Nitrate in vegetables

Scientific Opinion of the Panel on Contaminants in the Food chain

(Question No EFSA-Q-2006-071)

Adopted on 10 April 2008

PANEL MEMBERS

SUMMARY
Nitrate is a naturally occurring compound that is part of the nitrogen cycle, as well as an approved food additive. It plays an important role in the nutrition and function of plants. Nitrate is an important component of vegetables due to its potential for accumulation; this can be affected by a number of biotic and abiotic factors. Higher levels of nitrate tend to be found in leaves whereas lower levels occur in seeds or tubers. Thus leaf crops such as lettuce and spinach generally have higher nitrate concentrations. Human exposure to nitrate is mainly exogenous through the consumption of vegetables, and to a lesser extent water and other foods. Nitrate is also formed endogenously. In contrast exposure to its metabolite nitrite is mainly from endogenous nitrate conversion.

Nitrate per se is relatively non-toxic, but its metabolites and reaction products e.g., nitrite, nitric oxide and N-nitroso compounds, have raised concern because of implications for adverse health effects such as methaemoglobinemia and carcinogenesis. On the other hand recent research

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indicates that nitrite participates in host defence having antimicrobial activity, and other nitrate metabolites e.g. nitric oxide, have important physiological roles such as vasoregulation. Despite being a major source of nitrate, increased consumption of vegetables is widely recommended because of their generally agreed beneficial effects for health.

In order to provide a strategy to manage any risks to human health from dietary nitrate exposure resulting from vegetable consumption an updated risk assessment was requested from the Panel on Contaminants in the Food Chain (CONTAM) of the European Food Safety Authority (EFSA) by the European Commission. The opinion was to take into account the amounts of nitrate found in vegetables as consumed and any relevant considerations on the possible balance between risks and benefits.

As a response to a call for data on nitrate levels in vegetables, EFSA received 41,969 analytical results from 20 Member States and Norway. There was a large variation in median concentrations of nitrate in different vegetables from a low of 1 mg/kg (peas and Brussels sprouts) to a high of 4,800 mg/kg (rucola). Less than 5% of all samples were reported as being below the limit of detection (LOD) for nitrate. A reasonable approximation of European vegetable consumption was estimated from the GEMS/Food Consumption Cluster Diets database and consumption data submitted by EU Member States. In consequence, a base case of vegetable and fruit intake of 400 g/person/day, as recommended by the World Health Organization (WHO), was used, but considered to be all in the form of vegetables. In addition, from the data collected, different scenarios combining a range of consumption patterns with concentration of nitrates in the relevant food category were estimated. The scenarios demonstrated that the critical driver for a high dietary exposure to nitrate is not the absolute amount of vegetables consumed but the type of vegetable (e.g. leafy vegetables) and the concentration of nitrate related to the conditions of production.

An Acceptable Daily Intake (ADI) for nitrate of 3.7 mg/kg b.w./day, equivalent to 222 mg nitrate per day for a 60 kg adult was established by the former Scientific Committee on Food (SCF) and was reconfirmed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2002. The CONTAM Panel noted that no new data were identified that would require a revision of the ADI.

To assess any potential health impacts from the different vegetable intake scenarios the CONTAM Panel compared the nitrate exposure estimates with the ADI for nitrate of 222 mg/day for a 60 kg human. Additionally, to place these findings in context, exposures from other nitrate sources such as drinking water and cured meat, at an average of 35-44 mg/person per day, were also taken into account. As a conservative base case, a person eating 400 g of mixed vegetables at typical median nitrate concentration levels would on average receive a dietary exposure to nitrate of 157 mg/day. This is within the ADI even when the exposure to nitrate from other dietary sources is considered. Considering that for most people, fruit, which has low nitrate levels in the order of 10 mg/kg, comprises up to one half of the total recommended daily intake of 400 g of vegetables and fruit, actual nitrate intakes would be reduced to between 81-106 mg/day for the majority of the EU population. Further mitigation of nitrate intake may result from processing e.g. washing, peeling and/or cooking.
A small part of the population (2.5%) in some Member States eats only leafy vegetables and in high amounts, which can lead to the ADI being exceeded. Should the vegetables be produced under unfavourable growing conditions the ADI could be exceeded by approximately two fold. The Panel also noted that consumption of more than 47 g of rucola at the median nitrate concentration would lead to an excursion above the ADI without taking into account any other source of nitrate exposure.

Epidemiological studies do not suggest that nitrate intake from diet or drinking water is associated with increased cancer risk. Evidence that high intake of nitrite might be associated with increased cancer risk is equivocal.

The Panel compared the risk and benefits of exposure to nitrate from vegetables. Overall, the estimated exposures to nitrate from vegetables are unlikely to result in appreciable health risks, therefore the recognised beneficial effects of consumption of vegetables prevail. The Panel recognised that there are occasional circumstances e.g. unfavourable local/home production conditions for vegetables which constitute a large part of the diet, or individuals with a diet high in vegetables such as rucola which need to be assessed on a case by case basis.

**KEY WORDS**

Nitrate, nitrite, vegetables, ADI, risk assessment, risk benefit analysis, human health
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BACKGROUND AS PROVIDED BY REQUESTOR

The Scientific Committee for Food (SCF) set an Acceptable Daily Intake (ADI) for nitrate of 0-3.7 mg/kg body weight in 1995. It is possible that this ADI might be exceeded in some cases, for example based upon levels found in vegetables and quantities that are consumed.

The European Commission set maximum levels for nitrate in lettuce and spinach (Regulation (EC) 1881/2006). These levels are regularly reviewed using monitoring data from the Member States. In some cases, despite developments in good agricultural practice, the maximum levels can be exceeded, particularly for spinach. In addition, the implications for food safety from nitrate in other vegetables are unclear. For example, rucola (often called rocket in English) has been found to regularly contain high levels of nitrate, whereas potatoes often contain lower levels of nitrate, but are eaten more frequently and so dietary intake can be significant. Thus the amounts of nitrate in vegetables might sometimes lead to the ADI being exceeded, although it is unclear whether cooking could significantly lower the nitrate levels in the products as eaten.

The significance of the levels of nitrate found in vegetables (e.g. lettuce, spinach, rucola, potatoes and others) should be assessed, taking into account the effects of preparation, such as usual cooking procedures, on the levels of nitrate or relevant metabolites that may be present in the vegetables as normally consumed.

To provide an up-to-date scientific basis for the longer-term strategy for managing the risk from nitrate in vegetables, a scientific risk assessment is needed from the European Food Safety Authority taking into account new information generated since the opinion of the SCF in 1995. The assessment should take into account any relevant considerations on risks and benefits, for example to weigh the possible negative impact of nitrate versus the possible positive effects of eating vegetables, such as antioxidant activities or other properties that might in some way counteract or provide a balance to the risks from nitrate and the resulting nitroso-compounds.

TERMS OF REFERENCE AS PROVIDED BY REQUESTOR

In accordance with Art. 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to assess the risks to consumers from nitrate in vegetables. The assessment should take into account the amounts of nitrate found in vegetables as consumed and any relevant considerations on the possible balance between risks and beneficial health effects.

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ASSESSMENT

1. Introduction

Almost 80% of the earth’s atmosphere consists of nitrogen as the most abundant chemical element. Nitrogen is also a key component of essential biomolecules such as amino acids, vitamins, hormones, enzymes, and nucleotides. In living tissues, nitrogen is ranked quantitatively as the fourth most common element behind carbon, oxygen and hydrogen and is an integral part of the nitrogen cycle, which continuously interchanges nitrogen between organisms and the environment.

Nitrate is a naturally occurring compound and is an important component of vegetables because of its potential to accumulate. It is formed naturally in living and decaying plants and animals, including humans (Mensinga et al., 2003; Lundberg et al., 2004 and 2008; Camargo and Alonso, 2006). Nitrate is also used in agriculture as a fertilizer to replace the traditional use of livestock manure and in food processing as an approved food additive. Nitrate per se is relatively non-toxic, but its metabolites, nitrite, nitric oxide and N-nitroso compounds, make nitrate of regulatory importance because of their potentially adverse health implications. On the other hand recent research shows that its conversion to nitrite plays an important antimicrobial role in the stomach (McKnight, et al., 1999), and other nitrate metabolites also have important physiological/pharmacological roles (Lundberg et al., 2004, 2006 and 2008; Bryan et al., 2005).

The first international evaluation of the risks associated with the ingestion of nitrate and nitrite was conducted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1961 (FAO/WHO, 1962). The Scientific Committee for Food reviewed the toxicological effects of nitrate and nitrite and established an Acceptable Daily Intake (ADI) of 0-3.7 mg/kg b.w. for nitrate in 1990 (EC, 1992), retained the ADI in 1995 and derived an ADI of 0-0.06 mg/kg for nitrite (EC, 1997). The JECFA completed its most recent review in 2002 and reconfirmed an ADI of 0-3.7 mg/kg b.w. for nitrate and set an ADI of 0-0.07 mg/kg b.w. for nitrite (FAO/WHO, 2003a,b).

Nitrate predominately enters the human body exogenously from vegetables, water, and other foods, but is also formed to a limited extent endogenously (Lundberg et al., 2004 and 2008).

Some vegetables, particularly leafy vegetables, have been shown to have relatively high levels of nitrate, but implications for food safety are unclear. In order to provide a scientific basis for a longer-term strategy to manage any risks from dietary nitrate an updated risk assessment was considered necessary by the European Commission.
Nitrate and plants

Nitrogen is the main growth-limiting factor in most field crops and the major source in plants is mineralised nitrogen, as nitrate and ammonium. Farmers may therefore use manure and nitrogen-based fertilizers to boost crop yields. A range of leafy vegetables can accumulate high levels of nitrate. The concentrations depend on a range of factors including season, light, temperature, growing conditions, fertilizer use, and storage of the crop (Dich et al., 1996). In Europe there is a tendency for the concentrations to be higher in more northerly latitudes and during the winter, owing to the lower light intensity and fewer daylight hours.

Nitrate and water

If Good Agricultural Practice (GAP) is not followed, the application of nitrogen fertiliser and/or manures can result in increased concentrations of inorganic nitrogenous compounds in ground and surface waters. International efforts have been put in place to reduce and limit the occurrence of nitrate in water. In the European Union, the first harmonised standards concerning the quality of surface water intended to be used as drinking water were laid down in 1975 in the Council Directive 75/440/EEC3. The current maximum level of 50 mg nitrate/L in drinking water is laid down in Council Directive 98/83/EC4.

Sources of nitrate and nitrite exposure

While human exposure to nitrate is mainly exogenous, exposure to nitrite is mainly endogenous via nitrate metabolism. Some nitrite is consumed as a consequence of its use as a food preservative and to a lesser extent from its presence in vegetables. Figure 1 illustrates the estimated total daily dietary exposure for both nitrate and nitrite, expressed as a percentage of the total diet, for the UK as an example of a Northern European country (MAFF, 1998a,b) and France as an example of a Central/Southern European country (modified after Causeret, 1984). For both the UK and France the most important sources of dietary intake of nitrate are vegetables and fruit contributing 50 to 75% to the overall dietary intake (see Figures 1a and 1b). Also for both countries the largest source of nitrite is endogenous conversion from nitrate (see Figures 1e and 1f). A conservative factor of 7% for the ingested nitrate to nitrite conversion was used for the calculations (see chapter 8.1).

Although fruit and vegetables contribute 11-41% of exogenous nitrite dietary intake (see Figures 1c and 1d), this amount is overshadowed by the endogenous reduction of secreted salivary nitrate to nitrite. Thus the exposure assessment in this opinion will focus mainly on nitrate concentrations in vegetables and consider nitrite only when dealing with the total body burden of nitrate and its metabolites with regard to any implications for human health.

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Figure 1. Relative intake contribution for sources of nitrate and nitrite in the UK and France.
Nitrate in humans and laboratory animals

The toxicokinetics of nitrate are complex (see chapter 8.1). In humans, dietary nitrate is rapidly absorbed via the stomach and the proximal intestine into the plasma and at least 25% is transported into the saliva. The salivary nitrate concentration is approximately 10-fold that of plasma due to bio-concentration (McKnight et al., 1999). On the surface of the tongue, commensal bacteria reduce approximately 20% of the secreted nitrate into nitrite (Lundberg et al., 2004 and 2008) which is then swallowed along with the unconverted nitrate. Healthy adults have a salivary conversion of nitrate to nitrite of normally 5-7% of the total nitrate intake, whereas infants and patients with gastroenteritis who have a higher gastric pH can have a considerably greater conversion rate.

Nitrate is relatively non-toxic, the main toxicological endpoints in laboratory animals result from the formation of nitrite and its ability to react to form N-nitroso compounds. Several toxicological effects have been identified: methaemoglobin formation, hyperplasia of the zona glomerulosa of the adrenal cortex and gastric neoplasia.

Definition of vegetables

The focus of this opinion is on vegetables. The definition of the word vegetable is traditional rather than scientific and is somewhat arbitrary and subjective. All parts of herbaceous plants eaten as food by humans, whole or in part, are normally considered vegetables. Mushrooms, though belonging to the biological kingdom fungi, are also commonly considered vegetables. Tubers (like potato) are included in the definition of vegetables in some countries but not in others. Nuts, grains, herbs, spices and culinary fruits are normally not considered as vegetables. Botanically, fruits are reproductive organs, while vegetables are vegetative organs which sustain the plant. Nevertheless, several fruits, e.g. cucumbers, are also included in the term vegetables.

A formal definition of fruits and vegetables was attempted by the World Health Organisation for use in epidemiological studies (IARC, 2003). They are defined as “edible plant foods excluding cereal grains, nuts, seeds, tea leaves, coffee beans, cocoa beans, herbs and spices”. Fruits are “edible parts of plants that contain the seeds and pulpy surrounding tissue; have a sweet or tart taste; generally consumed as breakfast beverages, breakfast and lunch side-dishes, snacks or desserts.” Vegetables are “edible plant parts including stems and stalks, roots, tubers, bulbs, leaves, flowers, and fruits; usually include seaweed and sweet corn; may or may not include pulses or mushrooms; generally consumed raw or cooked with a main dish, in a mixed dish, as an appetiser, or in a salad”.

For this opinion vegetables were taken to be leaves (e.g. lettuce), stems (e.g. asparagus), roots (e.g. carrots), flowers (e.g. broccoli), bulbs (e.g. garlic), seeds (e.g. peas and beans) and botanical fruits such as cucumbers, squash, pumpkins, and capsicums. Tubers are included in the general definition but also presented separately. Herbs are presented in a separate table but not included in the overall definition of vegetables.
Risk benefit analysis

Risk-benefit analysis of foods with regard to human health is a developing area and no common paradigm on the general principles or approaches for conducting a quantitative risk-benefit analysis for food and food ingredients has been established yet at either a European or international level. Within the EU recently a number of projects such as Qalibra\(^5\), Beneris\(^6\) and Brafo\(^7\) have been initiated to progress the science, tools, methods and implications of risk-benefit analysis. At a recent EFSA Scientific Colloquium on Risk-benefit Analysis of Foods – Methods and Approaches (EFSA, 2007) it was concluded that risk-benefit analysis should be symmetrical and thus mimic the classical risk assessment and risk analysis paradigms. The same steps should be involved, namely health benefit identification, health benefit characterisation and exposure assessment in order to arrive at an overall benefit characterisation. The outcome of the respective risk characterisation and benefit characterisation would then drive an integrated risk-benefit analysis via risk-benefit assessment, risk-benefit management and risk-benefit communication.

2. Legislation on nitrate

Maximum levels for nitrate in vegetables were first set in the EU in 1997 by Commission Regulation (EC) No 194/97\(^8\). Since then, the Regulation has been amended several times. The current maximum levels are laid down in the Annex, Section 1 of Commission Regulation (EC) No 1881/2006\(^2\) of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs\(^9\) (Table 1) The Regulation on nitrate applies for the following five food commodities: fresh spinach, preserved, deep-frozen or frozen spinach, fresh lettuce (protected and open-grown lettuce), iceberg-type lettuce and processed cereal-based foods and baby foods for infants and young children. All maximum levels are expressed as mg nitrate/kg fresh weight.

Because of widely varying climatic conditions, production methods and eating habits in different parts of the European Union, maximum levels for fresh spinach and fresh lettuce are fixed depending on the season. The maximum levels for nitrate in those foodstuffs that are harvested between 1 October and 31 March are generally higher than the respective levels for samples harvested between 1 April and 30 September. Moreover, with respect to fresh lettuce the Regulation differentiates between lettuce grown under cover and lettuce grown in the open air with lower levels for the latter commodities.

\(^5\) Available at URL: [http://www.qalibra.eu/](http://www.qalibra.eu/)
\(^6\) Available at URL: [http://www.beneris.eu/](http://www.beneris.eu/)
\(^7\) Available at URL: [http://europe.ilsi.org/activities/ecprojects/BRAFO/default.htm](http://europe.ilsi.org/activities/ecprojects/BRAFO/default.htm)
\(^8\) OJ L 31, 1.2.1997, p. 48
Table 1. Maximum levels for nitrate as laid down in Commission Regulation (EC) No 1881/2006.

<table>
<thead>
<tr>
<th>Foodstuff</th>
<th>Maximum level (mg nitrate/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh spinach (Spinacia oleracea)</td>
<td></td>
</tr>
<tr>
<td>Harvested 1 October to 31 March</td>
<td>3,000</td>
</tr>
<tr>
<td>Harvested 1 April to 30 September</td>
<td>2,500</td>
</tr>
<tr>
<td>Preserved, deep-frozen or frozen spinach</td>
<td>2,000</td>
</tr>
<tr>
<td>Fresh lettuce (Lactuca sativa L.)</td>
<td></td>
</tr>
<tr>
<td>(protected and open-grown lettuce) excluding</td>
<td></td>
</tr>
<tr>
<td>lettuce listed below</td>
<td></td>
</tr>
<tr>
<td>Harvested 1 October to 31 March:</td>
<td></td>
</tr>
<tr>
<td>lettuce grown under cover</td>
<td>4,500</td>
</tr>
<tr>
<td>lettuce grown in the open air</td>
<td>4,000</td>
</tr>
<tr>
<td>Harvested 1 April to 30 September</td>
<td></td>
</tr>
<tr>
<td>lettuce grown under cover</td>
<td>3,500</td>
</tr>
<tr>
<td>lettuce grown in the open air</td>
<td>2,500</td>
</tr>
<tr>
<td>Iceberg-type lettuce</td>
<td></td>
</tr>
<tr>
<td>Lettuce grown under cover</td>
<td>2,500</td>
</tr>
<tr>
<td>Lettuce grown in the open air</td>
<td>2,000</td>
</tr>
<tr>
<td>Processed cereal-based foods and baby foods</td>
<td></td>
</tr>
<tr>
<td>for infants and young children</td>
<td>200</td>
</tr>
</tbody>
</table>

By way of derogation several Member States are allowed for a transitional period (until 31 December 2008) to authorize the marketing of spinach or lettuce grown and intended for consumption in their territory with nitrate levels higher than the levels fixed in Regulation (EC) No 1881/2006. However, lettuce and spinach producers in the Member States which have been given the aforementioned authorisation should progressively modify their farming methods in order to minimise the nitrate content by applying the good agricultural practices (GAP – see also chapter 4) recommended at the national level.

3. Sampling and methods of analysis

As sampling, sample preparation and analytical procedures play an important role for a reliable nitrate determination, general criteria were set in the EU in 1997 by Commission Regulation (EC) No 194/97. These provisions were amended several times and are presently fixed in Commission Regulation (EC) No 1882/2006 of 19 December 2006 laying down methods of sampling and analysis for the official control of the levels of nitrate in certain foodstuffs. Besides definitions and general provisions this Regulation stipulates detailed requirements for methods of sampling for the different food commodities. No requirements exist for the number of samples that have to be analysed. Article 9 of Commission Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs only requests that Member States shall monitor nitrate levels in vegetables which may contain significant levels, in particular green leaf vegetables, and communicate the results to the Commission by 30 June each year. As a general obligation, according to Regulation (EC) No 882/2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules,
Member States shall ensure that official controls are carried out regularly, on a risk basis and with appropriate frequency.

Commission Regulation (EC) No 1882/2006\(^9\) also contains strict requirements with which the methods of analysis have to comply in order to ensure that control laboratories use procedures with comparable levels of performance. The Regulation follows the “criteria approach”. This means that no prescribed fixed official methods have to be followed but laboratories can use each method of analysis, provided it can be demonstrated in a traceable manner that they strictly fulfil the analytical requirements laid down in the respective legislation. As a general requirement, methods for nitrate analysis used for food control purposes must comply with the provisions of items 1 and 2 of Annex III (characterisation of methods of analysis) to Regulation (EC) No 882/2004\(^{12}\) of the European Parliament and of the Council of 29 April 2004\(^{13}\) on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. While Regulation (EC) No 882/2004\(^{12}\) contains the general provisions, the specific requirements for the official control of nitrate in vegetables are laid down in Commission Regulation (EC) No 1882/2006\(^{9}\). The latter sets performance criteria for recovery and precision. The “recommended” recovery values for the concentration values <500 mg/kg and ≥500 mg/kg are set as 60-120% and 90-110%, respectively. Moreover, annex D.3.2 of Commission Regulation (EC) No 1882/2006\(^{9}\) states that the precision values have to be calculated at the concentration of interest from the Horwitz equation\(^{14}\). While the recommended value is the one derived from the Horwitz equation, the maximum permitted value is two times the value from the Horwitz equation.

