer who lives in an area with low drinking-water contamination, total exposure to nitrate from food and water is estimated to be about 60–90 mg per person per day, of which at least 90% is from food. For high consumers of vegetables, the intake of nitrate may reach 200 mg per person per day. Similar intakes could result from high consumption of water contaminated with more than 50 mg/L nitrate. The relative impact of contaminated water was confirmed in studies of total exposure to nitrate measured through 24-h urinary excretion.

For nitrite, the main source of exogenous human exposure is also food (but see Section 5.4 for endogenous formation). Important sources include cereal products, vegetables and cured meat. Over the last 30 years, the relative contribution of cured meat to dietary exposure to nitrite for an average consumer has decreased from about 40% to about 20%. For high consumers of cured meat, the relative contribution may have reached 90%. The total intake of exogenous nitrite is estimated to be about 0.75–2.2 mg per day for an adult with an average food consumption pattern. Nitrite may react with amines and amides to form *N*-nitroso compounds during the storage of food.

## 5.2 Human carcinogenicity data

Ingested nitrite and nitrate have been studied in relation to the occurrence of many cancers; however, with the exception of stomach and brain cancers, few case–control or cohort studies are available for any given cancer site. Ingestion of nitrite and nitrate can result in the endogenous formation of *N*-nitroso compounds, particularly in the presence of nitrosatable precursors and in the absence of inhibitors of nitrosation such as vitamin C. Some of the epidemiological studies evaluated nitrite intake from cured meats or animal sources separately. Others evaluated risk among persons who had higher nitrite and lower vitamin C intake, a dietary pattern that may result in increased endogenous formation of *N*-nitroso compounds. The Working Group gave these latter studies more weight in the evaluation of the literature.

Vegetables are the primary source of nitrate when levels of nitrate in the drinking-water are low (see Section 5.1). Because many vegetables contain vitamin C or other inhibitors of endogenous nitrosation, nitrate from these sources may result in less endogenous formation of *N*-nitroso compounds than nitrate in the drinking-water. The Working Group therefore considered the evaluation of nitrate ingested in the diet and in drinking-water separately.

### 5.2.1 Gastric and oesophageal tumours

#### (a) Ingested nitrate

The relationship between stomach cancer and nitrate in the drinking-water has been addressed in 15 ecological studies, two case–control studies and one cohort study. The two case–control studies (one in the USA and the other in Taiwan, China) used deceased cases and reported no association. A cohort study in the Netherlands addressed the contribution of nitrate from water as part of total nitrate intake separately from dietary sources; no association was observed. No clear evidence emerged from the ecological studies.

The association between the intake of nitrate from foods and stomach cancer was analysed in seven case–control and two cohort studies. Three case–control studies and the two cohort studies, one in the Netherlands and one in Finland, reported no association. Four case–control studies found a lower risk for stomach cancer with higher levels of dietary nitrate. Most of these studies were carried out in populations who used public water supplies with relatively low levels of nitrate; thus the majority of the ingested nitrate was from foods, of which vegetables were the main source. The inverse association found in the four studies may be attributed to the nutrients associated with high vegetable consumption.

One case–control study, conducted in the USA, assessed the relationship between oesophageal cancer and dietary intake of nitrate, for which an inverse association was found. The few ecological studies of nitrate in the drinking-water provided no clear evidence for an association between nitrate in the drinking-water and oesophageal cancer.

#### (b) Ingested nitrite

The evidence for an association between dietary intake of nitrite and cancer of the stomach is based on seven well-designed case–control studies and two cohort studies, all of which were carried out in Europe and North America.

Six of the seven case–control studies found a positive association, which was significant in four. Two studies considered the potential interaction between dietary intake of nitrite and potential inhibitors of nitrosation: a large case–control study conducted in several areas of Italy reported a fivefold increase in risk for subjects who ingested high levels of nitrite and proteins and low levels of antioxidant micronutrients (e.g., vitamin C,  $\alpha$ -tocopherol) compared with those who had a low intake of nitrite and proteins and a high intake of antioxidants. A multicentric study in the USA reported a significant

increase in risk for stomach tumours located in both the cardia and non-cardia regions among subjects who had a high intake of nitrite and a low intake of vitamin C compared with those who had a low intake of nitrite and high vitamin C consumption. Four of the seven case–control studies also assessed the association between stomach cancer and *N*-nitrosodimethylamine present in food: one study in Canada and a study in Italy found no association, while one study in Spain and another in France found that a high intake of *N*-nitrosodimethylamine was positively associated with risk for stomach cancer. None of the studies reported effect estimates for nitrite adjusted for *N*-nitrosodimethylamine.

