Report from the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) in relation to the risk assessment of infants and young children's exposure to nitrates resulting from the consumption of chard in Spain

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Abstract

The main via of exposure to nitrates for humans is through diet, as vegetables are the main dietary source making up between 80 and 85% of the daily intake. The relatively high levels found in vegetables such as rocket, lettuce and spinach is of note.

The European Food Safety Authority (EFSA) has recently assessed the possible effects on children of the nitrates found in certain leaf vegetables, and has concluded that the nitrate levels in lettuce do not pose a risk, although in the case of spinach, they indicate the possible existence of the risk of methaemoglobinemia in children between 1-3 years old if they eat more than one portion per day (EFSA 2010).

This report presents a risk assessment of the exposure of infants and young children to nitrates from eating chard in Spain, leaf vegetables with a high nitrate content, which have not been evaluated by the EFSA as consumption at European level is very low and localised.

Based on the data provided by the Spanish Agency for Food Safety and Nutrition (AESAN) corresponding to nitrate concentrations in chard during the period 2000-2009, a significant variability in the content was detected in a total of 1,018 samples, with a median concentration of 1,562 mg nitrate/kg of chard, and with higher nitrate levels than in spinach (median: 816 mg/kg) published by the EFSA at European level.

Assuming that the level of consumption of chard in children (from 3 months to 1 year old) is the same as that of spinach among young children in the European population, and accepting the consumption of chard in children aged between 7-12 years from the Spanish diet model as valid for children between the age of 1 and 3 (AESAN, 2006), the estimations for chronic exposure due to eating chard among children (from 1-3 years), with three levels of nitrate concentration, 1,562, 3,000 and 3,700 mg nitrates/kg (median, maximum permitted nitrate level in fresh spinach, and P95 content, respectively), are lower than the ADI of 3.7 mg/kg b.w. established.

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The estimations of acute exposure to nitrates indicate that in none of the cases considered, for the age groups of 3, 6 and 9 months, is the value of 15 mg/kg b.w./day exceeded, considered as a reference to prevent high levels of methaemoglobin in infants and young children. Only 12 month old children, in cases of extreme consumption and very high concentrations, would reach a higher exposure, although given the low percentage of chard samples that exceeded these content levels, the probability of acute toxicity would be very low.

In Spain, according to available data, this Committee recommends that consumption levels for spinach be extended to chard given the significant levels of consumption and the higher nitrate content.

The establishment of maximum nitrate levels in chard as exist for spinach is considered suitable.

Key words

Nitrates, chard, infants, young children, risk assessment.

Introduction

Nitrates are present in the environment, and therefore are found in air, food (mainly vegetables and fruits) and water. Their presence is a result of what is known as the nitrogen cycle, in which bacteria fix nitrogen to form nitrates before they are used by plants in protein synthesis. They are also used as fertilisers and food additives.

Humans are exposed to nitrates through endogenous synthesis and exogenous exposure through both dietary and non-dietary sources. The main channel for nitrate exposure in humans is diet, and the main sources of nitrates are vegetables, canned meats and drinking water. Overall, vegetables are thought to be the main dietary source of nitrates, providing between 80% and 85% of the daily amount consumed (Gangolli et al., 1994) (van Velzen et al., 2008). However, the daily consumption of nitrates depends on multiple factors, such as lifestyle, cultural considerations and geographic location (JECFA, 1995).

Nitrate content in vegetables ranges widely (between 1 and 10,000 mg/kg) depending on the type and their source, as well as their cultivation or storage conditions (JECFA, 1995). Relatively high concentrations are found in vegetables such as rucola, lettuce and spinach. For example, there have been cases of leafy vegetables with a nitrate content of more than 4,500 mg/kg (Table 1).

Table 1. Nitrate concentrations in leafy vegetables

Leafy vegetable	Mean concentration (mg/kg)	Leafy vegetable	Mean concentration (mg/kg)
Rucola	4,677	Belgian endive	1,465
Amaranth	2,167	Lettuce	1,324
Lamb's lettuce	2,104	Cos lettuce	1,105
Mixed lettuce	2,062	Spinach	1,066
Butterhead lettuce	2,026	Iceberg lettuce	875
Beet	1,852	Dandelion	605
Chard	1,690	Escarole	523
Curled lettuce	1,601	Radicchio	355
Oak-leaf lettuce	1,534	Water cress	136

Source: (EFSA, 2008).

As for nitrites, the exogenous route provides between 11% and 41%, given their low concentration in fresh vegetables and fruits (1-20 mg/kg). The metabolism of nitrates, especially the reduction of nitrates secreted in saliva, means that the main route of human exposure to nitrites is endogenous (Thomson, 2004) (Greer and Shannon, 2005) (EFSA, 2008).

Nitrates have undergone a series of assessments by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 1995, 2002, 2003), the Scientific Committee on Food (SCF) (SCF, 1992) and the European Food Safety Authority (EFSA) (EFSA, 2008, 2010). The EFSA assessment (2008) on risks arising from the presence of nitrates in vegetables examined different exposure scenarios with a variety of consumption patterns and nitrate concentrations in several types of vegetables. The critical element of exposure was shown to be the specific type of vegetable and its nitrate content, rather than the quantity of vegetables consumed.

Recently, EFSA (2010) evaluated the possible effects in children of nitrates found in some leafy vegetables such as spinach and lettuce, since these are the most widely consumed in Europe. One of its most important conclusions was that nitrate content in lettuce does not constitute a risk. In the case of spinach, however, the study indicated a potential risk of methaemoglobinaemia in children aged 1 to 3 years if they consume more than one portion per day. Furthermore, it is more likely that infants would be fed cooked spinach than lettuce.

However, children in Spain also consume chard. Chard is a leafy vegetable with a high nitrate content. However, it has not been evaluated by the EFSA because its consumption in Europe is low and restricted to certain geographic areas. As a result, there is currently no European standard establishing maximum nitrate level in chard (Table 2).

Table 2. Maximum nitrate levels established by Regulation (EC) No 1881/2006

Foodstuffs			**Maximum levels (mg NO_3/kg)**
1.1	Fresh spinach (*Spinacia oleracea*)	Harvested 1 October to 31 March	3,000
		Harvested 1 April to 30 September	2,500
1.2	Preserved, deep-frozen or frozen spinach		2,000
1.3	Fresh Lettuce (*Lactuca sativa L.*) (protected and open-grown lettuce) excluding lettuce listed in point 1.4	Harvested 1 October to 31 March: lettuce grown under cover	4,500
		Harvested 1 October to 31 March: lettuce grown in the open air	4,000
		Harvested 1 April and 30 September: lettuce grown under cover	3,500
		Harvested 1 April and 30 September: lettuce grown in the open air	2,500
1.4	Iceberg-type lettuces	lettuce grown under cover	2,500
		lettuce grown in the open air	2,000
1.5	Processed cereal-based foods and baby foods for infants and young children		200

Source: (EU, 2006).

In light of this situation, given the lack of European regulations on chard, the Executive Director of the Spanish Agency for Food Safety and Nutrition (AESAN) petitioned the Scientific Committee to assess the risk of exposure to nitrates through chard consumption in infants and small children (under 1 year and up to 3 years old), in order to establish appropriate risk management strategies.

Hazard identification

The nitrate ion is an inorganic compound formed by one nitrogen atom and three oxygen atoms (NO_3^-). Its molecular weight is 62 g/mol. It forms salts with different cations (Table 3).

