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DE SÉCURITÉ SANITAIRE
DES ALIMENTS

Maisons-Alfort, 11 July 2008

THE DIRECTOR GENERAL

OPINION

of the French Food Safety Agency on the assessment of the health risks associated with non-compliance with quality limits for nitrates and nitrites in water intended for human consumption

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Context of the request

The French Food Safety Agency (Afssa) received a request on 17 April 2003 from the Directorate General for Health to issue an opinion on the assessment of the health risks related to non-compliance with quality limits for nitrates and nitrites in water intended for human consumption and therefore set up an *ad hoc* working group. The "nitrates" and "nitrites" parameters were examined from the month of September 2006. The "Eau et rivières de Bretagne" [Brittany Water and Rivers] association has also requested Afssa for a similar opinion.

Method of expertise

The "Water" scientific panel was consulted on 4 December 2007 and 8 January 2008. The "Chemical and Physical Contaminants and Residues" scientific panel was consulted on 21 January 2008.

Context

Considering the World Health Organisation (WHO) guideline value of 50 milligrams per litre for nitrates;

Considering the quality limits of 50 milligrams per litre for nitrates and 0.5 milligrams per litre for nitrites in annex I of the decree of 11 January 2007 on quality limits and references for untreated water and water intended for human consumption stipulated in articles R. 1321-2, R. 1321-3, R. 1321-7 and R. 1321-38 of the French Public Health Code;

Considering Afssa's opinion of 2 December 2003 on the establishment of quality criteria for natural mineral waters and bottled spring waters enabling risk-free consumption by infants and young children, which states that **to protect the resource**, a guideline value of 10 milligrams per litre for nitrates could be set according to the terms of paragraph 3 of article 2 of directive 2003/40/CE of 16 May 2003 on the concentration limits in natural mineral waters;

Considering the initial assessment of health risks related to non-compliance with quality limits and references in water intended for human consumption presented in the Afssa report of September 2004;

Considering the assessment of health risks associated with non-compliance with quality limits in water intended for human consumption concerning nitrates and nitrites described in fact sheet 19;

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Argument

Concerning treatment processes

Considering that treatment processes which comply with the stipulated regulations can be used to reduce nitrate concentration in water leaving the water treatment plant;

Concerning SISE-Eaux data

Considering data available in the SISE-Eaux database which show that for nitrates, during the year 2006, 99.5% of mean values for the water supply outputs were compliant and that for more than 99% of the water output which did not comply, the mean concentration was between 50 and 75 milligrams per litre;

Considering the data available in the SISE-Eaux database which show that for nitrites, over the period from January 2003 to December 2006, the 50th percentile of non-compliant analytical results was 0.62 milligrams per litre and the 95th percentile was 1.85 milligrams per litre;

Concerning methaemoglobinaemia in infants

Considering the scientific uncertainties about the role of nitrates alone in the development of infantile methaemoglobinaemia and, in particular, the possible role of a microbiological factor and gastrointestinal tract inflammatory mechanisms;

Concerning IARC assessments

Considering that the International Agency for Research on Cancer (IARC) believes that:

- there is inadequate evidence in humans for the carcinogenicity of nitrate in drinking water;
- there is limited evidence in experimental animals for the carcinogenicity of nitrites *per se*;
- nitrates and nitrites are probably carcinogenic to humans following ingestion under conditions that result in endogenous nitrosation;

Concerning JECFA assessments

Considering the acceptable daily intake (ADI) for nitrates of 3.7 mg/kg b.w./d established by the FAO/WHO[1] Joint Expert Committee on Food Additives (JECFA) in 2003 for chronic exposure in rats[2];

Considering that in the above study (Lehman, 1958), the exposure period and target effects seen were different to those resulting in methaemoglobinaemia and that published data appear open to methodological criticism when establishing an acceptable daily intake;

Considering that the use of the rat as a toxicological model is not pertinent to assess the human health risk, particularly because of the absence of entero-salivary recirculation of nitrates in rats;

Considering the acceptable daily intake for nitrites of 0.07 mg/kg b.w./d established in rats by the FAO/WHO Joint Expert Committee on Food Additives (JECFA) in 2003;

[1] Food and Agriculture Organisation / World Health Organisation

[2] The JECFA ADI (2003) was adopted by EFSA (European Food Safety Authority) in its 2008 report

Concerning exposure data

Considering the estimated dietary intake (solid foodstuffs and drinking water) of nitrites;

Considering the current scientific uncertainties relative to quantification of endogenous N-nitroso compound formation from exogenous nitrate and nitrite intake;

Considering that the only TNS-Sofres survey of 2005 on tap water consumption in 64 people stated that up to three months of age no infants would consume water from the public mains water supply;

Conclusions and recommendations

The French Food Safety Agency recalls:

- that it is necessary to ensure maximum protection of the quality of raw water resources used to produce water intended for human consumption;

- that measures should be used to reduce nitrate and nitrite concentration in water intended for human consumption, to reach the quality limit, as soon as possible;

The French Food Safety Agency considers that:

- there are sufficient data currently available to state that the risk of infantile methaemoglobinaemia can be deemed to be negligible for water in which the nitrate concentration complies with the quality limit of 50 mg/L;

