Netherlands Food and Consumer
Product Safety Authority
Ministry of Economic Affairs

> Postbus 43006 3540 AA Utrecht The Netherlands

Office for Risk Assessment & Research

Catharijnesingel 59
3511 GG Utrecht
Postbus 43006
3540 AA Utrecht
The Netherlands
www.nvwa.nl

Contact
Dr Jacqueline Castenmiller

T +31 (0)88 223 3333
M +31 (6)27 083 115
F +31 (0)88 223 3334
j.j.m.castenmiller@nvwa.nl

Our ref.
NVWA/BuRO/2014/4365

Advice from the director Office for Risk Assessment & Research

To the Minister of Health, Welfare and Sport and Minister for Agriculture

Date 10 October 2014

Advisory report on dietary nitrate

Background

Nitrate is naturally present in drinking water and vegetables and is sometimes added to foods to extend shelf life or to enhance colour and aroma. The nitrate level in vegetables depends on their breed and increases if a large amount of fertiliser is used in their cultivation or when the growing plants are exposed to a limited amount of sunlight. In the human body, nitrate can be converted into nitrite. Storing, preparing or eating vegetables can also cause partial conversion of nitrate to nitrite. Nitrite can react with proteins in the body to form possibly carcinogenic N-nitroso compounds such as nitrosamines. To minimise the formation of nitrite and nitrosamines, the Netherlands Nutrition Centre has formulated recommendations[1].

On 4 December 2012, the director of the Office for Risk Assessment & Research (BuRO) of the Netherlands Food and Consumer Product Safety Authority (NVWA) was asked by the Ministry of Health, Welfare and Sport to assess the safety of nitrate and nitrite intake. Based on the outcome of this assessment, the Netherlands Nutrition Centre will then be able to review its recommendations.

Approach and action taken by BuRO

BuRO conducted a literature search and incorporated its findings into this advisory report. BuRO also took note of the fact that the NOC*NSF (Netherlands Olympic Committee*Netherlands Sport Federation) advises athletes to drink half a litre of beetroot juice, which is high in nitrate, three hours before exercising. Towards the end of 2012, the Health Council of the Netherlands and the Belgian Superior Health Council jointly published an advisory report on childhood leukaemia and recommended that pregnant women should avoid eating nitrite-cured meat, such as ham, bacon and sausages. This advisory report also deals with these issues. It has been evaluated by a number of internal and external experts.

Summary of the findings

- Based on the acceptable daily intake (ADI) for nitrate (0-3.7 mg/kg body weight; maximum 259 mg for a 70 kg adult) and nitrite (0-0.06 mg/kg body weight; maximum 4.2 mg for a 70 kg adult), guidelines have been established

[1] http://www.voedingscentrum.nl/encyclopedie/nitriet.aspx (in Dutch)

for the consumption of nitrate-rich vegetables and drinking water. The ADIs for nitrate and nitrite are based on 'no-observed-adverse-effect levels' (NOAEL) obtained from research on rats; however, rats cannot concentrate nitrate in their salivary glands and, unlike humans, do not excrete it via these glands.

- With a maximum nitrate intake and 5% conversion to nitrite, the nitrite intake is approximately 13 mg per day for a 70 kg adult, which is higher than the stipulated ADI for nitrite. A very small percentage of the Dutch population exceeds the ADI for nitrate (259 mg of nitrate for a 70 kg adult). Healthy individuals do not seem to suffer any adverse health effects as a result of this, however. Infants are at greatest risk of exceeding the ADI because of their low body weight, total nitrate intake and different physiology.
- To date, there is no evidence for the carcinogenicity of nitrate and nitrite to humans. Animal experiments have shown that nitrosamines may be carcinogenic, depending on the dietary intake of nitrosatable substances and inhibitors such as vitamin C. But N-nitroso compounds can also be ingested directly from the diet. The endogenous formation of N-nitroso compounds following the intake of nitrate in combination with fish rich in nitrosatable compounds such as amines significantly increased excretion of nitrosodimethylamine (NDMA) in urine. A meal containing nitrate-rich vegetables also resulted in a significant increase in urinary NDMA excretion (Vermeer 2000). Zeilmaker et al. (2010) concluded that acute exposure to endogenously formed NDMA appears to lead to negligible small increases of cancer risk for the Dutch population. The large number of epidemiological studies with humans has not shown a relationship between the intake of nitrate or nitrite and cancer incidence.
- It has been known since the 1980s that nitrite and nitric oxides (NO) are generated in the human body (the nitrate-nitrite-nitric oxide pathway). Some of the ingested nitrate is excreted in the saliva and converted into nitrite by bacteria in the oral cavity; nitrite is subsequently metabolised to nitrogen oxides in the stomach. Nitrite can react with, for example, proteins to form N-nitroso compounds. A few years later, the L-arginine-nitric oxide synthase (NOS) pathway was discovered. Besides NO formation from nitrite, oxidation of the amino acid L-arginine occurs in nearly all cells of the body, forming L-citrulline and nitric oxide. In the past few decades research has clearly shown that nitrosation reactions, in which nitrate, nitrite and nitrogen oxides are involved, are important fundamental processes for many bodily functions.
- The European Food Safety Authority (EFSA 2010) could not establish an Acute Reference Dose (ARfD), but did determine that the concentration methaemoglobin was not elevated in children or infants older than three months when exposure to nitrate from lettuce and spinach was below 15 mg/kg body weight per day.
- The EFSA Panel concluded in 2008 that the estimated exposures to nitrate from vegetables were unlikely to result in health risks and therefore the recognised beneficial effects of consumption of vegetables should prevail. However, a risk to some infants eating more than one spinach meal in a day could not be excluded. The EFSA Panel advised against feeding spinach to infants and children with bacterial infections of the gastrointestinal tract because such children are more sensitive to the effects of nitrate (EFSA 2010).
- The effect of a nitrate supplement (typically half a litre of beetroot juice) on sports performance has been studied. It was found that drinking beetroot juice decreased the amount of oxygen needed during exercise, increased 'time to exhaustion' and, in several cases, resulted in lowering of blood pressure. A high-nitrate diet, compared with a control diet, did not increase nitrate and nitrite concentrations in the blood, but intake of a single dose of a nitrate supplement (beetroot juice with at least 4 mmol of nitrate) resulted in elevated plasma nitrate and nitrite concentrations (Miller et al. 2012).

- Meat can be cured with nitrite to extend its shelf life, but the contribution to human nitrate and nitrite intake is small. Within the framework of the European Science Advisory Network for Health (EuSANH), a joint Committee of the Belgian Superior Health Council and the Health Council of the Netherlands published an advisory report in late 2012 on environmental factors and childhood leukaemia. The Committee said that it was advisable, "... given the possibility of a causal relation with childhood leukaemia, for pregnant women to avoid nitrite-cured meat, such as ham, bacon and sausages". This statement is based on a report by Kreis et al. (2011), which in turn made reference to Blot et al. (1999). A causal relation between eating nitrite-cured meat and the risk of childhood brain cancer and/or leukaemia could not be established. BuRO stresses the conclusion that the quality and execution of the studies left much to be desired: the consumption of fruit and vegetables or other factors was not considered and participants were not asked about the intake of other foods. Furthermore, the very small amount of nitrosamine in nitrite-cured meat (mainly dimethylnitrosamine) is not a carcinogen that crosses the placenta or causes tumours in the central nervous system in oral animal experiments.

Conclusions

- Nitrate and nitrite have long been considered toxic dietary components because of their role in the formation of nitrosamines and the possible association with cancer. Scientific evidence for adverse health effects for humans of dietary nitrate and nitrite is weak. The ADIs for nitrate and nitrite are determined based on laboratory animal experiments. Rats and humans have a completely different metabolism, however.
- A diet containing nitrate or nitrite in addition to nitrosatable substances can result in the endogenous production of N-nitroso compounds, including NDMA. The human body excretes these compounds in urine and sweat. Healthy individuals synthesise too few N-nitroso compounds to suffer any adverse health effects.
- High-risk groups, such as infants, pregnant women, patients with kidney diseases, people with bacterial infections and people with chronic oxygen deficiency (anoxia) should exercise caution in consuming nitrate-rich vegetables in combination with nitrosatable substances.
- Eating vegetables, which are a rich source of nitrate, is associated with positive health effects such as a lower blood pressure and a reduced risk of cancer and cardiovascular diseases.
- Nitrate and nitrite are essential nutrients for many physiological processes of the body, e.g. regulating blood pressure and platelet aggregation and protecting blood vessels.
- A risk-benefit assessment based on disability-adjusted life years (DALYs) shows that the positive effects of eating fish, fruit and vegetables far outweigh the risks of eating these foods, which contain nitrate.
- When the acceptable daily intake (ADI) for nitrate and nitrite were established, various factors were not taken into account: the health promoting effects of these compounds and their metabolites, the metabolic differences between between rats and humans, and the effects of other nutrients, such as antioxidants, consumed at the same time. The health-promoting effects of vegetables, which contain high levels of nitrate, should be considered.
- No studies have been found on the potential adverse health effects of long-term intake of high quantities of nitrate via food supplements, such as drinking half a litre of beetroot juice per day or taking nitrate tablets to enhance sports performances. Such research is needed.
- BuRO cannot conclude that consumption of nitrite-cured meat by mothers is linked to the risk of brain tumours and/or leukaemia in their children.