Table 3. Examples of nitrate salts

Nitrate salts	Chemical formula	CAS No	Uses (examples)
Sodium nitrate	$NaNO_3$	7631-99-4	Fertiliser, preservative in meat products, cement production
Potassium nitrate	KNO_3	7757-79-1	Fertilizer, preservative, gunpowder production
Ammonium nitrate	NH_4NO_3	6484-52-2	Fertilizer, explosives production
Calcium nitrate	$Ca(NO_3)_2$	10124-37-5	Fertilizer, wastewater treatment, concrete production
Magnesium nitrate	$Mg(NO_3)_2$	10377-60-3	Fertilizer
Silver nitrate	$AgNO_3$	7761-88-8	Antiseptic, disinfectant, cauterising agent

Source: (IPCS, 1999).

Nitrate is an important metabolite in the nitrogen cycle. It is formed through nitrite oxidation (NO_2^-) caused by the action of *Nitrobacter* bacteria. It occurs naturally in soil and vegetables, and it is a common metabolite in mammals. Its presence in soil and surface water is due to the mineralisation of organic matter and the use of fertilizers (IPCS, 1999).

Vegetables are noted for their ability to store nitrates, but their nitrate content depends on the type of vegetable. For example, leafy green vegetables may have high nitrate content. Nitrate content depends on several factors (Meah et al., 1994) (Thomson, 2004) (EFSA, 2008). These include:

- Soil characteristics. Nitrate accumulation depends on the soil type and its mineral content. Since nitrates move from the soil to the root surface by convection, lack of water can limit nitrate transport. In the same way, excessive humidity can dilute nitrates in the soil, thus limiting plant growth and leading to loss of nitrates by denitrification.
- Use of fertilizers. For example, intensive agriculture can lead to the use of large amounts of fertilizers, which are a source of nitrates.
- Sunlight intensity. This is the key factor which determines nitrate levels in leafy vegetables. High irradiation levels in summer tend to reduce nitrate content. In addition, the highest growth rates coincide with periods with high irradiance and warm temperatures (Kanaan and Economakis, 1992). For example, vegetables raised in northern Europe have higher nitrate content than those raised in southern Europe because they are exposed to lower levels of sunlight. Fruits and vegetables raised in greenhouses have higher nitrate content because of lower light intensity and high mineralisation in their environment (Gangolli et al., 1994).
- Storage conditions. Nitrate content in fresh vegetables may decrease during storage at room temperature. On the other hand nitrate levels, which tend to be very low, may increase during storage

depending on the vegetable species, the specific endogenous action of the *nitrate reductase* enzyme or bacterial contamination. When the stored vegetables are refrigerated (7 days at 5 ºC), nitrate content remains unchanged, which implies that *nitrate reductase* becomes inactive and bacterial activity is also inhibited.

- Processing. Nitrates are water soluble, and therefore washing leafy vegetables can lower their nitrate content by 10% to 15%. Peeling tubers and fruits can also lower nitrate content by 34%-61% (Dejonckheere et al., 1994).
- Cooking method. Nitrates in vegetables are not homogenously distributed. For example, removing the stems and midribs of lettuce and spinach leaves can decrease their nitrate content by 30%-40% (Dejonckheere et al., 1994). In the same way, several authors indicate that a vegetable's nitrate content can be decreased by between 16% and 79%, depending on the type of vegetable, when it is boiled in water (Abo Bakr et al., 1986) (Schuster and Lee, 1987) (Dejonckheere et al., 1994). In light of the current trend of consuming fresh foodstuffs, particularly different varieties of leafy vegetable, EFSA has adopted a conservative approach. It does not consider potential reductions in nitrate content due to processing and cooking in its calculations for initial exposure, although they may be taken into account as mitigating factors in several exposure scenerarios involving consumption of assorted vegetables (EFSA, 2008).

Hazard characterisation

1. Absorption, distribution, metabolism and excretion

Nitrates found in vegetables, whether raw or cooked, are absorbed efficiently, resulting in a bioavailability of nearly 100% (van Velzen et al., 2008). Once ingested, nitrates are absorbed rapidly through the proximal region of the small intestine. They then move through the bloodstream and are distributed in such a way that the highest concentrations of nitrates in humans are found in serum, saliva and urine (JECFA, 2003). On this subject, Bartholomew and Hill (1984) point out that about 65%-70% of nitrates administered by the oral route are excreted in urine.

Once nitrates have been distributed, they are actively secreted from the bloodstream to the saliva, as has been observed in both humans and in several types of experimental animals (except rats). In humans, approximately 25% of all nitrates consumed are secreted in the saliva. In turn, of the 25% of total nitrates secreted in saliva, 20% are reduced to nitrites due to a stable population of nitrate-reducing bacteria found at the base of the tongue (Gangolli et al., 1994) (JECFA, 2003). As a result, approximately 5%-7% of all ingested nitrates can be detected as nitrites in saliva in healthy adults (EFSA, 2008).

Nitrates can be reduced to nitrites through the activity of enteric bacteria, or by the *nitrate reductase* enzyme in mammals, since many of the micro-organisms occurring in the gastrointestinal tract perform this function. However, it must be stated that the location with the most marked reduction activity varies depending on the species, its microbial colonisation and nitrate absorption.

In the acidic environment of the stomach, nitrites present in saliva are rapidly transformed into nitrous acid. This in turn decomposes into nitrogen oxides such as nitric oxide (NO); at the same time,

endogenous synthesis of NO from L-arginine occurs in the urea cycle, through the action of the *NO-synthase* enzyme (NOS). Under physiological conditions, as mentioned above, most of the absorbed nitrates are excreted in urine; however, selective reabsorption from the kidney, along with biliary and salivary recirculation, are always present (EFSA, 2008).

2. Toxicity

Nitrate *per se* has relatively low toxicity to humans. However, its metabolites and reaction products which form in the human body (i.e. nitrites, nitric oxide and N-nitroso compounds) are associated with health problems such as methaemoglobinaemia and carcinogenesis. In addition, the normal conversion rate of nitrate to nitrite is between 5% and 7%, although it may reach 20% in some cases (JECFA, 1995, 2002) (EFSA, 2008).

As we stated before, nitrates have been evaluated on several occasions by both the Joint FAO/WHO Expert Committee on Food Additives and the European Food Safety Authority. The first important aspect indicated by these assessments is the need to use experimental animal species whose nitrate toxicokinetics and nitrite conversion processes are similar to those of humans (JECFA, 2002). Animal studies of nitrate metabolism and toxicokinetics confirmed that rats, for example, are not a good animal model, since they do not transport nitrate by saliva, and therefore their nitrate-to-nitrite conversion process is limited.

In addition, it is thought that nitrate, nitrite and N-nitroso compound toxicology must be taken into account in order to carry out such an assessment.

Acute and subchronic oral toxicity

Studies carried out to date allow us to observe that acute oral nitrate toxicity in animals is generally low, with LD_{50} values ranging between 300 mg/kg b.w./day in pigs to 6,250 mg/kg b.w./day in mice. Likewise, it has been observed that the lethal oral dose in adult humans is 330 mg/kg b.w. (Walker, 1990) (FAO/WHO, 1996), and therefore human sensitivity regarding acute toxicity is similar to that of pigs.

Observed results indicate that the toxicity of sodium nitrite is far greater than that of sodium nitrate, with LD_{50} values ranging between 180 mg/kg b.w. (in rats) and 214 mg/kg b.w. (in mice) (EFSA, 2008).

Methaemoglobinaemia

As stated before, acute nitrate toxicity is mainly attributed to nitrate-to-nitrite reduction; nitrites cause oxidation of haemoglobin (Hb) in red blood cells, creating methaemoglobin (MHb) and thus producing methaemoglobinaemia. In normal conditions, full-term newborns have a circulating MHb level of 2%, while in premature newborns the level is 2%-3% (Greer and Shannon, 2005). In adults, the level is $< 2\%$ (Gómez Lumbreras et al., 2008).