- the fragmentary information from the methaemoglobinaemia risk assessment in infants and absence of a sufficiently robust acceptable daily intake are such that it is not possible to propose a derogation value in the event of non-compliance with the quality limit for nitrates and that, as a result, it would be desirable to have access to toxicological studies conducted in relevant animal models;

- the dietary daily intakes of nitrites established on the basis of conservative estimates are higher than the acceptable daily intake proposed by JECFA in 2003 of 0.07 mg/kg b.w./d for children on the one hand and high consuming adults on the other; consumption of water containing a nitrite concentration above the quality limit of 0.5 mg/L is therefore not recommended;

- it is not possible from current knowledge to quantify endogenous formation of N-nitroso compounds from nitrate and nitrite intake and therefore to assess the carcinogenic risk related to such intake.

The Director General of the French Food Safety Agency

Pascale BRIAND

Key words: nitrates, nitrites, N-nitroso compounds, non-compliance with quality limits, drinking water.

Form 19: Assessment of the health risks associated with non-compliance with quality limits for nitrates and nitrites in water intended for human consumption

Nitrate quality limit: 50 mg/L[3]
Nitrite quality limit: 0.5 mg/L

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[3] Nitrate and nitrite concentrations in water are often expressed in the literature as “nitrate- nitrogen” and “nitrite-nitrogen”. The equivalents are: 1 mg/L (expressed as N) = 4.43 mg/L (expressed as NO_3), and 1 mg/L (expressed as N) = 3.29 mg/L (expressed as NO_2).

Nitrate (NO_3^-) and nitrite (NO_2^-) ions are natural ions present throughout the environment, and are the products of nitrogen oxidation by microorganisms in plants, soil or water. Nitrate is the most stable oxidised form of nitrogen, which can, however, be reduced to nitrite by microbial activity. This fact sheet therefore examines these two ions jointly.
In living organisms, nitrates and nitrites can lead to the formation of N-nitroso compounds, which are of major toxicological importance. For this reason the hazards associated with these N-nitroso compounds are included in this fact sheet and the toxicological and metabolic data are presented in the annex.
EFSA (European Food Safety Authority) provided an opinion[4] to the European Commission in April 2008 concerning the risks and benefits of nitrates in vegetables, taking into account variations in exposure seen in Europe.
Afssa's approach takes into account intake from water and in the diet (including vegetables) and therefore provides a precise assessment of nitrate and nitrite exposure in the French population.

1 – Origins and sources of contamination

1.1 Nitrates

Soil nitrates arise from the binding of atmospheric nitrogen by certain species of plants (leguminous) and are therefore always present, even in the absence of nitrogen-containing fertilisers, although the latter are the main source.
Nitrates from **groundwater** come from leaching of nitrates produced naturally in the superficial soil or added in the form of fertilisers. The natural nitrate concentration in groundwater is less than 10 mg/L.
There are two major sources of nitrates in **surface water:** introduction from groundwater resources associated with farming activities and urban wastewater, which also contains ammonium. Some industrial activities (for example in the agro-industrial sector) may discharge wastewater with a high nitrate concentration.
Leaching from farm soil by rain water, particularly in the winter or following heavy rains shortly after fertiliser spreading, can also represent a significant source of nitrates in surface water. Marked seasonal variations in nitrate concentrations are seen in numerous French water rivers, with high concentrations in winter and low concentrations in summer.

1.2 Nitrites

The nitrite ion can be present in trace amounts in groundwater or be formed in badly corroded piping although is very rapidly oxidised into the nitrate ion by free chlorine and it is therefore only very rarely found in drinking water.

2 – Treatments reducing the nitrate concentration in water

According to the article R.1321-50 of the French Public Health Code, treatment processes and products placed on the market and intended for human consumption must, under normal and predictable conditions of use, comply with the specific provisions defined by the order of the French Ministry of Health ensuring that (i) they are not liable, either intrinsically or as a result of their residues, to present a direct or indirect hazard to human health or lead to an alteration in the composition of water, defined by values set by this decree; (ii) they are sufficiently effective.
To date, pending the publication of an order on water treatment substances and processes, the specific terms to be observed are those defined by:

[4] Opinion of the Scientific Panel on Contaminants in the Food chain at the request of the European Commission to perform a scientific risk assessment on nitrates in vegetables, The EFSA Journal (2008), Journal number, 689, 1-79

- the circular of 28 March 2000[5],
- the circular of 16 March 1995 for processes implementing membrane filtration modules.

The following treatments reduce nitrate concentrations in water, although it is necessary to ensure that their use is authorised on a case-by-case basis.

Biological treatments: nitrification-denitrification

In the presence of biologically oxidisable substances, some bacteria can use oxygen from nitrates if the oxygen content in water is too low. The nitrates are then reduced firstly to nitrites and then to gaseous nitrogen. This explains why water must not be supplied when the system is first started up, for as long as nitrites are being produced.

- Processes are described as heterotrophic when the oxidisable substance is an easily biodegradable molecule: acetic acid and ethanol are authorised in France.
- Autotrophic processes use oxidisable mineral substances: sulphur and sulphides are authorised in France.