Two cohort studies were reviewed, one conducted in the Netherlands and the other in Finland. In the Finnish study, no association was found between dietary intake of nitrites or *N*-nitrosodimethylamine and the risk for stomach cancer. The Dutch study reported a significant increase in risk for stomach cancer at the highest level of nitrite intake; this increase became non-significant after adjustment for potential confounders, including vitamin C and  $\beta$ -carotene. Effect modification of nitrite by vitamin C was not assessed in this study.

Overall, the results for stomach cancer were consistent for the case–control studies, but neither of the cohort studies found a clear positive association. Furthermore, none of the studies that were reviewed had taken into account potential confounding or effect modification by *Helicobacter pylori*, an important risk factor for stomach cancer, when assessing the effect of nitrite.

For oesophageal cancer, two case–control studies, both conducted in the USA, assessed the association with nitrite intake. Both were well-designed and adjustment was made for the main risk factors for oesophageal cancer. Both studies reported a positive but non-significant association. When intake of vitamin C was taken into account, both studies observed a significant increase in risk for subjects who had a high intake of nitrite and a low intake of vitamin C compared with those who had low nitrite and high vitamin C consumption. The potential confounding effect of *N*-nitroso compounds in food was not investigated.

### 5.2.2 Brain tumours

#### (a) Ingested nitrate

Overall, the Working Group evaluated 10 case–control studies of brain tumours, six of which were conducted in adults and four in children. In one of the four case–control studies of childhood brain tumours, current levels of nitrate in the tap-water in homes in which the pregnancies had developed were estimated using non-validated measurements from semi-quantitative water test strips. No significant association was observed, although the risk for astroglial tumours showed a non-significant, twofold increase for the highest category of nitrate exposure ( $\geq 50$  mg/L).

Two case–control studies of adult brain tumours estimated levels of nitrate in the drinking-water by linking information on residence to public water quality monitoring data in regions that had mainly low to moderate levels of nitrate contamination. One of the studies also measured nitrate levels in a current tap-water sample from users of private wells. Nitrate in drinking-water was not associated with risk for brain tumours in either of these studies.

None of the studies that focused on dietary sources of nitrate observed a positive association with brain tumours among adults or children. Some observed a decreased risk for brain tumours in relation to dietary nitrate; however, in studies that further adjusted for vitamin C intake, this inverse association was attenuated or disappeared.

#### (b) Ingested nitrite

The Working Group evaluated 12 case–control studies that focused on nitrite in the diet or in drinking-water: five investigated brain tumours in children and four of these examined maternal diet during pregnancy as a possible risk factor for the development of brain tumours in the offspring. The largest

case–control study that was conducted in the western USA observed no association between estimated dietary intake of nitrite and the incidence of childhood brain tumours. However, when the source of dietary nitrite was considered, children born to mothers in the highest quartile of intake of nitrite from cured meat ( $> 1.28$  mg per day) had an almost twofold increased risk for brain tumours; nitrite intake from vegetable sources was not associated with the occurrence of brain tumours. A re-analysis of these data using better estimates of nitrite levels in cured meat in the year of the pregnancy suggested a stronger association (a threefold increase in the highest category of intake of nitrite from cured meat during pregnancy;  $\geq 3.0$  mg per day). No increased risk in relation to overall dietary nitrite was observed in studies in Israel or France or in a study in North America that focused on children under 6 years of age who had either astrocytic gliomas or primitive neuroectodermal tumours. These studies did not quantify nitrite intake from cured meat specifically.

Nitrite in the drinking-water was investigated in a combined analysis of the children of studies from the western USA and France together with children from Spain. Current levels of nitrite in the tap-water in homes in which the pregnancies had developed were estimated using non-validated measurements from semi-quantitative water test strips. This study reported a twofold increase in risk for brain tumours in the offspring for the two categories of detectable nitrite in the drinking-water. This association was stronger among children who had astroglial tumours and among those whose mothers did not rely on bottled water.

Seven studies of dietary intake of nitrite and adult brain tumours were conducted, six of which gave risk estimates for glioma. No significant associations were reported for dietary nitrite overall. The largest study in California, USA, observed a twofold increase in risk for glioma among men who consumed levels of nitrite above the median and levels of vitamin C below the median; this pattern did not occur among women. Two small studies in the USA, one in Ohio and one among women in California, observed a positive association with intake of nitrite from cured meat; a larger case–control study in Nebraska, USA, observed no association with nitrite from animal sources but a threefold increase in risk for glioma among persons who had high consumption of nitrite from plant sources. The study from Nebraska observed no effect modification by vitamin C. None of these studies reported risk estimates for meningioma or other tumour types.