In infants, this disease, also known as "blue baby syndrome" is characterised by a blue-grey skin colouration resulting from contact with oxidising agents or different reasons relating to diet, genetics, etc. (Herranz and Clerigué, 2003).

Methaemoglobinaemia occurs when the rate of oxidation of Hb to MHb exceeds the ability of the *NADH-cytochrome b5 methaemoglobin reductase* enzyme to reduce MHb to Hb once more (Sánchez-Echaniz et al., 2001) (Pérez-Caballero et al., 2005), or when this enzyme is insufficient (congenital methaemoglobinaemia (Da-Silva et al., 2003) (Laporta Báez et al., 2008).

According to several authors, the greatest incidence rate of methaemoglobinaemia in infants is observed in those younger than 4-6 months, particularly in infants younger than 3 months. This is due to several factors, such as a high proportion of foetal haemoglobin, which is more susceptible to oxidation and being converted to MHb due to nitrite exposure; high stomach pH which favours growth of nitrate-reducing bacteria, and therefore, increased transformation of nitrates to nitrites in the intestinal tract (this can cause gastroenteritis, which concomitantly increases nitrite formation); a 40% to 50% decrease in the activity of the *NADH-cytochrome b5 methaemoglobin reductase* enzyme (ATSDR, 2004) and increased risk of intestinal infections (Savino et al., 2006). However, it is also stated that this susceptibility disappears after the indicated age, since enzyme levels become similar to those of adults and almost no foetal haemoglobin remains (Herranz and Clerigué, 2003) (Greer and Shannon, 2005) (Gómez Lumbreras et al., 2008).

MHb formation causes red blood cells to be unable to capture oxygen, pass it along to tissues and transport carbon dioxide. This can lead to tissue hypoxia and cyanosis. The severity of the clinical profile depends on MHb concentration, so with MHb levels below 20% (10-15%), cyanosis appears as the first symptom (central cyanosis of the trunk, lips and mucous membranes, generally in patches) (Knobeloch et al., 2000) (Herranz and Clerigué, 2003) (Laporta Báez et al., 2008). However, Greer and Shannon (2005) point out that it is possible for the first symptoms to appear in infants with low Hb levels when MHb levels are at 3%. At MHb concentrations of more than 20%, increased mucocutaneous cyanosis, irritability, tachypnea and altered mental state appear. The most severe cases result in metabolic acidosis, cardiac arrhythmia, coma and generalised convulsions (Herranz and Clerigué, 2003) (Gómez Lumbreras et al., 2008) (Laporta Báez et al., 2008). On this subject, some authors state that MHb levels of more than 50% cause severe hypoxaemia and central nervous system depression, while MHb levels above 60% to 70% can cause death (Alonso Vega et al., 2007) (Gómez Lumbreras et al., 2008).

These symptoms also depend on prior clinical conditions; symptoms that are more numerous than would be expected with relatively low MHb levels may develop in patients with heart or respiratory failure, anaemia or acidosis (Alonso Vega et al., 2007). Other factors that may influence MHb formation are exposure to a list of medications, including topical local anaesthetics, silver nitrate, acetaminophen, sulphonamides, sodium valproate, etc.

There are not many studies linking the formation of MHb in infants and small children with consumption of vegetables and prepared baby foods containing vegetables such as spinach, carrots, etc., although several individual cases have been published (Sander and Jacobi, 1967) (Hack et al., 1983). (Greer and Shannon, 2005). For example, there has been a case of high MHb (25%) in conjunction with supraventricular tachycardia and perioral cyanosis in a six-month old infant following consumption of a mixed-vegetable puree which had been prepared for five days before and kept refrigerated (Bryk et al., 2003).

During the last few years in Spain, we have continued to see cases of methaemoglobinaemia. The main cause is consumption of homemade vegetable juices which are stored in improper conditions and have high nitrite levels, or by mixing infant formula with water used to boil vegetables. Although nitrite levels in raw, undamaged vegetables are generally very low, these levels increase during storage, due to nitrate reduction and the decrease in water content. This process is accelerated when the vegetables are prepared as a puree (Chung et al., 2004).

Sánchez-Echaniz et al. (2001) reported cases of methaemoglobinaemia (MHb levels between 10% and 58%) in infants following consumption of mixed vegetable purees early prepared and kept in the refrigerator during 12 to 27 hours. Chard was a common ingredient, and it is one of the vegetables with the highest nitrate content (mean: 3,200 mg/kg). More recently, Gómez-Lumbreras et al. (2008) recorded a similar case in a seven-month-old baby (MHb 21%) with peripheral cyanosis and mucocutaneous pallor, who had ingested a chard puree prepared the day before. After removing the source of exposure, the clinical symptoms resolved in 72 hours. Other authors (Alonso Vega et al., 2007) (Laporta Báez et al., 2008) have reported on cases of methaemoglobinaemia in 8 to 9-month-old babies with cyanotic lips and extremity pallor following ingestion of a mixed-vegetable puree containing chard, or chard puree, which had been left out the refrigerator for several hours before it was consumed. Therefore, inappropriate or prolonged storage of cooked vegetables is a risk factor for methaemoglobin formation.

EFSA (2010) revised the existing association between nitrate levels in drinking water and MHb levels in children, and indicated that methaemoglobin levels are not elevated when nitrate concentrations are lower than 100 mg/l.

Since nitrates may potentially cause methaemoglobinaemia after they are reduced to nitrites, and this may occur after a single exposure, EFSA states that it would be appropriate to establish an Acute Reference Dose (ARfD) in order to evaluate safety levels for acute nitrate exposure. Nonetheless, data available from experimental studies in animals and cases of human intoxication are still not sufficient to provide an adequate basis for establishing an ARfD (EFSA, 2008). According to EFSA (2010), currently available data indicate that MHb levels are not elevated in children or infants above three months old when their nitrate consumption through drinking water and vegetables is lower than 15 mg/kg b.w./day.

Genotoxicity

Results obtained from genotoxicity studies show that nitrates themselves are not genotoxic. Nor is there evidence that would warrant nitrites being classified as genotoxic (JECFA, 2002).

Chronic toxicity Carcinogenicity

From a toxicological point of view, nitrates act in the formation of nitrosamines, and therefore have carcinogenic potential (AESAN, 2008). The International Agency for Research on Cancer (IARC) recently completed an evaluation of nitrates and nitrites ingested through diet, and placed them in category 2A, indicating that they are probably carcinogenic in humans (IARC, 2010).

Carcinogenicity studies on nitrates were negative except when extraordinarily high doses of nitrates and nitrogen-enriched precursors were administered. Likewise, epidemiological studies in humans have

not shown evidence linking nitrate exposure through diet or drinking water to the risk of developing cancer (JECFA, 1995, 2002) (EFSA, 2008) (IARC, 2010). Nonetheless, a study on sodium nitrite carried out in rats and mice demonstrated equivocal evidence of carcinogenic activity (NTP, 2001). According to the IARC (2010), there is a limited body of evidence regarding the carcinogenicity of the nitrites present in food; they are associated with an increased incidence rate of stomach cancer in humans.

Regarding N-nitroso compounds, there is no quantitative evidence of their endogenous formation from nitrites and N-nitrosable compounds ingested through diet. It has therefore not been considered appropriate to evaluate nitrite risk based on the endogenous formation of N-nitroso compounds (JECFA, 2002). Furthermore, when nitrates are consumed through a normal, varied diet containing vegetables, other bioactive substances are ingested concomitantly which act as antioxidants (vitamins C and E, for example). These are efficient MHb formation inhibitors, and may partially inhibit the endogenous formation of nitrosamines (EFSA, 2010).