As these bacteria are very sensitive to low temperatures, only denitrification of groundwater is authorised for the production of water intended for human consumption.

Physicochemical treatments: ion exchange or electrodialysis

- Anion exchange resins replace the nitrate ion by the chloride ion. These resins retain the sulphate ion strongly and when the system is started up, some of the hydrogen–carbonate ions present, resulting in a change in pH. Under these conditions, particular attention must be paid to the calco-carbonic equilibrium in the water.
  Furthermore, if the system is stopped for more than 12 hours these resins can leach amines, which, in stagnated water, react with nitrites which have been newly formed from nitrates present in the water or exchanged onto the resin. Regeneration is therefore essential before restarting the system. For this reason the domestic use of these resins is not recommended.
  Finally, very careful attention must be paid to the discharge of eluates from these resins, which contain very high nitrate concentrations.

- Electrodialysis treatments use a nitrate permselective membrane.

Membrane treatments: nanofiltration or reverse osmosis

- Some nanofiltration membranes with a cut-off point of less than 100 Daltons retain up to 50% of nitrates.
- Reverse osmosis can demineralise water and therefore remove nitrates.

3 – Analytical methods

The order of 17 September 2003[6] relative to analytical methods for water samples and their performance characteristics states that:

- for nitrates, the accuracy, precision and limit of quantification must not exceed 10% of the parametric value (5 mg/L) and that the limit of detection must be below 4 mg/L,
- for nitrites, the accuracy, precision and limit of quantification must not exceed 10% of the parametric value (0.05 mg/L) and that the limit of detection must be below 0.05 mg/L.

Nitrates and nitrites may be quantified in water using the standardised methods listed below:

- NF EN ISO 10304-1 (June 1995): determination of dissolved fluoride, chloride, nitrite, orthophosphate, bromide, nitrate and sulphate ions by liquid phase ion chromatography – Part 1: method applicable to water with low contamination
- NF EN ISO 13395 (October 1996): Determination of nitrite nitrogen, nitrate nitrogen and the

[5] Circular DGS/VS 4 No. 2000-166 of 28 March relative to treatment substances and processes for water intended for human consumption, NOR: MESP0030113C

[6] Order of 17 September 2003 relative to analytical methods for water samples and their performance characteristics, NOR: SANP0323688A, JORF of 7 November 2003, p. 19027 to 19033

sum of both by flow analysis (CFA and FIA) and spectrometric detection.
- NF EN 26777 (May 1993) Water quality – Determination of nitrites – Molecular absorption spectrometric method.

Analytical uncertainty

Uncertainty of measurement can be estimated from inter-laboratory studies by determining the coefficient of variation of reproducibility (CVR%).

- For nitrates, the maximum concentration tested in inter-laboratory studies is 25 mg/L: the CVR% value at this concentration is 4.1% and the 95% confidence interval is ± 1 mg/L. The upper analytical limit is therefore in the region of 26 mg/L (AGLAE, 2003).

- For nitrites, the 95% confidence interval at the quality limit (0.5 mg/L) is ± 0.029 mg/L in the AGLAE inter-laboratory studies (2003).

Table 3.1: Evolution of uncertainty for different nitrite concentrations in water on the basis of the CVR% estimated by AGLAE from inter-laboratory studies, all analytical methods combined– Source: AGLAE, 2003

Concentration in water (mg/L)	0.2	0.3	0.4	0.5	0.6	0.7
CVR%	9.5	7.4	6.4	5.8	5.4	5.1
Estimated uncertainty* (mg/L)	± 0.02	± 0.02	± 0.03	± 0.03	± 0.03	± 0.04

**95% confidence interval for a measurement performed by a randomly selected quality controlled laboratory.*

4 – Exposure assessment

4.1 Nitrates

Air contamination contributes a minimal fraction of nitrate exposure compared to diet. Plants represent the main source if the nitrate concentration in water does not exceed the quality limit.

4.1.1 Exposure to nitrates from foodstuffs

4.1.1.1 General data

According to WHO, mean individual nitrate exposure is between 43 and 131 mg NO_3/day (duplicate meals study; WHO, 2007). Estimates based on urinary nitrate excretion are between 39 and 268 mg/day (WHO, 2007).
Food additives are minor contributors.
Nitrate intakes vary greatly from one country to another and between groups of people (vegetarians/non-vegetarians, for example – Schuddeboom, 1993; JECFA, 2003) and are between 43 and 154 mg NO_3/day in Europe – excluding intake from water (Schuddeboom, 1993; JECFA, 2003), i.e. 0.72 to 2.57 mg/kg b.w./d for an individual weighing 60 kg.
EFSA has defined five European exposure scenarios to cover the variations seen in levels of nitrate consumption and contamination in vegetables. The basic scenario shows estimated nitrate intake from vegetables to be 157 mg/person/d using a median vegetable nitrate concentration of 392 mg/kg and individual daily intake of 400 g/person/day of vegetables alone (scenario based on WHO recommendations concerning fruit and vegetable intake). Although extremely variable, daily nitrate exposure from non-vegetable sources is estimated to be 44 mg/person/day, of which 20 mg/person/day is attributable to water (EFSA, 2008).