Depending on type of foodstuff and concentration of interest a variety of analytical methodologies and principles are applicable. Some methods have already been standardized for the determination of nitrate and nitrite in various foodstuffs by the European Committee for Standardization (CEN). Within its technical committee CEN/TC 275 “Food analysis – horizontal methods” standards were elaborated for the determination of nitrate and nitrite in vegetables, vegetable products, including vegetable containing food for babies and infants as well as in meat and meat products. The respective methods are published in the standard series EN 12014, parts 1, 2, 3, 4, 5, and 7.

Finally, the Commission Regulation (EC) No 1882/2006\(^9\) sets requirements for the reporting of results and the assessment of compliance of the lot or sub lots. For this, the analytical result corrected for recovery shall be used for checking compliance. The analytical result must be reported as x ± U whereby x is the analytical result and U is the expanded measurement uncertainty, using a coverage factor of 2 which gives a level of confidence of approximately 95%.

\(^{13}\) OJ L 191, 28.5.2004, p. 1

\(^{14}\) The Horwitz equation is a generalised precision equation which has been found to be independent of analyte and matrix but solely dependent on concentration for most routine methods of analysis.
4. Factors influencing the concentration of nitrate in plants

Nitrogen is essential to the nutrition and function of plants, so plants exert a close metabolic control on the concentration of nitrate and other nitrogen compounds. Nitrate is mainly to be found in cell vacuoles and is transported in the xylem. The xylem carries water and nutrients from the roots to the leaves, whereas the phloem carries the products of photosynthesis from the leaves to the growth points of the plant. This affects the distribution of nitrate between the leaves and storage organs such as seeds or tubers. This means that leaf crops such as cabbage, lettuce and spinach have fairly large nitrate concentrations whereas storage organs such as potato tubers, carrots, leeks, onions, seeds and pods of pea and bean plants have relatively small concentrations.

Another consequence of the transport system is that young leaves have lower nitrate concentration than older leaves. Such a relation was shown for cabbage with greatest nitrate concentrations in the outer leaves and much smaller nitrate concentration in the innermost leaves (Greenwood and Hunt, 1986).

Both environmental and agricultural factors can influence the nitrate concentrations in vegetables. The former include soil moisture, light intensity and temperature and the latter fertilizers, variety and crop protection strategies. The codes of GAP take these factors into consideration, region by region, in order to minimise nitrate levels in crops.

Soil

Nitrate moves from the bulk soil to the root surface mainly by convection rather than diffusion, so shortage of water will restrict nitrate uptake. Excess soil water dilutes the nitrate in the soil solution and can make the soil anoxic, thereby restricting crop growth and causing loss of nitrate by denitrification. Soil type and mineral content can affect nitrate accumulation.

Light intensity

The coupling of nitrogen assimilation and photosynthetic electron transport in leaves implies that light intensity is the key factor in determining nitrate concentrations in leaf crops. Month to month differences in light intensity caused as much as threefold variations in nitrate concentrations in lettuce grown in Western Europe (Van Eysinga, 1984). Winter-sown crops have generally higher nitrate concentration than summer crops in the same environment and Northern European crops have higher nitrate levels compared to Southern European crops (Weightman et al. 2006, AFFSA, 2007). These differences can be explained by both higher irradiance in summer which tends to reduce nitrate, and also to higher growth rates which coincide with periods of high irradiance and warmer temperatures (Kanaan and Economakis 1992). Current UK crop assurance protocols therefore suggest that growers should avoid sampling lettuce during dull weather conditions or during a particular time of the day (Anonymous 2002). The maximisation of light availability influences also the level of nitrate when crops are produced under glasshouse conditions (Premuzic et al., 2002), this means e.g. shading of crops should be avoided.
Nitrogen fertilizer

Nitrogen fertilizer may contain nitrogen as nitrate, ammonium or urea and occasionally other forms. Once in the soil the other forms will mainly be converted to nitrate. Applying nitrogen fertilizer increases nitrate concentrations in the xylem but has virtually no effect on concentrations in the phloem. Leaf crops such as lettuce or cabbage therefore show an increased concentration of nitrate in response to nitrogen fertilizer, except in their very youngest leaves, while storage organs such as peas and beans that are fed by the phloem tend to show little effect. Nitrate concentrations in soil-growing storage roots that have very small nitrate concentrations will respond little to nitrogen.

Good agricultural practice for minimizing nitrate concentrations in vegetables

Schemes for GAP with regard to nitrate were developed to help farmers respond to the European nitrate regulations and the need to minimise nitrate concentrations in vegetables. They were produced by the Member States themselves, vegetable growers' organisations or commercial interests. Each GAP comprises an assembly of currently available knowledge, including recommendations based on experiments, and is intended to address environmental, agricultural, economic and social sustainability issues in on-farm production and the processing of produce beyond the farm gate.

A range of different GAP schemes operate within the Member States, each of which takes into account the particular climatic conditions in that Member State. All the schemes focus on abiotic factors shown to have a significant effect on nitrate concentrations in plants. First among these in almost all GAP schemes is adequate light intensity, particularly for vegetables grown under glass or plastic sheeting. GAP for nitrogen nutrition also aims to minimize 'untimely nitrate', that is, nitrate that is in the soil when it is not needed by the crop. The finding that nitrate concentrations in the outer leaves of lettuce are greater than those in inner leaves leads to another common element in GAP, the recommendation that growers should aim for large head weights to allow some trimming where appropriate. Other advice concerns inter alia analyses of the growing medium, choice of cultivars and the time between harvest and sale. The latter needs to be as short as possible to prevent water loss, which would be expected to increase the nitrate concentration on a weight for weight basis in the produce.

5. Occurrence of nitrate in vegetables

A call for detailed information on nitrate concentrations in individual vegetable samples was issued by the European Commission to EU Member States in November 2006. In total, EFSA received 41,969 analytical results from 20 Member States and Norway covering the period from 2000 to 2007. The sample distribution across countries is shown in Figure 2.
Germany contributed almost 34% of the results, with Romania the second largest contributor at close to 20% and Spain the third largest at just over 9%. The country of origin of the vegetables was indicated for 72.3% of the data and varied considerably from the testing country (Figure 3). Vegetables imported from 37 third countries (excluding Norway) comprised only 1% of the data, with 98 samples from Morocco, 80 from Turkey, 58 from Israel and 49 from Egypt being the highest numbers.

There was a considerable flow of product across Member State borders. Examples of the submitted data with known country of origin showed Germany produced 63% of the vegetables within its borders and imported 12% from Italy, 7% from Spain, 6% from the Netherlands, 5% from Belgium, and another 7% from other countries. Denmark produced 74% of the vegetables within its borders and imported 7% from Italy and Spain, respectively, 4% from the Netherlands, 3% from Germany and a further 5% from other countries.

**Figure 2.** Number of results for vegetables submitted by each Member State.
Results reported covered 92 different vegetable varieties, although for 23 of those less than 10 samples of each had been tested comprising 60 in total. They have been excluded from the further analysis because of the uncertainty associated with isolated results. It is Nevertheless worth mentioning that some leafy herbs like mint, oregano and thyme contained nitrate levels higher than 5,000 mg/kg, but would on the other hand be consumed only in low amounts. Less than 5% of all samples (1,934 sample results) were reported as below the limit of detection (LOD) for nitrate, and no further information was provided on how data were reported at the LOD. The LOD for the methods used to analyse these samples varied between 1 and 500 mg/kg as reported by the different laboratories, however 60% of these analytical results were reported with a LOD of 5 mg/kg or below and less than 2% with a LOD above 100 mg/kg.

Nitrate concentrations for vegetables where 10 or more samples have been analysed are presented in Tables 2 to 10 grouped into product categories as defined in regulation EC 178/2006\textsuperscript{15} of 1 February 2006 for maximum levels of pesticide in food and feed. Statistical descriptors include median and mean concentrations as well as the 5\textsuperscript{th} and 95\textsuperscript{th} percentile concentrations (abbreviated as P5 and P95, respectively). Overall there are 41,415 datapoints, whereas 554 were excluded from the calculations as they comprised less than 10 samples or belonged to herbs and spices.

Vegetables were not proportionally tested to reflect their true part of the diet, but sampling disproportionately targeted species with maximum legislated limits. To correctly calculate the dietary nitrate impact of each species the relative proportion of each type of vegetable in the diet was estimated and used as a weighting factor calculated by means of dividing the estimated relative dietary proportion by the relative sample proportion, applied when calculating the overall median presented in Table 11. No such corrections were applied to the individual vegetable group calculations. It proved very difficult to get accurate consumption figures for all vegetable

\textsuperscript{15} OJ L29 2.2.2006
varieties and production volumes were thus used as proxies for consumption. The GEMS/Food regional European cluster diet was used as a starting point with some broad categories further split to species level by applying European horticultural production statistics (WHO, 2003a; EuroStat, 2007). The calculated weighting factors applied for each species in 9 different vegetable groups are given in Tables 2 to 10. The weighted overall result for vegetables including roots and tubers, but excluding herbs, is given in Table 11.

Table 2. Nitrate concentrations in brassica vegetables.

<table>
<thead>
<tr>
<th>Brassica vegetables</th>
<th>Number of samples (weighting factor)</th>
<th>Nitrate concentration (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P5</td>
<td>Median</td>
</tr>
<tr>
<td>Broccoli</td>
<td>227 (*0.54)</td>
<td>16</td>
</tr>
<tr>
<td>Brussels sprouts</td>
<td>130 (*0.54)</td>
<td>1</td>
</tr>
<tr>
<td>Cabbage</td>
<td>1,198 (*3.99)</td>
<td>47</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>289 (*2.06)</td>
<td>7</td>
</tr>
<tr>
<td>Chinese cabbage</td>
<td>469 (*0.02)</td>
<td>77</td>
</tr>
<tr>
<td>Curly kale</td>
<td>169 (*0.10)</td>
<td>19</td>
</tr>
<tr>
<td>Kohlrabi</td>
<td>135 (*0.02)</td>
<td>142</td>
</tr>
<tr>
<td>Red cabbage</td>
<td>196 (*0.60)</td>
<td>35</td>
</tr>
<tr>
<td>Sauerkraut</td>
<td>37 (*0.02)</td>
<td>37</td>
</tr>
<tr>
<td>Savoy cabbage</td>
<td>342 (*0.02)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>3,192</strong></td>
<td><strong>7</strong></td>
</tr>
</tbody>
</table>

Samples below the LOD were expressed as upper bound value which means the actual LOD was used in the calculations.

Brussels sprouts had particularly low nitrate concentrations. Most of the vegetables in the brassica group had median nitrate concentrations of approximately 40 to 200 mg/kg except Chinese cabbage and kohlrabi with concentrations around 900 mg/kg. The maximum recorded level of 4,900 mg/kg originated from ordinary cabbage imported from China. A further 349 samples or 11% had values above 1,000 mg/kg.

Table 3. Nitrate concentrations in bulb vegetables.

<table>
<thead>
<tr>
<th>Bulb vegetables</th>
<th>Number of samples (weighting factor)</th>
<th>Nitrate concentration (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P5</td>
<td>Median</td>
</tr>
<tr>
<td>Garlic</td>
<td>13 (*0.60)</td>
<td>8</td>
</tr>
<tr>
<td>Onions</td>
<td>230 (*5.55)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>243</strong></td>
<td><strong>1</strong></td>
</tr>
</tbody>
</table>

Samples below the LOD were expressed as upper bound value which means the actual LOD was used in the calculations.

Bulb vegetables were generally low in nitrate as shown in Table 3, although there were very few test results.
Table 4. Nitrate concentrations in fruiting vegetables.

<table>
<thead>
<tr>
<th>Fruiting vegetables</th>
<th>Number of samples (weighting factor)</th>
<th>P5 Median</th>
<th>Mean</th>
<th>P95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aubergine</td>
<td>182 (*0.46)</td>
<td>29</td>
<td>303</td>
<td>314</td>
</tr>
<tr>
<td>Capsicum</td>
<td>455 (*0.40)</td>
<td>1</td>
<td>28</td>
<td>108</td>
</tr>
<tr>
<td>Chili pepper</td>
<td>152 (*2.08)</td>
<td>4</td>
<td>62</td>
<td>67</td>
</tr>
<tr>
<td>Courgette</td>
<td>159 (*0.02)</td>
<td>11</td>
<td>297</td>
<td>416</td>
</tr>
<tr>
<td>Cucumber</td>
<td>898 (*0.90)</td>
<td>22</td>
<td>156</td>
<td>185</td>
</tr>
<tr>
<td>Gherkin</td>
<td>88 (*0.90)</td>
<td>11</td>
<td>40</td>
<td>69</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>32 (*0.70)</td>
<td>8</td>
<td>392</td>
<td>894</td>
</tr>
<tr>
<td>Tomato</td>
<td>856 (*6.96)</td>
<td>1</td>
<td>26</td>
<td>43</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2,822</td>
<td>1</td>
<td>83</td>
<td>149</td>
</tr>
</tbody>
</table>

Samples below the LOD were expressed as upper bound value which means the actual LOD was used in the calculations.

The fruiting vegetables group, although disparate, had the third lowest median concentration of nitrate after the legume and bulb vegetable groups (not counting the few samples in the fungi group). There were some surprisingly high concentrations reported for a few pumpkin samples with the maximum at 5,665 mg/kg found in France and a few courgette samples over 1,000 mg/kg, but most other samples were below this latter level.

Table 5. Nitrate concentrations in fungi.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Number of samples (weighting factor)</th>
<th>P5 Median</th>
<th>Mean</th>
<th>P95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mushroom</td>
<td>12 (*0.80)</td>
<td>31</td>
<td>43</td>
<td>61</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>12</td>
<td>31</td>
<td>41</td>
<td>59</td>
</tr>
</tbody>
</table>

Samples below the LOD were expressed as upper bound value which means the actual LOD was used in the calculations.

Only a few sample results from fungi testing were reported, generally on the low side.

Table 6. Nitrate concentrations in herbs.

<table>
<thead>
<tr>
<th>Herbs</th>
<th>Number of samples</th>
<th>P5 Median</th>
<th>Mean</th>
<th>P95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basil</td>
<td>68</td>
<td>94</td>
<td>1,827</td>
<td>2,292</td>
</tr>
<tr>
<td>Borage</td>
<td>15</td>
<td>200</td>
<td>1,536</td>
<td>1,918</td>
</tr>
<tr>
<td>Chives</td>
<td>83</td>
<td>1</td>
<td>307</td>
<td>748</td>
</tr>
<tr>
<td>Coriander</td>
<td>20</td>
<td>1,135</td>
<td>2,468</td>
<td>2,445</td>
</tr>
<tr>
<td>Dill</td>
<td>57</td>
<td>13</td>
<td>1,123</td>
<td>1,332</td>
</tr>
<tr>
<td>Parsley</td>
<td>249</td>
<td>10</td>
<td>480</td>
<td>958</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>492</td>
<td>10</td>
<td>791</td>
<td>1,240</td>
</tr>
</tbody>
</table>

Samples below the LOD were expressed as upper bound value which means the actual LOD was used in the calculations.

Several products belonging to the herbs group had high median nitrate concentrations and also the 95th percentile approached the concentration found in leafy vegetables. However, there is less concern in relation to this group since the volume consumed will only be small. It is therefore not included in the calculation of the overall vegetable nitrate statistics thus no weighting factors are given.
Table 7. Nitrate concentrations in leafy vegetables.

<table>
<thead>
<tr>
<th>Leafy vegetables</th>
<th>Number of samples (weighting factor)</th>
<th>Nitrate concentration (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P5</td>
<td>Median</td>
</tr>
<tr>
<td>Amaranth</td>
<td>12 (*0.01)</td>
<td>439</td>
</tr>
<tr>
<td>Beet</td>
<td>12 (*0.002)</td>
<td>84</td>
</tr>
<tr>
<td>Belgian endive</td>
<td>1,006 (*0.41)</td>
<td>63</td>
</tr>
<tr>
<td>Butterhead lettuce</td>
<td>3,426 (*1.44)</td>
<td>53</td>
</tr>
<tr>
<td>Cos lettuce</td>
<td>124 (*1.03)</td>
<td>167</td>
</tr>
<tr>
<td>Curled lettuce</td>
<td>301 (*0.10)</td>
<td>16</td>
</tr>
<tr>
<td>Dandelion</td>
<td>23 (*0.02)</td>
<td>5</td>
</tr>
<tr>
<td>Escarole</td>
<td>73 (*0.002)</td>
<td>6</td>
</tr>
<tr>
<td>Iceberg lettuce</td>
<td>1,980 (*2.06)</td>
<td>210</td>
</tr>
<tr>
<td>Lamb's lettuce</td>
<td>710 (*0.10)</td>
<td>121</td>
</tr>
<tr>
<td>Lettuce</td>
<td>7,749 (*2.06)</td>
<td>56</td>
</tr>
<tr>
<td>Mixed lettuce</td>
<td>89 (*2.01)</td>
<td>281</td>
</tr>
<tr>
<td>Oak-leaf lettuce</td>
<td>470 (*0.10)</td>
<td>8</td>
</tr>
<tr>
<td>Radicchio</td>
<td>40 (*0.10)</td>
<td>5</td>
</tr>
<tr>
<td>Rucola</td>
<td>1,943 (*0.10)</td>
<td>1,528</td>
</tr>
<tr>
<td>Silverbeet (chard)</td>
<td>666 (*0.10)</td>
<td>178</td>
</tr>
<tr>
<td>Spinach</td>
<td>6,657 (*0.52)</td>
<td>64</td>
</tr>
<tr>
<td>Water cress</td>
<td>25 (*0.02)</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>25,306</td>
<td>66</td>
</tr>
</tbody>
</table>

Samples below the LOD were expressed as upper bound value which means the actual LOD was used in the calculations.

Leafy vegetables clearly had the highest median value of all groups. The highest nitrate value recorded in the group, 19,925 mg/kg, belonged to an oak-leaf lettuce sample grown under cover in Norway. However, rucola had the highest median concentration of nitrate (4,800 mg/kg) of any vegetable with 56% of values over 4,500 mg/kg, followed by amaranth and Lamb’s lettuce. Butterhead lettuce, a common salad vegetable, had a median nitrate concentration just below 2,000 mg/kg and 2% of the samples exceeded 4,500 mg/kg. The median for spinach at 785 mg/kg was well below the maximum level allowed with 5% of the samples exceeding 3,000 mg/kg.

Table 8. Nitrate concentrations in legumes.

<table>
<thead>
<tr>
<th>Legumes</th>
<th>Number of samples (weighting factor)</th>
<th>Nitrate concentration (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P5</td>
<td>Median</td>
</tr>
<tr>
<td>Beans</td>
<td>48 (*0.24)</td>
<td>6</td>
</tr>
<tr>
<td>French beans</td>
<td>52 (*0.24)</td>
<td>4</td>
</tr>
<tr>
<td>Green beans</td>
<td>362 (*2.39)</td>
<td>9</td>
</tr>
<tr>
<td>Peas</td>
<td>407 (*2.79)</td>
<td>1</td>
</tr>
<tr>
<td>String beans</td>
<td>13 (*0.24)</td>
<td>170</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>882</td>
<td>1</td>
</tr>
</tbody>
</table>

Samples below the LOD were expressed as upper bound value which means the actual LOD was used in the calculations.

Legume results were generally fairly low except for 27% of the French bean results, which had nitrate concentrations over 1,000 mg/kg.
Table 9. Nitrate concentrations in stem vegetables.

<table>
<thead>
<tr>
<th>Stem vegetables</th>
<th>Number of samples (weighting factor)</th>
<th>Nitrate concentration (mg/kg)</th>
<th>P5</th>
<th>Median</th>
<th>Mean</th>
<th>P95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asparagus</td>
<td>260 (*0.30)</td>
<td>1</td>
<td>24</td>
<td>209</td>
<td>1,459</td>
<td></td>
</tr>
<tr>
<td>Celery</td>
<td>387 (*0.40)</td>
<td>18</td>
<td>693</td>
<td>1,103</td>
<td>3,319</td>
<td></td>
</tr>
<tr>
<td>Fennel</td>
<td>116 (*0.002)</td>
<td>25</td>
<td>783</td>
<td>1,024</td>
<td>3,047</td>
<td></td>
</tr>
<tr>
<td>Leek</td>
<td>558 (*0.40)</td>
<td>5</td>
<td>257</td>
<td>345</td>
<td>975</td>
<td></td>
</tr>
<tr>
<td>Rhubarb</td>
<td>58 (*0.40)</td>
<td>28</td>
<td>2,808</td>
<td>2,943</td>
<td>6,550</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1,379</strong></td>
<td><strong>3</strong></td>
<td><strong>302</strong></td>
<td><strong>698</strong></td>
<td><strong>2,923</strong></td>
<td></td>
</tr>
</tbody>
</table>

Samples below the LOD were expressed as upper bound value that which the actual LOD was used in the calculations.