Taking a NOEL (Non Observed Adverse Effects Level) of 370 mg/kg b.w./day as a reference, expressed as nitrite ion, and a safety factor of 100, JECFA (1995) established an acceptable daily intake (ADI) of 5 mg/kg b.w. expressed as sodium nitrate, or 3.7 mg/kg b.w. expressed as nitrite ion. This ADI was re-evaluated in the JECFA's fifty-ninth session based on recently published studies. It was concluded that the toxic effects observed result from *in vivo* conversion of nitrates to nitrites, but that data were insufficient to warrant modifying the established ADI. Similarly, an ADI of 0.07 mg/kg b.w. was established for nitrites, expressed as nitrite ion (JECFA, 2002), and attention was called to their ability to cause methaemoglobinaemia. Following that, the EFSA Panel on Contaminants in the Food Chain (CONTAM) (EFSA, 2008) concluded that in the absence of significant new toxicological data, it was not necessary to reconsider the ADIs.

Exposure Assessment

1. Nitrate levels in vegetables

The EFSA Panel on Contaminants in the Food Chain published a scientific study on nitrate content in vegetables in 2008. This study compares the risks and benefits of exposure to nitrates in vegetables (EFSA, 2008), and its most significant data is revealed below.

To elaborate its opinion, the Panel received and evaluated 41,969 data sheets, prepared by 20 European member states and Norway, containing nitrate levels in 92 different varieties of vegetables. Spain participated and provided 3,811 data sheets on nitrate content in vegetables. The study showed enormous variation amongst the median nitrate levels in vegetables. Levels were as low as 1 mg/kg for peas and Brussels sprouts, and as high as 4,800 mg/kg in rucola, being the leafy vegetable species consistently shown to have the highest nitrate concentration (EFSA, 2008).

Most vegetables had a median nitrate content of 392 mg/kg, and the median levels in the vegetables or vegetable families that EFSA used in its different nitrate exposure scenarios are listed here in ascending order: 106 mg nitrate/kg in potatoes, 785 mg nitrate/kg in spinach, 1,388 mg nitrate/kg in various types of lettuce and 4,800 mg nitrate/kg in rucola. For its evaluation, the Panel also considered the highest (median) nitrate levels in certain geographic areas for spinach (1,745 mg/kg) and for several varieties of lettuce (2,652 mg/kg).

The Panel examined the variability of nitrate content in vegetables due to geographic differences and the factors listed in section 2. For example, nitrate levels in lettuces grown in southern Europe were lower than those in lettuces grown in central or northern Europe.

Nitrite levels in the vegetables that were analysed were much lower than nitrate levels. The panel did not believe that they would directly contribute to exposure in humans in a significant way, compared with endogenous formation from nitrates.

In December 2010, EFSA then published a statement addressing the possible health effects of nitrates in certain vegetables, such as spinach and lettuce, in children. The EFSA's statement, based on 13,391 data sheets on the nitrate content in lettuces and 7,358 in spinach, found median nitrate levels of 1,260 mg/kg in lettuces and 816 mg/kg in spinach (EFSA, 2010).

2. Nitrate levels in chard

EFSA's 2008 scientific opinion stated that the mean concentration of nitrates in chard (n=666) was 1,690 mg/kg, with a similar, but somewhat lower median of 1,510 mg nitrate/kg.

This scientific opinion drawn up by the AESAN's Scientific Committee uses data provided by the AESAN itself and corresponding to nitrate concentrations in chard (mg nitrate/kg sample) between 2000 and 2009. A total of 1,018 results were collected from across the country, using the regional community control programmes. There were only 19 cases (1.8% of the total) in which values were below limits of detection (LOD) and/or limits of quantification (LOQ), so they would have little impact on the overall statistical results. A numerical value equal to LOD/LOQ was therefore assumed, as per EFSA (2008, 2010) criteria.

There is no European legislation stipulating nitrate levels in chard, but knowing that, as with spinach, there are seasonal differences in nitrate content, and that content varies between fresh and frozen vegetables, basic statistical calculations were carried out with data categorised as follows:

- Fresh chard.
  - Harvested in summer (1 April-30 September).
  - Harvested in winter (1 October-31 March).
- Tinned, frozen or deep-frozen chard.

Table 4 summarises the basic statistics for the samples that were analysed. The P5 (Percentile), P95, mean and standard deviation and median and maximum value were recorded for each of the categories. To do so, the following was taken into account:

1. The dates determining summer or winter harvest are not always available. For that reason, two options were used for presenting data: a global category for which summer/winter was not specified, and separate categories specifying summer/winter harvest when that information was included in the database.
2. Specifying fresh or frozen chard was possible since this information is generally indicated.

However, since in terms of consumption, there are no differences between the time of harvest (summer/winter), the cultivating method (fields/greenhouses) and the processing of the sample (fresh/frozen, etc.) we will use total nitrate levels (mg/kg) without breaking them down by type.

Table 4. Basic statistics on nitrate levels (mg/kg) in chard in Spain, provided by AESAN between 2000 and 2009

Foodstuff category	N	<LOD	P5	Median	Mean (SD)	P95	Max
Fresh chard (Summer)	424	11	102.75	**1,420.00**	1,606.03 (1,188.89)	**3,706.65**	6,634.73
Fresh chard (Winter)	232	0	231.00	**1,904.00**	1,977.02 (1,096.75)	**3,909.47**	4,770.00
Fresh chard total	751	11	121.00	**1,597.00**	1,735.71 (1,156.09)	**3,780.00**	6,634.73
Frozen, refrigerated or tinned chard total	137	1	372.00	**1,386.00**	1,485.76 (778.74)	**2,701.76**	5,119.00
Chard total	1,018	19	172.02	**1,562.00**	1,691.12 (1,110.46)	**3,700.00**	6,634.73

The reported concentrations were highly variable with a wide range among levels. For this reason, scenarios in the exposure calculations are based on median nitrate content assuming that a consumer will choose chard from the market at random, according to the normal methodology used by EFSA (2008). The (median) nitrate content of 1,562 mg/kg in the samples analysed in Spain between 2000 and 2009 according to AESAN data is similar to the median content of 1,510 mg/kg reported by EFSA (2008) on a Europe-wide level.

Taking into account the harvest season (summer/winter) for samples whose season is specified in the database, a higher nitrate level in chard picked in winter (1,904 mg/kg) than in chard picked in summer (1,420 mg/kg) has been observed. This corroborates the way that sunlight influences nitrate content in this type of leafy vegetable.

It must be stated that although there are no maximum permitted nitrate limits for chard, the data evaluated in this report shows that 126 chard samples out of the total, or 12.4%, exceed the maximum nitrate level permitted in spinach 3,000 mg/kg. In 69 samples (6.8%), nitrate content was above 3,500 mg/kg, the proposed maximum limit currently being debated by European regulators (EFSA, 2010).

Data included in Table 5, which compares nitrate content in chard and spinach according to different sources, indicate that the NO_3^- content in chard is similar to that in spinach. According to the 2000-2009 data provided by AESAN for this report, the median nitrate content in chard is approximately 90% higher than that of the spinach analysed by EFSA in 2010. As a result, keeping in mind that chard is widely consumed in Spain, it seems logical to evaluate the risk involved in chard consumption as was done with spinach, even if the assessment is only carried out on a national level.