Advice of NVWA-BuRO

To the Minister of Health, Welfare and Sport and the State Secretary for Economic Affairs

- Ask EFSA via the European Commission to review the acceptable daily intakes (ADIs) of nitrate and nitrite, taking into consideration the scientific information that has come to light in recent years.
- Ask Netherlands Nutrition Centre's
  1. to retract recommendations regarding the consumption of nitrate-rich vegetables by adults because there are no indications that the estimated and recommended nitrate intakes from vegetables will result in adverse health effects;
  2. to amend the advice not to give infants younger than six months any nitrate-rich vegetables in an advice to infants younger than three months; it may, however, be assumed that infants younger than three months will not be fed vegetables;
  3. to maintain the advice not to use nitrate-rich water from private wells for bottle-feeding; and
  4. to generalise the recommendations on storing and preparing (washing) nitrate-rich vegetables to apply to all foods.

Yours sincerely,

Prof. Dr Antoon Opperhuizen
Director Office for Risk Assessment & Research

Appendix 1. Evidence base

Background
Nitrate is naturally present in vegetables and drinking water and is sometimes added to foods to extend shelf life. In the human body, nitrate can be converted into nitrite. Storing or preparing vegetables can also cause partial conversion of nitrate to nitrite. Nitrite can react with proteins to form harmful N-nitroso compounds such as nitrosamines.

Questions
To minimise the formation of nitrite and nitrosamines, the Netherlands Nutrition Centre has been advising the following guidelines for adults for some considerable time[2]:

- Do not eat nitrate-rich vegetables more than twice a week. Eating a small plate of green salad as a side dish four times a week counts as once.
- Do not store nitrate-rich vegetables for longer than two days after purchase. Bacterial growth causes the nitrite content to rise after two days.
- Wash spinach and lettuce before use and remove the midribs and outer leaves of lettuce. This can reduce the nitrate content by up to 30%.
- Quickly cool heated leftovers of nitrate-rich vegetables and immediately put them in the fridge. They can be reheated later without increasing the nitrite content.
- Nitrate-rich vegetables should preferably not be combined with fish (except for salmon and mackerel), crustaceans or shellfish.

The following additional guidelines apply for children:
- Infants younger than six months should preferably not be fed any nitrate-rich vegetables, and those aged six months and over, no more than twice a week.
- Nitrate-rich vegetables should preferably not be served with fish.
- Do not use water from private wells to bottle-feed babies. Due to runoff of fertiliser from surrounding farmland, the water may contain higher nitrate concentrations. Use tap water or still mineral water with a low nitrate content for bottle-feeding.

Based on the studies by Vermeer (2000) and Krul et al. (2004), the Netherlands Nutrition Centre issued the following advice in 2003: "Fish, crustaceans and shellfish should preferably not be combined with nitrate-rich vegetables. Salmon and mackerel are exceptions and can be combined with nitrate-rich vegetables". This advice enjoyed wide support at the time, because the risk involved was considered to be avoidable (Peters 2008).

On 4 December 2012, the director of the Office for Risk Assessment & Research (BuRO) of the Netherlands Food and Consumer Product Safety Authority (NVWA) was asked by the Ministry of Health, Welfare and Sport to assess the safety of nitrate and nitrite intake. Based on the outcome of this assessment, the Netherlands Nutrition Centre would then be able to review its recommendations.

The study
The request from the Ministry of Health, Welfare and Sport prompted an up-to-date overview of studies on the effects of nitrate intake. BuRO conducted a

[2] http://www.voedingscentrum.nl/encyclopedie/nitriet.aspx (in Dutch)

literature search and incorporated its findings into this advice. BuRO also took note of the fact that the NOC*NSF (Netherlands Olympic Committee*Netherlands Sport Federation) advises athletes to drink beetroot juice as a nitrate supplement. Towards the end of 2012, the Health Council of the Netherlands and the Belgian Superior Health Council (Health Council 2012) jointly published an advisory report on childhood leukaemia and recommended that pregnant women should avoid eating nitrite-cured meat, such as ham, bacon and sausages. Both of these issues are also addressed in this advice, which has been reviewed by peers.

Research results

Dietary nitrate

Vegetables, especially leafy vegetables such as lettuce and spinach, and drinking water are important sources of nitrate. 50-85% of dietary nitrate comes from vegetables; in the Netherlands 7-9% comes from drinking water (Geraets et al. 2011). The bioavailability of nitrate is close to 100% (EFSA 2008, van Velzen et al. 2008). The nitrate levels in vegetables partly depend on their breed and can increase if a large amount of chemical fertiliser or manure is used in their cultivation or if the growing plants are exposed to limited sunlight. Low-nitrate breeds are usually selected for freezing vegetables and for baby food in jars. Before being frozen, spinach is quickly blanched and then chilled; this process results in very little or no nitrite formation.

Nitrate in plants plays a crucial role in their nutrition and function. Nitrate is taken up from the soil and accumulates in the leaves. The nitrate concentration is highest in the stem, leaf stalk and leaf veins, lower in the mesophyll and very low in fruits and flowers. This also explains the higher nitrate levels in leafy vegetables compared with other types of vegetables (Weitzberg and Lundberg 2013). Research has shown that removing the outer leaves and the main veins in lettuce and discarding the stems and veins in spinach reduces the nitrate content by 20-40% (Chan 2011). Nitrate dissolves readily in water so washing vegetables - and certainly cooking them - reduces the nitrate level.

Kinetics of nitrate, nitrite and N-nitroso compounds

It has been known since the 1980s that nitrate can be converted into nitrite in the human body (the nitrate-nitrite-nitric oxide pathway). Nitrate derived from food or drinking water is completely absorbed in the upper part of the small intestine. Nitrate circulates in plasma, distributes over the tissues and has a half-life of approximately five hours. About 25% (20-28%) of the nitrate ingested is excreted in saliva (Hord et al. 2009, van Velzen et al. 2008), while the rest is excreted by the kidneys. Around 20% of the nitrate in saliva, 4-8% of the nitrate ingested, is converted into nitrite by bacteria in the oral cavity; nitrite is then metabolised to nitric oxide (NO) and nitroso compounds in the acidic environment of the stomach. N-nitroso compounds contain a nitrogen atom (N), to which a nitroso group (NOx) is attached. The half-life of NO is in the order of milliseconds and it is rapidly oxidised to nitrite and other N-oxides and N-nitroso compounds. Nitrite can be converted to nitrate in the plasma and excreted in saliva or urine. Humans produce more than a litre of saliva per day. Nitrite concentrations in fasting saliva are a thousand times higher than in plasma. This highlights the fact that substantial amounts of nitrite are constantly being formed in the body. Normal nitric oxide synthesis is estimated to be approximately 1 mg/kg body weight per day; there is consequently an endogenous flux of around 60 mg of nitric oxide per day for adult humans through the conversion of nitrite to nitrate (Archer 2002). Besides nitric oxide formation from nitrite, oxidation of the amino acid L-arginine occurs in nearly all cells of the body, forming L-citrulline and nitric oxide (l'Hirondel and l'Hirondel 2001). Hord et al. (2009) estimate that a 60 kg adult produces 1.44 mmol (approximately 43 mg) nitric oxide per day. The L-arginine-nitric oxide synthase (NOS) pathway is oxygen-dependent, whereas the nitrate and nitrite-mediated nitric oxide production occurs at lower oxygen tensions. The nitrate-nitrite-nitric oxide pathway can therefore be viewed as a back-up system to ensure sufficient nitric oxide levels under conditions of reduced oxygen availability.