The median for stem vegetables was higher than for fruiting vegetables as would be expected from plant physiology. Rhubarb showed particularly high values.

Table 10. Nitrate concentrations in roots and tubers.

<table>
<thead>
<tr>
<th>Roots and Tubers</th>
<th>Number of samples (weighting factor)</th>
<th>Nitrate concentration (mg/kg)</th>
<th>P5</th>
<th>Median</th>
<th>Mean</th>
<th>P95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artichokes</td>
<td>65 (*1.10)</td>
<td>1</td>
<td>21</td>
<td>174</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Beetroot</td>
<td>1,013 (*0.40)</td>
<td>110</td>
<td>1,100</td>
<td>1,379</td>
<td>3,670</td>
<td></td>
</tr>
<tr>
<td>Black radish</td>
<td>19 (*0.002)</td>
<td>233</td>
<td>1,245</td>
<td>1,271</td>
<td>2,302</td>
<td></td>
</tr>
<tr>
<td>Black salsify</td>
<td>12 (*0.002)</td>
<td>1</td>
<td>12</td>
<td>43</td>
<td>230</td>
<td></td>
</tr>
<tr>
<td>Carrot</td>
<td>2,383 (*4.39)</td>
<td>21</td>
<td>125</td>
<td>296</td>
<td>1,574</td>
<td></td>
</tr>
<tr>
<td>Celeriac</td>
<td>41 (*0.002)</td>
<td>20</td>
<td>263</td>
<td>390</td>
<td>975</td>
<td></td>
</tr>
<tr>
<td>Parsnip</td>
<td>22 (*0.40)</td>
<td>2</td>
<td>16</td>
<td>83</td>
<td>349</td>
<td></td>
</tr>
<tr>
<td>Potato</td>
<td>2,795 (*48.05)</td>
<td>10</td>
<td>106</td>
<td>168</td>
<td>340</td>
<td></td>
</tr>
<tr>
<td>Radish</td>
<td>788 (*0.40)</td>
<td>115</td>
<td>735</td>
<td>967</td>
<td>2,515</td>
<td></td>
</tr>
<tr>
<td>Turnip</td>
<td>241 (*0.40)</td>
<td>10</td>
<td>312</td>
<td>663</td>
<td>3,400</td>
<td></td>
</tr>
<tr>
<td>White radish</td>
<td>200 (*0.02)</td>
<td>135</td>
<td>1,256</td>
<td>1,416</td>
<td>3,488</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>7,579</strong></td>
<td><strong>15</strong></td>
<td><strong>152</strong></td>
<td><strong>506</strong></td>
<td><strong>2,302</strong></td>
<td></td>
</tr>
</tbody>
</table>

Samples below the LOD were expressed as upper bound value which means the actual LOD was used in the calculations.

Although the median concentration of nitrate for the roots and tuber group is low at 152 mg/kg, the median values for the different product groups ranged from 12 to 1,256 mg/kg. Potatoes and carrots are both major components in the diet of many countries and the medians are just above 100 mg/kg. Beetroot, on the other hand, is almost ten times higher at a median of 1,100 mg/kg, but is consumed much less frequently except in a few countries. The mean is more than three times higher than the median indicating some high values at the tail end of the distribution. This could influence local intake, particularly in self-production systems with little variation in source of produce. There are 64 results higher than 4,000 mg/kg with more than half belonging to beetroot and also turnip being over-represented. The samples with high concentrations were produced in seven countries with The Netherlands and to some extent Hungary having more samples with high concentrations than would be expected from the number of overall samples tested.

In summary, there is a large variation in median concentrations of nitrate in different vegetables from a low of 1 mg/kg (peas and Brussels sprouts) to a high of 4,800 mg/kg (rucola).
To avoid sampling bias when calculating an overall median for the vegetable group, results for each vegetable variety were adjusted according to approximated consumption volumes as estimated by production volumes. The weighted overall result for the nitrate content in vegetables including roots and tubers but excluding herbs was a median best estimate of 255 mg/kg (Table 11).

**Table 11.** Overall nitrate concentration used in the assessment of vegetables calculated by applying a weighting factor to individual results according to relative production volumes for the variety.

<table>
<thead>
<tr>
<th>Overall vegetables</th>
<th>Number of samples</th>
<th>Nitrate concentration (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P5</td>
</tr>
<tr>
<td>All (including roots and tubers)</td>
<td>41,415</td>
<td>27</td>
</tr>
</tbody>
</table>

Samples below the LOD were expressed as upper bound value that means the actual LOD was used in the calculations.

**Variation of nitrate levels due to geographical differences**

Apart from the bias introduced by the number of samples analysed for each type of vegetable, uncertainty in the estimated overall concentration of nitrate in vegetables is also associated with the representativeness of geographical coverage and season, as well as the production and processing methods utilised.

Since there is a complex interaction between season, production method and location in relation to the amount of sunlight and the accumulation of nitrate in vegetables as has been described earlier, an attempt was made to disaggregate those factors. Countries were allocated to one of three regions:
- North including Finland, Sweden, Norway, Iceland, Denmark, the United Kingdom, Ireland, Estonia, Latvia and Lithuania;
- Central including Poland, Germany, The Netherlands, Belgium, Luxembourg, Czech Republic, Slovakia, Austria and Hungary; and
- South including France, Portugal, Spain, Italy, Malta, Greece, Slovenia, Rumania and Bulgaria.

Samples were also recorded as being produced during winter or summer and in open air or under cover.

Lettuce varieties were treated as a group including butterhead lettuce, cos lettuce, curled lettuce, iceberg lettuce, Lamb’s lettuce, lettuce (unspecified), mixed lettuce and oak-leaf lettuce. Rucola and spinach were studied separately. A detailed analysis of the influence of season, production method and location was performed on those products. Samples lacking such details were omitted from the analysis. The lettuce varieties group was stratified using a modelling approach applying estimated market share as indicated above to the respective variety and curve fitting to calculate median and percentile values. A logistic equation was applied for all varieties as the best fit equation using @Risk (Palisade Corporation). Generally nitrate levels found in lettuce varieties produced in southern Europe were lower than levels found in central or northern Europe (Figure 4).
Production under cover (potentially reduced light intensity) increased nitrate levels irrespective of season except for lettuce varieties produced in southern Europe during winter. Variations in median concentrations of nitrate in lettuce varieties across all production conditions of more than eight times were recorded, with the lowest of 625 mg/kg found in summer field production in the south and the highest of 2,652 mg/kg found in covered winter production in central Europe.

Rucola was a staple component of the Roman diet in ancient times. This is borne out by poets like Horace who credited his friend Martial, who spoke of it as a magical herb, with having discovered its aromatic and flavouring properties. However, its popularity decreased and it was almost absent in common diets for many centuries. Now the use of rucola as part of a leafy salad mix or by itself again seems to be increasing in several European diets. Currently rucola is used in cooking as an herb, a side dish to accompany meat dishes and as a topping for first courses. Rucola is grown in the open during the summer season, but is also grown in greenhouses during the winter season, which could result in increased nitrate levels. The nitrate levels of rucola as influenced by season and region are shown in Figure 5.

Variations in nitrate levels found in rucola under different production conditions were much less than for the lettuce varieties with a maximum 68% difference. However, sample numbers were low for most groups. Rucola is not captured by the current legislation limiting nitrate concentrations in some vegetables.
Spinach has often been seen as a major vegetable source for nitrate in the diet and home made baby food including spinach stored under inappropriate conditions has been involved in some cases of methaemoglobinaemia, (Filer *et al*., 1970; Sánchez-Echaniz and Benito-Fernández, 2001). Median levels of nitrate in spinach were mainly below 2,000 mg/kg (Figure 6).
It is of interest to note that production under cover of spinach during winter in southern Europe achieved the lowest levels of nitrate indicating that this is a well controlled system. The low number of sample results in the rest of the matrix does not allow further conclusions to be drawn.

Table 12 summarises the occurrence data for nitrate in vegetables and vegetable groups which are used in the exposure assessment. The median concentrations given for other vegetables and for lettuce varieties have been adjusted by applying a weighting factor calculated according to estimated volume of production/consumption for each vegetable.

Table 12. Median occurrence data for nitrate in vegetables and vegetable groups by applying a weighting factor to individual results according to relative production volumes for the variety. These values have been used in the exposure assessment. N= number of results reported.

<table>
<thead>
<tr>
<th>Nitrate concentration mg/kg</th>
<th>Most vegetables a)</th>
<th>Lettuce varieties</th>
<th>Spinach</th>
<th>Rucola</th>
<th>Potato</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>392</td>
<td>1,338</td>
<td>785</td>
<td>4,800</td>
<td>106b)</td>
</tr>
<tr>
<td>Median range</td>
<td>-</td>
<td>625-2,652</td>
<td>386-1,745</td>
<td>3,805-6,400</td>
<td>-</td>
</tr>
<tr>
<td>N</td>
<td>33,836</td>
<td>14,849</td>
<td>6,657</td>
<td>1,943</td>
<td>2,795</td>
</tr>
</tbody>
</table>

a) All vegetable products including the also separately shown lettuce varieties, spinach and rucola but excluding roots and tubers, and herbs.
b) Potatoes representing roots and tubers.

The median range reflects the different production conditions as illustrated in Figures 4 - 6. The upper limits represent adverse growing conditions (less sunlight, under cover etc) or excess nitrogen fertilisation and can be a worst case scenario under local circumstances e.g., dominating the diet for some time if consuming self-produced product exclusively.

Nitrite information was not requested from Member States. Data from the literature (Jakszyn et al., 2004) showed that vegetables in general contribute only approximately by 2 to 6% of daily dietary intake to the total nitrite exposure (including endogenous conversion from nitrate). This low amount is overwhelmed by the endogenous conversion of dietary nitrate from vegetable consumption to nitrite through entero-salivary recirculation. The direct nitrite intake from vegetables is thus well within the margins of error for the occurrence analysis and nitrite occurrence was not studied in detail.

5.1 Influence of storage and food processing on nitrate levels

Levels of nitrate and nitrite in raw agricultural commodities can be influenced by a number of factors such as storage time and conditions (i.e. ambient, refrigerated, frozen), and food processing (i.e. washing, peeling, blanching, boiling). Overall, there is a paucity of published data in this area.
5.1.1 Storage

Ambient temperature

Nitrate levels in raw vegetables kept at ambient temperatures can decrease during the period of storage. On the contrary, nitrite levels in fresh, undamaged plant tissues are usually very low but post-harvest storage and wilting processes favours its increase. The increase in nitrite levels may be dependent on species differences, specific endogenous nitrate reductase activities (Pate, 1973; Andrews, 1986; Wallace, 1986) and the amount of bacterial contamination (Phillips, 1968; Ezeagu and Fafunso, 1995; Ezeagu, 1996; Chung et al., 2004).

Studies on nitrate and nitrite levels in spinach (Phillips, 1968; Chung et al., 2004), Nigerian leaf vegetables (Ezeagu and Fafunso, 1995; Ezeagu, 1996) and Chinese cabbage (Chung et al., 2004) under storage at ambient temperature indicated that nitrate content decreased whereas nitrite tended to increase over time. This process was accelerated when the produce was pureed.

Refrigerated

Under refrigerated storage (7 days) at 5ºC, nitrate levels were almost unaffected in Chinese cabbage and spinach, respectively. Nitrite concentrations remained low over the whole storage period (Chung et al., 2004). This implies inactivation of endogenous nitrate reductase under cold storage conditions as well as prevention of bacterial activity.

On the other hand, high levels of nitrite have been found in home-made vegetable purees even after refrigerated storage for only 12 hours or more (Sánchez-Echaniz and Benito-Fernández, 2001). Presumably pureeing releases endogenous nitrate reductase causing excessive formation of nitrite particularly in vegetables containing high level of nitrate like spinach and silverbeet. The authors recommended that infant food should be prepared for immediate use or kept frozen when consumption is delayed for more than 12 hours.

Frozen

Nitrite accumulation is inhibited under frozen storage (Phillips, 1968). Schuster and Lee found no significant changes in nitrate or nitrite content of spinach, beet, carrot, parsley-root, celery or potatoes during frozen storage for up to 12 weeks, (Schuster and Lee, 1987).

5.1.2 Processing

Washing

Nitrate is soluble in water and washing of leafy vegetables (lettuce, Lamb’s lettuce, endives) can reduce nitrate levels by 10-15% (Dejonckheere et al., 1994). Mozolewski and Smoczynski (2004) showed that levels of nitrate and nitrite in potatoes can also be decreased by 18 to 40 % and 25 to 75%, respectively after preliminary processing methods (washing, peeling and rinsing).
These findings are in line with other studies (Czarniecka-Skubina and Golaszewska, 2001; Golaszewska and Zalewski, 2001).

**Peeling**

The nitrate content in two potato varieties (Innowator and Santana) before peeling was 258 and 349 mg/kg dry matter, respectively, and it decreased considerably during French fries production. About 30% of the nitrate was removed during peeling. Preheating and cutting reduced the nitrate content by a further 20% and blanching by 30%. After final frying only 5-6% of the original nitrate content remained or 16-18 mg/kg dry matter (Rytel et al., 2005).

After peeling of potatoes, bananas and melons the nitrate content decreased by 34%, 62% and 41% (Dejonckheere et al., 1994) and only by 20% or 6.6% in beetroots for nitrate and nitrite, respectively (Czarniecka-Skubina et al., 2003).

**Cooking**

The distribution of nitrate in vegetables is not even across the product. For lettuce and spinach elimination of the stem and midrib resulted in a decrease of the nitrate content of 30-40% (Dejonckheere et al., 1994). The “flesh” makes up the bulk of the carrot, but had a significantly lower concentration of nitrate than the core tissue (Schuster and Lee, 1987). The largest amount of nitrate in potatoes is found in and just under the skin, however nitrite is more evenly distributed (Marin et al., 1998).

Different studies have shown reduction of nitrate levels when vegetables are cooked in water. Peas, cabbage, beans, carrots, potatoes and spinach, endives and celery leaves lost between 16 to 79%, of the nitrate, respectively, during cooking (Abo Bakr et al., 1986, Schuster and Lee, 1987, Dejonckheere et al., 1994). The content of nitrate and nitrite decreased similarly after boiling by about 50% in carrot, parsley-root, celery and potatoes (Roszczenko et al., 2001). Varoquax et al. (1986) showed that the diffusion of nitrate from carrots depended on water temperature, surface area (thickness of the carrot slice) and ratio of carrot to water with the total content of nitrate as measured in the carrot and water combined remaining constant (Schuster and Lee, 1987).

During thermal processing of potato tubers with different heating methods (boiling, microwave, steaming, and deep frying) losses of nitrate (16-62%) and nitrite (61-98%) have been reported (Mozolewski and Smoczynski, 2004). The greatest decrease in reducing nitrate (36-58%) and nitrite (82-98%) was observed when peeled potatoes were boiled in water compared to steaming methods. Deep frying of potatoes resulted also in considerable losses of nitrate (50-62%). However other studies reported that frying and baking of potatoes did not affect nitrate concentrations (MAFF, 1998a,b). Overall the losses of nitrite were greater then for nitrate when applying different preliminary processing and heating methods. Differences in nitrate and nitrite
losses were observed between potato varieties subjected to the same processing conditions (Mozolewski and Smoczynski, 2004).

**Other ways of food processing**

Limited data are available on nitrate and nitrite levels in canned vegetables. One study found that canned vegetables contained much higher amounts of nitrite (450 mg/kg) than those reported in the raw commodity (Jakszyn, et al., 2004).

In red beet and kohlrabi, nitrate was reduced by fermentation by up to 50% and in white cabbage by up to 87% (Preiss et al., 2002).

In summary, handling, storage, processing including washing, peeling and cooking can significantly reduce the amount of nitrate in vegetables. This holds true for vegetables eaten cooked, like potato, spinach and cabbage. For vegetables eaten raw, only handling and storage would impact nitrate levels. Since there is a trend nowadays towards consumption of fresh produce, and in particular leafy vegetable varieties, a conservative approach was adopted. Thus, the potential decreases in nitrate concentrations due to processing were not considered for the initial exposure calculations but can be considered as mitigating factors in a range of mixed vegetable consumption scenarios.

6. **Consumption of vegetables**

The World Health Organisation is responsible for the Global Environment Monitoring System - Food Contamination Monitoring and Assessment Programme, commonly known as GEMS/Food. As part of its mandate to assess the potential exposure of populations to chemicals in food, GEMS/Food from 1989 onwards produced estimates of regional dietary patterns of raw and semi-processed food commodities. The latest GEMS/Food European Regional Diet from 2003 was previously discussed when stratifying occurrence data in Chapter 5 since it provides a Europe wide estimate (WHO, 2003a). In 1997, GEMS/Food was given the mandate to refine the regional diets and in 2006 introduced the GEMS/Food Consumption Cluster Diets database (WHO, 2006). The GEMS/Food Consumption Cluster Diets are based on national food balance sheets of annual food production as well as import and export for individual countries aggregated into clusters according to similar consumption behaviour. The main advantage of the data is the good comparability between different countries because the same methodology and standardised food classification system of the Codex Alimentarius were used. There are 13 cluster diets in total and because different EU Member States are part of four clusters (Table 13) there are some indications of variability in intake patterns. However, data from food balance sheets do not give information on consumption at the individual level, so that only a “per capita” mean consumption amount of a population can be derived. Information on high percentiles of the population and on selected population subgroups (age-groups, vulnerable subgroups) cannot be derived from these data.
Table 13. Composition of GEMS/Food Consumption Cluster Diets that include European Member States and Norway.

<table>
<thead>
<tr>
<th>Cluster B</th>
<th>Cluster D</th>
<th>Cluster E</th>
<th>Cluster F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyprus</td>
<td>Albania</td>
<td>Austria</td>
<td>Estonia</td>
</tr>
<tr>
<td>Greece</td>
<td>Armenia</td>
<td>Belgium</td>
<td>Finland</td>
</tr>
<tr>
<td>Israel</td>
<td>Azerbaijan</td>
<td>Croatia</td>
<td>Iceland</td>
</tr>
<tr>
<td>Italy</td>
<td>Belarus</td>
<td>Czech Republic</td>
<td>Latvia</td>
</tr>
<tr>
<td>Lebanon</td>
<td>Bosnia and Herzegovina</td>
<td>Denmark</td>
<td>Lithuania</td>
</tr>
<tr>
<td>Portugal</td>
<td>Bulgaria</td>
<td>France</td>
<td>Norway</td>
</tr>
<tr>
<td>Spain</td>
<td>Georgia</td>
<td>Germany</td>
<td>Sweden</td>
</tr>
<tr>
<td>Turkey</td>
<td>Iran, Islamic Rep of</td>
<td>Hungary</td>
<td></td>
</tr>
<tr>
<td>United Arab Emirates</td>
<td>Kazakhstan</td>
<td>Ireland</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kyrgyzstan</td>
<td>Luxembourg</td>
<td></td>
</tr>
<tr>
<td>Moldova, Republic of</td>
<td>Romania</td>
<td>Netherlands</td>
<td></td>
</tr>
<tr>
<td>Russian Federation</td>
<td>Serbia and Montenegro</td>
<td>Slovakia</td>
<td></td>
</tr>
<tr>
<td>Tajikistan</td>
<td>The former Yugoslav</td>
<td>Slovenia</td>
<td></td>
</tr>
<tr>
<td>Republic of Macedonia</td>
<td>Turkmenistan</td>
<td>United Kingdom</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ukraine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uzbekistan</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Consumption of vegetables in the four cluster diets including EU Member States is illustrated in Table 14 as well as the GEMS/Food European regional diet.

Table 14. Consumption of vegetables as estimated in the GEMS/Food Consumption Cluster Diets for the four clusters relevant to the EU and the European Regional Diet.

<table>
<thead>
<tr>
<th>Food Group</th>
<th>GEMS/Food - consumption g/person per day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cluster diets</td>
</tr>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td>Roots and tubers</td>
<td>246</td>
</tr>
<tr>
<td>Other vegetables</td>
<td>525</td>
</tr>
</tbody>
</table>

Consumption of roots and tubers are fairly similar between the different diets. However, there is considerable variation in overall vegetable intake with the cluster B estimated other vegetable consumption more than double the amount of cluster F. The average European regional diet of 372 g seems to be a good overall approximation of an average of the four cluster diets for other vegetable consumption.