### 5.2.3 *Tumours of the urinary tract*

#### (a) *Ingested nitrate*

Five ecological studies, one cohort and one case–control study of tumours of the urinary tract in association with nitrate intake in the diet and drinking-water were reviewed. These included investigations of cancers of the urinary bladder, kidney or of all tumours of the lower urinary tract combined, among which bladder cancer predominated. The cohort and case–control studies were conducted in the Iowa, USA and estimated exposure to nitrate among people who used public water systems; average levels were generally below 50 mg/L. In the cohort study which included only women, risk for cancer of the urinary bladder was positively associated with average concentrations of nitrate in the water. No association was observed with kidney cancer. This study did not evaluate any potential interactions between nitrate and inhibitors of nitrosation such as vitamin C. The largely non-overlapping case–control study of bladder cancer examined nitrate intake from the diet and drinking-water. No association with estimates of nitrate intake from the diet or drinking-water was detected, and no interaction was observed between intake of vitamin C and ingestion of nitrate in water. The five ecological studies, conducted in Europe, were uninformative.

#### (b) *Ingested nitrite*

Two well-designed case–control studies of tumours of the urinary tract assessed dietary intake of nitrite; both used a food-frequency questionnaire to ascertain dietary history and both considered potentially confounding factors. The case–control study of cancers of the lower urinary tract from Oahu, Hawaii, USA, found an increased risk for cancer of the urinary bladder with greater dietary

intake of nitrite among men of Japanese ancestry. There was no association among women of Japanese ancestry or among Caucasian men or women. In the study of cancer of the urinary bladder from Iowa (largely Caucasian), dietary intake of nitrite was not associated with cancer risk. Neither of the studies evaluated interactions with vitamin C.

#### 5.2.4 *Non-Hodgkin lymphoma*

##### (a) *Ingested nitrate*

One cohort study and two case-control studies, all conducted in agricultural regions of the USA, evaluated ingestion of nitrate in the diet and drinking-water and risk for non-Hodgkin lymphoma. Another case-control study in the USA only evaluated intake of nitrate in drinking-water. The four studies that evaluated nitrate in drinking-water linked historical data on levels of nitrate in public water supplies to a residential history of water source. Increasing quartiles of the average nitrate level in public supplies were associated with an increased risk for non-Hodgkin lymphoma in the case-control study in Nebraska where the highest average nitrate quartile was > 4.0 mg/L nitrate-N. The case-control and cohort studies in Iowa had slightly lower average exposures to nitrate (highest quartiles > 2.5 and  $\geq$  2.9 mg/L nitrate-N); increasing quartiles of exposure were not associated with cancer risk. The case-control study in Minnesota had the lowest exposure levels (highest quartile > 1.5 mg/L nitrate-N) and observed an inverse association with risk for exposure at this level. The case-control studies in Nebraska and Iowa evaluated risk by comparing high and low strata of nitrate intake in water with strata of vitamin C intake. In the study in Nebraska, there was a statistically significant threefold increased risk among persons who had a higher nitrate intake from water and lower vitamin C intake compared with those who had a lower nitrate intake from water and high vitamin C intake. There was no evidence of such a pattern in risk in the case-control study in Iowa that used similar exposure categories. Five ecological studies evaluated nitrate levels in public water supplies and risk for non-Hodgkin lymphoma. The Working Group considered that these studies provided little information for the evaluation because levels of nitrate were mainly below 10 mg/L nitrate-N and because of the limitations of the ecological study design.

Dietary intake of nitrate was associated with non-significant inverse risks for non-Hodgkin lymphoma in the Iowa cohort study and the Nebraska case-control study; a significant inverse association with higher intake of dietary nitrate was reported in the Iowa case-control study.

##### (b) *Ingested nitrite*

The relationship between ingested nitrite and non-Hodgkin lymphoma was evaluated in two case-control studies in the USA. Dietary nitrite was not associated with risk for this cancer in one study but there was an increase in risk with increasing quartiles of nitrite intake in the other study. When plant and animal sources of dietary nitrite were evaluated separately, the positive association was observed only for plant sources.

#### 5.2.5 *Cancers of the colon and rectum*

##### (a) *Ingested nitrate*

One case-control study and two cohort studies evaluated the intake of nitrate from drinking-water and dietary sources in relation to risk for cancers of the colon and rectum.