4.1.1.2 French data

Duplicate meals study - 1991

In 1991, the Ministry of Health conducted a study– the nitrates diagonal – intended to establish the amounts of nitrates actually ingested by the French population using a direct assay method in food servings[7]. (Ministry of Health, 1991). The results are shown in table 4.1.1.2-1:

Table 4.1.1.2-1: Estimate of dietary nitrate intake in the French population - 1991
Source: Ministry of Health 1991

	School	Company	Hospital	Retirement home	Mean adult	Mean general
Mean Food mg/meal	27.7	57.7	78	42.4	59.4	51.45
Total (food + water) mg/meal	42.4	74	93	54.8	73.9	66.05

Assuming that the daily intake represents the equivalent of two meals taken in community canteen facilities, the average amount ingested is in the region of 103 mg/day, i.e. 1.72 mg/kg b.w./d (excluding intake from water), which falls within the European interval [43 mg NO_3/day,154 mg NO_3/day] defined above. This represents 78% of total intake.

The main contributing foodstuffs are "concentrating" vegetables: radishes, beetroot, spinach, lettuce, chard, lambs lettuce, celery and turnips (48% of intake). Foodstuffs which are "non-concentrating" but are eaten in large amounts are also important food vectors (34%): potatoes, carrots, cabbage, courgettes, green beans and leeks.

SCOOP Task Force - 1997 (EC, 1997)

Exposure of the population of France to nitrates from plants was also estimated by the SCOOP task force in 1997 (Table 4.1.1.2-2). The following data were used:

- consumption data derived from SECODIP panel purchase data (1991), which provides information on the general population and on consumers, including the extreme percentiles (95^{th} percentile);
- contamination data from DGCCRF (Directorate General for Competition, Consumer Affairs and Fraud Control) control and surveillance programmes.

Table 4.1.1.2-2: Estimate of dietary nitrate intake in the French population - 1997
Source: European Commission, 1997

Daily nitrate intake (mg/kg b.w./d)	General population	Consumers alone
Mean	0.6	0.97
95^{th} percentile	2.8	3.3

It should be noted that these exposure values do not take into account endogenous nitrate and nitrite formation or the endogenous reduction of ingested nitrates into nitrites (approximately 5% in saliva, according to Schuddeboom, 1994).

Estimate of nitrate exposure– Afssa, 2007

The study conducted by Afssa (Afssa – Paser, 2007a) combined individual food consumption data from the INCA1 survey with concentration data from the DGCCRF control and surveillance programmes during the years 2000 to 2006, i.e. 13657 data on 138 foodstuffs.

[7] In order to bypass food storage and cooking methods which are important variables for nitrate concentration it was decided to perform the analysis directly on the consumer's plate in community canteen facilities (1/4 of French people eating this type of meal at lunchtime; this figure was increasing by 1% per year in 1990). This study was conducted on:
- 12 *départements* representative of the diverse range of situations in France
- 4 major types of community canteen facilities (nursery and primary schools, retirement homes, hospitals, company canteens)
- 42 canteens and 400 meals analysed

In order to take into account data below the limit of detection or limit of quantification, upper and lower estimates of daily intake were made. The calculation method is shown in annex 1 and the results are presented in table 4.1.1.2-3.

Table 4.1.1.2-3: Estimated dietary nitrate intake (excluding water) in the French population - 2007
source: Afssa, 2007

Daily nitrate intake (mg/kg b.w./d)	Adults > 15 years old		Children between 3 and 14 years old	
	Lower estimate	Upper estimate	Lower estimate	Upper estimate
Mean	1.3	1.3	1.7	1.8
95th percentile	2.7	2.75	3.8	3.9

Limitations of the nitrate exposure estimate: data obtained from the DGCCRF surveillance programmes only relate to foodstuffs known to contribute to dietary intake. In addition, difficulties were encountered coding some of the foodstuffs analysed. Finally, a very variable number of analyses were performed per foodstuff.

The national data in this survey showed that the main foodstuffs contributing to population exposure were “concentrating” vegetables (60% of intake): salads (41%), radishes, peas, green beans and celery sticks. Potatoes which are “non-concentrating” but extensively eaten are also important vector foodstuffs, representing 13% of intake.

4.1.1.3 Summary: exposure to nitrates from foodstuffs

Table 4.1.1.3 summarises the available studies assessing dietary nitrate intake.