Consumption information was also supplied by 11 Member States and Norway including different ranges of vegetables depending on the data available as shown in Table 15. The information was extracted from the most recent national food consumption surveys undertaken within the last ten years. Different survey methodologies used make a direct comparison between countries unreliable, but the information can be used to identify high consumers.
Table 15. Individual vegetable consumption information as supplied by MS and Norway.

<table>
<thead>
<tr>
<th>Product</th>
<th>Country</th>
<th>Whole population</th>
<th>Eaters only</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>P95</td>
<td>P97.5</td>
<td>Mean</td>
</tr>
<tr>
<td>Broccoli</td>
<td>Germany 4</td>
<td>19</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Ireland 6</td>
<td>24</td>
<td>34</td>
<td>13</td>
</tr>
<tr>
<td>Cabbage</td>
<td>Czech Republic 23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Estonia 25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Germany 45</td>
<td>106</td>
<td>128</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>Ireland 11</td>
<td>39</td>
<td>46</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Norway 4</td>
<td>16</td>
<td>22</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Spain 2</td>
<td>18</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Sweden 5</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cauliflower</td>
<td>Czech Republic 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Estonia 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Germany 11</td>
<td>33</td>
<td>42</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Ireland 3</td>
<td>19</td>
<td>26</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Norway 1</td>
<td>3</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Spain 4</td>
<td>30</td>
<td>35</td>
<td>47</td>
</tr>
<tr>
<td>Celery</td>
<td>Czech Republic 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Germany 0</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Ireland 1</td>
<td>6</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Spain 0</td>
<td>5</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Chard leaves</td>
<td>Spain 5</td>
<td>36</td>
<td>42</td>
<td>55</td>
</tr>
<tr>
<td>Ch. cabbage</td>
<td>Norway 4</td>
<td>15</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Courgette</td>
<td>Ireland 1</td>
<td>4</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Spain 3</td>
<td>25</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td>Cucumber</td>
<td>Czech Republic 18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Estonia 19</td>
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<td>9</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Norway 6</td>
<td>22</td>
<td>31</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Spain 2</td>
<td>16</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Sweden 9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curly kale</td>
<td>Germany 1</td>
<td>9</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td>Green onion</td>
<td>Czech Republic 25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Estonia 16</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Ireland 16</td>
<td>42</td>
<td>48</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Norway 0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Spain 13</td>
<td>40</td>
<td>45</td>
<td>18</td>
</tr>
<tr>
<td>Iceberg lettuce</td>
<td>Germany 1</td>
<td>2</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Ireland 2</td>
<td>10</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>Lettuce</td>
<td>Czech Republic 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Germany 17</td>
<td>63</td>
<td>79</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Ireland 1</td>
<td>8</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Spain 36</td>
<td>103</td>
<td>116</td>
<td>57</td>
</tr>
<tr>
<td>Parsley</td>
<td>Czech Republic 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Germany 1</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Potato</td>
<td>Cyprus 144</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Czech Republic 199</td>
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<tr>
<td></td>
<td>Estonia 224</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Germany 119</td>
<td>244</td>
<td>280</td>
<td>121</td>
</tr>
<tr>
<td></td>
<td>Ireland 247</td>
<td>608</td>
<td>768</td>
<td>249</td>
</tr>
<tr>
<td></td>
<td>Lithuania 243</td>
<td></td>
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<td></td>
<td>Spain 57</td>
<td>143</td>
<td>160</td>
<td>70</td>
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<tr>
<td></td>
<td>Sweden 142</td>
<td>144</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 15. continued.

<table>
<thead>
<tr>
<th>Product</th>
<th>Country</th>
<th>Whole population</th>
<th>Eaters only</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>P95</td>
<td>P97.5</td>
</tr>
<tr>
<td>Spinach</td>
<td>Czech Republic</td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Ireland</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Spain</td>
<td>5</td>
<td>37</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>Sweden</td>
<td>1</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>UK</td>
<td>11</td>
<td>44</td>
<td>44</td>
</tr>
</tbody>
</table>

P95 and P97.5 indicate the 95th and 97.5th percentile of consumption, respectively. Consumption amounts are given distributed across the whole population and across eaters only. The proportion of eaters is indicated in the last column and is a measure of consumers of the respective product during the survey period. Not all of the 11 Member States provided a full breakdown of the consumption statistics.

The information supplied was aggregated at different levels by Member States as shown in Table 16. The aggregated information is an approximation only. Some Member States aggregated their information into a group called leaf and stem vegetables, others into all lettuce or salad vegetables. There are considerable overlaps between those groups and the information should be taken as an indication only of the actual consumption.

The “most vegetable” grouping excludes potato consumption. The custom of including or excluding potato consumption when calculating total vegetable intake vary between regions.

The “eaters’ only” frequency for other vegetables in Table 16 indicates consumption of at least one vegetable during the survey period explaining the almost 100% frequency.

Comparing the information provided through GEMS/Food based on vegetable volumes for production and trade with the individual Member State information based on reported consumption it is clear that as expected the GEMS/Food data is on the high side since it does not include wastage. Using the GEMS/Food information as an upper limit for mean consumption would thus prove to be a conservative approach.
Table 16. Aggregated vegetable consumption information as supplied by Member States and Norway.

<table>
<thead>
<tr>
<th></th>
<th>Whole population</th>
<th>Eaters only</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>P95</td>
<td>P97.5</td>
</tr>
<tr>
<td><strong>Most vegetables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Austria</td>
<td>148</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Czech Republic</td>
<td>213</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lithuania</td>
<td>136</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norway</td>
<td>135</td>
<td>317</td>
<td>393</td>
</tr>
<tr>
<td>Sweden</td>
<td>99</td>
<td>100</td>
<td>99</td>
</tr>
<tr>
<td><strong>Leaf &amp; stem vegetables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyprus</td>
<td>64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>7</td>
<td>28</td>
<td>38</td>
</tr>
<tr>
<td>Lithuania</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>All lettuce</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>8</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Norway</td>
<td>4</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>UK</td>
<td>10</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td><strong>Salad vegetables</strong></td>
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</tr>
<tr>
<td>Germany</td>
<td>20</td>
<td>71</td>
<td>97</td>
</tr>
<tr>
<td><strong>Cabbages</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyprus</td>
<td>58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>3</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td><strong>Herbs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estonia</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Summarising the information the following can be noted:

- For “most vegetables”, excluding potato, the highest 97.5th percentile daily consumption of 393 g per person was recorded in Norway (Table 16) and this was also close to the food balance information established by the GEMS/Food Regional Diet for a mean of 372 g per capita. A minimum amount of 400 g is also recommended by WHO for fruit and vegetable intake combined, which could be satisfied by consuming the whole amount as vegetables. In consequence and taking a conservative approach a daily consumption figure for “most vegetables” of 400 g was used in this opinion as the recommended target figure (exposure scenario S1, see chapter 7).

- For potatoes the highest 97.5th percentile daily consumption of 771 g per person was recorded in Ireland (see Table 15). This was almost three times higher than the mean recorded in the GEMS/Food Regional Diet or Cluster Diets. To estimate the upper limit for the potential contribution of potato consumption to nitrate exposure a figure of 771 g was used in a separate exposure scenario (S2).

- For leafy vegetables (lettuce) the highest 97.5th percentile daily consumption of 133 g per person was recorded in Spain (Table 15). To estimate the upper limit for the potential contribution of leafy vegetable consumption to nitrate exposure the figure of 133 g was used
for one scenario as the only vegetable and in a further scenario combined with the remainder of the recommended target case consumption allocated to other vegetables.

7. Exposure scenarios

The following exposure scenarios only include nitrate intake from vegetable sources. On the basis of the data collected, scenarios combining levels of consumption with concentration of nitrate in the relevant food category were elaborated. In summary, the base case assumed the consumption of vegetables, other than potatoes, at a level compatible with international dietary recommendations (400g/day) where the whole recommended intake was in the form of vegetables (no fruit). This represents a conservative approach. The influence of potatoes on the dietary exposure to nitrate was estimated in a separate scenario by using high percentile consumption. Since leafy vegetables exhibited high nitrate levels in general, high percentile consumption of leafy vegetables was applied to either spinach or “lettuce varieties” (Table 12) in two separate sub-scenarios, A and B respectively, see below. Those two sub-scenarios were expanded by also including the contribution of other vegetables using the remaining dietary intake from the base case. The impact of the trend to include rucola as a significant component of a leafy salad mix or by itself as a salad side dish or as a main pizza topping was tested in sub-scenario C. Finally, the highest recorded regional median concentrations of nitrate in spinach and “lettuce varieties” were applied to the previous sub-scenarios A and B to provide an upper estimate of nitrate dietary exposure. In calculating high percentile consumption, information from the GEMS/Food database or data submitted by the Member States were considered. The following presents a detailed description of the scenarios (S) used.

- **S1** (“recommended vegetable and fruit intake scenario”): The first scenario is a base case founded on the daily consumption of 400 g of fruits and vegetables recommended by WHO to reduce the risk of coronary heart disease, stroke, high blood pressure and cancer (WHO, 2003b), but with the whole amount allocated to vegetables. This figure also corresponds very closely to the highest consumption of “most vegetables” reported by Norway as the 97.5th percentile of the distribution (Table 16) as well as GEMS/Food Regional Diet indicating a mean intake of 372 g per capita (Table 14). The concentration of nitrate used is the median of 392 mg/kg for “most vegetables” as presented in Table 12. It should be noted that some vegetables will be consumed only once a day or less while others might be consumed on several occasions. Because of the potential for chronic effects of nitrate, use of the median for “most other vegetables” stratified according to volumes consumed should cater for such variations.

- **S2** (“potato scenario”): The second scenario was developed to separately determine the potential influence of potato consumption on nitrate intake at the upper level. It is based on the highest 97.5th percentile of consumption of potatoes of 771 g reported by Ireland (Table 15). The concentration of nitrate is the median for potatoes (Table 12). The result can be added to other scenarios, although it is considered unlikely that at this consumption level there is potential for further vegetable consumption.

- **S3** (“green/leafy vegetables scenario”): The third scenario explores the influence of consumption of vegetables only from the “leafy vegetable” group containing the highest levels of nitrate recorded. It is based on the highest 97.5th percentile for consumption of
“leafy vegetables” (lettuce) of 133 g reported by Spain (Table 15). The median concentrations of nitrate in spinach and all combined lettuce varieties are used alternatively to provide respective scenarios S3A and S3B. In scenario S3C rucola is assumed to comprise one third of a leafy vegetable mix with lettuce varieties as the remaining two thirds.

- **S4 (“combined S1 and S3 scenario”):** In the fourth scenario it is assumed that while consuming leafy vegetables at the highest percentile volume as in scenario S3, other vegetables will be consumed as well. It is thus based on the recommended daily consumption of 400 grams as outlined in S1 by splitting consumption into 133 g of leafy vegetables, alternatively with the median concentrations of nitrate in spinach and lettuce varieties, and 267 g of other vegetables with their median concentration of nitrate to provide respectively scenarios S4A and S4B.

- **S5 (“regional scenario”):** The fifth scenario is similar to the fourth but the overall EU median concentration for leafy vegetables was replaced by the highest “regional” median concentration reported to estimate the impact of regional differences.

The main potential adverse effect of nitrate results from long-term exposure, and therefore the median or mean value is used for dietary exposure assessments. All the scenarios are based on median content of nitrate assuming that a consumer is randomly choosing vegetables on the market. Nevertheless the fifth scenario (S5) assumes that a consumer is choosing randomly vegetables from a “regional” market in which the median concentration for nitrate is higher than the overall European median concentration for the same categories of vegetables.

Results from calculating dietary exposure to nitrate in the different scenarios using a deterministic approach are presented in Table 17.

Table 17. Various dietary exposure scenarios based on nitrate from vegetables only.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Vegetable consumption g/person/day</th>
<th>Vegetable</th>
<th>Overall median (S5 - highest regional median) nitrate concentration mg/kg a)</th>
<th>Calculated exposure mg/person/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Potato</td>
<td>Spinach</td>
<td>Lettuce</td>
</tr>
<tr>
<td>S1</td>
<td>400</td>
<td>Most</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td>771</td>
<td>Potato</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S3</td>
<td>133 (44/89) e)</td>
<td>Leafy</td>
<td>106</td>
<td>785</td>
</tr>
<tr>
<td>S4</td>
<td>133/267</td>
<td>Leafy/Most</td>
<td></td>
<td>785</td>
</tr>
<tr>
<td>S5</td>
<td>133/267</td>
<td>Leafy/Most</td>
<td></td>
<td>1,745 b)</td>
</tr>
</tbody>
</table>

a) See Table 12; b) Highest regional median; c) Spinach at 133 g; d) Lettuce varieties at 133 g; e) 1/3 of a leafy vegetable mix as rucola (44 g) and 2/3 as lettuce varieties (89 g); f) Spinach at 133 g and other vegetables at 267 g; g) Lettuce varieties at 133 g and other vegetables at 267 g

A= spinach; B= all combined lettuce varieties; C= a mix of rucola (1/3) and lettuce varieties (2/3)

Table 17 indicates that consuming vegetables only at the levels found in many dietary recommendations for the combined consumption of vegetables and fruit (S1) could result in a nitrate exposure of 157 mg/person/day. A focus on potato consumption could for a high percentile consumer (S2) lead to nitrate exposure of 82 mg/person/day at the most. A high-level
consumer of either spinach (S3A) or lettuce varieties (S3B) could record nitrate exposure levels of 104 and 178 mg/person/day, respectively. However, by replacing a third of the lettuce varieties by rucola (S3C) the nitrate exposure would jump to 330 mg/person/day or almost double the contribution of lettuce varieties alone. In the event that the consumer from scenario S1 consumed spinach or lettuce varieties at the 97.5th percentile level as part of the 400 g vegetable mix, a third of the vegetables would be consumed as spinach (S4A) or lettuce (S4B) and the nitrate exposure would increase to 209 and 283 mg/person/day, respectively. Finally, should the last two scenarios occur in regions reporting the highest median nitrate occurrence levels, a nitrate exposure of 337 and 457 mg/person/day for spinach (S5A) and lettuce varieties (S5B), respectively, would be possible. However, only a very small proportion of the European population would even theoretically reach such level, it would be for sporadic periods only, and a number of mitigating factors would generally apply.

The various exposure scenarios demonstrated that the critical driver for a high dietary exposure to nitrate is not the absolute amount of vegetables consumed but the type of vegetable (i.e. leafy vegetables) and the concentration of nitrate related to the conditions of production. Thus consumption of a variety of vegetables, as promoted in dietary recommendations, contributed to less nitrate than lettuce varieties at an almost three times lower consumption level. Leafy vegetables grown under less favourable conditions had the potential to increase nitrate dietary exposure by 50-60%. The contribution of potato consumption to nitrate dietary exposure is lower or much lower than the contribution from a mix of other vegetables.

The above scenarios used nitrate concentrations as determined at retail for fresh commodities. Very few analytical results were available for nitrate in vegetables ready to eat. Washing of vegetables and heating have been shown to reduce nitrate concentrations to varying extents. A 40% reduction during cooking as indicated in a previous section would alone reduce the dietary nitrate intake in scenario S4B from 283 to 241 mg per day. A further mitigating factor is the role of fruit as part of fruit and vegetable consumption. Across 14 European countries examined, fruit contributed from a third to slightly more than half of the total fruit and vegetable consumption (EFSA, 2008). Fruit in general contains low levels of nitrate of the order of 10 mg/kg according to a review by White (1975) of three previous reports. Thus, a mixed fruit and vegetable diet can be estimated to reduce the base case scenario (S1) from 157 mg nitrate per day to between 81-106 mg with an estimated substitution of vegetables for fruit of 133-200 g of the 400 g daily total consumption.

The potentially high levels seen during winter in leafy vegetables in certain regions would not be sustained during the summer months. Thus, applying and combining the above mitigating factors that may occur from processing losses together with typical levels of fruit consumption as well as a six-month winter influence only, scenario S5A would be reduced to 171 mg per day and scenario S5B to 210 mg per day on an annualised basis.
Population subgroups

Accurate data are not available for children’s fruit and vegetable consumption although it is known that overall they significantly favour the fruit component (Gregory et al. 2000; Richter et al., 2008). Considering that nutritional recommendations are also valid for children, and in the absence of actual data the estimate was made that children could consume half the amount of adults. Thus 200 g of vegetables was considered to be a reasonable figure for children high consumers. Therefore, taking a body weight of 20 kg, the daily nitrate exposure for children would range from 2 to 12 mg/kg b.w./day under the different scenarios when calculating dietary intakes at half the adult levels and without considering mitigation factors.

Vegetarians and vegans are estimated to make up to 5% of the population of different Member States. Vegetarians might be suspected to have higher nitrate intakes than the general population. However, due to the physiological requirement for proteins, products from animal origin are likely to be substituted by cereals, nuts and pulses, generally low in nitrate, and not with excessive amounts of vegetables. Therefore, vegetarians and vegans are not considered likely to exceed significantly the 400g vegetables and fruit scenario. This was confirmed in a study conducted by the UK Ministry of Agriculture, Fisheries and Food (MAFF, 2000) where mean dietary exposure of vegetarians to nitrate was 83 mg/day and the highest 209 mg/day.

Nitrite contribution

Regarding nitrite, the evidence shows that actual nitrite levels in vegetables are not a major direct contributor to human exposure (see Figure 1 c, f). Similarly to nitrate, the dietary exposure should be based on the central tendency of the distribution of nitrite in vegetables (i.e. the median or the mean). Nitrite levels up to 45 mg/kg crop have been reported occasionally in the literature (Jakszyn et al., 2004). A mean concentration of 0.5 mg/kg was found in the United Kingdom’s 1997 total diet study (MAFF, 1998b) for all vegetables. Combining those 2 figures with the recommended amount of vegetables (400 g/day) results in a dietary exposure ranging from 0.2 to 0.8 mg/day corresponding to 0.003 to 0.013 mg/kg b.w./day assuming 60 kg body weight. This is low compared to systemic amounts of nitrite resulting from the bioconversion of nitrate (see Figure 1 c, f).

8. Hazard identification and characterisation

This section presents a historical perspective and a summary, mainly of human data, for both nitrate and nitrite as other data principally from animals have recently been subject to a detailed review by the JECFA (FAO/WHO, 2003a,b). Where new studies have been published these are included together with the existing core safety studies.

8.1 Toxicokinetics

The fate of nitrate has been the subject of a great number of studies and the results have been compiled in several reviews (e.g. FAO/WHO, 2003a). Nitrate undergoes a number of metabolic
interconversions, and is recycled between the saliva and the gut and the bile and the gut. Lately appreciation of the complexity of its metabolic handling has increased rapidly as the research area of nitric oxide physiology has expanded (e.g. Gladwin et al., 2005).

**Absorption**

**Nitrate**

Nitrate is quickly and effectively absorbed from the upper part of the small intestine in humans after ingestion in food or water (Bartholomew and Hill, 1984; Ellen et al., 1982; Spiegelhalder et al., 1976; Turek et al., 1980). For example, no or very little nitrate or nitrite is found in ileostomic fluid from persons who have ingested 250 mg of nitrate, suggesting that nitrate does not reach the large intestine (Ellen and Schuller, 1983). In humans, an average 25-fold increase in plasma nitrate was found 10 min after ingestion of nitrate, and intake peaked in blood after 40 min (Cortas and Wakid, 1991). In the rat, more than 50% of an oral dose was detected in the eviscerated carcass within 1 h (cited in Walker, 1990). In humans and most laboratory animals plasma nitrate is selectively absorbed by the salivary glands and concentrated 10-fold, resulting in a salivary secretion that represents approximately 25% of the ingested dose (Witter and Balish, 1979; Fritsch et al., 1985). In humans the dose-dependent increase in salivary nitrate secretion, peaking 1-3 hours after oral ingestion (Bartholomew and Hill, 1984), is mediated by an active transport system that is shared also by iodide and thiocyanate (Forman et al., 1985a,b).

Nitrate can also be absorbed via inhalation e.g. from cigarette smoke and car exhausts (Ellen and Schuller, 1983; Lundberg et al., 2004 and 2008) although in absolute terms the quantitative amount is of minor importance compared to the oral route via the diet.

**Nitrite**

In humans, gastrointestinal (GI) absorption of sodium nitrite is rapid, with maximum plasma nitrite concentrations observed 15-30 min after dosing. Moreover, nitrite disappeared rapidly from plasma, with an average elimination half-life of 30 min. It was concluded that under fasting conditions 90-95% of orally administered sodium nitrite is absorbed from the gastrointestinal tract (Kortboyer et al., 1997). However, extensive pre-systemic metabolism in the GI tract, results in a considerable part of the nitrite that enters the GI tract potentially being transformed to other N-containing species before absorption takes place (Speijers et al., 1987).

**Distribution**

**Nitrate**

Absorbed nitrate is rapidly transported by the blood and selectively secreted by the salivary glands, and probably other exocrine glands, resulting in high salivary nitrate levels. After intravenous administration of $^{15}$N-labelled nitrate in one volunteer, the labelled compound was rapidly distributed in the bloodstream throughout the body. The radioactivity accumulated almost
linearly with time in a small region of the abdomen, which probably was due to the swallowing and entero-salivary recirculation of nitrate/nitrite (Witter et al., 1979).