The case-control study found no overall association between average levels of nitrate in drinking-water from public water supplies and risk for either type of cancer. However, for cancer of the colon, a significant twofold elevated risk was observed among persons who had a higher intake of nitrate from water and low vitamin C intake. A significant twofold elevated risk was also observed with higher intake of nitrate from water and high meat intake. Average nitrate levels in public water supplies were

not associated with an increase in risk for cancer of the colon or rectum in one of the cohort study; however, elevated risks for colon cancer were observed in the intermediate exposure categories.

Dietary nitrate intake was not associated with risk for colorectal cancer in either cohort studies. Higher dietary intake of nitrate was associated with a decreased risk for colon cancer in the Iowa case–control study, whereas there was no association with rectal cancer.

(b) *Ingested nitrite*

One case–control study in the USA and one cohort study in Finland evaluated dietary nitrite intake and risk for cancers of the colon and rectum. The case–control study found a 50% increased risk for colon cancer and a 70% increased risk for rectal cancer. Dietary intake of nitrite was not associated with cancer risk in the cohort study.

5.2.6 *Other cancers*

Several studies investigated the potential association between ingested nitrate or nitrite and other cancers (breast, corpus uteri, larynx, lung, nasopharynx, oral cavity, pancreas and testis). For each site, however, only few studies were available and the results were of insufficient quality, consistency or statistical power to permit a conclusion.

### 5.3 **Animal carcinogenicity data**

5.3.1 *Nitrate*

In three studies in mice, no evidence of carcinogenic activity of nitrate alone was observed whether it was administered in the drinking-water or in the diet at high concentrations. One study showed that less than 2.5% of the administered nitrate was reduced to nitrite in mice. In four studies in rats, no increased incidences of tumours were observed when sodium nitrate alone was administered in the drinking-water or in the diet.

5.3.2 *Nitrite*

In most of the studies, mice or rats that were exposed to nitrite alone in the diet, by gavage or in the drinking-water did not have higher incidences of tumours compared with untreated controls. It was noted that the negative findings may be due to the low doses of nitrite used, the short duration of exposure or the instability of nitrite. In some instances, when a carcinogenic activity of nitrite was observed, the investigators noted the formation of *N*-nitroso compounds in the diet mix or in the stomach contents.

One study in female mice treated with nitrite in the drinking-water showed an increased trend in the incidence of forestomach papillomas and carcinomas combined. In one study in mice that were exposed to nitrite in the drinking-water *in utero* (exposure of dams) and throughout life (until natural death), increased incidences of lymphoma and lung tumours were observed.

Many studies in mice tested nitrite in combination with specific secondary or tertiary amines or amides, administered in the diet, in drinking-water or by gastric intubation. Most combinations resulted in increased tumour incidences when compared with the amine or amide alone or with nitrite alone. The amines and amides that led to positive results included dibutylamine, *N*-methylaniline, piperazine, morpholine, butylurea, ethylurea, ethylthiourea, methylurea, and carbendazim. The tumours included lung tumours, malignant lymphomas, forestomach tumours, urinary bladder papillomas or uterine adenocarcinomas. In one of these studies, when piperazine was administered at a constant level in the diet with varying levels of sodium nitrite in the drinking-water, the increase in the incidence of lung adenomas was directly proportional to the levels of nitrite intake.

In one study, female rats that were fed nitrite in the diet had an increased incidence of hepatocellular carcinomas. The Working Group attributed the findings to the fact that some ingredients in the rat chow (e.g. fishmeal) may have contained high levels of nitrosatable compounds. In one study in which rats were exposed to dietary nitrite *in utero* (exposure of dams) and throughout life, increased incidences of lymphoreticular tumours were observed. In one study, rats that received nitrite in the drinking-water had an increased incidence of forestomach papillomas.

Many studies in rats tested nitrite in combination with specific secondary or tertiary amines or amides, administered in the diet, in drinking-water or by gastric intubation. Most combinations resulted in increased tumour incidences when compared with the amine or amide alone or with nitrite alone. The amines and amides that led to positive results included aminopyrine, bis(2-hydroxypropyl)amine, chlorpheniramine, diphehydramine, heptamethylenimine, morpholine, *N,N*-dimethyldodecylamine-*N*-oxide, butylurea, and disulfiram. The tumours included oesophageal tumours, haemangiosarcomas, hepatocellular adenomas and carcinomas, lung squamous-cell carcinomas and nasal cavity tumours. In some of these studies, at a constant level of sodium nitrite, the tumour incidence induced was directly related to the levels of amine. When the level of amine was kept constant, tumour yield was also directly related to the level of sodium nitrite. When pregnant rats were given ethylurea and sodium nitrite in the drinking-water, neurogenic tumours developed in the offspring.