Table 4.1.1.3: summary of dietary nitrate exposure

Country	Date	Mean intake mg/ kg b.w./d	Intake for high consumers mg/ kg b.w./d	Comments
Europe		0.72 to 2.57		Intake excluding water Extremely variable between countries
Europe	2008	3.0	8.0	Intake from vegetables + 24 mg/person/day for other sources excluding water Extremely variable from one country to another Specific scenarios are proposed to take into account the variability of exposure parameters
France – Duplicate meals	1991	1.72 (general population)		Intake excluding drinking water
France –SCOOP task force contamination x Consumption	1997	0.6 (general population)	2.8 (general population) (95th percentile)	Intake from vegetables
France – Afssa Contamination x Consumption	2007	1.3 (adults) 1.8 (children)	2.75 (adults) 3.9 (children) (95th percentile)	Intake excluding drinking water and heated water (tea, coffee, soup)

The results of the studies conducted in 1991 and 2007 are similar, although the methods used to estimate nitrate intake differ. The method combining French foodstuff consumption and foodstuff contamination data provides the most representative estimate of intake in the French population. The duplicate meals method takes into account degradation or migration of nitrates during cooking. In terms of the effect of cooking foodstuffs, the study conducted by the United Kingdom showed a 75% reduction in nitrate concentration in most vegetables (Ministry of Agriculture, Fisheries and Food, 1998). It should be noted, however, that salads are generally eaten raw and account for more than 40% of nitrate intake.

4.1.2 Intake from drinking water

4.1.2.1 Drinking water quality

The health control regulatory programme defined in the order of 11 January 2007 stipulates that analyses be performed on this parameter at the draw point (from once every 5 years to 12 times per year) and after treatment (from 1 to more than 144 times per year).

According to data from the SISE-Eaux database (Ministry of Health - DDASS) shown in table 4.1.2.1, 46.8% of supplies tested had mean nitrate concentrations of 10 mg/L or less and 99.5% were below 50 mg/L.

Table 4.1.2.1: Distribution of samples tested in 2006 according to mean nitrate concentration in water.

NO_3 concentration in (mg/L)	≤ 10	> 10 and ≤ 25	> 25 and ≤ 40	> 40 and ≤ 50	> 50 and ≤ 60	> 60 and ≤ 75	> 75 and ≤ 100	>100	Total
Controlled flows (millions m^3/d)	7.57	6.07	2.09	0.38	0.06	0.02	0.0008	0.0	16.2
No. of water treatment plants controlled	12438	4024	2428	722	270	83	16	1	19982
Number of measurements (health controls stipulated by the *prefectorial* * order alone without an analysis of re-test data)	29343	12722	7952	2202	682	210	33	1	53145

Of those situations which did not comply with the quality limit:

- in more than 74.3% of the non-compliant water flows, the mean nitrate concentration was between 50 and 60 mg/L,
- in more than 99% of the non-compliant water flows, the mean nitrate concentration was between 50 and 75 mg/L.

4.1.2.2 Ingestion of water apart from meals

Data from the INCA 1 survey (table 4.1.2.2), which provides information about dietary consumption in the general French population, show that the consumption of heated and unheated drinking water in France, excluding meals, represents an average of 38% of total intake.

Table 4.1.2.2: Water consumption from the public water supply (percentage of total intake). Source: Afssa – Paser, 2007b

Daily consumption of heated and unheated water (ml/j)	General population		Consumers alone	
	Total	Excluding meals	Total	Excluding meals
Mean	433	166 (38 %)	474	182 (38 %)
95th percentile	1211	619 (51 %)	1256	637 (51 %)

4.1.2.3 Ingestion of water by infants (children under 12 months old)

The Observatory of Food Consumptions – Nutritional Epidemiology (Afssa) used dietary consumption data for young French infants and children (between 1 and 36 months old) from a 2005 study conducted by TNS-SOFRES in collaboration with the University of Burgundy, for the French Union for Childhood Food (SFAE).

This dietary survey conducted over short periods of 3 consecutive days was conducted on 447 infants and young children[8] under 12 months old. Only infants and young children who were not being breast-fed (either exclusively or partially) and who were not attending a community nursery or school during the days of the survey were included. Average water consumption was estimated over 3 days. The results are shown in table 4.1.2.3.

Table 4.1.2.3: Proportion of infants and young children between 1 and 12 months old drinking tap water over 3 days and amount drunk in mL/d

Age (months)	Number of children	Frequency of drinking tap water		Amount of tap water drunk by consumers only in mL/d		
		n	%	Mean ± s-d	Min	Max
1-3	64	0	0.00%	Not est.	Not est.	Not est.
4-6	187	2	1.07%	148.3 ± 157.9	36.7	260.0
7-12	196	14	7.14%	73.4 ± 80.8	10.0	236.7
Total 1-12 months	447	16	3.58%	82.7 ± 89.3	10.0	260.0

In the only survey which is available (TNS - Sofres, 2005), tap water does not appear to be drunk by infants under three months old.

4.1.3 Contribution from exposure sources to total dietary daily intake

The study conducted by Afssa (2007a) provided estimates of cumulative nitrate intake from drinking water for different exposure scenarios. Only the upper estimates are shown in this table[9].