**Nitrite**

Plasma nitrite levels are normally much lower than nitrate levels, firstly because of the lower exposure and secondly due to the rapid oxidation from nitrite to nitrate by oxygenated haemoglobin in the blood. Therefore, the sum of nitrate and nitrite in blood is almost identical to the nitrate levels (Lundberg and Weitzberg, 2005). This is also seen in body fluids and tissues of laboratory animals, where nitrite in the normal situation is practically absent, except in saliva where it increases as nitrate levels decrease (Witter and Balish, 1979; Fritsch et al., 1985; Cortas and Wakid, 1991). In mice and rabbits, intravenous injection of labelled nitrite resulted in a homogenous distribution of radioactivity to a number of organs, including liver, kidneys and urinary bladder (Parks et al., 1981).

**Metabolism**

There are some species differences in the metabolism of nitrate. In the case of humans, dogs and mini-pigs nitrate is concentrated from the plasma to the saliva and then commensal bacteria present on the back of the tongue reduce approximately 20% of the secreted nitrate to nitrite, which is then swallowed into the stomach. Nitrate is also secreted in the gut (Fritsch et al., 1985; McKnight et al., 1999, Xia et al., 2003).

Although, *in vitro* studies with rat tongue section have shown that nitrate reduction occurs in the back of the tongue with Staphylococcus sciuri and Staphylococcus intermedius as the major nitrate reducing bacterial species (Li et al., 1997). In the rat, oral reduction of nitrate to nitrite in the saliva is limited and nitrate is mainly secreted in the gastric and intestinal fluid by active transport involving entero-systemic recirculation as observed in man (McKnight et al., 1999; FAO/WHO., 2003a; Mensinga et al., 2003).

In humans, about 25% of ingested nitrate is secreted in the saliva and approximately 20% of the secreted salivary nitrate is then converted to nitrite by microorganisms on the tongue and thus for normal individuals about 5-7% of ingested nitrate can be detected as salivary nitrite. However, for individuals with a high rate of conversion this figure may be up to 20% (Eisenbrand et al., 1980; Speijers et al., 1987; Kortboyer et al., 1995; Lundberg et al., 1994; FAO/WHO, 2003a). The major site for nitrate reduction is at the base of the tongue where a stable, nitrate-reducing microflora is present (McKnight et al., 1999, Duncan et al., 1995). The concentration of salivary nitrite is directly related to orally ingested nitrate (Stephany and Schuller, 1978; Spiegelhalder et al., 1976), but the conversion may become saturated at high nitrate intakes (Tannenbaum et al., 1976). Oral reduction of nitrate is the most important source of nitrite for humans, and will account for approximately 70-80% of the human total nitrite exposure (Stephany and Schuller, 1980; Bos et al., 1985). Factors that may influence the oral microbial flora are, e.g., nutritional status, infection, environmental temperature and age. Salivary nitrite levels were generally higher in older age groups, although considerable variation between individuals was noted (Eisenbrand
After transport to the stomach, the acidic conditions will rapidly transform nitrite to nitrous acid, which in turn will spontaneously decompose to nitrogen oxides including nitric oxide. Compared to the enzymatically produced nitric oxide in mammalian cells (from L-arginine by nitric oxide synthases, see below), the concentration of nitric oxide in the upper intestine is up to 10,000 times higher (McKnight et al., 1997).

A low pH in the fasting stomach (pH 1-2) is considered too low for microbial growth and, as a consequence, for bacterial nitrate reduction. However, in normal healthy adults a significant proportion (30-40 %) of the population was found to have a fasting pH over 5, which results in increased bacterial activity and hence nitrite levels (Ruddell et al., 1976; Müller et al., 1984). Infants younger than 3 months are highly susceptible to gastric bacterial nitrate reduction to nitrite because they have very little production of gastric acid (Ellen and Schuller, 1983; Kross et al., 1992). Gastrointestinal infections in infants may produce an additional increase in the reduction of nitrate to nitrite.

Nitrate undergoes active secretion in humans not only in the salivary duct cells but also in the gastric parietal cells and occurs at a number of other sites leading to enterosystemic cycling of nitrate and nitrite. Additionally nitrate biotransformation is complex and involves nitrate reduction, nitrite formation, nitrite reoxidation to nitrate, and resulting methaemoglobin in a dynamic equilibrium (Lundberg et al., 2004 and 2008; Gladwin, et al., 2005). Nitrite appears to have a transient role with nitrate being the normal state.

**Endogenous nitrate/nitrite formation**

There are many reports of an excess of urinary nitrate excretion compared with that ingested at low nitrate intakes (Bartholomew and Hill, 1984; Lee et al., 1986; Gangolli et al., 1994). Ellen and Schuller (1983) suggested that a part of this excess excretion could originate from the inhalation of nitrate and nitrite from indoor and outdoor air and cigarette smoke, although the main part most probably originates from endogenous synthesis.

The main source of endogenous nitrate in mammals is the L-arginine-NO synthase pathway, which is constitutively active in numerous cell types throughout the body. Nitric oxide is produced from the amino acid L-arginine and molecular oxygen by nitric oxide synthetase (NOS). Under basal conditions, the metabolites of endogenous nitric oxide in plasma are mainly derived from the L-arginine-NO pathway in the endothelium of blood vessels and possibly neuronal tissue. However, during systemic inflammatory reactions or infections, white blood cells express an inducible NOS, which produces large amounts of nitric oxide and ultimately, by the binding to oxidised haemoglobin, results in methaemoglobin and a considerable increase in the concentrations of nitrate in plasma (Lundberg et al., 2004 and 2008). In tissues other than the blood, nitrite is formed by reductive pathways and further oxidation produces nitrate (Jensen,
2005; Gladwin, et al., 2005). For example, in the dog large quantities of nitrate were excreted in the bile when the dogs received $^{15}$N-labelled nitrite, indicating endogenous oxidation of nitrite (Fritsch et al., 1985).

In recent years, the function of nitric oxide in vascular physiology has become better understood and nitrite is now considered to be a nitric oxide donor under physiological conditions. Thus, low oxygen pressure, low pH and high nitrite concentration favour nitric oxide formation from nitrite, and in mammalian red blood cells nitrite is thus reduced to nitric oxide by deoxyhaemoglobin (Cosby et al., 2003; Gladwin et al., 2004). On the other hand, oxidized haemoglobin will react with nitrite to form nitrate and methaemoglobin (Kosaka and Tyuma, 1987). Normal levels of methaemoglobin in human blood are 1-3 %, and reduced oxygen transport has been noted clinically when methaemoglobin concentration reaches 10 % or more (Walker, 1990; FAO/WHO, 2003a,b). The balance between the two different haemoglobin reactions produces nitric oxide at low oxygen pressure and the vasodilation induced by nitric oxide will increase blood flow to reverse the situation (Jensen, 2005).

**Nitrosamine formation**

In healthy human volunteers, N-nitrosomorpholine was detected in stomach samples, and the level increased after ingestion of nitrate. Radioactive labelled nitrogen confirmed that the nitrosamine-nitrogen originated from nitrate, demonstrating in situ formation of N-nitrosamine from dietary nitrate via nitric oxide (Winter et al., 2007). Nitrosamines were formed in the gastrointestinal tract of Sprague Dawley rats after feeding a normal rat chow, and also in rats fed semipurified diets mixed with meat or hot dogs. In the latter case, the nitrosamine levels increased 2-3 times above control (semipurified diet alone). In a dynamic in vitro gastrointestinal model, the formation of N-nitrosodimethylamine (NDMA) was observed after gradually adding nitrite to food samples (fish). For some of the samples the model produced measurable NDMA levels, and the addition of orange juice or tea (antioxidants) generally decreased the NDMA formation (Krul et al., 2004).

Thus overall, when nitrate is consumed as part of a normal diet containing vegetables, other bioactive substances concomitantly consumed such as the antioxidant vitamin C can reduce the amount of nitrosamine formed by up to half (Brambilla and Martelli, 2007).

**Excretion**

About 25 % of an oral nitrate dose was secreted in the saliva (Eisenbrand et al., 1980; Speijers et al., 1987; Kortboyer et al., 1995; Lundberg et al., 1994; FAO/WHO, 2003a), but there were marked inter-individual and diurnal variations in this secretion (Bartholomew and Hill, 1984; Cortas and Wakid, 1991). In a study on healthy volunteers that were administered an oral dose of 10 mg sodium nitrate/kg b.w. and monitored for one day, the cumulative salivary nitrate excretion, expressed as percentage of the ingested dose, was 28 % (Kortboyer et al., 1995). In the minipig, an appropriate model for humans in terms of salivary secretion, bilateral removal of the
parotid glands led to a significant decrease of nitrate secretion from blood to saliva, and thus low nitrite levels. The study suggests that the parotid salivary glands play an important role in the balance of nitrate and nitrite levels in the body (Xia et al., 2003).

Single oral gavage of varying doses of potassium nitrate gave a urinary nitrate excretion of 65-70% irrespective of dose. Excretion was maximal 5 h after ingestion and returned within 18 h to baseline levels, which in fasting subjects were 10-20 mg/litre (Bartholomew and Hill, 1984; Tannenbaum and Green, 1981; Wagner et al., 1983). Results indicate a predominantly tubular excretion of nitrate (Ellen et al., 1982). In a study on healthy infants, the urinary excretion of nitrate (average 8.7 mg nitrate/day) was as high as, or even higher, than a low (average) intake of 2-7 mg nitrate plus nitrite per day. The authors concluded that excretion probably included endogenously formed nitrate (Hegesh and Shiloah, 1982). In the anaesthetized dog, urinary excretion rates of nitrate increase progressively in response to increases in the circulating levels without exhibiting a maximum; however, there was a progressive decrease in fractional reabsorption with increasing dose (Godfrey and Majid, 1998). It should be noted that a major part of the primary urinary nitrate (ca 80%) is pumped back to the blood by an active transport mechanism (Kahn et al., 1975). This salvaging of nitrate from the urine, in addition to the known recycling of nitrate from saliva and also from the intestines (after biliary excretion) strongly suggests that the body is acting to conserve a substance of physiological importance.

In faeces, low levels of nitrate and nitrite are present (Saul et al., 1981; Wagner et al., 1983). However, the observed conversion of nitrate to nitrite by the faecal microflora suggests that biliary excretion of nitrate may be higher than the amount detected in the faeces (Archer et al., 1982; Saul et al., 1981). In a model developed by Schultz and co-workers (1985) the bacteria of the large intestine were suggested to be responsible for about half of the extrarenal removal of nitrate from the body.

Levels up to 5 mg nitrate/kg breast milk have been reported (Sugekawa and Matsumoto, 1975). Nitrate levels in milk from lactating women after a normal meal did not exceed the simultaneously measured maternal plasma nitrate levels (Green et al., 1982).

**Summary**

In conclusion, once nitrate is ingested it is quickly absorbed from the gastrointestinal tract into the plasma in humans. About 25% of the plasma nitrate is taken up by the salivary glands, bioconcentrated approximately 10-fold and secreted into the saliva. In the mouth, bacterial reduction of approximately 20% of the secreted nitrate to nitrite occurs, normally constituting 5-7% of the absorbed nitrate dose in healthy adults. In the stomach, under acidic conditions, nitrite will be transformed to nitric oxide and other metabolites. Most of the absorbed nitrate is ultimately excreted in the urine, but considerable salvage takes place in advance through selective reabsorption from the kidney together with biliary and salivary recirculation.
8.2 General toxicology

The toxicity of nitrate is known to be low and adverse effects have been shown to arise from its metabolic conversion to nitrite (EC, 1997). This section presents a short summary of the toxicology of nitrate and nitrite with particular emphasis on the potential for human health effects. A full account of all toxicological studies will not be provided since this has been carried out previously by the JECFA (FAO/WHO, 2003a,b) and no new significant data have been found.

8.2.1 Acute toxicity

8.2.1.1 Nitrate

The acute oral toxicity of nitrate in animals is generally low with LD$_{50}$ values of approximately 2500-6250 mg/kg b.w./day in mice, 3300-9000 mg/kg b.w./day in rats, 1900-2680 mg/kg b.w. in rabbits and 300 mg/kg b.w. in pigs (Walker, 1990, Speijers, et al., 1987). It has been observed that the oral lethal dose of nitrate in humans is around 330 mg/kg b.w (Walker, 1990).

8.2.1.2 Nitrite

Sodium nitrite is approximately 10-fold more toxic than sodium nitrate depending on the species with LD$_{50}$ values of 214 mg/kg b.w. in mice, 180 mg/kg b.w. in rats and 186 mg/kg b.w. in rabbits (NIOSH, 1987).

8.2.2 Sub-chronic toxicity

8.2.2.1 Nitrate

No new subchronic studies have been identified for nitrate. Historically, no adverse effects were observed in two dogs after dosing sodium nitrate in the diet at a level of 2% for 105 and 125 days (Lehman, 1958) calculated to be equivalent to 500 mg/kg b.w./day corresponding to 370 mg/kg b.w. nitrate (Walker, 1990). Short term studies in rats dosed up to 10% sodium nitrate in drinking water over 6 weeks showed slight elevation of methaemoglobin.

8.2.2.2 Nitrite

A 14 week study was conducted in B6C3F1 mice (10 males and 10 females/group) with dose levels of 0, 375, 750, 1500, 3000, or 5000 ppm sodium nitrite (equivalent to average daily doses of approximately 90, 190, 345, 750, or 990 mg sodium nitrite/kg b.w. to males and 120, 240, 445, 840, or 1230 mg sodium nitrite/kg b.w. to females) in drinking water. Overall at the highest dose, body weight, spleen weight and sperm counts were lower in males compared to controls and in females, absolute and relative organ weights (heart, kidney, liver and spleen), together with the length of estrous cycle, were impaired. Histopathological examination showed that squamous cell hyperplasia of the forestomach and extramedullary haematopoiesis were more frequent at the two highest dose levels in both sexes. Degeneration of the testis was seen in...
males at 750 mg/kg b.w. and above. The NOAEL was concluded to be 190 mg/kg b.w./day (NTP, 2001).

A 14 week study was conducted in male and female rats (10 males and 10 females/group) at dose levels of 0, 375, 750, 1500, 3000, or 5000 ppm sodium nitrite (equivalent to average daily doses of approximately 30, 55, 115, 200, or 310 mg sodium nitrite/kg b.w. to males and 40, 80, 130, 225, or 345 mg sodium nitrite/kg b.w. to females) in drinking water. Elevated methaemoglobin (metHb) was observed at all dose levels. Sperm motility was the endpoint related to a no observed effect level (NOEL) of 55 mg/kg for sodium nitrite corresponding to 37 mg/kg for nitrite (NTP, 2001).

Methaemoglobin results from the reaction of nitric oxide with oxyhaemoglobin at the same time forming nitrate. A number of factors are critical to metHb formation including the presence of increased nitrite, intestinal infection together with inflammation of the stomach lining and NADH-cytochrome b5 methaemoglobin reductase (which converts methaemoglobin back to haemoglobin). Methaemoglobin is produced normally with background levels of 1-3%. Levels of 10% or more have been shown clinically to reduce oxygen transport. At levels above 20%, cyanosis and hypoxia can occur and an increase to 50% methaemoglobin can prove fatal (Mensinga et al., 2003). Infants younger than 3 months of age are more susceptible to methaemoglobinaemia than adults due to a 40-50% lower activity of NADH-cytochrome b5 methaemoglobin reductase r (which converts methaemoglobin back to haemoglobin) and their increased risk for intestinal infections (Savino et al., 2006).

Hypertrophy of the adrenal zona glomerulosa has been investigated in a 13-week study using Wistar rats. A no observed adverse effect level (NOAEL) of 5.4 mg/kg b.w./day for the nitrite was found (Til et al., 1997). The mechanism is considered to involve nitrite-induced vasodilatation via nitric oxide production and a reduction in blood pressure activating the renin-angiotensin system in the kidney. Consequential mechanisms to restore the physiological blood pressure result in the production of the vasoconstrictor angiotensin II and release of aldosterone from the adrenal zona glomerulosa, resulting in hypertrophy of the zona glomerulosa (Boink et al., 1998; Mensinga et al., 2003).

8.3 Genotoxicity

From the peer-reviewed literature, sodium nitrate was not found to be mutagenic in in vitro tests. For nitrite, in vitro mutagenic potential was shown in Salmonella typhimurium strain TA100 both with and without metabolic activation but not in strain TA98 (NTP, 2001).

In vitro culture of peripheral blood lymphocytes has been used to evaluate the ability of nitrate and nitrite to produce chromosome aberrations in mammalian cells. Sodium nitrate did not increase aberrations (17.6-70.6 mM) but high doses of sodium nitrite (14.4 mM) resulted in a slight increase in micronucleated cells and chromatid gaps (Balimandawa et al., 1993). However, in vivo no micronuclei induction occurred in the bone marrow of rats and mice after intraperitoneal injection and a test for micronuclei in peripheral blood from mice in the 14-week
study (described above) also gave negative results suggesting that overall sodium nitrite is not genotoxic in vivo (NTP, 2001, FAO/WHO, 2003a,b).

A significant increase in the mean number of chromatid/chromosome breaks was reported in a group of Greek children exposed to nitrate concentrations above 70.5 mg nitrate/L in drinking water compared to a control group exposed to very low nitrate concentrations (i.e., 0.7 mg/L). There was no significant increase in the mean number of sister chromatid exchanges per cell (Tsezou et al., 1996).

Overall, the JECFA concluded that there was no evidence for the reclassification of either nitrate or nitrite as genotoxic compounds (WHO/FAO, 2003a,b).

### 8.4 Chronic toxicity/ carcinogenicity

#### 8.4.1 Nitrate

A number of long-term toxicity/carcinogenicity studies have been performed. Firstly, rats were given 0, 0.1, 1, 5 and 10 % sodium nitrate in the diet for 2 years (Lehman, 1958; Walker, 1990) equivalent to 0, 50, 500, 2,500 and 5,000 mg/kg b.w./day (Walker, 1990). A NOEL of 500 mg/kg b.w./day was established for sodium nitrate based on a slight depression in growth rate and inanition at higher doses. No adverse histological changes or increase in tumour frequency were found (Lehman, 1958 as cited by WHO/FAO, 1962). Secondly, rats were dosed with 0 or 0.5% sodium nitrate in drinking water over 84 weeks (Lijinsky et al., 1973) calculated to be equivalent to 0 and 500 mg/kg b.w./day (Walker, 1990). No histopathological effects of treatment were observed. Thirdly, in a more recent 2 year study rats were given 0, 2.5 and 5 % sodium nitrate in drinking water (Maekawa et al., 1982) calculated to be equivalent to 0, 2,500 and 5,000 mg/kg b.w./day (Walker, 1990). At 5,000 mg/kg b.w./day, slight to moderate reduced body weight gain was observed. From this study a NOAEL of 2,500 mg/kg/b.w/day for nitrate was derived. Overall, these studies demonstrate a low chronic toxicity of nitrate.

#### 8.4.2 Nitrite

In a two year chronic toxicity study in rats given nitrite in the drinking water equivalent to doses of 0, 10, 100, 200 and 300 mg/kg b.w./day no significant differences between control and treated groups were shown for growth, mortality and total haemoglobin levels. At the highest three doses, methaemoglobin increased to 5, 12 and 22% and lung toxicity was observed with dilatation of the bronchi with infiltration of lymphocytes and emphysema. At the highest dose, focal degeneration and fibrosis of the heart muscle as well as dilatation of coronary arteries were also observed. Based on heart and lung toxicity the NOAEL for sodium nitrite was 10 mg/kg b.w./day and hence the NOAEL for the nitrite ion was 6.7 mg/kg b.w./day (Gruener and Shuval, 1973).

More recently, two year carcinogenicity studies for sodium nitrite were conducted under the National Toxicology Programme (NTP, 2001) in B6C3F1 mice and F344/N rats. In the mouse study, 50 male and 50 female B6C3F1 mice were exposed through drinking water to daily doses
equivalent to 0, 60, 120, or 220 mg/kg b.w./day and 0, 45, 90, or 165 mg/kg b.w./day respectively. Overall, there was no difference in survival between exposed groups compared to controls although mean body weights were lower in females treated with the highest dose. Exposed groups generally consumed less water than the control groups. The incidences of squamous cell papilloma or carcinoma (combined) in the forestomach of female mice occurred with a “positive dose-related trend” (not statistically significant) with respective frequencies of 1/50, 0/50, 1/50 and 5/50 at 0, 45, 90, or 165 mg/kg b.w./day respectively. The incidence of hyperplasia of the glandular stomach epithelium was significantly greater in males treated at the highest dose. In females, the authors concluded that there was equivocal evidence\(^\text{16}\) for carcinogenic activity (NTP, 2001) based on the trend in the combined incidence of squamous cell papilloma and carcinoma of the forestomach.