Of all N-nitroso compounds, nitrosodimethylamine (NDMA) is most frequently present in the human environment. The formation of an N-nitroso compound requires two reaction partners: a nitrosating agent such as nitrite in an acid environment or nitric oxide and a nitrosatable substance such as an amine or amide. In the human stomach, the conditions for nitrosamine formation reactions are optimal after a meal.

Health-promoting properties

The discovery that nitric oxide was the long-studied 'endothelium-derived relaxing factor' resulted in a paradigm shift in the understanding of control of physiological processes. In 1992 Science Magazine declared it to be 'molecule of the year' and in 1998 the pioneering researchers in this field were awarded the Nobel Prize in Physiology or Medicine. Nitrosation is a fundamental physiological process and involves adding a nitrosonium ion (NO+) to an organic compound, primarily thiols (S–NO) or amines (N-NO). Primary amines (R–NH2) react with nitrite to the very unstable N-nitrosamines that degrade into alcohols. However, secondary amines (R1-NH-R2) react to stable N-nitrosamines, most of which have been shown to be carcinogens in rodent bioassays after activation by cytochrome P-450 enzymes (Bryan et al. 2012). The first report back in the 1950s on the liver carcinogenicity of N-nitrosodimethylamine (NDMA) sparked interest in N-nitrosamines and their possible association with cancer. There are numerous effective inhibitors of N-nitrosation reactions in biological systems, such as vitamins C and E.

Although the formation of N-nitrosamines is generally considered harmful, biomedical science over the past 20 years has recognised nitrosation reactions as important fundamental processes (Bryan et al. 2012) that play a part in the following (see also Weitzberg and Lundberg 2013).

- The conversion of nitrate to nitrite and nitric oxide contributes to preventing and combating gastrointestinal infections and increasing mucus production and gastric blood flow (Gilchrist et al. 2011). Nitrite kills bacteria under acidic conditions, such as in the stomach or mouth.
- S-nitrosothiols are natural carriers of nitric oxide and potent inhibitors of blood platelet aggregation and hence of blood clot formation (McKnight et al. 1999).
- NO can cause conversion of *Heliobacter pylori* from spiral to coccoid form. The coccoid organisms are non-replicating and, unlike the spiral form, are incapable of inducing interleukin-8 secretion by gastric epithelial cells, with the result that there is no inflammatory response (Cole et al. 1999). *H. pylori* infection plays a role in nearly all cases of stomach cancer; moreover, it reduces the availability of vitamin C.
- De Bont and Van Larebeke (2003) documented the following processes:
  - An increase in the production of nitric oxide can contribute to vasodilation during exercise;
  - Nitric oxide is involved in sperm function and hormone concentrations in women;
  - The nitrate concentration in breast milk is higher than in plasma during the first five days following childbirth. It is probable that nitric oxide is

synthesised in the breast itself. It may stimulate lactation or may be involved in the process of adapting to breastfeeding;
- Many pathological conditions, such as gastroenteritis, stomach cancer, infectious diarrhoea, hypertension, hepatitis C and depression, are associated with an increase in plasma nitrate concentration.

- Nitrite can increase blood flow, thereby facilitating oxygen delivery to hypoxic tissues.
- Nitric oxide is a key regulator of renal and cardiovascular function. Oxidative stress and subsequent nitric oxide deficiency in the kidney are associated with the development of hypertension and other forms of cardiovascular disease (Lundberg et al. 2011, Sindelar and Milkowski 2012). An aspect of vascular endothelial dysfunction is a reduced ability to make nitric oxide via the L-arginine-dependent pathway by endothelial nitric oxide synthase (Miller et al. 2012).
- Continuous generation of nitric oxide is important for communication in the nervous system and nitric oxide is a critical molecule in the immune system (Milkowski et al. 2010, Sindelar and Milkowski 2012).
- Nitric oxide has a role in wound repair (Sindelar and Milkowski 2012).
- A diet rich in fruit and vegetables lowers blood pressure and reduces the risk of cardiovascular disease and certain forms of cancer. Dietary nitrate is a likely contributor to these effects (Joris and Mensink 2013, Lundberg et al. 2006, Machha and Schechter 2012, Milkowski et al. 2010, Tang et al. 2011, Webb et al. 2008). Weitzberg and Lundberg (2013) concluded that the blood pressure-lowering effect of nitrate is confirmed by research, particularly among healthy young people, but also among older adults. There appears to be a dose-related effect of nitrate on blood pressure.
- The effect of fruit and vegetables on the development of type 2 diabetes has been studied. A meta-analysis showed that there was no effect from higher fruit and/or vegetable intakes. However, incidence of the disease was 14% lower in people who consumed larger quantities of green leafy vegetables. Similar results were recently presented by the European Prospective Investigation into Cancer - InterAct cohort study (Cooper et al. 2012): green leafy vegetable intake was inversely associated with diabetes. Further research on this association is needed, ensuring that a clear distinction is made between fruit and vegetables and between different kinds of vegetables (Weitzberg and Lundberg 2013).

Harmful properties

Methaemoglobinemia
This blood disorder can be caused by exposure to nitrate and/or nitrite through food or drinking water or as a result of enteritis. Nitrite reacts with iron (II), converting it to iron (III) in haemoglobin; this results in the formation of methaemoglobin, which interferes negatively with oxygen transport. The sensitivity of babies to nitrate intake decreases rapidly from the age of three months. In newborn infants, some haemoglobin is still in the form of foetal haemoglobin, which is more readily oxidised to methaemoglobin by nitrite than adult haemoglobin. Furthermore, the enzyme responsible for the reduction of methaemoglobin back to haemoglobin (NADH-dependent methaemoglobin reductase) is 40-50% less active in babies. As a result of low lactic acid production, infants have a relatively high gastric pH, which promotes the growth of nitrate-reducing bacteria. This can lead to gastroenteritis and increased nitrite formation. These factors increase the risk of methaemoglobin formation in infants and young children (De Bont and Van Larebeeke 2003, EFSA 2008), which may result in an oxygen deficiency ('blue baby syndrome'). Clinical symptoms may occur if 3% or more of the haemoglobin is in the form of methaemoglobin. Fifty

percent methaemoglobin can be fatal (Mensinga et al. 2003). Methaemoglobinemia is now virtually non-existent in Western Europe, because water from private wells, which may contain -high nitrate concentrations due to runoff of fertiliser from surrounding farmland, is no longer used for bottle-feeding. Neonates and infants up to the age of six months are not usually fed nitrate-rich vegetables either. EFSA (EFSA 2010) could not establish an acute reference dose (ARfD) but did determine that methaemoglobin was not elevated in children or infants above three months old when exposure to nitrate was below 15 mg/kg body weight per day.

Nitrate intake during pregnancy

Brender et al. (2013) carried out a case-control study on the relationship between nitrate concentrations in drinking water and the occurrence of birth defects and found that women who had babies with neural tube defects, defects of limbs or a cleft palate had a greater chance than mothers in the control group to consume 5 mg nitrate or more per day via drinking water. The authors concluded that the formation of N-nitroso compounds is probably not the underlying mechanism. For these American women, about 6% of the total daily exposure to nitrate came from drinking water. Given the small contribution of nitrate from drinking water to total nitrate intake, it is unlikely that the increase in birth defects is due to the nitrate levels in drinking water. The (small) differences in birth defects that were found, can be caused by many other factors (EFSA 2008, Wolterink et al. 2013). In Netherlands, the contribution from drinking water is 9% of the total nitrate intake, assuming a nitrate concentration in drinking water of 5.1 mg/l, the average of 1118 samples from 2006 (Geraets et al. 2011). Also this data shows that the nitrate intake from food is significantly higher than from drinking water.

Carcinogenicity of N-nitroso compounds

An expert working group convened by the International Agency for Research on Cancer (IARC) in June 2006 found that dietary nitrate or nitrite is probably carcinogenic to humans (Group 2A). This was based on conclusions that there is no adequate evidence of the carcinogenicity of nitrate in experimental animals; sufficient evidence of the carcinogenicity of nitrite in experimental animals in combination with amines or amides; and limited evidence of the carcinogenicity of nitrite *per se* in experimental animals. However, the methodological aspects and conclusions of animal and *in vitro* testing can be disputed. Consideration should be given to, amongst others, the use of preformed N-nitrosamines, differences between experimental animals and humans (rodents have a rumen, for example) and the high concentrations of the substances tested.