Table 4.1.3: Upper estimates of nitrate intake for different scenarios of water intake. The contribution provided by water is shown in brackets. Source: Afssa – Paser, 2007a

Adults > 15 years old				
Daily nitrate intake (mg/kg b.w./d)	C° NO_3 = 20 mg/L	C° NO_3 = 50 mg/L	C° NO_3 = 60 mg/L	C° NO_3 = 70 mg/L
Mean	1.5 (11%)	2 (34%)	2.15 (38%)	2.3 (42%)
95th percentile	3	3.7	3.9	4.1
97.5th percentile	3.3	4.1	4.3	4.6
Children 3 to 14 years old				
Daily nitrite intake (mg/kg b.w./d)	C° NO_3 = 20 mg/L	C° NO_3 = 50 mg/L	C° NO_3 = 60 mg/L	C° NO_3 = 70 mg/L
Mean	2 (10%)	2.8 (34%)	2.95 (39%)	3.1 (42%)
95th percentile	4.3	5.5	5.8	6.1
97.5th percentile	4.9	6.1	6.5	6.9

Once the nitrate concentration in drinking water approaches 20 mg/L, intake from water makes up approximately 10% of the intake of the French population. If the concentration in water is 50 mg/L (quality limit), the proportion from water would then represent 34% of nitrate exposure.

[8] These infants and children were selected using the TNS-SOFRES quota method designed to ensure that the cohort study is, theoretically, representative of the French population.

[9] Note that if the JECFA toxicological value of 3.7 mg/kg b.w./d is adopted, daily nitrate intake exceeds the ADI in part of the population. This toxicological reference value and the studies on which it is based are described in paragraph 6.1.1

4.2 Nitrites

No data concerning nitrite concentrations in air were listed. This exposure pathway is considered to be negligible.

4.2.1 Exposure to nitrites from foodstuffs

Mean nitrite intake per person is:

- according to WHO, between 1,2 and 3 mg/d (duplicate meals) (WHO, 2007),
- according to JECFA, between 0.21 and 0.63 mg/d (JECFA, 2002),
- according to the US National Research Council, between 0.3 and 2.6 mg/d (NAS, 1981 in WHO, 2007).

Nitrites have different origins: they are naturally present in foodstuffs or may be introduced as food additives in the form of sodium or potassium nitrite[10].

4.2.1.1 Intake excluding additives

Mean nitrite intake from the diet (excluding additives) is estimated to be 1.7 mg/d (0.028 mg/kg b.w./d) for an individual weighing 60 kg. This estimate is based on mean contamination data provided by MAFF (Ministry of Agriculture, Fisheries and Food - UK) during the years 1997, 1998 and 2001 and from European consumption data. The main contributors are cereals (35%) and water (20%), assuming 2 litres of water containing a concentration of 0.3 mg/L to be ingested daily.
EFSA has identified the small contribution of nitrite exposure via vegetables in view of exposure due to biotransformation of nitrates into nitrites (EFSA, 2008).

4.2.1.2 Estimated intake from food additives

A study conducted by Afssa in 2005 combined consumption data provided by the Observatory of food consumptions (OCA) and industrial food recipe and consumption data obtained by the DGCCRF from manufacturers.
This study did not take into account nitrites naturally present in foodstuffs. This more recent information supports the results obtained from the older study (Hoellinger *et al.*, 1999).
An estimate of actual food additive intake was calculated from individual consumption data from the INCA survey. This was estimated using a calculation hypothesis which took into account the mean value in mg/kg estimated on the basis of data provided by DGCCRF as the concentration, including zero values identified in the recipes for the selected products. This scenario assumes that consumers randomly eat foodstuffs which may or may not contain additives. According to this hypothesis the mean nitrite intake from food additives is 0.034 mg/kg b.w./d for adults over 15 years old and 0.058 mg/kg b.w./d for children between 3 and 14 years old.

4.2.1.3 Total nitrite intake

Different European studies have sought to estimate total nitrite intake from the diet:

- ***A total diet study (TDS) was conducted by the United Kingdom*** in 1997. Its results are shown in table 4.2.1.3-1:

[10] EFSA produced an opinion on 26 November 2003 relative to the effects of nitrates and nitrites on the microbiological safety of meat products (Opinion of the Scientific Panel on Biological Hazards at the request of the Commission related to the effects of Nitrites/Nitrates on the Microbiological Safety of Meat Products, *The EFSA Journal* (2003) 14, 1-34)

Table 4.2.1.3-1: Estimate of nitrite intake in the United Kingdom (1997)

	Adults Food excluding water	Adults Food and water	General population Low estimate
Mean mg/kg b.w./d	0.022	0.023	0.012 lower limit
97.5th percentile mg/kg b.w./d	0.037	0.038	0.021 upper limit

- In the **Netherlands, a duplicate meal study** was conducted in 1994. This study provided an estimate of nitrite intake in water and foodstuffs for 123 adults between 18 and 74 years old. The data were collected over a year in order to take into account seasonal variations. The median intake was less than 0.003 mg/kg b.w./d with a maximum of 0.23 mg/kg b.w./d.

- **Estimate of nitrite exposure– Afssa, 2007**
  The study conducted by Afssa (Afssa – Paser, 2007a) combined the individual foodstuff consumption data from the INCA1 survey with concentration data from the DGCCRF control and surveillance programmes in the years 2000 to 2006, i.e. 13657 data on 109 foodstuffs.
  In order to take into account data below the limit of detection or limit of quantification, upper and lower estimates of daily intake were made. The calculation method for these intake estimates is shown in annex 1 and the results are presented in table 4.2.1.3-2.