8.5 Endocrine toxicity

Nitrate intake could have the potential to adversely affect thyroid function as nitrate shares the same transport mechanism as iodide. This inhibition could lead to a decrease in circulating thyroid hormone levels with feedback resulting in compensatory thyroid gland enlargement (goitre). To investigate this a four week oral study performed in human volunteers showed that sodium nitrate exposure of three times the ADI (15 mg/kg b.w./day in water) did not cause changes in the thyroid gland function (Lambers et al., 2000).

8.6 Derivation of the acceptable daily intakes for nitrate and nitrite

The former SCF and the JECFA both derived ADIs for nitrate and nitrite. The SCF reviewed the toxicological effects of nitrate and nitrite and established an ADI of 0-3.7 mg/kg b.w. for nitrate in 1990 (EC, 1992), retained the ADI in 1995 and derived an ADI of 0-0.06 mg/kg for nitrite (EC, 1997). The most recent assessment of nitrate and nitrite in 2002 by the JECFA reconfirmed the ADI of 0-3.7 mg/kg b.w. for nitrate and set an ADI of 0-0.07 mg/kg b.w. for nitrite based on a long term NTP rat study (FAO/WHO, 2003a,b). In the absence of significant new toxicological and toxicokinetic data, the Panel concluded that there was no need to re-consider these ADIs.

The key studies used to derive the ADIs are summarized in Table 18. A NOEL of 500 mg/kg b.w./day sodium nitrate corresponding to 370 mg/kg b.w. nitrate was derived from long term studies in rats and the subchronic toxicity study in dogs. Applying an uncertainty factor of 100 resulted in ADIs of 0-5 and 0-3.7 mg/kg b.w./day for sodium nitrate and nitrate, respectively. It has been argued that the rat may not be a good model for humans due to its low conversion of nitrate into nitrite in the saliva. However, because of the importance of the chronic toxicology, the rodent toxicokinetics and similar NOAELs found in the dog (a relevant model for humans) these studies continue to be considered to be relevant for risk assessment.

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\(^{16}\) The term equivocal evidence of carcinogenic activity is defined in NTP as studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
The JECFA (FAO/WHO, 1995) also considered the conversion of nitrate to nitrite in the saliva in its assessment using the calculation developed by the SCF (EC, 1997) to derive a transposed NOAEL for nitrate based on the NOAEL for nitrite. These “transposed” NOAELs can be compared with the current ADI of nitrate. The JECFA (FAO/WHO, 1995) applied an uncertainty factor of 50 to the “transposed” NOAEL for normal converters of 160 mg/kg b.w day which resulted in an ADI of 3.2 mg/kg b.w day. Because this was in the same range as the ADI for nitrate (3.7 mg/kg per day), there was no justification to amend this ADI in the JECFA 2002 assessment (FAO/WHO, 2003a).

Table 18. Summary of the NOELs from toxicological studies used to derive ADI values for nitrate and nitrite in the latest the JECFA (FAO/WHO, 2003a,b) evaluation.

<table>
<thead>
<tr>
<th>Type of study</th>
<th>Toxicological Endpoint</th>
<th>NOEL(^{17}) sodium salt/anion mg/kg b.w./day</th>
<th>ADI sodium salt/anion mg/kg b.w./day</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate</td>
<td>Subchronic study in dogs (125 days)</td>
<td>Growth depression</td>
<td>500/370</td>
<td>5.0/3.7</td>
</tr>
<tr>
<td></td>
<td>2 year chronic study in rats</td>
<td>Growth depression</td>
<td>500/370</td>
<td>5.0/3.7</td>
</tr>
<tr>
<td>Nitrite</td>
<td>2 years study in rats</td>
<td>Heart and lung toxicity</td>
<td>10/6.7</td>
<td>0.1/0.07</td>
</tr>
</tbody>
</table>

\(^{a}\) mg/kg body weight per day

### 8.7 Human data

#### 8.7.1 Introduction on epidemiological studies

Epidemiological study designs can be ranked according to increasing strength of evidence: ecologic (or correlation) studies, cross-sectional, case-control, cohort studies, intervention trials. A classification of the different epidemiologic study designs with respect to their potential for bias to occur and, consequently, the strength of evidence they provide, and the costs involved has been described (van den Brandt et al., 2002). It indicates that intervention trials provide the strongest evidence for a causal relationship on risk and (due to the ability of the design to control for confounding and bias), have the lowest chance for potential bias to occur. However, they are the most expensive and usually the least feasible studies. The less expensive cohort studies assess exposure and select study participants before the health outcome of interest occurs and thus provide relatively strong evidence. Although the cheaper case-control studies generally assess

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\(^{17}\) The term NOEL has been used by the JECFA until 2007. See URL: http://www.fao.org/ag/agn/agns/files/jecfa68_final.pdf
exposures retrospectively in subjects with and without the health outcome, the resultant evidence is more debatable. This is particularly true in the case of dietary exposures, due to the possibility of selection bias, recall bias and/or presence-of-disease bias to occur. The lowest costs are associated with correlation (ecological) studies but, as mentioned previously, they provide weak evidence and are much more susceptible for bias. Some investigators have stated that observational studies cannot, by definition, establish causality of a relationship based on a statistical association. However, if several high quality studies, such as those in which biases are shown to be minimal are available, and these consistently show a dose-response association, then observational studies may very well contribute to conclusions about causality.

Because vegetable consumption may confer some degree of protection against cancer, negative confounding may be present in studies linking nitrate exposure from vegetables to a cancer risk.

8.7.2 Relationship between nitrate (and nitrite) intake and possible health effects in humans

The JECFA report 2003

The relationship between nitrate and nitrite intake and human health has been considered in earlier reviews (e.g., Gangolli et al., 1994) and by the JECFA at its 59th meeting, which included literature until 2002 (FAO/WHO, 2003a,b). The part of the JECFA report on nitrate intake, methaemoglobinemia risk and human cancer risk can be summarized as follows.

The results of studies in humans on the potential of a high nitrate intake to cause methaemoglobinemia were equivocal. Some of the studies showed an association between a high nitrate concentration in drinking-water and methaemoglobinemia, and others indicated that gastrointestinal infections, inflammation and the ensuing overproduction of nitric oxide are major factors in infantile methaemoglobinemia. No increase in methaemoglobin concentration was seen in volunteers after a single administration of sodium nitrate in drinking water providing a dose of 7.3 mg/kg b.w., expressed as nitrate.

Several studies were reviewed on the effect of administration of nitrate on the release of nitric oxide at the junction of the oesophagus and the stomach in humans, which, it had been speculated, might be associated with an increased incidence of cancer at this site. However, no such association has been observed in epidemiological studies.

Six ecological (correlation) studies were reported on nitrate in drinking-water and mortality from or incidence of cancer. Elevated risks were found for prostate cancer and for brain tumours (each in one study), but the results of six studies on gastric cancer were equivocal. Furthermore, most of the ecological studies were based on limited data on nitrate concentrations and on cancer mortality rates (rather than incidence rates), and none took an induction period for cancer into account.

Three of the studies were cross-sectional, involving measurement of, e.g., salivary nitrate in cancer patients and healthy subjects. Because cross-sectional studies do not take into account the
time between exposure and disease, any observed differences in biomarkers of exposure might also be a consequence of the disease; therefore these studies cannot contribute to a causal interpretation of the results of studies of nitrate intake and cancer risk.

Seven case–control studies on nitrate in drinking-water and/or food and cancers at various sites were reviewed. In the studies on nitrate in drinking-water, equivocal results were reported with regard to an association with non-Hodgkin lymphoma, and no association was found with brain tumours. In the studies on dietary nitrate, no association was found with oral, oesophageal, gastric or testicular cancer. No other cancer sites have been studied.

Three prospective cohort studies have been conducted on nitrate intake and cancer risk. A cohort study in the Netherlands, with 6 years of follow-up, found no significant association between the incidence of gastric cancer and intake of nitrate from food or drinking-water, with relative risks for increasing quintiles of total nitrate intake of 1.0 (reference quintile), 1.2, 0.7, 0.9 and 0.9 for mean intakes of 60, 85, 100, 120 and 180 mg/day, respectively. Neither the relative risks nor the trend across relative risks was significant. A further analysis of the effect of nitrate within tertiles of vitamin C intake also did not reveal a positive association between nitrate intake and gastric cancer (van Loon et al., 1998). A Finnish cohort study on dietary nitrate, with 24 years of follow-up, reported no association with the risks for tumours of the stomach, colorectum or head and neck. The average nitrate intake in this cohort was reported to be 77 mg/day (Knekt et al., 1999).

A cohort study in Iowa, USA, with 11 years of follow-up, revealed no consistent association between intake of nitrate from drinking-water and the risks for cancers at many sites, and an inverse association was reported with cancers of the uterus and rectum. Positive associations with nitrate intake were observed only for cancers of the ovary and urinary bladder, although it was not possible to determine whether other factors in drinking-water were responsible for these associations. In addition, no evidence of a dose–response relationship was found for any of the cancer sites addressed in the study in Iowa (Weyer et al., 2001). The cohort studies included control for various potential confounders, such as intake of vegetables, age and smoking.

Overall, the epidemiological studies reviewed by the JECFA at its 59th and 44th meeting, did not provide evidence that nitrate is carcinogenic to humans (FAO/WHO, 2003a,b).

The part of the JECFA report on nitrite intake and human cancer risk regarding literature until 2003 can be summarized as follows. A number of epidemiological studies of the relationship between the intake of nitrite and cancer risk had been published since the 44th meeting. The JECFA ranked the study designs according to their capacity to provide evidence of a relationship.

Nine case–control studies on previous nitrite intake and various cancer types were reviewed. For oral and laryngeal cancer, no association was found with nitrite intake. One study conducted in the USA reported a positive association with oesophageal cancer, with Odds Ratio (ORs) of 1.0 (reference category), 1.2 and 1.6 for persons with a daily nitrite intake of < 1.1 mg, 1.1–1.6 mg and > 1.6 mg, respectively. The ORs and the trend across ORs were not statistically significant, however. The association between nitrite intake and oesophageal cancer was stronger, and it was significant for persons with a history of cancer (as an indicator of possible endogenous nitrosation) (Rogers et al., 1995). Another study in the USA, however, found no association
between nitrite intake and oesophageal cancer, nor with the subtypes adenocarcinoma and squamous-cell carcinoma; a positive association was found only with gastric cancer other than of the cardia (Mayne et al., 2001). A positive association with gastric cancer was reported in an Italian case–control study (average consumption, 2.4 mg/day) (La Vecchia et al., 1997), while no association was found in a French study (average consumption, 1.9 mg/day) (Pobel et al., 1995).

An association of borderline significance was found between nitrite intake and urinary bladder cancer in men but not women of Japanese descent, nor in whites of either sex, in Hawaii, USA (Wilkens et al., 1996). Although a positive association was reported from a study in the USA between brain tumours in children and their mothers’ consumption of processed meat (Preston-Martin et al., 1996), no association was found with nitrite intake during gestation or in childhood in a recent case–control study from Israel (Lubin et al., 2000). One study on nasopharyngeal cancer among Taiwanese reported no association with nitrite intake in adulthood, but a positive association was found with childhood nitrite intake as recalled by the mothers of the cases and controls (Ward et al., 2000).

Two prospective cohort studies have been conducted on nitrite intake and cancer risk. A cohort study from the Netherlands, with 6 years of follow-up, on dietary nitrite and gastric cancer risk reported relative risks of 1.0 (reference category), 1.2, 1.2, 0.9 and 1.4 for increasing mean quintiles of nitrite intake of 0.01, 0.04, 0.09, 0.16 and 0.35 mg/day, respectively. Neither the relative risks nor the trend was significant (van Loon, et al., 1998). A Finnish cohort study, with 24 years of follow-up, reported no association with the incidence of stomach, colorectal, or head-and-neck tumours. The average nitrite intake by this cohort was reported to be 5.3 mg/day (Knekt et al., 1999).

Thus, some studies indicated increased risks for oesophageal and gastric cancer; however, other studies – particularly prospective cohort studies – revealed no such association. The results for brain tumours in children and for urinary bladder cancer in adults were equivocal. Wide variation between the studied populations in the recorded intake of nitrite was noted. In none of these studies was a possible interaction between nitrite and nitrosatable amines evaluated in respect of cancer risk. The results of these studies and those of the epidemiological studies considered by the JECFA at its 44th meeting did not provide evidence that nitrite is carcinogenic to humans (FAO/WHO, 2003a,b).

**New studies since the JECFA 2003 report**

In the following, new epidemiological studies that have been published from 2002 onwards will be summarized, categorized according to study design and strength of evidence.
Nitrate (and nitrite) and methaemoglobinaemia

In a case-control study nested in a cohort, risk factors for methaemoglobinaemia in 71 children were investigated in the Transylvania region of Romania, where wells are a very important water source. Univariate and multifactorial analysis of risk factors for methaemoglobinaemia emphasised that, for this population, methaemoglobinaemia is most strongly associated with nitrate/nitrite exposure through the dietary route (p = 0.0318), via feeding of infant formula and tea made with water containing high levels of nitrate (253 mg/L in the exposed group versus 28 mg/L in the control group). Moreover, breast-feeding was found to be protective in infants younger than 6 months of age (p = 0.0244). Mean reported nitrate intake levels among case and controls were 103.6 and 11.2 mg/kg/b.w/day, respectively. The findings also raise questions about the role of diarrhoeal disease in the development of methaemoglobinaemia, as multifactorial analysis indicated a significant role for diarrhoeal disease for some individuals (Zeman et al., 2002).

Relationship between chronic nitrate (and nitrite) intake and possible risk of cancer in humans

Ecologic studies

An ecologic study on nitrate levels in drinking water and non-Hodgkin lymphoma (NHL) and cancers of the digestive and urinary tracts was conducted in an agricultural district (Trnava District; population 237,000) of the Slovak Republic. Routinely collected nitrate data (1975-1995) for villages using public water supplies were computerized and linked to cancer incidence ascertained for the period 1986-1995. Increasing standardized incidence ratios (SIRs) for villages with low average levels of total nitrate in drinking water (0-10 mg/L), medium (10.1-20 mg/L), or high (20.1-50 mg/L) were seen for colorectal cancer in women (0.64, 1.11, 1.29; P for trend <0.001) and men (0.77, 0.99, 1.07; P for trend=0.051), and non-Hodgkin lymphoma in women (0.45, 0.90, 1.35; P for trend=0.13) and men (0.25, 1.66, and 1.09; P for trend=0.017). There were no associations for kidney or bladder cancer. These ecologic data support the hypothesis that there is a positive association between nitrate in drinking water and NHL and colorectal cancer (Gulis et al., 2002).

In an ecologic study, Cocco and co-workers compared the NHL incidence in 1974-1993 with nitrate monitoring data from community water supplies from 1971-1994 available for 75% of the 376 communes in Sardinia, Italy. Among the study communes, the average nitrate concentration in 2003 was 4.57 mg/L (SE 0.35; median 3.27). The relative risks (RRs) for NHL for men and women combined did not increase with increasing 1993 nitrate level. Among men, the RRs were significantly increased in some nitrate concentration categories. Among women, the RRs were not increased in any exposure category. There was limited evidence among men for an association with NHL, but not among women (Cocco et al., 2003).
Cross-sectional studies
No new studies were reported since 2002.

Case-control studies

Stomach cancer
A case-control study was conducted in Korea to assess gastric cancer (GC) risk in relation to dietary intake of nitrate. Trained dieticians interviewed 136 patients diagnosed with GC, and the same number of controls was selected by matching sex, age and hospital. Intake of citrus fruits rather than total fruits was shown to have a protective effect on the risk of GC, but was not significant. Intake of citrus fruits rather than total fruits was shown to have a protective effect on the risk of GC with ORs of 0.60 and 0.66 in medium and high consumers (95% confidence interval [CI] = 0.33-1.10 and 0.31-1.41, but was not significant (P for trend=0.27). In this study, intake of total vegetables was shown to have a protective effect with OR for GC of 0.43 and 0.64 in medium and high consumers respectively (95% confidence interval [CI] = 0.23-0.80 and 0.31-1.32, P for trend=0.025). However, an increased risk for GC was shown in medium (OR=1.67, [CI] =0.87-3.2) and high consumers (OR=2.17 [CI] =1.02-4.65) of high nitrate-containing vegetables but it did not reach statistical significance (P for trend=0.18) (Kim et al., 2002).

Other gastrointestinal cancers
The association of nitrate in public water supplies with incidence of colon and rectum cancers was studied in a case-control study conducted in Iowa, USA, from 1986 to 1989. Nitrate levels in Iowa towns were linked to the participants' water source histories. Analyses were focused on the period from 1960 onward, during which time nitrate measurements were more frequent, and analyses were restricted to those persons with public water supplies that had nitrate data (actual or imputed) for greater than 70% of this time period (376 colon cancer cases, 338 rectum cancer cases, and 1244 controls). There were no overall associations of colon or rectum cancers with measures of nitrate in public water supplies, including average nitrate and the number of years with elevated average nitrate levels. For more than 10 years with average nitrate greater than 5 mg/L, the OR for colon cancer was 1.2 ([CI] = 0.9-1.6) and for rectum the OR was 1.1 (CI = 0.7-1.5). However, nitrate exposure (>10 years with average nitrate >5 mg/L) was associated with increased colon cancer risk among subgroups with low vitamin C intake (OR = 2.0; CI = 1.2-3.3) and high meat intake (OR = 2.2; CI = 1.4-3.6). These patterns were not observed for rectum cancer. (De Roos et al., 2003).

Non-Hodgkin Lymphoma (NHL)
A population-based case-control study of NHL was conducted in 1998 to 2000 in Iowa, Detroit, Seattle, and Los Angeles. Monitoring data for public supplies were linked to water source histories from 1960 onward. Nitrate was measured at interview homes with private wells. For those in the diet arm, dietary nitrate and nitrite intake were estimated using a 117-item food-frequency questionnaire that included foods high in nitrate and nitrite. In multivariate analyses,
no overall association was found with the highest quartile of average drinking water nitrate (> 2.90 mg/L nitrate-N: odds ratios = 1.2; 95% confidence interval = 0.6-2.2) or with years > or = 5 mg/L (10+ years: 1.4; 0.7-2.9). No evidence of an interaction was seen between drinking water nitrate exposure and either vitamin C or red meat intake, an inhibitor and precursor, respectively, of N-nitroso compound formation. Among those in the diet arm, dietary nitrate was inversely associated with risk of NHL (highest quartile: 0.54; 0.34-0.86). Dietary nitrite intake was associated with increasing risk (highest quartile: 3.1; 1.7-5.5) largely due to intakes of bread and cereal sources of nitrite. Average drinking water nitrate levels below 3 mg/L were not associated with NHL risk (Ward et al., 2006).

**Brain tumours in adults**

A population-based case-control study of adult glioma in eastern Nebraska, USA, was carried out with 236 glioma cases and 449 controls using information obtained from a food-frequency questionnaire. After adjusting for potential confounders, inverse associations with risk of adult glioma were observed for intakes of dark yellow vegetables (highest quartile versus lowest: OR = 0.6, P trend = 0.03) and beans (OR = 0.4, P trend = 0.0003), but no associations were seen for dietary sources of preformed nitrosamines or high-nitrate vegetables. No significant associations were observed with risk of adult glioma for intakes of nitrate, nitrite, vitamin C, vitamin E, saturated fat, cholesterol, dietary fibre from grain products, or fibre from vegetables and fruit. The authors concluded that this study did not support the N-nitroso compound hypothesis for adult glioma (Chen et al., 2002).

In a further extension of this case-control study of adult glioma in Nebraska, drinking water nitrate and nitrite were also considered. Water utility nitrate measurements were linked to residential water source histories. Average nitrate exposure over a 20-year period was computed. A food frequency questionnaire was used to assess dietary nitrate and nitrite. Increasing quartiles of the average nitrate level in drinking water were not significantly associated with risk (adjusted odd ratios: 1.4, 1.2, 1.3). Risk was similar among those with both higher and lower intakes of vitamin C. Dietary nitrite intake was not associated with risk. The authors concluded that this study did not support a role for drinking water and dietary sources of nitrate and nitrite in risk of adult glioma (Ward et al., 2005).

**Childhood brain tumours**

Pogoda and Preston-Martin (2001), building on the earlier case-control study with 540 cases and 801 controls from the USA by Preston-Martin et al., (1996), reported a further analysis with refined nitrite intake calculations. They found a positive association between childhood brain tumours (CBT) and their mothers’ intake of nitrite from cured meat, which was only significant in the highest nitrite intake category: they observed a 2-3 fold increased risk in the offspring of mothers who consumed on average at least 3 mg nitrite from cured meat per day during pregnancy. Distinction between total nitrite intake and cured meat was not available from the study and no further subdivision of CBT was made in the analysis.
Mueller et al., (2004) conducted a multicentre case-control study in France, Italy, Spain, Canada and the USA on drinking water levels of nitrate and nitrite and risk of CBT, with 836 CBT cases and 1485 controls. They found no increased CBT risk with increasing nitrate from drinking water. However, the risk of astrocytoma was significantly positively associated with increasing nitrite levels in residential drinking water during pregnancy: the odds ratios (and 95% CI) were 4.3 (1.4 – 12.6) for nitrite levels of 1 - <5 mg/L nitrite and 5.7 (1.2 – 27.2) for nitrite levels ≥5 mg/L. There was no association with other CBT.