NDMA is toxic after acute oral administration to rats, with LD50 ranging from 23 to 40 mg/kg body weight per day. After repeated oral exposure over a short period (3-4 weeks), hepatic effects, often associated with reduced survival, have been observed in a number of mammalian species (such as rats, mice, hamsters, guinea pigs and monkeys). In addition, congestion in various organs (i.e. kidneys, lungs, spleen and myocardium) and gastrointestinal haemorrhages have been observed in rats. In humans, two deaths linked to acute ingestion of NDMA, as well as a third attributed to the consumption of at least four doses of approximately 250-300 mg NDMA over a two-year period, have been reported. Liver failure was observed in three cases and in two acute cases cerebral haemorrhage was diagnosed (FAVV 2010). There is ample evidence that NDMA is carcinogenic both *in vitro* and *in vivo* (WHO 2008). NDMA increases the incidence of tumours in liver and Leydig cells in rats ingesting nitrosamine from drinking water (5 mg/l) or food (10 mg/kg body weight). Hepatic, pulmonary and renal tumours were observed in mice following administration of NDMA via drinking water (0.01 - 5 mg/l). Moreover, in some cases, the period of exposure was relatively short (e.g. three weeks). NDMA is also genotoxic both *in vitro* and *in vivo* (WHO 2008). Nitrosamines require metabolic activation to become reactive and to cause DNA damage and mutations that may ultimately result in cancer. Toxic doses of some nitrosamines in rats can damage insulin-producing cells in the pancreas and hence cause diabetes mellitus.

The large number of human epidemiological studies has failed to demonstrate a causal association between nitrate and nitrite intake and stomach cancer incidence or mortality rates (Bryan et al. 2012, l'Hirondel and l'Hirondel 2001) or the risk of other types of cancer (EC 1995, NRC 1995). Since people are also exposed to harmful nitrosamines from, for example, cigarette smoke or cosmetics, the specific risk of nitrate and nitrite in food is difficult to establish. The relationship between nitrate, nitrite and NDMA intake and risk of cancer of the gastrointestinal tract was studied in a cohort of 9,985 adult Finnish men and women. A positive association between intake of NDMA from smoked and salted fish and risk of colorectal cancer was observed. No relationship was found between cancer incidence and nitrate or nitrite intake (Knekt et al. 1999).

The endogenous formation of N-nitroso compounds after intake of a quantity of nitrate (dissolved in drinking water) at the ADI level, in combination with a fish meal rich in nitrosatable compounds such as amines, has been studied in humans. The results showed a significant increase in NDMA excretion in urine (Vermeer 2000). This implied that there is a risk of increased formation of carcinogenic nitrosamines after nitrate intake (Vermeer 2000). A meal containing nitrate-rich vegetables (274 mg nitrate) also resulted in a significant (240%) increase in urinary NDMA excretion, demonstrating that the dietary level of nitrosation inhibitors is not sufficient to prevent endogenous formation of N-nitroso compounds after intake of a quantity of nitrate at the ADI level. In this study, a detectable base level of NDMA was always present in urine. Some of this NDMA is probably directly derived from the diet. Subsequently, the inhibition of endogenous nitrosamine formation in humans through vitamin C supplements and consumption of green tea was studied. Urinary NDMA excretion was significantly decreased after intake of 250 mg nitrate, 1 gram vitamin C and four cups of green tea (Krul et al. 2004, Vermeer 2000). Intakes of higher doses of vitamin C had no additional effect. Consumption of 4 grams (eight cups) of green tea, which contains oxidising phenolic compounds, resulted in a significant increase in NDMA excretion in urine (Vermeer 2000).

Tricker (1997) concluded that the concentration of N-nitroso compounds at which there is no longer any clearly demonstrable carcinogenic effect is around 1-5 ppm and that the daily ingestion of an average consumer is clearly below this limit (approximately 1.10 µmol/day), making the potential negative impact of continuous exposure difficult to assess (ATSDR 2004). In the US, an MRL (maximum residue limit) has been determined for drinking water, ranging from 6×10^{-4} µg/l to 2.4×10^{-2} µg/l (Valentine et al. 2006).

At the request of the former VWA, the National Institute for Public Health and the Environment (RIVM) studied the formation of NDMA using an *in vitro* gastrointestinal model to simulate NDMA formation upon intake of fish and vegetables rich in nitrate, based on the results of the Dutch National Food Consumption Survey 1997-1998. The 95th percentile of the long-term exposure distribution was approximately 4 and 0.4 ng/kg body weight for young children and adults, respectively. About 50% of the children and 5% of the adults had a long-term exposure above the acceptable value. In terms of cancer risk, the effects of exceeding the exposure limit were found to be limited, however: the 95th percentile of these exposures was 6×10^{-6} extra risk for five-year-old children and 8×10^{-7} for adults. Estimated in this way, 1% of five-year-olds exceeded the acute exposure limit of 110 ng/kg (derived in this study) on 2% of the consumption days. For an extra risk of 10^{-6}, the decrease in 'time to tumour' was estimated at 3.8 minutes in rats, equivalent to 0.1 days in humans. The conclusion of the study was that acute exposure to NDMA leads to a negligible small cancer risk for the Dutch population (Zeilmaker et al. 2010).

A Panel commissioned by the World Cancer Research Fund (WCRF/AICR 2007) concluded in 2007 that there was limited evidence suggesting that red meat and processed meat were causes of cancer. Meat can be processed in many ways. However, processing usually refers to red meat preserved by smoking, salting or the addition of nitrite or other preservatives. The term is not used consistently and is often not clearly defined in epidemiological studies. The process by which nitrite reacts with degradation products of amino acids to form N-nitroso compounds can occur in meat during the nitrite curing process or in the body (particularly in the stomach) as a result of nitrite or nitrate intake. Eating red meat increases the levels of N-nitroso compounds in the body, which may be partially due to its high haem content. Haem promotes the formation of N-nitroso compounds and contains iron. Free iron can lead to the production of free radicals. The Panel concluded that processed meat was a cause of colorectal cancer and that there was limited evidence for a causal association between processed meat intake and oesophageal, lung, stomach and prostate cancer. On the other hand, there was limited evidence suggesting that eating fish protects against colorectal cancer and that fruit and vegetables protect against oesophageal and stomach cancer (WCRF/AICR 2007).

In a prospective study involving more than 23,000 men and women aged 40-79 years in Norfolk (UK), the relationship between the intake of N-nitroso compounds (NDMA) through diet (about 0.06 µg/day), the endogenous N-nitroso compounds index, nitrite from the diet and cancer incidence was investigated. After a study period of 11.4 years a relationship was found between intake of NDMA from the diet and a higher incidence of gastrointestinal cancer. A significant interaction was found between the plasma concentration of vitamin C and the intake of NDMA on cancer incidence. The N-nitroso compounds index, an estimate of the endogenously formed N-nitroso compounds, and the intake of dietary nitrite were not significantly associated with cancer risk (Loh et al. 2011). The index for the endogenously formed N-nitroso compounds is based on an increased production of N-nitroso compounds in the colon after consumption of red meat.

Intake

Vegetables contribute 50-70% to the overall intake of nitrate (EFSA 2008). In the case of children (1-19 years old), chronic dietary nitrate intake varied between 0.77 and 1.39 mg/kg body weight per day, and the 97.5th percentile (P97.5) of the exposure between 2.95 and 4.76 mg/kg body weight per day (EFSA 2010). The highest levels were seen in young children. The median intake of children aged 1-4 was 1.39 and the P97.5 intake was 4.76 mg/kg body weight per day. Acute intake was estimated to vary between 0.8 and 3.8 mg/kg body weight per day, assuming nitrate levels in spinach of 816 mg/kg. A possible nitrate intake of 13.8 mg/kg body weight per day from spinach consumption was estimated for the 12-month-old age group at the current maximally permitted legal level of nitrate in spinach. Dietary intake of nitrate was higher in the younger age groups, and was estimated to be at most 23.3 and 27.2 mg/kg body weight per day at nitrate concentrations of 3,000 and 3,500 mg/kg, respectively, in six-month-old infants. This did not include nitrate intake from water (EFSA 2010).