Table 4.2.1.3-2: Estimated dietary nitrite intake in France (excluding water)
source: Afssa, 2007

Daily nitrite intake (mg/kg b.w./d)	Adults > 15 years old		Children between 3 and 14 years old	
	Lower estimate	Upper estimate	Lower estimate	Upper estimate
Mean	0.020	0.044	0.035	0.080
95th percentile	0.038	0.076	0.071	0.145

Limitations of the nitrite exposure estimate: the data from the DGCCRF surveillance programmes only relate to foodstuffs known to contribute to dietary intake. Difficulties were also encountered in identifying some of the foodstuffs analysed and a very variable number of analyses were performed per foodstuff. Uncertainties about exposure from “other cured or tinned products” are still difficult to assess and should be explained because of a lack of detail about names and lack of individual consumption data for these processed foodstuffs.

The major foodstuffs which contribute to population exposure are the "other cured or tinned foodstuffs” (34% of the upper intake estimate) and cooked pork meats (11% of intake). The contribution from vegetables and potatoes is up to 24% of the upper intake estimate.

4.2.1.4 Summary: Exposure to nitrites from foodstuffs

Table 4.2.1.4 summarises the studies available used to assess dietary nitrite intake.

Table 4.2.1.4: Summary of nitrite exposure from foodstuffs

Country	Date	Mean intake mg/ kg b.w./d	Intake for high consumers mg/ kg b.w./d	Comments
		0.02 to 0.05		WHO, 1985
		< 0.001 to 0.145		JECFA, 1995
		0.005 to 0.043		NAS 1991
Europe		0.0035 to 0.0063	0.07	Total intake – TDS or duplicated meals
UK	1997	0.012	0.021	General population
		0.022	0.037	Adults excluding drinking water
Europe	1997/1998/2001	0.028		Intake from food excluding additives UK contamination data
France	2007	0.020 to 0.043 (adults) 0.035 to 0.078 (children)	0.038 to 0.075 (adults) 0.071 to 0.143 (children) (95th percentile)	Foodstuffs (intake excluding drinking water) Consumption x contamination
Europe	2008	0.003 to 0.013		Intake from vegetables only EFSA, 2008

4.2.2 Exposure to nitrites from water: summary of data from the SISE-Eaux database

The health control regulatory programme defined in the order of 11 January 2007 stipulates that analyses be performed on this parameter at the draw point (from once every 5 years to 12 times per year) and after treatment (from 1 to more than 144 times per year).
The study of data available from the SISE-EAUX database (Ministry of Health– SISE-Eaux) for the period between January 2003 and December 2006 shows that:

- analyses[11] are available for more than 99.9% of the water supply units (UDI) (i.e. 26840 UDI),
- nitrites can be detected in the water from some UDI. In the absence of chlorine, nitrites are detected intermittently in localised sites. Chlorine concentrations in the network make the presence of nitrites in water extremely rare.
- at least one non-compliant result was found in 0.4% of the UDI serving a maximum of 548 000 people,
- The 50th percentile of the results of 112 non-compliant analyses was 0.62 mg/L (the 95th percentile was 1.85 mg/L).

4.2.3 Contribution of exposure sources to total dietary intake

The study conducted by Afssa (Afssa – Paser, 2007a) provides estimates of cumulative nitrite intake using different water exposure scenarios. Only the upper estimates (cf. 4.1.1, presentation of the Afssa study 2007) are shown in table 4.2.3.

Table 4.2.3: Upper estimates of nitrite intake for different scenarios of water intake. The contribution provided by water is shown in brackets. Source: Afssa – Paser, 2007a

Adults > 15 years old			
Daily nitrite intake (mg/kg b.w./d)	C° NO_2 = 0 mg/L	C° NO_2 = 0.5 mg/L	C° NO_2 = 1.68 mg/L
Mean	0.043	0.050 (14%)	0.066 (35%)
95th percentile	0.075	0.081	0.106
97.5th percentile	0.083	0.090	0.116
Children 3 to 14 years old			
Daily nitrite intake (mg/kg b.w./d)	C° NO_2	C° NO_2 = 0.5 mg/L	C° NO_2= 1.68 mg/L
Mean	0.078	0.088 (10%)	0.110 (29%)
95th percentile	0.143	0.158	0.201
97.5th percentile	0.169	0.183	0.223

[11] Analyses performed on samples taken either during production or from the mains supply.

When the nitrite concentration in water is 0.5 mg/L (quality limit), the contribution from water represents 10 to 14% of nitrite exposure.

5 – Health effects

5.1 – Metabolism of nitrates and nitrites[12]

Nitrates and nitrites are rapidly absorbed after oral ingestion (in less than one hour) in the proximal small intestine (Walker, 1996). Unlike rodents, no nitrate absorption can be detected in the stomach in humans. Nitrate and nitrite bioavailability is close to 100%. Nitrates diffuse widely throughout the entire extracellular compartment (Jungerstern L. *et al.*, 1996). Plasma nitrate concentrations are generally between 30-60 µmol/L and may rise to 200 µmol/L in response to increased intake (Bednar and Kies 1994). The metabolism depends on the dose of nitrates ingested (Cohen and Myant, 1959; Spiegelhalder *et al.*, 1976; Fritsch *et al.*, 1985).