Other cancers

A population-based case-control study of bladder cancer (men and women) and nitrate in drinking water was conducted in Iowa, USA, using 808 cases and 1259 controls. Among controls, the median average nitrate level for their Iowa residences with public water supplies was 1.3 mg/litre nitrate-nitrogen (interquartile range = 0.6-3.0). After adjustment for confounders, no increased risk of bladder cancer was found with increasing average nitrate levels in drinking water; the highest quartile odds ratio for women was 0.8 (95% confidence interval = 0.4-0.8), and for men 0.5 (0.4-0.8). In addition, no association was observed among those with high water nitrate exposure (>median) and low (<median) vitamin C intake compared with those who had low water nitrate and high vitamin C intake. These data suggested according to the authors that long-term exposure to nitrate in drinking water at levels in this study (90th percentile 5.5 mg/litre nitrate-nitrogen) is not associated with risk of bladder cancer. Moreover, no increased risk in bladder cancer was concluded when taking into account dietary nitrate levels (>119 mg/day) and dietary nitrite levels (>1.4 nitrite mg/day) in men and women (Ward et al., 2003).

Cohort studies

The association between nitrate exposure from diet and drinking water and bladder cancer risk was investigated in The Netherlands Cohort Study, conducted among 120,852 men and women, 55-69 years of age at entry. Information on nitrate from diet was collected via a food frequency questionnaire in 1986 and a database on nitrate content of foods. Individual nitrate exposures from beverages prepared with tap water were calculated by linking the postal code of individual residence at baseline to water company data. After 9.3 years of follow-up and after excluding subjects with incomplete or inconsistent dietary data, 889 cases and 4,441 subcohort members were available for multivariate analyses. The multivariate RRs for nitrate exposure from food, drinking water, and estimated total nitrate exposure were 1.06 (95% CI, 0.81-1.31), 1.06 (95% CI, 0.82-1.37), and 1.09 (95% CI, 0.84-1.42), respectively, comparing the highest to the lowest quintiles of intake. Dietary intake of vitamins C and E (low/high) and cigarette smoking (never/ever) had no significant impact on these results, i.e. there was no interaction. The authors concluded that this study did not support an association between nitrate exposure and bladder cancer risk (Zeegers et al., 2006)

The association between intake of nitrite and nitrosamines and gastric cancer (GC) and oesophageal cancer (OC) was evaluated in a recent systematic review. All published case-control
and cohort studies analyzing the relationship between nitrosamines and nitrite intake (and related foods) and GC or OC risk were reviewed. There were 11 cohort studies and 50 case-control studies. Evidence from case-control studies supported an association between nitrite and nitrosamine intake and gastric cancer risk, but was insufficient regarding oesophageal cancer risk. Evidence from cohort studies did not support significantly positive associations (Jakszyn et al., 2006).

Conclusions regarding nitrate, nitrite and human cancer risk

Several ecologic, case-control and cohort studies have been published since the JECFA report (FAO/WHO, 2003a,b; Cogliano et al., 2008). For nitrate, some studies suggest a positive association with risk of NHL, gastric and colon cancer. However, these were mostly studies with a weak study design and limited strength of evidence; other case-control studies and cohort studies (which provide stronger evidence) find no increased risk with increasing nitrate intake after multivariate adjustment. It should be borne in mind however, that the measurement of dietary nitrate intake is not without error and could result in an effect being underestimated. In general, misclassification is nondifferential, leading to attenuation of dose-response relationships. This attenuation applies equally to positive and inverse associations that have been reported for nitrate and cancer, which means that both observed inverse and positive associations might in reality be stronger. Some validation studies have been conducted on nitrate intake measurement; these indicate that the questionnaires are able to rank individuals according to intake, and that the possible attenuation is likely to be moderate. Since the observed associations are often very weak or even null, the CONTAM Panel concluded that, when the newly published data are considered together with studies previously summarized in the JECFA report FAO/WHO, 2003a,b), the evidence does not suggest that nitrate intake from diet or drinking water is associated with increased cancer risk.

For nitrite in food and drinking water, two case-control studies have found that high maternal intakes of nitrite from cured meat or drinking water might be associated with risk of childhood brain tumours. No further cohort studies have been reported on nitrite since the JECFA 2003 report. Taken together, more evidence is available now that a high nitrite intake might be associated with risk of childhood brain tumours and possibly gastric and oesophageal cancer. This evidence is only based on retrospective case-control studies; cohort studies found no significantly increased risks.

8.7.3 Relationship between nitrate (and nitrite) intake and non-cancer health effects

The JECFA 2003 report

The relationship between nitrate and nitrite intake and non-cancer health effects in humans has been considered by the JECFA at its 59th meeting, which included literature until 2002 (FAO/WHO, 2003a,b). The part of the JECFA report on nitrate intake and non-cancer health effects can be summarized as follows.
A number of studies were performed to determine whether there are associations between nitrate intake in drinking-water and insulin-dependent diabetes mellitus, neural tube defects or sudden infant death syndrome. In none of these studies was a hypothesis proposed for the mechanism of an association. Two studies were conducted on the incidence of insulin-dependent diabetes mellitus and nitrate intake via drinking-water. One study in Yorkshire, United Kingdom, suggested a positive association (McKinney et al., 1999). A study in the Netherlands with a larger number of subjects did not show a positive association. The two studies on nitrate intake and neural tube defects also showed no association (van Maanen et al., 2000). In an ecological study in Sweden, a correlation was reported between the nitrate concentration in drinking-water and the occurrence of sudden infant death syndrome; however, no confounding factors were taken into account (George et al., 2001). The JECFA considered that it would be premature to include these observations in its safety assessment (FAO/WHO, 2003a,b).

New studies since the JECFA 2003 report

In an ecological study in Finland, the association between geographical variation of Type 1 diabetes (IDDM) and its putative environmental risk factors, zinc and nitrate, were investigated. The association was evaluated using Bayesian modelling and the geo-referenced data on diabetes cases and population. Neither zinc, nor nitrate, nor the urban/rural status of the area had a significant effect on the variation in incidence of childhood Type 1 diabetes, although there was a tendency to increasing risk of Type 1 diabetes with the increasing concentration of nitrate in drinking water (Moltchanova et al., 2004).

In a case-control study of Mexican American women, the amine-containing (nitrosatable) drug exposure and neural tube defect (NTD)-affected pregnancies were examined in relation to dietary nitrite and total nitrate intake. A total of 184 women with NTD-affected pregnancies and 225 women with normal live births were interviewed, including questions on periconceptional drug exposures and dietary intake. For 110 study participants, nitrate was also measured in the usual source of drinking water. Women who reported taking drugs classified as nitrosatable were 2.7 times more likely to have an NTD-affected pregnancy than women without this exposure (95% CI = 1.4-5.3). The effect of nitrosatable drugs was observed only in women with higher intakes of dietary nitrite and total nitrite. Women within the highest tertile (greater than 10.5 mg/day) of total nitrite were 7.5 times more likely to have an NTD-affected pregnancy if they took nitrosatable drugs (95% CI = 1.8-45.4). The association between nitrosatable drug exposure and NTDs was also stronger in women whose water nitrate levels were higher. The findings suggested that effects of nitrosatable drug exposure on risk for neural tube defects in offspring could depend on the amounts of dietary nitrite and total nitrate intake (Brender et al., 2004).

Drinking water disinfection by-products have been associated with an increased risk for congenital defects including cardiac defects. Using Swedish health registers linked to information on municipal drinking water composition, individual data on drinking water characteristics were obtained for 58,669 women. Among the infants born, 753 had a cardiac defect. The risk for a cardiac defect was determined for ground water versus surface water, for different chlorination procedures, and for trihalomethane and nitrate concentrations. Ground
water was associated with an increased risk for cardiac defect when crude rates were analyzed but after suitable adjustments this excess rate was found to be associated by chlorination procedures including chlorine dioxide (Cedergren et al., 2002).

In a review of maternal exposure to nitrate in drinking water and adverse reproductive and developmental effects, it was concluded that the current literature does not provide sufficient evidence of a causal relationship (Manassaram et al., 2006).

Conclusions regarding nitrate, nitrite and non-cancer health effects in humans

Taken together with the studies that were reviewed in the JECFA report FAO/WHO, 2003a,b), the CONTAM Panel concluded that there is no clear evidence of an effect of nitrate or nitrite on non-cancer health effects. Most of the evidence is based on methodologically weak ecologic studies, and the lack of individual exposure measurement entails little control for confounding by other causes in the reported studies.

9. Risk characterisation

Vegetables contain higher levels of nitrate than other foods and contribute the most to dietary nitrate exposure. Plants have different storage capacities for nitrate with spinach and lettuce often containing more significant amounts, and rucola having the highest. Some assumptions about vegetable consumption were made in Chapter 6, but detailed information at the individual species level is scarce across Europe. There is anecdotal evidence that rucola consumption is especially popular in Italy and increasing. However, no firm data are available. Results reported to EFSA indicated that the coverage of certain species in relation to production method, season, and region left some gaps. It was possible to generalise overall dietary exposure to arrive at average nitrate intakes, however, some uncertainty remains about regional and individual variations.

The CONTAM Panel estimated dietary exposure to nitrate from vegetables by calculating different exposure scenarios (S1 to S5). Scenario S1 is based on a consumption of 400 g vegetables excluding roots and tubers and herbs. This represents a conservative approach as the international dietary recommendation of 400 g/day is for the combined consumption of vegetables and fruit. Scenario S2 is based on the potential contribution of potato consumption at the 97.5th percentile to nitrate exposure. Scenario S3 is based on the highest 97.5 percentile of leafy vegetable consumption at the median level of nitrate recorded with spinach, lettuce and rucola consumption as subscenarios. Scenario S4 explored the impact of splitting overall vegetable consumption at the 400 g/day level into the 97.5th percentile consumption level of leafy vegetables and the remainder as mixed vegetables. Finally, scenario S5 is similar to S4 but takes regionally reported concentrations into account.

The scenario calculations presented in Chapter 7 demonstrate that the critical driver for a high dietary exposure to nitrate is not the absolute amount of vegetables consumed but the type of vegetable and its nitrate concentration. There are a number of factors which can alter the amounts of nitrate consumed. On the one hand preparation such as handling, processing and cooking may
go someway to reducing the concentration of nitrate in vegetables, whereas general dietary recommendations encouraging an increase in the consumption of vegetables and fruit could potentially lead to increased nitrate exposure.

Based on the five scenarios presented in Chapter 7 and also shown in Table 17 the calculated exposure ranged from 82 mg/person/day to 457 mg/person/day when no account was taken of other sources or mitigating factors.

The Panel noted that there were no new hazard data that would alter the JECFA 2002 evaluation and used the ADI for nitrate as derived by the JECFA (FAO/WHO 2003a,b). The ADI of 3.7 mg/kg b.w. is equal to 222 mg of nitrate per person per day at a body weight of 60 kg. Although highly variable, dietary exposure to nitrate from sources other than vegetables is estimated to be on average in the range of 35-44 mg/person/day of which some 20 mg/person/day is contributed by water (see Figure 1). The higher end of this range has been added to the nitrate exposure from vegetables in Table 17 in order to estimate total dietary nitrate exposure for comparison with the ADI in Table 19.

Table 19. Comparison of the ADI for nitrate with different vegetable consumption scenarios including estimates of dietary exposure to nitrate from other sources.

<table>
<thead>
<tr>
<th>Vegetable consumption g/person/day</th>
<th>Vegetable</th>
<th>Overall median (S5 - highest regional median) nitrate concentration mg/kg a)</th>
<th>Calculated total exposure mg/person/day b)</th>
<th>% of ADI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Potato</td>
<td>Spinach</td>
<td>Lettuce</td>
<td>Rucola</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1 400</td>
<td>Most</td>
<td>106</td>
<td>392</td>
<td>201</td>
</tr>
<tr>
<td>S2 771</td>
<td>Potato</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S3 133</td>
<td>Leafy</td>
<td>785</td>
<td>1338</td>
<td>4800</td>
</tr>
<tr>
<td>S4 133/267</td>
<td>Leafy/most</td>
<td>785</td>
<td>1338</td>
<td>392</td>
</tr>
<tr>
<td>S5 133/267</td>
<td>Leafy/most</td>
<td>1745 b)</td>
<td>2652 b)</td>
<td>392</td>
</tr>
</tbody>
</table>

a) See Table 12
b) Highest regional median
c) Spinach at 133 g
d) Lettuce varieties at 133 g
e) Spinach at 133 g and other vegetables at 267 g
f) Lettuce varieties at 133 g and other vegetables at 267 g
h) Including background exposure from sources other than vegetables (44 mg/person/day).

A = spinach
B = all combined lettuce varieties
C = a mix of rucola (1/3) and lettuce varieties (2/3)

The Panel noted that a high-level consumer of “most vegetables” (S1) or of potato (S2) would not exceed the ADI, neither would a high-level consumer of spinach or lettuce varieties by themselves (S3A or S3B). However, by replacing a third of the leafy vegetables by rucola the ADI would be exceeded (S3C). Indeed, due to its high nitrate content consuming more than 47 g of rucola would result in exceeding the ADI without taking into account any other sources of nitrate exposure. In the event that a high-level consumer of vegetables also consumes lettuce varieties at the 97.5th percentile level (i.e. a third of the vegetables consumed as lettuce, or spinach and lettuce) at the highest regional median levels seen during the winter months, the ADI would also be exceeded. Although the average consumer would not exceed the ADI through vegetable consumption, individuals consuming vegetables produced under unfavourable growing conditions...
conditions as in scenario S5 would exceed the ADI by approximately two fold. However, there are a number of mitigation factors (such as fruit consumption and processing) which make this an unlikely regular event. A small part of the population that consume only vegetables, particularly leafy vegetable consumption in high amounts as reported by 2.5% of the population in some Member States (that is the 97.5th percentile consumption level) also can exceed the ADI. Overall, the Panel concluded that there would be no appreciable health risk.

The Panel noted that there can be local situations where drinking water may also significantly contribute to the nitrate exposure, particularly at levels close to the regulatory limit of 50 mg nitrate/L.

**Population subgroups**

Considering that nutritional recommendations of eating 400 g of fruit and vegetables per day are also valid for children and in the absence of actual data for EU, the estimate was made that children could consume approximately half the amount of adults. Thus 200 g of vegetables was considered to be a reasonable figure for children high consumers and correspond to the 95th percentile of consumption in Germany (Richter *et al.*, 2008). This would result in a nitrate exposure of 78 mg/day. In this case, the ADI of 3.7 mg/kg b.w., corresponding to an acceptable nitrate intake of 74 mg/child/day, based on a bodyweight of 20 kg, would be exceeded by 5%. This does not take into account other sources of nitrate exposure for which good data are not available for children. This could also increase if the vegetable intake consists mainly of leafy vegetables. Nevertheless, the CONTAM Panel recognises that up to one half of the vegetable allocation (Gregory *et al.*, 2000; Richter *et al.*, 2008) is likely to be in the form of fruit, which typically contains low levels of nitrate (normally below 10 mg/kg), and thus for the majority of children the nitrate exposure is likely to be below the ADI.

As outlined in chapter 7 vegetarians and vegans do not significantly differ from the general population in their dietary exposure to nitrate.

**10. Benefit identification and characterisation**

When benefits are discussed one has to differentiate between the physiological effects, the potential beneficial effects of nitrate and its metabolites and the benefits which can, despite the potential risks of high nitrate levels, be attributed to the consumption of vegetables and fruits because of their composition and nutrients.

Vegetable are considered beneficial in human nutrition as a source of fibre, vitamins and trace elements. In addition, vegetables may contain additional bioactive molecules, such as antioxidants which may serve as chemoprotective agents against chronic diseases and cancer. These positive effects are generally acknowledged by nutritionists and physicians, and the consumption of vegetables is therefore promoted in education programs directed to a balanced nutrition.
10.1 Physiological and pharmacological role of nitrate, nitrite and nitric oxide

Endogenous nitrate synthesis occurs through the L-arginine-NO synthase pathway. Endogenous conversion from nitrate is approximately 1 mg/kg b.w. per day for a 70 kg adult (Archer, 2002). Nitric oxide (NO) is produced from the amino acid L-arginine and this reaction is catalyzed by the NO-synthase (NOS) for which 3 different isoforms have been characterized (Lerzynski et al., 2006). The neuronal NOS1 (nNOS) and endothelial NOS3 (eNOS) forms produce NO as a signalling molecule and the inducible form (NOS 2, iNOS) mediates primarily host inflammatory response and its expression is up-regulated by a number of pathological and inflammatory conditions. NO is then oxidized to nitrite, which in turn reacts with oxidized haemoglobin to form nitrate and methaemoglobin. Endogenous NO has essential physiological functions, including the control of blood pressure and regional blood flow, and the limitation of adhesion and aggregation of platelets. In the central nervous system (non-adrenergic, non-cholinergic NANC fibres), NO is involved in neurotransmission, long term potentiation and plasticity (memory, appetite, nociception). In the peripheral nervous system, NO plays a role in neurotransmission, for example, in the regulation of gastric emptying and in blood flow regulation associated with penile erection. The vasodilatory effects have been attributed to the NO-dependent increase of cGMP resulting in a decrease in the intra-cellular Ca++ availability. Major therapeutic indications for the use of nitric oxide donors (i.e. nitroprusside and organic nitrovasodilators such as glyceryl trinitrate) are obstructive coronary heart diseases, pulmonary hypertension, pyloric stenosis in children, and erectile dysfunction.

In inflammatory diseases, upregulation of iNOS results in excessive amounts of NO (and associated radical species), which then contribute to the clinical symptoms of inflammation (vasodilatation and formation of oedemas (Schopfer et al., 2003)). In turn, excessive NO is converted into toxic ONOO⁻ radicals, which contribute to the non-specific host defence mechanisms against numerous pathogens, including bacteria, fungi, protozoa and parasites, and controversially also to tissue damage due to their cytotoxic effects. The key feature of these mechanisms is that protein tyrosine nitration is part of the inflammation process and moves the physiological role of NO towards an oxidative, nitrative and pathological one, depending on the actual tissue concentration. Nitrogen radicals are also effective against tumour cells (Ying and Hofesth (2007).

There is evidence that enteropathogens can survive for a surprisingly long time in acid alone, but the combination of acid and nitrite results in effective killing. This led to the finding that NO and solutions of acidified nitrite, mimicking gastric conditions, have antimicrobial activity against a wide range of organisms including a variety of gastrointestinal pathogens such as Yersinia and Salmonella (Duncan et al., 1995; Dykhuizen et al., 1996; Vallance, 1997; McKnight et al., 1997; 1999). Thus nitrate, in the form of nitric oxide may play a role in host defence, (Lundberg et al., 2008).

All nitrogen species, including NO, nitrite (NO₂⁻), and nitrogen dioxide (‘NO₂) may lead to increased concentrations of nitrate in the plasma (Schopfer et al., 2003, Lundberg et al., 2004 and 2008, Cui et al., 2006; Wright et al., 2006).
In humans, a large proportion of exogenous nitrate exposure (60 – 80%) arises from the consumption of vegetables and fruits. Nitrate is converted to nitrite in the human saliva (see chapter 9) and both nitrate and nitrite may be absorbed from the gastrointestinal tract. The contribution of these dietary sources to NO formation (which is regulated in tissues by a negative feedback mechanism involving the control of NOS by intracellular calcium-calmodulin) remains currently unknown.

In this context, a recent study hypothesised that the high nitrate content of beetroot juice represented a source of vasoprotective nitric oxide via bioactivation. In healthy volunteers, approximately 3 hours after ingestion of 500 mL of beetroot juice, a dietary nitrate load of 2.9 g/L, a significant reduction of blood pressure was observed (-10.4/8 mm Hg) and this effect was correlated with peak increases in plasma nitrite concentration. In the human forearm, dietary nitrate load prevented endothelial dysfunction induced by an acute ischemic insult and significantly attenuated ex vivo platelet aggregation in response to collagen and ADP. Interruption of the enterosalivary conversion of nitrate to nitrite prevented the rise in plasma nitrite, blocked the decrease in blood pressure and abolished the platelet aggregation inhibition thus confirming that such vasoprotective effects were mediated via nitrite converted from dietary nitrate (Webb et al., 2008).