In the Netherlands, based on data from the Dutch National Food Consumption Survey 1997-1998, the dietary intake of children aged between 1 and 3 years (n=156) was calculated. The median intake was 2.3 mg/kg body weight per day; the P97.5 was 5.1 mg/kg body weight per day (Westenbrink et al. 2005). Twenty per cent of the one-year old children exceeded the ADI for nitrate (van Asselt et al. 2008). Based on the intake data from the children's Dutch National Food Consumption Survey 2005-2006, the median intake was 1.9 mg/kg body weight per day for 2-year-olds (Boon et al. 2009). According to the 2007-2010 survey, the long-term intake (P50) for children aged 7-16 was 1.2 mg/kg body weight per day; the P95 was 2.0 mg/kg body weight per day. The corresponding figures for adults were 0.9 and 1.8 mg/kg body weight per day, respectively (Geraets et al. 2011). It was found that 0.04% of the children (7-15 years old) and 0.03% of the adults (16-69 years old) exceeded the ADI for nitrate. Occasionally exceeding the ADI does not *per se* indicate a health risk, because the ADI for nitrate was derived from subchronic and chronic studies and relates to a lifelong intake (EFSA 2010).

N-nitroso compounds have also been shown to occur in foods. These compounds are formed in foods high in nitrite and protein, such as meat, fish, cheese and spinach, by binding of nitrite to proteins when these foods are heated at high temperature, e.g. when roasted, baked or smoked. The intake of N-nitroso compounds was estimated at 36-140 µg per day in the UK, the average intake of NDMA at between 0.1 and 101 µg per day (Gangolli et al. 1994). Based on a rough mass balance of exogenous exposure and excretion via the urine and faeces, it was found that 45-75% of the N-nitroso compounds (nitrosamines) were formed endogenously (Tricker 1997). When N-nitrosamines were found in foods over the past forty years, production methods and ingredients were altered or omitted so as to eliminate or minimise the formation of these compounds. Examples include new techniques for brewing alcoholic beverages and the use of nitrosation inhibitors in nitrite-cured and processed meat. This is also the objective of a European project under the Seventh Framework Programme, the PHYTOME project[3].

It is worth noting that a daily diet of the recommended 200 grams of vegetables and two pieces of fruit will exceed the ADI for nitrate. For a 70 kg adult, the ADI is 259 mg nitrate, which can be provided by less than 200 grams of spinach, beetroot, rocket or lettuce. The recommendations for a healthy diet are based on the health-promoting properties of fruit and vegetables. Green leafy vegetables, which have the highest nitrate concentrations, are foods that also offer the highest protection against coronary heart disease, stroke and type 2 diabetes (Weitzberg and Lundberg 2013).

<u>EU maximum levels of nitrate</u>
Limits have been set for nitrate concentrations in food and drinking water. Nitrate and nitrite, mixed with table salt or potassium salt, may be used as an additive in, for example, semi-hard or hard cheeses and prepared meat products to increase shelf life or to enhance colour and aroma. Permitted additives are E249 (potassium nitrite), E250 (sodium nitrite), E251 (sodium nitrate) and E252 (potassium nitrate). The maximum amounts that may be added during manufacture to various foods range from 100 to 500 mg/kg and maximum residual levels vary from 10 to 250 mg/kg food (2006/52/EC). This maximum amount is well below the (permitted) nitrate levels in vegetables, which may contain up to 4,500 mg nitrate per kg fresh weight. Nitrite protects primarily against the bacterium *Clostridium botulinum*, which produces a virulent toxin.

3 http://www.phytome.eu/v2/

A European standard of 50 mg nitrate per litre of water applies for drinking water, though the Netherlands has an even lower target level of 25 mg/l. Spring water sold in bottles may contain no more than 45 mg nitrate per litre. In practice, however, nitrate levels in spring water are usually much lower.

European environmental policy on fertiliser and minerals is specified in the 1991 Nitrates Directive (91/676/EEC). Maximum levels for nitrate have been set for two products: fresh lettuce (with separate levels for iceberg-type lettuce) and fresh spinach (with a separate level for deep-frozen/preserved spinach) (Commission Regulation (EC) No. 1881/2006). Member States may request derogation from the European Directive.

Acceptable daily intake (ADI)
FAO/WHO (1996) mentions human lethal doses of 4-50 g nitrate. Methaemoglobin formation was reported at intakes of 33-150 mg nitrate per kg body weight. However, in a study in which twelve volunteers received 9.5 g sodium nitrate intravenously in one hour, no toxic symptoms were observed. In another study, 7-10.5 g ammonium nitrate administered orally in one dose caused vomiting and diarrhoea in two of twelve people. FAO/WHO (1996) assumed that the lethal dose of nitrate in adults is probably around 20 grams. The acute oral lethal dose of nitrite reported for humans varies from 33 to 250 mg nitrite per kg body weight. Toxic doses that lead to methaemoglobinemia are between 0.4 and 200 mg/kg body weight (FAO/WHO 1996).

The ADI for nitrate is set at 0-3.7 mg/kg body weight (EC 1992, EC 1995, FAO/WHO 2003a). For nitrite, the Scientific Committee for Food (SCF) established an ADI of 0-0.06 mg/kg body weight (EC 1997), and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) an ADI of 0-0.07 mg/kg body weight per day (FAO/WHO 2003b). This is the maximum amount a person can ingest on a daily basis over a lifetime without a health risk. The ADIs are based on a no-observed-adverse-effect level (NOAEL) (heart and lung abnormalities) obtained from tests on rats; a safety factor of 100 was applied. However, rats cannot concentrate nitrate in their salivary glands and, unlike humans, do not excrete it via these glands. Moreover, JECFA argued in 2003 that there was an increased cancer risk with increasing nitrate intake. However, there is no evidence of a relationship between intake of nitrate, nitrite or N-nitroso compounds and stomach-, brain-, oesophageal- and throat cancer (Archer 2002, Bryan et al. 2012, Gilchrist et al. 2011, Milkowski et al. 2010). WCRF/AICR (2007) concluded that processed meat was a cause of colorectal cancer and that there was limited evidence of a causal relationship between processed meat intake and oesophageal-, lung-, stomach- and prostate cancer. It should be pointed out that 'processed meat' is not always clearly defined in studies.

The US Environmental Protection Agency (EPA) calculated the so-called reference dose (RfD) corresponding to the ADI. In 1991, EPA established an RfD for chronic oral exposure for nitrate based on the risk of infant methaemoglobinemia. This RfD of 7 mg nitrate per kg body weight per day (1.6 mg nitrate-nitrogen per kg body weight per day) is higher than the ADI derived by FAO/WHO and EU (EPA 1991).

EFSA issued a scientific opinion on 'Nitrate in vegetables' in 2008 and an additional opinion in 2010 on acute effects of nitrate intake from lettuce or spinach in children. In its 2008 opinion, the EFSA Panel concluded that the estimated nitrate intakes from vegetables were unlikely to result in health risks and therefore the recognised beneficial effects of consumption of vegetables should prevail. EFSA concluded in 2010 that some vegetables, particularly leafy vegetables such as lettuce and spinach, contain relatively high levels of nitrate, especially when grown under cover or in conditions of reduced lighting. The nitrate exposure of infants and young children is unlikely to be a health concern, although a risk for some infants eating more than one spinach meal in a day could not be excluded. EFSA (2010) could not establish an acute reference dose (ARfD), but did conclude that methaemoglobin was not elevated in children or infants above three months old when intakes of nitrate from lettuce and spinach were below 15 mg/kg body weight per day. The EFSA Panel advised against feeding spinach to infants and children with bacterial infections of the gastrointestinal tract because such children are more sensitive to the effects of nitrate (EFSA 2010).

Risk-benefit assessment
In a 2004 RIVM report entitled 'Our food, our health' (van Kreijl et al. 2004), the possible health gain of avoiding exposure to nitrate and nitrite and the associated formation of nitrosamines was estimated at 100-500 DALYs (disability-adjusted life years, a health indicator that combines effects on illness and death, using a disability weighting factor). RIVM commented that the calculation of the cancer risk posed by genotoxic carcinogens, such as nitrosamines, was somewhat conservative due to the hypotheses applied and may represent an overestimate of the actual risk. Based on a more accurate estimate in 2010, it was concluded that acute exposure to NDMA appeared to lead to a negligible small cancer risk for the Dutch population (Zeilmaker et al. 2010).

In 'Our food, our health' (van Kreijl et al. 2004), the health gain to be achieved in terms of avoidable illness or death from cardiovascular diseases if everyone in the Netherlands were to eat fish at least once a week was estimated to be 82,000 DALYs. Following the recommendations for fruit and vegetables (200 grams per day of each) would lead in 2004 to a health gain of 47,000 and 95,000 DALYs, respectively. Foodborne infections from eating raw vegetables resulted in a health loss of approximately 50-200 DALYs. It can be concluded from these estimates that the positive effects of eating fruit and vegetables far outweigh the risks (van Kreijl et al. 2004).