Table 5. Comparison of nitrate content (mg/kg) in spinach and chard, according to different sources

Sample	Source	N	Median	Mean	Range or P5/P95
Spinach	AESAN (2006)**	367	1,153	1,368	14-5,837
Spinach	AESAN (2007)**	333	1,068	1,261	14-5,712
Spinach	(Menard et al., 2008)	266	Not given	1,681.7	?-8,700
Spinach	(EFSA, 2008).	6,657	785	1,066	64/3,048 (P5/P95)
Spinach	(EFSA, 2010)*	7,358	816	1,092	?-10,470
Chard	AESAN (2006)**	143	1,454	1,628	80-6,634
Chard	AESAN (2007)**	142	1,400	1,548	50-4,690
Chard	(Menard et al., 2008)	7	Not given	1,354	?- 3,500
Chard	(EFSA, 2008)	666	1,510	1,690	178/3,685 (P5/P95)
Chard	**AESAN (2000-2009)**	**1,018**	**1,562**	**1,691**	**10-6,634**

*Samples collected from 21 countries.

**Results from nitrate testing in vegetables during 2006 and 2007. AESAN internal report.

3. Chard consumption and resulting ingestion of nitrates in children under 1 year and up to 3 years old

Foodstuff consumption patterns are fundamental to establishing exposure to a certain contaminant. The problem arises when the issue hinges upon determining the exposure in specific population groups (in this case, children aged 0-3), since consumption data for the adult population is more commonly available. Furthermore, in this case, most of the available databases do not contain data on chard consumption, since it is not widely consumed in Europe, as stated before.

EFSA's recent opinion on health risks to children arising from the presence of nitrates in vegetables does not include the group of infants younger than three months, as they do not consume vegetables (EFSA, 2010). In a similar way, in this report of the Scientific Committee the following age groups have been considered: children aged 3 months, 6 months, 9 months, 12 months and 1-3 years.

Table 6 contains the available data on chard and spinach consumption according to different sources.

Table 6. Consumption of chard and spinach among different age groups (grams/person/day)

Consumption (g/person/day)					
Children (Mean weight: 34.48 kg. Age: 7-12 years)					
	Whole population	**Consumers only**			**Source**
	Mean±SD	**% consumers**	**Mean±SD**	**P97.5**	
Chard	2.61±10.69	10.19	25.62±23.17	71.04	AESAN. Spain (1)
Spinach	2.69±11.3	8.19	32.84±23.95	79.77	AESAN. Spain (1)
Adults (Mean weight: 68.48 kg. Age: ≥17 years)					
	Whole population	**Consumers only**			**Source**
	Mean±SD	**% consumers**	**Mean±SD**	**P97.5**	
Chard	5.21±18.94	9.43	55.26±32.28	118.53	AESAN. Spain (1)
Spinach	4.87±19.31	9.34	52.17±39.18128.96		AESAN. Spain (1)
Adults (Age: >18 years)					
	Whole population	**Consumers only**			**Source**
	Mean				
Chard	4.74	–			CAPV (2)
Spinach	2.16	–			CAPV (2)
	Children (Age: 3-14 years)	**Adults (Age: >15 years)**			**Source**
	Mean	**Mean**			
Chard	0.2	0.6			France (3)
Spinach	3.2	4.1			France (3)
Adults (Mean weight: 60 kg)					
	Whole population	**Consumers only**			**Source**
	Mean (g/day)				
Chard	2.2	–			GEMS-Food (4)
Spinach	5	–			GEMS-Food (4)
Adults (Mean weight: 60 kg)					
	Whole population	**Consumers only**			**Source**
		Mean (g/kg/day)			
Chard	–	9.03			GEMS-Food (Netherlands) (4)
Spinach	–	13.01			GEMS-Food (Netherlands) (4)
Children Age ≥ 6 years (Mean weight: 16.5 kg)					
	Whole population	**Consumers only**			**Source**
		Mean (g/kg/day)			
Chard	–	2.5			GEMS-Food (France) (4)
Spinach	–	29.60			GEMS-Food (South Africa) (4)

(1) Model Spanish diet to determine consumer exposure to chemical substances (AESAN, 2006). (2) Quantitative study of foodstuffs consumed in the Autonomous Community of the Basque Country (CAPV) (2008). (3) (Menard et al., 2008). (4) GEMS/FOOD Regional Diets (WHO, 2003).

According to the data from the AESAN's model Spanish diet (AESAN, 2006), chard consumption is similar to that of spinach for both the general population and the "consumer only" group for the two populations under study (children aged 7-12 years and individuals over 17 years of age). However, no consumption information is provided for children under 7, the most vulnerable group and the one being studied here.

In the Autonomous Community of the Basque Country (CAPV), adults consume twice as much chard they do spinach (Gobierno Vasco, 2008). Available data from France show greater consumption of spinach than of chard in both adults and children (Menard et al., 2008); in this last study, the children's ages range from 3 to 14 years, which is outside of the interval considered in our report.

Meanwhile, data from GEMS-Food (Food Contamination Monitoring and Assessment Programme) show the same chard consumption in adults as that in the model diet used by AESAN, while spinach consumption is higher (nearly twice as high). The same source gives chard consumption in France (P97.5) for "consumers only" in children older than 6 years as 41.25 g/day. In the Spanish model diet (AESAN, 2006), the same value for children aged 7 to 12 is 71.04 g/day.

With regards to consumption by young children, a study carried out in the Basque Country in 2001 analysed the diet of 282 children aged 8 months to 12 months. Of these children, 97.5% consumed vegetable purees on a daily basis, with a mean daily portion of 260.0 ± 9.1 g. The same study recorded that the typical puree consumed by these children contained 6% chard, amounting to 15.6 g/day (Gobierno Vasco, 2003).

Data recorded in the 2010 EFSA report to evaluate acute exposure to nitrates were also revealing with regard to spinach consumption in three age groups: 1-3 years, 4-6 years and older than 7. These values are summarised in Table 7. The Agency's opinion states that the median (shown in the table) and mean consumption values are quite similar across the three age groups. From this, we conclude that children aged 1-3 years can be assumed to eat the same quantities of chard as children aged 7 and up, according to the available data for chard consumption in the model Spanish diet (AESAN, 2006).

Table 7. Spinach consumption (grams/day) leading to acute nitrate exposure in different age groups

Age	Bodyweight (kg)[1]	Spinach consumption (g/day)	Spinach consumption (g/kg/day)	Source
3 months	6.1	6.1-7.9	1.0-1.3	(EFSA, 2010)[2]
6 months	7.7	10.8-15.4	1.4-2.0	
9 months	8.8	18.5-29.9	2.1-3.4	
12 months	9.7	27.2-44.6	2.8-4.6	
1-3 years	14	44.2 (median)	3.0	(EFSA, 2010)[3]
4-6 years	20	33.1 (median)	1.6	
≥ 7 years	35	46.6 (median)	1.2	

Adapted from (EFSA, 2010).

[1]The listed weight coincides with the median values for different age groups as recorded by the WHO. [2]Based on the study carried out by Kersting et al. (1998), we consider that three-month-old infants receive two meals per day containing spinach, with this vegetable representing 2/3 thirds of the food weight. For children aged 6, 9 and 12 months, spinach content in food amounts to 50%, and they receive one meal with spinach per day. Consumption (g/day) is indicated in this table based on the above assumptions. The maximum exposure in theory (in children aged 12 months) would be two meals per day with a spinach content of 100%. [3]These values correspond to "consumers only". Only 9.6% of all children consume spinach. For the group aged 1-3 years, N=266.

Only recently data on consumption amongst children aged 0-3 years have become available, following the development of certain databases such as EXPOCHI, which was used in the EFSA report (2010). In this report, EFSA recognises the lack of Member State data regarding food consumption in children aged 12 months and younger. After examining the results of several studies (Kersting et al., 1998), it provides an estimate for acute nitrate exposure due to spinach consumption in children aged 3 to 12 months (Table 7), considering two scenarios: high and low food consumption.

In summary, when assuming that chard consumption is equal to spinach consumption amongst Spanish children, and using the same criteria that EFSA (2010) has employed to measure consumption in young children, chard consumption for each of the different age groups would be as shown in Table 7.