10.2 Potential beneficial health effects of nitrate, nitrite and metabolites

Nitrate and nitrite are used as food additives particularly for their anti-bacterial properties against the potentially lethal pathogen Clostridium botulinum, and good endogenous efficacy against bacterial gastroenteritis (McKnight et al., 1997; 1999; Duncan et al., 1995; Dykhuizen et al., 1996; Vallance, 1997). This should not be considered a direct health benefit of consuming nitrate. EU food law specifies that food shall not be placed on the market if it is unsafe, and that food is deemed to be unsafe if it is considered to be injurious to health. Hence if, for example, cured ham and bacon products were dependent upon the use of nitrate or nitrite to prevent contamination with C. botulinum, they could not be legally marketed if they did not contain nitrate or nitrite.

While the maintenance of human physiological activity is essential for normal health, it is not a health benefit per se. In consequence while typical dietary exposure to nitrate should not be considered harmful it cannot be considered to be a health benefit just because it has a range of physiological roles.

It may be, that in certain situations, a diet containing nitrate at levels typically within the ADI may beneficially support the body’s endogenous nitrate and nitrite ‘pools’ (Lundberg, et al., 2008).

10.3 Potential beneficial health effects of fruits and vegetables

Vegetables provide biologically active substances as well as nutrients like pro-vitamin A, vitamin C, calcium, iron, folate, potassium, magnesium, digestible carbohydrates and non-digestible carbohydrates (fibre), protein. A large range of these is listed in an overview provided in the International Agency for Research on Cancer (IARC) handbooks of cancer prevention (IARC, 2003). In addition, vegetables lack saturated fat and trans fatty acids and are low in sodium which confer them beneficial nutritional properties.

The following is a non-exhaustive list of nutrients and “bioactive substances” found in vegetables and fruit (IARC, 2003).

- **Allyl sulfides**
  - Allin
- **Carotenoids**
  - Alpha-carotene
  - Beta-carotene
  - Beta-cryptoxanthine
  - Lycopene
  - Lutein
  - Zeaxanthine
- **Citric acid**
- **Flavonoids**
  - Anthocyanins
  - Flavanols
    - Catechins
    - Proanthocyanidins
  - Flavanones
    - Hesperidin
    - Naringenin
    - Neohesperidin
  - Flavones
    - Apigenin
    - Luteolin
  - Flavonones
    - Quercetine, Rutin
    - Myricetin
    - Kaempherol
    - Isorhamnetin
  - Isoflavones
    - Genistein, genistin
    - Daidzein, daidzin
    - Glycitein, glycitin
    - Biochanin A
    - Coumestrol
    - Formononetin
  - Flavonols
    - Quercetine, Rutin
    - Myricetin
    - Kaempherol
    - Isorhamnetin
  - Isoflavones
    - Genistein, genistin
    - Daidzein, daidzin
    - Glycitein, glycitin
    - Biochanin A
    - Coumestrol
    - Formononetin
- **Fiber**
  - Pectin
  - Inulin
- **Pre-biotics Glucosinolates, and breakdown products**
  - Isothiocyanates
  - Indoles
  - Sulphoraphane
- **Lignans**
- **Minerals**
  - Potassium
  - Magnesium
- **Phenolic acids**
  - Cinnamic acids
    - Caffeic acid
    - Chlorogenic acid
    - Ferulic acid
    - Para-coumaric acid
  - Ellagic acid
  - Gallic acid
- **Plant sterols**
  - Beta-sitosterol
  - Campesterol
  - Stigmasterol
- **Resveratrol**
- **Salicylates**
- **Terpenes/terpenoids**
  - Limonene
- **Vitamins**
  - Folate
  - Vitamin C
  - B-vitamins
  - Vitamin K
  - Vitamin E
  - Pro-vitamin A

### Relationship between vegetables and health

Fruits and vegetables are frequently considered as one category. The recent reports by the WHO are instrumental in describing the relationship between fruits, vegetables and health. Data are typically derived from observational studies rather than intervention studies. Whereas evidence from observational studies can never provide definitive proof, these data are regarded adequate to support a relationship provided that the data are good and the study designs are appropriate.

Recently, the WHO concluded that “non-communicable diseases” (NCDs), i.e., “chronic diseases” such as cardiovascular diseases (CVDs), cancer, obesity and type 2 diabetes, currently kill more people than any other cause. Four lifestyle factors in the epidemiology of these diseases
(poor diet, physical inactivity, tobacco and alcohol use) are of overwhelming importance to public health (WHO, 2002).

Vegetables and fruit are important components of a healthy diet and, if consumed daily in sufficient amounts, could help prevent major diseases such as CVDs and certain cancers. According to The World Health Report 2002, low fruit and vegetable intake is estimated to cause about 31% of ischaemic heart disease and 11% of stroke worldwide (WHO, 2002). Overall it is estimated by the WHO that up to 2.7 million lives could potentially be saved each year if fruit and vegetable consumption was sufficiently increased (see also Lock et al., 2005).

The 2003 Joint FAO/WHO Expert Consultation on diet, nutrition and the prevention of chronic diseases, recommended the intake of a minimum of 400g of vegetables and fruit per day (excluding potatoes and other starchy tubers) for the prevention of chronic diseases, as well as for the prevention and alleviation of several micronutrient deficiencies, especially in less developed countries (WHO, 2003b).

The scientific database describing the evidence for the health benefits of fruit and vegetable consumption is large and growing.

The WHO and the World Cancer Research Fund (WCRF) have reviewed the evidence for the impact of fruits and vegetables on the development of the major chronic diseases (cancer, obesity/management of body weight, cardiovascular disease and diabetes), whereas the International Fruit and Vegetable Alliance (IFAVA) summarised data for other diseases.

An itemised overview of the most relevant diet and health relationships for fruits and vegetables and health is provided below. It should be noted that these chronic diseases are linked: diabetes is associated with cardiovascular disease, overweight and obesity is positively associated with cardiovascular disease, diabetes and cancer.

**Impact on cancer**

In 1997, the WCRF published their first expert report on “Food, Nutrition and the Prevention of Cancer: a global perspective” (WCFR/AICR, 1997). In its conclusions, the WCRF/AICR stated that “The epidemiological and experimental evidence that the recommended diets decrease the risk of cancer is strong and consistent for many sites. Over time, the implementation of one recommendation – consumption of 400 g/day or more of a variety of vegetables and fruits – could, by itself, decrease overall cancer incidence by at least 20%. The evidence is convincing or probable that diets high in vegetables and/or fruits protect against cancers of the mouth and pharynx, oesophagus, lung, stomach, colon and rectum, larynx, pancreas, breast and bladder.” The WCRF/AICR has recently published an updated expert report outlining the extent to which food, nutrition, physical activity, and body composition modify the risk of cancer (WCRF/AICR, 2007).
In the 2003 Handbook on Cancer Prevention the WHO/IARC indicated that approximately one in ten cancers in western populations are due to an insufficient intake of vegetables and fruit. The clearest evidence of a cancer-protective effect of eating more fruits is for stomach and oesophageal cancers. Similarly, a higher intake of vegetables probably reduces the incidence of cancer of oesophagus and colon-rectum (IARC, 2003, WCFR/AICR, 2007).

**Impact on overweight and obesity**

The WHO has published a review on dietary intake of fruits and vegetables and management of body weight (WHO, 2005a).

Short-term intervention studies, studies with an advice to increase consumption of fruits and vegetables, and studies with a dietary advice only (up to 1 year) showed that in general a diet high in fruits and vegetables and low in fat resulted in significant weight loss in males and females.

**Impact on cardiovascular disease**

The WHO has published a review on dietary intake of fruits and vegetables and risk of cardiovascular diseases (WHO 2005b). Data from both intervention and observational studies indicate that the consumption of fruits and vegetables may play an important role in the prevention of ischaemic heart disease and stroke. An estimate of the potential contribution of the increased intake of fruits and vegetables are 26,000 deaths prevented annually in the EU before the age of 65.

In the recent report “Our Food our Health” (RIVM, 2006) it is estimated that the relative risks (RR\(^{19}\)) for coronary heart disease is 0.8, when comparing high (> 200 g per day) versus low (< 50 g per day) consumption of fruit and vegetables. When refining the relatively risk for different age groups, it was shown that the relative risk is higher in older age groups (RR = 0.86, 70-79 years; RR = 0.91, > 80 years).

**Impact on diabetes**

The WHO has published a review on dietary intake of fruits and vegetables and risk of diabetes (WHO, 2005b). A small but growing body of evidence links a diet rich in fruits and vegetables with a lower risk of type 2 diabetes mellitus. The available studies support a role for fruits and vegetables independent of other diet and lifestyle factors in the prevention of type 2 diabetes.

**Effects of antioxidants in fruits and vegetables**

It has been debated whether the beneficial effects of fruits and vegetables can be broken down into their individual constituents. These compounds have been characterised, tested *in vitro, in vivo*.

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\(^{19}\) A relative risk smaller than 1 (R<1) means a reduced risk; a RR>1 means an increased risk.
**vivo** in animals and/or in humans. This reductionist science has made it possible to identify how bioactive substances might affect biological processes using more accurate and sophisticated endpoints. However, the CONTAM Panel concurs with the WHO conclusion (WHO, 2003b) that “The benefit of fruits and vegetables cannot be ascribed to a single or mix of nutrients and bioactive substances. Therefore, the food category was included rather than the nutrients themselves.....”.

These conclusions are in accord with other recent reviews (Verhagen et al., 2006; Huang et al., 2006; 2007; Bjelakovic et al., 2007; and NIH, 2007).

**11. Risk/benefit characterisation**

Consumption of various food types varies significantly at different population levels according to age, ethnicity, and dietary habits across different regions within the EU. Nevertheless, there is a growing recognition of the effects of diet as a major lifestyle factor. While vegetables can impact health positively the Panel also noted, that there can be risks associated with the consumption of some vegetables *per se* such as from antinutrients or allergens.

Risk-benefit analysis of foods with regard to human health is a developing area and the EU is now sponsoring a number of EU projects to progress the science, tools, methods and implications, Qalibra20, Beneris21 and Brafo22. This opinion follows the outline proposed by the EFSA Scientific Colloquium on risk-benefits analysis of foods (EFSA, 2007).

The CONTAM Panel concluded overall, that the estimated exposures to nitrate from vegetables are unlikely to result in appreciable health risks, therefore the recognised beneficial effects of consumption of vegetables prevail. The Panel recognised that there are occasional circumstances e.g. unfavourable local/home production conditions for vegetables which constitute a large part of the diet, or individuals with a diet high in vegetables such as rucola which need to be assessed on a case by case basis.

**12. Uncertainty analysis**

The evaluation of the inherent uncertainties in the assessment of exposure to nitrate has been performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006). In addition, the draft report on “Characterizing and Communicating Uncertainty in Exposure Assessment”, which is in preparation to be published as a World Health Organization/International Programme on Chemical Safety (WHO/IPCS) monograph, has been considered (WHO/IPCS, 2007).

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According to the guidance provided by the EFSA (EFSA, 2006) the following sources of uncertainties have been considered: Assessment objectives, exposure scenario, exposure model, and model input (parameters).

**Assessment objectives**

The objectives of the assessment were clearly specified in the terms of reference and the Panel prepared a risk assessment including the consideration of the ADI. The uncertainty in the assessment objectives is considered to be negligible.

**Exposure scenario / exposure model**

Several exposure scenarios have been considered to estimate the exposure to nitrate. All scenarios are based on the raw products. The possible changes of the nitrate content due to processing of the food commodities, such as washing, peeling and/or cooking could not be considered due to lack of representative data. However, overall, the data indicate that processing is likely to reduce nitrate levels and thus the non-consideration of the quantitative impact of food processing on nitrate levels may lead to an overestimation of the exposure.

**Model input (parameters)**

A number of uncertainties can be identified regarding the selection of parameters, such as characterisation of levels in food commodities and selection of consumption data.

First of all, the samples reported from the Member States differ greatly regarding the number of vegetables tested as well as concentrations determined in the respective products. Moreover, occurrence data on nitrate in fruits are scarce, although the overall tendency is lower than for vegetables. Thus the conservative base case, that the amount of 400 g of fruits and vegetables recommended by WHO is only allocated to vegetables, results in a high uncertainty and probable overestimate concerning the overall exposure to nitrate.

The estimation of European vegetable consumption from the GEMS Food Consumption Cluster Diet database, which is based on national food balance sheets of annual food production as well as import and export for individual countries aggregated into clusters according to similar consumption behaviour, adds another uncertainty to the exposure assessments.

The Panel used the ADI as established by international bodies and recognised the inherent uncertainties in using animal data to derive health based guidance values, but acknowledged the in-built conservatism.

There is uncertainty regarding the optimal amount of vegetable consumption for health benefits. The recommendation of WHO have been used in this assessment.
In Table 20 a summary of the uncertainty evaluation is presented, highlighting the main sources of uncertainty and indicating an estimate of whether the respective source of uncertainty might have led to an over- or underestimation of the exposure or the resulting risk. The magnitude is related to the source of uncertainty and should not be compared/summed from one source to another.

Table 20. Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of the dietary exposure to nitrate from vegetables.

<table>
<thead>
<tr>
<th>Sources of uncertainty</th>
<th>Direction &amp; Magnitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncertainty due to type of sampling, as samples are mostly collected in order to check for compliance with legal limits and not for monitoring purposes aimed at estimation of human exposure</td>
<td>++ / -a)</td>
</tr>
<tr>
<td>Uncertainty about the representativeness of most samples concerning, country of origin, size, regional and seasonal differences, specific type of vegetable</td>
<td>++ / --</td>
</tr>
<tr>
<td>Estimation of recommended intake of vegetables and fruits based only on vegetables because of lack of representative nitrate level data for fruits</td>
<td>++</td>
</tr>
<tr>
<td>Consumption data from only a number of Member States in combination with data from GEMS Food Consumption Cluster Diet database</td>
<td>++ /-</td>
</tr>
<tr>
<td>Uncertainties regarding the influence of food processing and/or cooking on the nitrate levels in the processed food</td>
<td>++</td>
</tr>
<tr>
<td>Uncertainties regarding the influence of storage on the nitrite level in food</td>
<td>-</td>
</tr>
<tr>
<td>Limitations in certain of the toxicological models e.g. rodents to establish health based guidance values</td>
<td>++</td>
</tr>
</tbody>
</table>

a) +, ++, +++= these are used in a semi-quantitative way to indicate the potential to cause small, medium or large over-estimation of exposure/risk.
-,-, --, --- = uncertainty with potential to cause small, medium or large under-estimation of exposure/risk (EFSA, 2006).

The Panel considered the impact of the uncertainties on the exposure to nitrate due to consumption of leafy vegetables and concluded that the exposure scenarios used tend to overestimate the intake of nitrate and that the risk assessment is likely to be conservative i.e. more likely to over- than to underestimate the risk.
CONCLUSIONS

General

- Nitrate is a naturally occurring compound as well as an approved food additive. Nitrate is also used as a fertiliser and consequently can be an environmental contaminant.

- There are different routes of nitrate exposure for humans: endogenous formation, and exogenous exposure from dietary and non-dietary sources.

- The main dietary sources of nitrate are vegetables, preserved meat and drinking water.

Exposure assessment

- In total nearly 42,000 analytical results originating from 21 European countries concerning 92 vegetable varieties were considered in this assessment.

- While there is a large variation in the median concentration of nitrate in different vegetables from 1 mg/kg (peas and Brussels sprouts) to 4,800 mg/kg (ruco la), green leafy vegetables have been shown consistently to have the highest levels.

- Several factors such as light intensity, storage, processing and/or cooking of vegetables influence nitrate concentrations.

- Different exposure scenarios calculated on the basis of the recommended daily intake of 400 g vegetables and fruit for adults (including vegetarians), but all consumed in the form of vegetables, showed that it is not the amount of vegetable eaten but the type of vegetable and its nitrate content that is the critical driver for consumer exposure.

- Exposure to nitrate from eating 400 g of mixed vegetables per day at typical median nitrate concentrations was estimated to be 157 mg/day.

- Nitrite is also found in vegetables but generally at much lower concentrations than nitrate. These levels are not a major direct contributor to human exposure compared with endogenous formation from nitrate.

Hazard characterisation

- A toxicological endpoint of concern for nitrate is nitrosamine formation and the potential for tumour formation. However, when nitrate is consumed in a normal diet containing vegetables, other bioactive substances concomitantly consumed, such as the antioxidant vitamin C, may inhibit the endogenous formation of nitrosamines.

- Epidemiological studies relating to nitrate and human cancer risk do not suggest that nitrate intake from diet or drinking water is associated with increased cancer risk.
Evidence that high intake of nitrite might be associated with increased cancer risk is equivocal.

No new data were identified that would require a revision of the ADI values of 0-3.7 mg/kg body weight for nitrate and 0-0.07 mg/kg b.w. for nitrite as reconfirmed by the Joint FAO/WHO Expert Committee on Food Additives in 2002.

**Risk characterisation**

Dietary exposure estimates showed that the ADI for nitrate would not be exceeded by an adult eating 400 g of mixed vegetables. However, high level consumers, of vegetables grown under unfavourable local production conditions may exceed the ADI approximately two fold. In these calculations the nitrate concentrations were not corrected for mitigation factors e.g. fruit consumption and processing and may overestimate exposure. Overall, the Panel concluded that there would be no appreciable health risk.

Consumption of more than 47 g of rucoila at the median nitrate concentration would lead to an excursion above the ADI without taking into account any other sources of nitrate exposure.

**Benefit Characterisation**

A range of physiological roles of nitrate and its metabolites are increasingly appreciated as a result of recent research. However, the extent to which exogenous nitrate contributes to human physiology in healthy individuals remains to be established.

There is a general consensus that a balanced diet high in vegetables and fruit confers significant health benefits in terms of a reduction of the risk for a range of diseases.

**Risk/benefit characterisation**

Overall, the estimated exposures to nitrate from vegetables are unlikely to result in appreciable health risks, therefore the recognised beneficial effects of consumption of vegetables prevail.

The Panel recognised that there are occasional circumstances e.g. unfavourable local/home production conditions for vegetables which constitute a large part of the diet, or individuals with a diet high in vegetables such as rucoila which need to be assessed on a case by case basis.
RECOMMENDATIONS

- There is a need for research into the factors that influence nitrate and nitrite concentrations and alterations during productions, storage and processing.

- Member States should submit individual analytical data on those crops regularly found to contain high levels of nitrate.

- Some vegetables such as rucoila can make a disproportionate contribution to overall nitrate exposure, and hence changing dietary habits need to be closely monitored.

- Continued efforts to progress methodology for the risk-benefit analysis of foods remain a high priority.

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**DOCUMENTATION PROVIDED TO EFSA**

Committee of Professional Agricultural Organisations in the EU (COGECA), Belgium. Letter including a paper from the UK lettuce growers.

Food Standards Agency (FSA), United Kingdom. Letter.

National Farmers Union (NFU), United Kingdom. Letter including a paper from the National Farmers Union on behalf of the UK lettuce growers.

Mechelse Veilingen, Belgium. Letter including scientific articles.

Section Nationale Salades. Email including scientific articles.
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADI</td>
<td>Acceptable Daily Intake</td>
</tr>
<tr>
<td>CBT</td>
<td>Childhood brain tumours</td>
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<tr>
<td>CEN</td>
<td>European Committee for Standardization</td>
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<tr>
<td>CONTAM</td>
<td>Panel on Contaminants in the Food chain</td>
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<tr>
<td>CVDs</td>
<td>Cardiovascular diseases</td>
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<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
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<tr>
<td>GAP</td>
<td>Good agricultural practices</td>
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<tr>
<td>GC</td>
<td>Gastric cancer</td>
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<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
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<tr>
<td>IFAVA</td>
<td>International Fruit and Vegetable Alliance</td>
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<tr>
<td>JECFA</td>
<td>Joint FAO/WHO Expert Committee on Food Additives</td>
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<tr>
<td>LOD</td>
<td>Limit of detection</td>
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<tr>
<td>ML</td>
<td>Maximum level</td>
</tr>
<tr>
<td>NDMA</td>
<td>N-nitrosodimethylamine</td>
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<tr>
<td>NHL</td>
<td>Non-Hodgkin lymphoma</td>
</tr>
<tr>
<td>NOAEL</td>
<td>No-observed-adverse-effect level</td>
</tr>
<tr>
<td>NOEL</td>
<td>No-observed-effect-level</td>
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<tr>
<td>NOS</td>
<td>Nitric oxide synthetase</td>
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<tr>
<td>NTD</td>
<td>Neural tube defects</td>
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<tr>
<td>OC</td>
<td>Ooesophageal cancer</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
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<tr>
<td>RRs</td>
<td>Relative risks</td>
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<tr>
<td>SCF</td>
<td>Scientific Committee for Food</td>
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<tr>
<td>SIRs</td>
<td>Standardized incidence ratios</td>
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<tr>
<td>TOR</td>
<td>Terms of reference</td>
</tr>
<tr>
<td>WCRF</td>
<td>World Cancer Research Fund</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>WHO/IPCS</td>
<td>World Health Organization/International Programme on Chemical Safety</td>
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