Effects of nitrate supplements (beetroot juice)
The effect on sports performance of dietary nitrate supplementation in the form of beetroot juice is recommended (NOC*NSF 2012) and has been studied. Drinking beetroot juice decreased the amount of oxygen needed during exercise and increased 'time to exhaustion'. Compared with a placebo, consuming a daily beetroot juice supplement (500 ml/day, approximately 8 mmol nitrate per day) for six consecutive days lowered the average VO_2 values (oxygen uptake) during submaximal exercise and improved the 10-km performance of trained cyclists (Cermak et al. 2012), runners (Lansley et al. 2011) and athletes (Bailey et al. 2009). Eight cyclists drank half a litre of beetroot juice per day (5.2 mmol nitrate per day) or were given a placebo for 4-5 consecutive days. Nitrate intake resulted in a reduction in oxygen consumption, enhanced performance and a lowering of blood pressure (Lansley et al. 2011, Vanhatalo et al. 2010). Nitrate supplementation with potassium nitrate capsules of 4 versus 12 mmol (1488 mg nitrate) or 24 mmol potassium nitrate versus 24 mmol potassium chloride or 250 ml beetroot juice (5.5 mmol nitrate) versus 250 ml water caused a dose-dependent increased plasma nitrite concentration and decreased blood pressure in healthy volunteers (Kapil et al. 2010). In 15 men and 15 women (aged 23-68 years), the ingestion of 500 g beetroot and apple juice (15 mmol nitrate/l) as a supplement to a normal diet only led to a statistically significant decrease in blood pressure in men six hours after drinking the juice. The average age of the men was 36 years, compared with 49 years for the women (Coles and Clifton 2012). In

a four-period (each period lasting three days) crossover study, eight older adults with an average age of 72.5 years were given either a control diet (3 mg nitrate and 28 µg nitrite per day) or a high-nitrate diet (155 mg nitrate and 246 µg nitrite per day) with or without supplement (500 ml beetroot juice with 527 mg nitrate (8.5 mmol) and 15.9 µg nitrite (0.3 µmol)). The intake from the high-nitrate diet with supplement was 8.4 mg nitrate/kg body weight. Plasma nitrate and nitrite levels were elevated in the groups that received the supplement. There was no difference in plasma concentrations between intake from the high-nitrate diet plus supplement and that from the control diet plus supplement and no effects on blood pressure were observed (Miller et al. 2012). The authors reported that a single dose of 4 mmol nitrate caused physiological effects and increased plasma nitrate and nitrite concentrations. This might explain why no effects in plasma concentrations were measured in the study with a nitrate dose of 2.5 mmol in the high-nitrate diet (Miller et al. 2012).

Effects of curing meat with nitrite
In the 1950s and 1960s the potential of forming nitrosamines from nitrate and nitrite was discovered and it ignited a debate about the safety of ingested nitrite, which ultimately focused on cured meats (Sindelar and Milkowski 2012). Meat can be cured with nitrite to prevent botulism and extend its shelf life. The contribution of cured meats to the human intake of nitrate and nitrite is small. Moreover, meat curing practices have been drastically changed to prevent or minimise nitrosamine formation (Archer 2002, Sindelar and Milkowski 2012).

Within the framework of the European Science Advisory Network for Health (EuSANH), a joint Committee of the Belgian Superior Health Council and the Health Council of the Netherlands (Health Council 2012) published an advisory report in late 2012 on childhood leukaemia and environmental factors. In evaluating scientific knowledge and in formulating recommendations, the Committee was guided by the precautionary principle. The Committee said that it was advisable, "...given the uncertainty of a causal relation with childhood leukaemia, that pregnant women avoid consuming nitrite-cured meat, such as ham, bacon and sausages". This recommendation was substantiated by referring to research on experimental animals exposed to N-nitroso compounds via the placenta. It was argued that these compounds are also sometimes found in cured meat and that consumption of meat cured with nitrite is a potential risk factor for the development of childhood cancer. Overall, the Committee considered a causal relation between the consumption of cured meat during pregnancy and childhood leukaemia to be uncertain, but did recommend limiting the intake of nitrite-cured meat (for example ham, bacon and sausages) by pregnant women. The analysis was based on a study by Kreis et al. (2011), which in turn was based on Blot et al. (1999).

Blot et al. (1999) reviewed fourteen epidemiological studies, of which thirteen were case-control studies that examined the relationship between the consumption of cured meat during pregnancy and the subsequent risk of brain tumours and other cancers in the offspring. Most of the studies showed no statistically significant relationship between total cured meat intake and childhood cancer risk. A statistically significant trend for the association between cured meat and brain cancer was found in four studies, but six studies showed no such trend. In the other four studies other cancers were examined and none reported an association. In the four studies in which a possible association with brain cancer was found, there was no clear association with nitrite-cured meat. For example, an association was found with the mother's intake of hot dogs, bacon and sausages, but not with luncheon meat or ham; or a higher risk for hot dogs and ham but not for bacon; for bacon but not for hot dogs or ham; for hot dogs but not for bacon/ham, bacon/salami or luncheon meat. In the case of leukaemia, an increased risk was found for consumption of bacon/ham but not for hot dogs or luncheon meat. Another study reported an increased risk for the mother's consumption of hot dogs but not of bacon/ham/sausages. In these studies, the intake of fruit and vegetables was not controlled for, it was difficult to estimate the actual consumption of the meat products studied and the effect of socio-economic status and the time interval between cancer diagnosis and interview was not clear. Most of the studies found no link with the mother's smoking. Other factors significantly related to childhood cancer risk included low birth weight; short height-for-age; use of incense, marihuana, antihistamines, pesticides, fly and tick spray; hairdryers; traffic density; paternal exposure to creosote or employment in the chemical industry; and maternal exposure to pigs and horses. From a methodological point of view, there is much that can be said about the studies reviewed (Blot et al. 1999). For example, the intake of cola was considered to be a 'neutral' indicator in two studies. Thus, no association was expected with the incidence of cancer, yet a positive association with cola intake was found. This might be explained by the fact that the mothers of children with cancer considered cola to be unhealthy and reported its intake more often than the mothers of children in the control group. Moreover, only the intake of a limited number of foods was included in the study. Besides cured meat, participants were only asked about two other types of red meat and nothing about, for example, fish consumption or preparation methods. A number of studies showed a positive association between maternal consumption of hamburgers and childhood cancer; however, hamburgers do not usually contain nitrite or other preservatives.

The research was prompted by studies showing transplacental exposure to N-nitroso compounds, nitrosamides and mainly nitrosoureas resulting in brain tumours in laboratory animals. The carcinogenic effect cannot be extrapolated to *in utero* exposure to nitrosamines. The very small amount of nitrosamines in nitrite-cured meat consists mainly of dimethylnitrosamine (NDMA), which is not a carcinogen that crosses the placenta or causes tumours in the central nervous system in oral animal experiments (Blot et al. 1999). Furthermore, the incidence of childhood brain cancer in the US rose by more than 20% between the 1970s and 1990s, with higher rates reported for white children than for black children. During the same period, the nitrite levels in cured meat dropped sharply (by up to 80%) and consumption fell by 17% in the US. Blot et al. (1999) therefore report that it cannot be concluded that eating cured meat has increased the risk of childhood brain cancer or any other cancers, but that an association cannot be definitely ruled out.

Many processed meats contain high levels of salt and nitrite. Meat cooked at high temperatures can contain heterocyclic amines and polycyclic aromatic hydrocarbons, which can have harmful health effects. Red meat also contains haem, which promotes the formation of N-nitroso compounds and contains iron. Free iron can lead to the production of free radicals (WCRF 2007). Antioxidants such as vitamins C and E inhibit the formation of nitrosating compounds by promoting the formation of nitric oxide from nitrite and are therefore often added to processed meat. If all of these factors are not known, it is difficult to draw unequivocal conclusions from studies.

Conclusions

- Whereas nitrate and nitrite were considered harmful food additives, they are now regarded as indispensable nutrients important for, amongst others, cardiovascular health by promoting nitric oxide production. Nitrate and nitrite are needed, for example, to regulate blood pressure and platelet aggregation and to protect blood vessels.