To estimate acute dietary exposure to nitrates due to chard consumption amongst children in different age groups between 3 months and 3 years, the above mentioned chard consumption values (Table 7) have been considered, and three scenarios relating to nitrate content in all chard samples analysed in Spain (AESAN 2000-2009) (Table 4): 1) the median of 1,562 mg/kg (the parameter generally used for chronic exposure), 2) a level of 3,000 mg/kg, which is currently the maximum permitted nitrate level in fresh spinach (most samples) and 3) a content of 3,700 mg/kg, corresponding to the P95 of the data obtained for chard nitrate content (worst case scenario).

Estimates nitrate exposures, expressed in mg nitrate/kg of body weight per day have been shown in Table 8.

According to the calculated estimates, nitrate exposure due to chard consumption in infants and young children (under 3 years of age) varies between 1.6 and 7.2 mg/kg b.w./day (9.5 and 69.7 mg of nitrate/person/day) allowing for a median concentration of 1,562 mg of nitrate/kg of chard.

In the worst-case scenario, children aged 12 months who consume large quantities of chard with nitrate levels at 3,700 mg/kg (P95) may be exposed to 17.0 mg/kg b.w./day (133.8 mg of nitrate/person/day).

A potential dietary exposure of 13.8 mg/kg b.w./day is estimated based on chard consumption amongst children aged 12 months, in the hypothetical case that the maximum nitrate level in chard be established at 3,000 mg/kg as it is for spinach. This level of exposure is the same as that which the EFSA calculated previously (2010).

The previous calculations do not consider exposure to nitrates from water used to reconstitute infant formulae. It is thought that water is a frequent and significant contributor to nitrate exposure in children. In its two opinions, (2008, 2010), EFSA has not considered water as a source of exposure since it did not have reliable information on nitrate content in the water used to prepare infant formulae.

The estimates for acute nitrate exposure in children due to consuming chard, considering the median level of 1,562 mg/kg of nitrates in chard obtained in this report (1.6-7.2 mg/kg b.w./day) are approximately double the exposure estimates due to spinach consumption which EFSA evaluated in 2010. These estimates ranged between 0.8 and 3.8 mg/kg b.w./day, owing to the median nitrate content of spinach being nearly half that of chard (816 mg/kg of nitrates). Consuming a single portion of spinach containing the maximum allowable nitrate level or higher (3,500 mg/kg of nitrates) resulted in an exposure of nearly 15 mg/kg b.w., similar to that obtained by consuming chard in the worst-case scenario.

Since lettuce makes up a smaller part of the diets of children in these age groups than boiled spinach and chard do, EFSA estimated total dietary exposure in children aged 1-18 years between 1.7 and 4.2 mg of nitrates/kg b.w., considering mean consumption and up to 16 mg nitrate/kg b.w. for the highest level of lettuce consumption, combined with high nitrate content data (EFSA, 2010).

Consumption data in Table 7 represent the "consumers only" group. Following the same hypothesis, consumption data for chard amongst children aged 7 to 12 years (AESAN, 2006) may be considered valid for the general population aged 1 to 3 years with values of 2.61±10.69 g/day (0.186 g/kg/day). According to this premise, the estimated chronic intake of nitrates in children aged 1 to 3 years due to exclusive consumption of chard (general population: consumers plus non-consumers) would be 0.29, 0.56 and 0.69 mg/kg/day, respectively, for the three levels of nitrates considered (1,562; 3,000; and 3,700 mg/kg).

In adults, chronic nitrate exposure through consumption of 400 g of mixed vegetables per day, considering typical median nitrate levels, was 157 mg of nitrate/day (EFSA, 2008). In infants and young children, median chronic nitrate exposure (through consumption of vegetables in general and other foodstuffs) ranged between 0.77 and 1.39 mg/kg b.w./day (EFSA, 2010).

Table 8. Estimates for dietary nitrate exposure in children (3 months-3 years) by age group, considering various scenarios based on different nitrate levels in chard, comparing levels with the established ADI (3.7 mg nitrates/kg b.w./day)

Consumers only							General population				
Age	Body-weight (kg)	Chard consumption (g/day)[1]	(g/kg/day)[1]	Nitrate exposure mg/kg b.w./day Nitrate content (mg/kg) 1,562	3,000	3,700	Chard consumption (g/day)[2]	(g/kg/day)[2]	Nitrate exposure mg/kg b.w./day (% ADI) Nitrate content (mg/kg) 1,562	3,000	3,700
3 months	6.1	6.1-7.9	1.0-1.3	1.6-2.0	3.0-3.9	3.7-4.8	–	–	–		
6 months	7.7	10.8-15.4	1.4-2.0	2.2-3.1	4.2-6.0	5.2-7.4	–	–	–		
9 months	8.8	18.5-29.9	2.1-3.4	3.3-5.3	6.3-10.2	7.8-12.6	–	–	–		
12 months	9.7	27.2-44.6	2.8-4.6	4.4-7.2	8.4-13.8	10.4-17.0	–	–	–		
1-3 years	14	44.2 (median)	3.15	4.9	9.4	11.6	2.61±10.69	0.186	0.29 (7.8%)	0.56 (15.1%)	0.69 (18.6%)

[1]Two food intake levels are considered: low (mean minus the standard deviation) and high (mean plus the standard deviation (Kerstin et al., 1998) (EFSA, 2010).[2]Model Spanish diet (AESAN, 2006).

Risk characterisation

According to the data obtained in Table 8 on estimated nitrate intake through chard consumption, and considering the models for estimated ingestion by children aged 1-3 years in the general population, chronic exposure estimates for only chard consumption for the three scenarios that are described (1,562; 3,000; and 3,700 mg/kg) are lower than the ADI of 3.7 mg/kg b.w.. They represent 7.8%, 15.1% and 18.6% of that ADI, respectively. These results support the values for vegetable consumption in the whole population that were obtained by the EFSA (2008), and those found in its opinion on risks in infants and young children associated with consumption of spinach and lettuce (EFSA, 2010).

The fundamental purpose of this report, like that of the EFSA opinion (2010), is to provide estimates for acute exposure due to chard consumption. Although some estimates for chronic exposure to nitrates approached or occasionally exceeded the ADI, this would not indicate a health risk *per se*, since the ADI for nitrates is drawn from both subchronic and chronic studies.

Acute exposure to nitrates, considering the median nitrate concentration in available data (1,562 mg/kg) and only the consumer group, would result from daily intake of 1.6-7.2 mg/kg b.w./day in the age groups considered in the study. Consumption in the other two scenarios (3,000 and 3,700 mg nitrates/kg) would lead to higher levels of exposure, between 3.0 and 13.8 mg/kg b.w./day, and as much as 17 mg/kg b.w./day, without calculating for other nitrate sources, such as water.

According to EFSA criteria (2010) in the risk characterisation of acute nitrate exposure, 15 mg/kg/day is taken as the reference value, since available data indicate that MHb levels in infants and children are not elevated at exposure levels below that value. Therefore, that dose would not be exceeded in any of the studied scenarios (1,562; 3,000; and 3,700 mg nitrates/kg) for the 3-month, 6-month and 9-month age groups, which are the ones with the highest risk of suffering methaemoglobinaemia.

Only children aged 12 months, in cases of excessive consumption and nitrate concentrations of 3,700 mg/kg (P95) would reach an exposure level of 17 mg/kg b.w./day, which slightly exceeds the

dose of 15 mg/kg b.w.. Taking into account AESAN data, only 6.8% of the samples collected between 2000 and 2009 had nitrate levels higher than 3,500 mg/kg, and for that reason, the probability of acute toxic symptoms would be very low.