A dose-related increase in the incidence of renal-cell carcinoma was observed when rats were administered nitrite in the drinking-water in combination with varying amounts of fishmeal in the diet. Levels of *N*-nitrosodimethylamine in the stomach contents also showed a dose-related increase.

In one study, nitrite in the drinking-water marginally enhanced the carcinogenic effects of two different leukaemia viruses in infected mice.

Studies with antioxidants in mice showed that ascorbate (vitamin C) or soy bean prevented the combination of nitrite and dibutylamine from producing liver and urinary bladder tumours. Thioproline, a nitrite scavenger, decreased the incidence of forestomach carcinomas produced by the combination of nitrite and *N*-benzylmethylamine in rats. Ascorbate delayed the induction of liver tumours produced by concurrent administration of nitrite and morpholine, but enhanced the incidence of forestomach tumours in the same experiment. Several other antioxidants ( $\alpha$ -tocopherol, propyl gallate, *tert*-butylhydroquinone, 1-*O*-hexyl-2,3,5-trimethylhydroquinone) or catechols, concurrently administered with nitrite, also enhanced the incidence of forestomach tumours in rats following administration of a tumour initiator.

In one study in hamsters, the incidence of liver-cell carcinomas was increased when sodium nitrite was fed together with morpholine.

## **5.4 Mechanistic and other relevant data**

### **5.4.1 *Absorption, distribution, metabolism and excretion***

In humans, nitrate and nitrite participate in a dynamic interchange — the human nitrogen cycle — that involves ingestion, endogenous synthesis and ultimate excretion mainly as nitrate in the urine. Exposure by ingestion is primarily to nitrate, approximately 5% of which is reduced by oral bacteria to nitrite. The nitrate/nitrite mixture enters the gastrointestinal system in swallowed saliva. Nitrate and nitrite are absorbed from the upper intestine into the general circulation where the nitrite is oxidized by haemoglobin to nitrate that then re-enters the cycle. Endogenous synthesis is mainly via the arginine-to-nitric oxide pathway followed by ultimate conversion of nitric oxide to nitrate. Nitrate and nitrite are widely distributed in the body, where nitrate predominates. Under some physiological conditions, e.g. hypoxia, nitrite can be converted in the reverse direction to nitric oxide; this pathway is suggestive of an important biological role for this anion.

#### 5.4.2 Genotoxic effects

In one study, high intake of nitrate was associated with an increased frequency of *HPRT* mutants in peripheral blood lymphocytes in human populations who had been exposed to various levels of nitrate in their drinking-water. In another study, high intake of nitrate was not associated with an increase in the frequency of sister chromatid exchange. A third study showed an increase in the number of chromosomal aberrations, but not of sister chromatid exchange, in lymphocytes of children exposed to concentrations of nitrate in the drinking-water that exceeded 70 mg/L.

Sodium nitrate gave negative results in the *Salmonella typhimurium* reverse mutation assay. Potassium nitrate caused chromosomal aberrations in Chinese hamster ovary cells *in vitro* in one study. In two *in vivo* studies, sodium nitrate increased the frequency of chromosomal aberrations in rats and mice. Sodium nitrate did not cause chromosomal aberrations, micronuclei, 8-azaguanine-resistant mutations, ouabain-resistant mutations or morphological transformation in the cells of hamster embryos after transplacental exposure. It was also negative in the mouse sperm-head abnormality test.

Sodium nitrite gave generally positive results in the *Salmonella* mutagenicity assay (seven of nine studies), but was negative in the SOS chromotest. It did not induce mutations in bacteria recovered in the host-mediated assay from rats and mice of various strains. In a single study, sodium nitrite induced somatic mutations in the wing-spot test in *Drosophila melanogaster*. It gave a positive response in a number of assays for chromosomal aberrations and micronucleus formation, both *in vitro* and *in vivo*. In a number of *in vitro* studies, sodium nitrite was consistently positive in inducing aneuploidy, cell transformation, 8-azaguanine-resistant mutations, 6-thioguanine-resistant mutations and ouabain-resistant mutations. Similarly, sodium nitrite induced 8-azaguanine-resistant mutations, ouabain-resistant mutations and morphological transformation in cells of hamster embryos after transplacental exposure, but did not induce chromosomal aberrations or micronuclei in this assay. It was also positive in the mouse sperm-head abnormality test.