- Evidence for adverse health effects of dietary nitrate and nitrite is weak, and intakes above the ADI might well be harmless (Katan 2009).
- The intake of vegetables, which contain vitamin C and other antioxidants as well as nitrate, lowers blood pressure and reduces the risk of cancer and cardiovascular diseases.
- Nitrite can react *in vitro* with nitrosatable substances to form nitrosamine, and nitrosamine causes cancer in rats (Zeilmaker et al. 2010). Formation of harmful N-nitroso compounds such as NDMA can also occur in humans, depending on the intake of nitrosatable substances and dietary inhibitors (Vermeer et al. 1998).
- The current scientific evidence indicates that usual dietary intake and endogenous formation of nitrate and nitrite do not entail an increased risk of (stomach) cancer (Bryan et al. 2012).
- The risk of methaemoglobinemia in children is limited and is not reported at nitrate intakes below 15 mg/kg body weight per day.
- When the acceptable daily intakes (ADIs) for nitrate and nitrite were established, various factors were not taken into account: the health-promoting properties of these substances and their metabolites, the metabolic differences between rats and humans, and the effects of other nutrients, such as antioxidants, consumed at the same time.
- A very small percentage of the Dutch population exceeded the ADI for nitrate (259 mg nitrate for a 70 kg adult). Infants are at greatest risk of exceeding the ADI because of their low body weight, total nitrate intake and different physiology. At the recommended intake for adults of 200 grams of vegetables, the ADI can be exceeded.
- Little is known about dietary nitrite intake. It should be noted that nitrate is converted into nitrite in the mouth. At a maximum nitrate intake and 5% conversion rate to nitrite, nitrite intake is approximately 13 mg per day for a 70 kg adult. This is above the current ADI for nitrite.
- High-risk groups, such as infants, pregnant women, kidney patients, people with bacterial infections and people with chronic oxygen deficiency (anoxia) should exercise caution in consuming nitrate-rich vegetables in combination with nitrosatable substances, such as amines in fish.
- A risk-benefit assessment based on DALYs shows that the positive effects of consuming sufficient fish, fruit and vegetables far outweigh the risks associated with consuming these foods that contain nitrate.
- Half a litre per day of beetroot juice high in nitrate (a supplement of approximately 500 mg) is recommended by NOC*NSF for athletes to enhance their performance. There are no studies on the potential adverse effects of long-term intake of this high, additional amount of nitrate.
- Further research is needed into the possible adverse health effects of long-term intake of high amounts of nitrate, such as drinking half a litre of beet juice a day to enhance sport performances.
- A causal relationship between maternal intake of cured meat and childhood brain tumours and/or leukaemia cannot be concluded based on the available evidence.

Prof. Dr Antoon Opperhuizen
Director Office for Risk Assessment & Research

References

- Archer DL. Evidence that ingested nitrate and nitrite are beneficial to health. Review. J Food Protect 2002;65(5):872-875.
- Asselt ED van, de Mul A, van Klaveren JD. Inname aan zware metalen en nitraat door jonge kinderen. Wageningen: RIKILT, mei 2008. Rapport 2008.003.
- ATSDR (Agency for Toxic Substances and Disease Registry). Interaction profile for cyanide, fluoride, nitrate, and uranium. Atlanta: US Department of Health and Human Services, 2004.
- Bailey SJ, Winyard P, Vanhatalo A, Blackwell JR, DiMenna FJ, Wilkerson DP, Tarr J, Benjamin N, Jones AM. Dietary nitrate supplementation reduces the O_2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. J Appl Physiol 009;107:1144-1155.
- Blot WJ, Henderson BE, Boice JD Jr. Childhood cancer in relation to cured meat intake: Review of the epidemiological evidence. Nutr Cancer 1999;34(1):111-118.
- Boon PE, Bakker MI, van Klaveren JD, van Rossum CTM. Risk assessment of the dietary exposure to contaminants and pesticide residues in young children in the Netherlands. Bilthoven, The Netherlands: RIVM, 2009. Report 350070002/2009.
- Brender JD, Weyer PJ, Romitti PA, Mohanty BP, Shinde MUuong AM, Sharkey JR, Dwivedi D, Horel SA, Kantamneni J, Huber JC Jr, Zheng Q, Werler MM, Kelley KE, Griesenbeck JS, Zhan FB, Langlois PH, Suarez L, Canfield MA. National Birth Defects Prevention Study. Prenatal nitrate intake from drinking water and selected birth defects in offspring of participants in the National Birth Defects Prevention Study. Environ Health Perspect 2013;121(9):1083-1089. doi: 10.1289/ehp.1206249. Epub 2013 Jun 11.
- Bryan NS, Alexander DD, Coughlin JR, Milkowski AL, Bofetta P. Ingested nitrate and nitrite and stomach cancer risk: An updated review. Food Chem Toxicol 2012;50:3646-3665, http://dx.doi.org/10.1016/j.fct.2012.07.062.
- Cermak NM, Gibala MJ, van Loon LJC. Nitrate supplementation's improvement of 10-km time-trial performance in trained cyclists. Internat J Sport Nutr Excercise Metab 2012;22:64-71.
- Chan TYK. Vegetable-borne nitrate and nitrite and the risk of methaemoglobinaemia. Toxicol Letters 2011;200:107-108.
- Cole SP, Kharitonov VF, Guiney DG. Effect of nitric oxide on Heliobacter pylori morphology. J Infectious Diseases 1999;180:1713-1717.
- Coles LT, Clifton PM. Effect of beetroot juice on lowering blood pressure in free-living, disease-free adults: a randomized, placebo-controlled trial. Nutr J 2012.
- Cooper AJ, Forouhi NG, Ye Z, et al. Fruit and vegetable intake and type 2 diabetes: EPIC-InterAct prospective study and meta-analysis. Eur J Clin Nutr 2012;66:1082-1092.
- De Bont R, Van Larebeke N. Literatuuronderzoek. Nitraten en nitrieten. Steunpunt beleidsrelevant onderzoek Milieu en Gezondheid, 2003.
- EC (European Commission). Opinion on nitrate and nitrite. Reports of the Scientific Committee for Food (SCF) 26th Series, 21-28, 1992. Available from http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_26.pdf.
- EC (European Commission). Opinion on nitrate and nitrite. Reports of the Scientific Committee for Food (SCF) 38th Series, 1-33, 1997. Available from http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_38.pdf.
- EC. European Commission Directorate-General III Industry. Scientific Committee for Food. Opinion on nitrate and nitrite expressed on 22 September 1995. Annex 4 to Document 111/5611/95.
- EFSA. EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion. Statement on possible health risks for infants and young children from