Consumption calculated for daily intake (Table 7) is based on the assumption that children aged 12 months consume chard once daily as part of a food preparation containing 50% chard, using the same criterion as EFSA did for spinach (2010). In exceptional cases, we may consider a consumption of two meals a day with double the chard content, and a maximum nitrate content of 3,000 mg/kg. This would give us a "maximum theoretical exposure" of 55.3 mg/kg b.w./day, up to three times higher than the dose of 15 mg/kg b.w./day.

Following the same reasoning, the "maximum theoretical exposure" in infants aged 6 months would be 24 mg/kg b.w./day given a nitrate concentration of 3,000 mg/kg, and 29.6 mg/kg b.w./day for a nitrate concentration of 3,700 mg/kg.

In these "maximum theoretical exposure" scenarios, a dose of more than 15 mg/kg/day would be received, and could give rise to methaemoglobinaemia.

Uncertainty

Throughout the Risk Assessment process for nitrates in chard consumption, the following elements of uncertainty were detected:

- Analytical uncertainties:
  a) The analytic methods used by the various laboratories that contributed data were not specified. This information is crucial to evaluating the reliability of results. Some parameters of the analytical methods were given (recovery and uncertainty), but this is clearly insufficient. The LOD/LOQ are not specified for each method, although some may be deduced from the data that was contributed. The laboratory performing the analysis is not always indicated.
  b) Representativeness of samples with respect to origin, regional and seasonal differences (summer/winter) and cultivation method (fields/greenhouses).
- Influence of processing/cooking on nitrate contents in the processed samples.
- Maximum limits: there are no established maximum limits for chard, and therefore the limits for spinach are often used. However, values in chard are generally higher.
- Intake: lack of data on chard consumption in the population under study.

In addition, there are uncertainties and limitations which the EFSA already specified (2010) for the presence of nitrates in leafy vegetables.

Consumption recommendations

Some recommendations with respect to leafy vegetable consumption are as follows:

Infants and children with bacterial gastrointestinal infections should not eat spinach because of increased nitrate sensitivity (EFSA, 2010).

These vegetables should be prepared shortly before eating, and if they are going to be consumed more than 12 hours after they are prepared, they should be kept frozen (EFSA, 2008). This is because

improper storage of these cooked foods may lead to nitrates being reduced to nitrites, thus increasing the risk of methaemoglobinaemia.

Furthermore, certain institutions such as the Spanish Paediatric Association (AEP) recommend feeding pureed vegetables to children 6 months and older and not introducing spinach, cabbage and beets until the child is 12 months old, although they do not make specific recommendations for chard (AEP, 2002).

Conclusions of the Scientific Committee

- Based on the evaluation of AESAN data for nitrate content in chard (between 2000 and 2009), we observe a high degree of variability in nitrate content, with a median value of 1,562 mg nitrate/kg of chard and a value of 3,700 mg/kg for P95. These are higher than the nitrate levels in spinach across Europe (median 816 mg/kg) published by EFSA (2008). Although there are no maximum permissible limits for nitrates in chard, 126 samples (12.4%) exceeded the maximum content of 3,000 mg/kg permitted in spinach, and 69 samples (6.8%) exceeded 3,500 mg nitrates/kg.
- To undertake a more precise evaluation of chard consumption leading to nitrate-related risks in infants and children aged 1-3 years, considering that this group is more likely to suffer methaemoglobinaemia following acute exposure, it would be advisable to have specific data regarding their consumption habits.
- We can assume that chard consumption in children aged 3 months to 1 year is equal to spinach consumption in young children in the European population studied by EFSA (2010), and that chard consumption data amongst children aged 7 to 12 in the model Spanish diet study (AESAN, 2006) can be extended to the population aged 1 to 3 years. In these cases, the estimates of chronic exposure due to consumption of chard only in children aged 1-3 years, considering three nitrate concentrations of 1,562; 3,000; and 3,700 mg nitrates/kg (the median, the maximum permitted nitrate concentration in fresh spinach, and the P95 concentration, respectively) are lower than the established ADI of 3.7 mg/kg b.w., with each of the above figures representing 7.8%, 15.1% and 18.6% of the ADI, respectively.
- Estimates for acute nitrate exposure caused by chard consumption (median: 1,562 mg nitrates/kg) that were obtained in this report ranged between 1.6 and 7.2 mg/kg b.w./day, approximately double that caused by spinach according to the 2010 EFSA study (0.8 to 3.8 mg/kg b.w. per day). If we adopt EFSA's reference value of 15 mg/kg b.w./day as the limit for preventing elevated MHb levels in infants and young children, our estimates indicate that this dose would not be exceeded in any of the scenarios that were studied amongst groups aged 3, 6 and 9 months which have the highest likelihood of developing methaemoglobinaemia. Only children aged 12 months, in cases of excessive consumption and very high nitrate concentrations (3,700 mg/kg) would attain an exposure level of 17 mg/kg b.w./day, although the probability of acute toxic symptoms would be extremely low given the small percentage of chard samples with such high nitrate levels.
- According to the data obtained, the nitrate content of chard in Spain is higher than that of spinach. For that reason, the Committee recommends including chard in the consumption guidelines for spinach, since chard is widely consumed in Spain.

- This Committee believes it appropriate to establish maximum nitrate limits for chard as has been done for spinach. A maximum concentration of 3,000 mg nitrates/kg of chard will not lead to health risks in infants and young children (under 1 year and up to 3 years).

References

Abo Bakr, T.M., El-Iraqui, S.M. and Huissen, M.H. (1986). Nitrate and nitrite contents of some fresh and processed Egyptian vegetables. *Food Chemistry*, 19, pp: 265-275.

AEP (2002). Asociación Española de Pediatría. Protocolos de Nutrición. Alimentación del lactante sano. Available at: http://www.aeped.es/documentos/protocolos-nutricion [accessed: 14-4-11].

AESAN (2006). Agencia Española de Seguridad Alimentaria y Nutrición. Modelo de dieta española para la determinación de la exposición del consumidor a sustancias químicas. Available at: http://www.aesan.msc.es/AESAN/docs/docs/notas_prensa/modelo_dieta_espanola.pdf [accessed: 21-6-11].

Alonso Vega, L.A., Gutiérrez Conde, M.L.G., Canduela Martínez, V.C., Hernández Herrero, M.H., Tazón Varela, M.T. and Pérez Mier, L.A.P. (2007). Metahemoglobinemia en una lactante por consumo de puré vegetal. *Emergencias*, 19, pp: 283-285.

ATSDR (2004). Agency for Toxic Substances and Disease Registry. Interaction profile for cyanide, fluoride, nitrate, and uranium. Atlanta: US Department of Health and Human Services. Available at: http://www.atsdr.cdc.gov/interactionprofiles/IP-09/ip09-a.pdf [accessed: 21-6-11].

Bartholomew, B. and Hill, M.J. (1984). The pharmacology of dietary nitrate and the origin of urinary nitrate. *Food and Chemical Toxicology*, 22 (10), pp: 789-795.

Bryk, T., Zalzstein, E. and Lifshitz, M. (2003). Methemoglobinemia induced by refrigerated vegetable puree in conjunction with supraventricular tachycardia. *Acta Paediatrica*, 92, pp: 1214-1215.

Chung, J.C., Chou, S.S. and Hwang, D.F. (2004). Changes in nitrate and nitrite content of four vegetables during storage at refrigerated and ambient temperatures. *Food Additives and Contaminants*, 21, pp: 317-322.

Da-Silva, S.S., Sajan, I.S. and Underwood, J.P. (2003). Congenital methemoglobinemia: a rare cause of cyanosis in the newborn-A case report. *Pediatrics*, 112 (2), pp: e158-e161.