#### 5.4.3 Other toxic effects

Nitrate, via reduction to nitrite as noted above, causes methaemoglobinaemia, especially in infants. Epidemiological evidence suggests a possible association between nitrate in the drinking-water and spontaneous abortions, intrauterine growth restrictions, birth defects, childhood onset of diabetes mellitus, thyroid effects, hypertension and recurrent diseases (respiratory tract infection, diarrhoea, stomatitis) in children. Animal studies with nitrite have demonstrated haematological changes and pathological changes in the lung and heart. Reproductive and developmental effects of nitrite include increased mortality, reduced growth and behavioural changes. No teratogenic effects were observed with nitrate or nitrite.

#### 5.4.4 Endogenous nitrosation and the carcinogenicity of N-nitroso compounds

Nitrosamines and nitrosamides are recognized animal carcinogens and some, e.g. the tobacco-specific nitrosamines *N*-nitrosornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNN and NNK), are human carcinogens. Nitrosamines need to be activated metabolically by cytochrome P450 enzymes to electrophilic intermediates in order to exert a carcinogenic effect, while nitrosamides are direct-acting carcinogens. Nitrosation of amines produces electrophiles that can alkylate nucleophilic sites on biological molecules. Nitrosation of primary exocyclic amino groups on DNA, followed by deamination, may lead directly to mutations.

Nitrosating agents — e.g. nitrous acid ( $\text{HNO}_2$ ) and nitrogen oxide ( $\text{N}_2\text{O}_3$ ) — that arise from nitrite under acidic gastric conditions react with amines or amides to form nitrosamines or nitrosamides, and the induction of tumours in animals via endogenous synthesis of *N*-nitroso compounds has been demonstrated. Ascorbic acid (vitamin C), a known inhibitor of nitrosation reactions, lowers the incidence of tumours in these experiments. The effect of ascorbic acid in the reduction of the risk for

cancer that is associated with ingested nitrite has also been shown in epidemiological studies (see Section 5.2). These observations support the role of endogenous nitrosation in tumorigenesis.

Nitrosation of proline to form the non-carcinogenic nitrosamine *N*-nitrosoproline has been used as a test of endogenous nitrosation, and a large number of studies have now demonstrated the nitrosation of proline in humans. In a typical experiment, proline and nitrate are administered and the nitrite that arises from oral nitrate reduction can then nitrosate proline in the stomach. Gastric nitrosation of proline in humans can be inhibited by vitamin C, as seen in animal experiments. Examples of endogenously formed carcinogenic nitrosamines in humans are *N*-nitrosodimethylamine and *N*-nitrosopyrrolidine. Nitrite-cured meat contains substantial amounts of nitrosatable compounds (*N*-nitroso compound precursors). Other pathways for endogenous nitrosation include bacterially mediated and nitric oxide-related nitrosation.

Thus, there is an active endogenous nitrogen cycle in humans that involves nitrate and nitrite. In the presence of amines or amides, endogenous nitrosation takes place in the acidic environment of the human stomach. The nitrosating reactions are enhanced following ingestion of additional nitrate, nitrite or nitrosatable compounds. Some of the *N*-nitroso compounds that are formed in humans under these conditions are known carcinogens.

## 6. Evaluation and rationale

There is *limited evidence* in humans for the carcinogenicity of nitrite in food. Nitrite in food is associated with an increased incidence of stomach cancer.

There is *inadequate evidence* in humans for the carcinogenicity of nitrate in food.

There is *inadequate evidence* in humans for the carcinogenicity of nitrate in drinking-water.

There is *sufficient evidence* in experimental animals for the carcinogenicity of nitrite in combination with amines or amides.

There is *limited evidence* in experimental animals for the carcinogenicity of nitrite *per se*.

There is *inadequate evidence* in experimental animals for the carcinogenicity of nitrate.

### Overall evaluation

Ingested nitrate or nitrite under conditions that result in endogenous nitrosation is *probably carcinogenic to humans (Group 2A)*.

The underlying mechanism is endogenous nitrosation, which in the case of nitrate must be preceded by reduction to nitrite. Nitrate and nitrite are interconvertible *in vivo*. Nitrosating agents that arise from nitrite under acidic gastric conditions react readily with nitrosatable compounds, especially secondary amines and alkyl amides, to generate *N*-nitroso compounds. Many *N*-nitroso compounds are carcinogenic.