the presence of nitrates in leafy vegetables. EFSA J 2010;8(12):1935, 42 pp. Question No EFSA-Q-2010-01037, adopted on 1 December 2010. doi:10.2903/j.efsa.2010.1935.
- EFSA. EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion. Nitrate in vegetables. EFSA J 2008;689:1-79. Question No EFSA-Q-2006-071, adopted on 10 April 2008.
- EPA. Integrated Risk Information System. Nitrate (CASRN 14797-55-8), 10 January 1991, http://www.epa.gov/iris/subst/0076.htm.
- FAO/WHO (Food and Agriculture Organisation of the United Nations/World Health Organization). Nitrate. In: Safety evaluation of certain food additives (Food additives Series 35). Geneva: World Health Organization, 1996. Available from http://www.inchem.org/documents/jecfa/jecmono/v35je14.htm
- FAO/WHO (Food and Agriculture Organisation of the United Nations/World Health Organization). Nitrate (and potential endogenous formation of N-nitroso compounds). Geneva: World Health Organisation, 2003a. WHO Food Additive series 50. http://www.inchem.org/documents/jecfa/jecmono/v50je06.htm.
- FAO/WHO (Food and Agriculture Organisation of the United Nations/World Health Organization). Nitrite (and potential endogenous formation of N-nitroso compounds). Geneva: World Health Organisation, 2003b. WHO Food Additive series 50. http://www.inchem.org/documents/jecfa/jecmono/v50je05.htm.
- FAVV. Fiche 1.9. Nitrosamines, Versie 22/03/2010. Brussel: FAVV, Wetenschappelijk Comité, adviezen 2010. http://www.favv.be/wetenschappelijkcomite/adviezen/_documents/Bijlage1-fiche1.9.nitrosamines.pdf.
- Gangolli SD, van den Brandt PA, Feron VJ, Janzowsky C, Koeman JH, Speijers GJ, Spiegelhalder B, Walker R, Wisnok JS. Nitrate, nitrite and N-nitroso compounds. Eur J Pharmacol Environ Toxicol Pharmacol Section 1994;292:1-38.
- Geraets L, te Biesebeek JD, van Donkersgoed G, Koopman N, Boon P. The intake of acrylamide, nitrate and ochratoxin A in the population aged 7 to 69 years living in the Netherlands. Bilthoven: RIVM, 2011. RIVM briefrapport 1294A01/2011.
- Gilchrist M, Shore AC, Benjamin N. Inorganic nitrate and nitrite and control of blood pressure. Cardiovascular Res 2011;89:492-498.
- Health Council of the Netherlands, Superior Health Council Belgium, EuSANH. Childhood leukaemia and environmental factors. Den Haag/Brussel: GR/SHC, December 2012.
- Hord NG, Tang Y, Bryan NS. Food sources of nitrates and nitrites: the physiologic context for potential health benefits. Am J Clin Nutr 2009;90:1-10.
- Joris PJ, Mensink RP. Beetroot juice improves in overweight and slightly obese men postprandial endothelial function after consumption of a mixed meal. Atherosclerosis 2013;231(1):78-83.
- Kapil V, Milsom AB, Okorie M, et al. Inorganic nitrate supplementation lowers blood pressure in humans. Role for nitrite-derived NO. Hypertension 2010;56:274-281.
- Katan MB. Nitrate in foods: harmful or healthy? Am J Clin Nutr 2009;90:11-12.
- Knekt P, Järvinen R, Dich J, Hakulinen T. Risk of colorectal and other gastro-intestinal cancers after exposure to nitrate, nitrite and N-nitroso compounds: a follow-up study. Internat J Cancer 1999;80(6):852-856.
- Kreijl CF van, Knaap AGAC, Busch MCM, Havelaar AH, Kramers PGN, Kromhout D, van Leeuwen FXR, van Leent-Loenen HMJA, Ocke MC, Verkley H. Ons eten gemeten. Gezonde voeding en veilig voedsel in Nederland. Bilthoven: RIVM, Bohn, Stafleu, Van Loghum, 2004, 365 p. RIVM Rapport 270555007.
- Kreis IA, van Rongen E, Kremer L. Aetiology of childhood leukaemia: summary of reviews that did not separate acute lymphoblastic and myeloid leukaemia. The Hague: Health Council of the Netherlands, 2011.

- Krul CAM, Zeilmaker MJ, Schothorst R, Havenaar R. Intragastric formation and modulation of N-nitrosodimethylamine in a dynamic in vitro gastrointestinal model under human physiological conditions. Food Chem Toxicol 2004;42:51-63.
- L'Hirondel J, l'Hirondel J-L. Nitrate and man, toxic, harmless or beneficial? Wallingford, UK: CABI Publishing, 2001.
- Lansley KE, Winyard PG, Fulford J et al. Dietary nitrate supplementation reduces the O_2 cost of walking and running: a placebo-controlled study. J Appl Physiol 2011;110:591-600.
- Loh YH, Jakszyn P, Luben RN, Mulligan AA, Mitrou PN, Khaw K-T. N-nitroso compounds and cancer incidence: the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Study. Am J Clin Nutr 2011;93:1053–61.
- Lundberg JO, Carlström M, Larsen FJ, Weitzberg E. Roles of dietary inorganic nitrate in cardiovascular health and disease. Cardiovascular Res 2011;89:525-532.
- Lundberg JO, Feelisch M, Björne H, Jansson EA, Weitzberg E. Cardioprotective effects of vegetables: Is nitrate the answer? Nitric Oxide 2006;15:359-362.
- Machha A, Schechter AN. Inorganic nitrate: a major player in the cardiovascular health benefits of vegetables? Nutr Rec 2012;70(6):367-372. doi: 10.1111/j.1753-4887.2012.00477.
- McKnight G, Duncan CW, Leifert C, Golden MH. Dietary nitrate in man: friend or foe? Br J Nutr 1999;81:349-358.
- Mensinga TT, Speijers GJ, Meulenbelt J. Health implications of exposure to environmental nitrogenous compounds. Toxicological Reviews 2003;22:41-51.
- Milkowski A, Garg HK, Coughlin JR, Bryan NS. Nutritional epidemiology in the context of nitric oxide biology: A risk-benefit evaluation for dietary nitrite and nitrate. Nitric Oxide 2010;22:110-119.
- Miller GD, Marsh AP, Dove RW, Beavers D, Presley T, Helms C, Bechtold E, King SB, Kim-Shapiro D. Plasma nitrate and nitrite are increased by a high-nitrate supplement but not by high-nitrate foods in older adults. Nutr Res 2012;32:160-168.
- Nederlands Olympisch Comité, Nederlandse Sport Federatie (NOC*NSF). Factsheet no. 31 – Rode bietensap. Juni 2012, http://www.nocnsf.nl/home.
- NRC. National Research Council. Subcommittee on Nitrate and Nitrite in Drinking Water. Nitrate and nitrite in drinking water. Washington DC: National Academy Press, 1995, 63 pp.
- Peters S. Combineer vis liever met worteltjes, bloemkool of prei. Voeding Nu 2008;(4):9-11.
- Sindelar JJ, Milkowski AL. Human safety controversies surrounding nitrate and nitrite in the diet. Nitric Oxide 2012;26:259-266.
- Tang Y, Jiang H, Bryan NS. Nitrite and nitrate: cardiovascular risk-benefit and metabolic effect. Curr Opin Lipidol 2011;22(1):11-15. doi: 10.1097/MOL.0b013e328341942c.
- Tricker AR. N-nitroso compounds and man: sources of exposure, endogenous formation and occurrence in body fluids. 1997. Eur J Cancer Prev. 6(3):226-68).
- Valentine R L, Zho Chen JC, Barrett SE, Hwang C, Guo Y, Wehner M, Fitzsimmons S, Andrews SA, Werker AG, Brubacher C, Kohut K. Factors affecting the formation of NDMA in water and occurrence. Denver CO: American Water Works Research Foundation, 2006, pp. 99–138.
- Vanhatalo A, Bailey SJ, Blackwell JR, DiMenna FJ, Pavey TG, Wilkerson DP, Benjamin N, Winyard PG, Jones AM. Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise. Am J Physiol Regul Integr Comp Physiol 2010;299:R1121-R1131.

- Velzen AG van, Sips AJAM, Schothorst RC, Lambers AC, Meulenbelt J. The oral bioavailability of nitrate from nitrate-rich vegetables in humans. Toxicol Letters 2008;181:177-181.
- Vermeer ITM, Pachen DMFA, Dallinga JW, Kleinjans JCS, van Maanen JMS. Volatile N-nitrosamine formation after intake of nitrate at the ADI level in combination with an amine-rich diet. Environ Health Perspect 1998;106:459-463.
- Vermeer ITM. Nitrate exposure and endogenous formation of carcinogenic nitrosamines in humans. Proefschrift. Maastricht: Universiteit Maastricht, 2000.
- Webb AJ, Patel N, Loukogeorgakis S, Okorie M, Aboud Z, Misra S, Rashid R, Miall P, Deanfield J, Benjamin N, MacAllister R, Hobbs AJ, Ahluwalia A. Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. Hypertension 2008;51:784-790.
- Weitzberg E, Lundberg JO. Novel aspects of dietary nitrate and human health. Annu Rev Nutr 2013;33:16.1-16.31.
- Westenbrink S, Jansen MCJF, Ter Doest D, Bausch-Goldblom RA. De inneming van nitraat en nitriet in Nederland. Zeist: TNO, 2005. TNO-rapport V6372.
- Wolterink G, Slob W, Boon P, Jeurissen S. Commentaar op: Brender et al. Prenatal nitrate intake from drinking water and selected birth defects in offspring of participants in the National Birth Defects Prevention Study. Environ Health Perspect 2013;121(9):1083-1089.
- World Cancer Research Fund (WCRF)/American Institute for Cancer Research (AICR). Food, nutrition, physical activity and the prevention of cancer, a global perspective. Second Expert Report. Washington D.C.: AICR, 2007.
- World Health Organization (WHO). N-Nitrosodimethylamine in drinking-water. Background document for preparation of WHO Guidelines for drinking-water quality. Geneva: World Health Organization, 2008. WHO/HSE/AMR/08.03/8, http://www.who.int/water_sanitation_health/dwq/chemicals/ndma_2add_feb2008.pdf.
- Zeilmaker MJ, Bakker MI, Schothorst R, Slob W. Risk assessment of N-nitrosodimethylamine formed endogenously after fish-with-vegetable meals. Toxicol Sci 2010;116(1):323-335.