Dejonckheere, W., Steurbaut, W., Drieghe, S., Verstraeten, R. and Braeckman, H. (1994). Nitrate in food commodities of vegetable origin and the total diet in Belgium 1992-1993. *Microbiologie-Aliments-Nutrition*, 12, pp: 359-370.

EFSA (2008). European Food Safety Authority. Nitrate in vegetables. Scientific Opinion of the Panel on Contaminantsin the Food Chain. *The EFSA Journal*, 689, pp: 1-79. Question Nº EFSA-Q-2006-071.

EFSA (2010). Statement on possible public health risks for infants and young children from the presence of nitrates in leafy vegetables. *The EFSA Journal,* 8 (12), pp: 1935.

EU (2006). Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20 of December 2006, pp: 5-34.

FAO/WHO (1996). Food and Agriculture Organization/World Health Organization. Nitrate. Safety evaluation of certain food additives. Food Additives Series 35. Available at: http://www.inchem.org/documents/jecfa/jecmono/v35je14.htm [accessed: 21-6-11].

Gangolli, S.D., van den Brandt, P.A. and Feron, V.J. (1994). Nitrate, nitrite and *N*-nitroso compounds. *European Journal of Pharmacology*, 292, pp: 1-38.

Gobierno Vasco (2003). Estudio sobre consumo de alimentos e ingesta de plaguicidas y nutrientes por niños/ niñas de 8 a 12 meses de edad de la CAPV (proyecto Montecarlo). Informe técnico, pp: 1-24. Available at: https://www6.euskadi.net/r33-2709/es/contenidos/informacion/sanidad_alimentaria/es_1247/adjuntos/proyecto Montecarlo_c.pdf [accessed: 9-6-11].

Gobierno Vasco (2008). Departamento de Agricultura, Pesca y Alimentación. Estudio cuantitativo del consumo de alimentos en la CAPV. Guías Elika, 8. Available at: http://www.elika.net/pub_otras.asp?publicacion=39&seleccionado=10 [accessed: 9-6-11].

Gómez Lumbreras, A.G., Solaz Moreno, L.S. and Villar Rubin, S.V. (2008). Intoxicación por puré de acelgas. *Anales de pediatría*, 69 (3), pp: 279-291.

Greer, F.R. and Shannon, M. (2005). Infant methemoglobinemia: the role of dietary intake in food and water. *Pediatrics*, 16, pp: 784-786.

Hack, W.W., Douwes, A.C. and Veerman, A.J. (1983). Spinach: A source of nitrite poisoning in young children. *Ned Tijdschr Geneeskd*, 127, pp: 1428-1431.

Herranz, M. and Clerigué, N. (2003). Intoxicación en niños. Metahemoglobinemia. *Anales del Sistema Sanitario de Navarra*, 26 (1), pp: 209-223.

IARC (2010). International Agency of Research in Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Ingested Nitrate and Nitrite and Cyanobacterial Peptide Toxins. Volume 94, pp: 1-325. Available at: http://monographs.iarc.fr/ENG/Monographs/vol94/index.php [accessed: 21-6-11].

IPCS (1999). International Programme on Chemical Safety. Nitrates and nitrites. Poisons Information Monograph (Group Monograph) G016.

JECFA (1995). Joint FAO/WHO Expert Committee on Food Additives. Nitrate and nitrite. Evaluation of Certain Food Additives. Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. Who Technical Report Series 859. TRS 859-JECFA 44/29,32.

JECFA (2002). Joint FAO/WHO Expert Committee on Food Additives. Nitrate and nitrite. Evaluation of Certain Food Additives. Fifty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Who Technical Report Series 913. TRS 913-JECFA 59/75.

JECFA (2003). Joint FAO/WHO Expert Committee on Food. Nitrate (and potential endogenous formation of N-nitroso compounds). Safety evaluation of certain food additives. Who Food Additives Series: 50.

Kanaan, S.S. and Economakis, C.D. (1992). Effect of climatic conditions and time of harvest on growth and tissue nitrate content of lettuce in nutrient film cultura. Acta Horticulturae, 323, pp: 75-80.

Kersting, M., Alexy, U., Sichert-Hellert, W., Manz, F, and Schoch, G. (1998). Measured consumption of commercial infant food products in German infants: results from the DONALD study. Dortmund Nutritional and Anthropometrical Longitudinally Designed. *Journal of Pediatric Gastroenterology and Nutrition*, 27, pp: 547-552.

Knobeloch, L., Salna, B., Hogan, A. and Postle, J. (2000). Blue babies and nitrate-contaminated well water. *Environmental Health Perspectives*, 108, pp: 675-678.

Laporta Báez, Y.L., Goñi Zaballo, M.G., Pérez Ferrer, A.P., Palomero Rodríguez, M.A.P., Suso, B. and García Fernández, J.G. (2008). Metahemoglobinemia asociada a la ingesta de acelgas. *Anales de pediatría*, 69 (2), pp: 191-192.

Meah, M.N., Harrison, N. and Davies, A. (1994). Nitrate and nitrite in foods and the diet. *Food Additives and Contaminants*, 11 (4), pp: 519-532.

Menard, C., Heraud, F., Volatier, J.L. and Leblanc, J.C. (2008) Assessment of dietary exposure of nitrate and nitrite in France. *Food Additives and Contaminants*, 25 (8), pp: 971-988.

NTP (2001). National Toxicology Program.Toxicology and carcinogenesis studies of sodium nitrite in F344/N rats and B6C3F1 mice (drinking water studies). *National Toxicology Program Technical Report Series*, 495, pp: 7-273.

Pérez-Caballero, C., Pérez, A. and Moreno, L. (2005). Probable metahemoglobinemia tras la administración de EMLA. *Anales de pediatría*, 63, pp: 179-180.

Sánchez-Echaniz, J., Benito, J. and Mintegui, S. (2001). Methemoglobinemia and comsuption of vegetables in infants. *Pediatrics*, 107, pp: 1024-1028.

Sander, C. and Jacobi, H. (1967). Methemoglobin poisoning in a 2-year old boy after eating spinach. *Zeitschrift fur Kinderheilkunde*, 98, pp: 222-226.

Savino, F., Maccario, S., Guid, C., Castagno, E., Farinasso, D., Cres, F., Silvestro, L. and Mussa, G.C. (2006.) Methemoglobinemia caused by the ingestion of courgette soup given in order to resolve constipation in two formula-fed infants. *Annals of Nutrition & Metabolism,* 50, pp: 368-371.

SCF (1992). Scientific Committee for Food. Nitrates and nitrites. Reports of the Scientific Committee for Food. Twenty-sixth series.

Schuster, B.E. and Lee, K. (1987). Nitrate and nitrite methods of analysis and levels in raw carrots, processed carrots and in selected vegetables and grain products. *Journal of Food Science*, 52 (6), pp: 1632-1636.

Thomsom, B. (2004). Nitrates and nitrites dietary exposure and risk assessment. Institute of Environmental Science & Research Limited. Available at:
htpp: //www.foodsmart.govt.nz/elibrary/nitrates_nitrites_dietary.pdf [accessed: 29-3-11].

Van Velzen, A.G., Sips, A.J.A.M., Schothorst, R.C., Lambers, A.C. and Meulenbelt, J. (2008). The oral biovailability of nitrate rich vegetables in humans. *Toxicological Letters*, 181 (3), pp: 177-181.

Walker, R. (1990). Nitrates, nitrites and N-nitrosocompounds: a review of the occurrence in food and diet and the toxicological implications. *Food Additives and Contaminants*, 7 (6), pp: 717-768.

WHO (2003). World Heatlh Organization. GEMS/FOOD Regional Diets.Food Safety Department. Geneva, Switzerland.