An important and specific feature of nitrate metabolism is the existence of an entero-salivary cycle which is specific to nitrates (Muramatsu *et al.*, 1979). Twenty-five per cent of ingested nitrates are secreted in saliva (Spiegelhalder *et al.*, 1976; Tannenbaum *et al.*, 1978). Five per cent of ingested nitrates are biotransformed from salivary nitrates into nitrites by nitrate-reductases from the buccal microflora (Walker, 1996, Pannala *et al.*, 2003). Oral reduction of nitrates into nitrites is believed to be the most important source of nitrites in humans (70-80% of nitrite exposure). Nitrates can also be reduced into nitrites by enteric bacteria and by certain cells (erythrocytes) that have a nitrate-reductase activity. Nitrites can be metabolised into nitric oxide (NO), which is very unstable (half life < 1 sec.) and which may itself react with thiol and amine groups, forming nitrosothiols (RSNOs), as summarised in Rassaf's review in 2004. Nitrites resulting from the reduction of ingested nitrates are very rapidly absorbed and oxidised into nitrates in the blood by an oxidation reaction coupled with oxyhaemoglobin, resulting in the formation of methaemoglobin. Endogenously formed plasma nitrates and nitrites arise from the oxidation of L-Arginine by NO synthetase [NOS].

Endogenous nitrate synthesis due to the degradation of NO produced by endothelial cells also exists and is estimated to represent approximately 1 mg/kg b.w./day under normal physiological conditions. This is therefore equivalent to mean daily dietary nitrate intake. Endogenous amine and amide nitrosation reactions are difficult to quantify and it is not possible, therefore, to differentiate between the dietary contribution and endogenous metabolism. In conclusion, endogenous nitrate metabolism is an important contributor to the production of nitrates by the body.

Nitrates and nitrites are excreted mostly in the urine, representing 65% of ingested nitrates (Pannala AS *et al.*, 2003, Green *et al.*, 1981 Wagner *et al.*, 1983). Marked tubular re-absorption occurs (Suto *et al,* 1995). Peak urinary excretion occurs after 5 hours and is complete after 18 hours, mostly in the form of nitrate (Bartholomew and Hill, 1984). Less than 2% of nitrates are recovered in faeces. A small amount is excreted in sweat [40 µmol/L of nitrates and 3 µmol/L of nitrites] (Weller *et al.*, 1996). Nitrates pass into breast milk through a passive diffusion mechanism without accumulation (Green *et al.*, 1981).

The major stages of nitrate, nitrite and nitric oxide metabolism are summarised in the diagram (Fig 1. nitrate metabolism, annex 2), alongside the exogenous intake compared to endogenous synthesis, tissue retention and excretion pathways. Systemic circulation is represented by the circle entitled plasma. An endothelial cell-erythrocyte relationship for biotransformation into nitrates is shown. There are uncertainties about this metabolism in humans concerning the mechanism of active transport. Some are beginning to be better defined for human cells (the mechanisms are better known for plants), in the kidneys, salivary glands and erythrocytes.

5.2 – Methaemoglobinaemia

Methaemoglobin is a form of haemoglobin in which haem iron is oxidised in the ferric form and therefore unsuitable for oxygen transport. Beyond a critical amount of methaemoglobin in blood (generally 15% of total haemoglobin), cyanosis onset occurs.

[12] This mechanism is not seen in rodents.

There are various causes of methaemoglobinaemia: methaemoglobin reductase enzyme deficiencies, genetic haemoglobin abnormalities that render haemoglobin more sensitive to oxidation, and exposure to oxidising medicinal products or oxidising chemical compounds, such as nitrites.

Children under 6 months old and particularly those under 3 months old are more susceptible to methaemoglobinaemia as they do not have the key enzyme NADH-cytochrome b5 reductase, which coverts methaemoglobin into haemoglobin. Levels of this enzyme approach those in adults once the child reaches the age of around 6 months old.

Although it has been suggested that persistent high foetal haemoglobin plays a part in increased susceptibility to methaemoglobinaemia, this form of haemoglobin has the same redox potential and the same auto-oxidation yield as haemoglobin A. It therefore does not contribute to increased vulnerability in children (Lukens, 1987).

The original publication by Hunter Comly in 1945 in JAMA raised the problem of the relationship between methaemoglobinaemia and water contamination by nitrates. According to Comly, nitrates in water are converted into nitrites in the gastrointestinal tract: as many children are not sensitive to the action of nitrates in water, Comly suggested that transformation of nitrates into nitrites only occurred in the event of bacterial infection at the upper gastrointestinal tract prior to nitrate ingestion. The nitrites formed could react with haemoglobin to form methaemoglobin, which in sufficient amounts, would result in the characteristic cyanotic appearance. This hypothesis was strengthened by other studies revealing associations between methaemoglobinaemia and high nitrate levels in well water.

Subsequently, a study conducted by the American Public Health Association on 278 cases of methaemoglobinaemia, including 39 deaths, showed that the incidence of methaemoglobinaemia increased with increasing nitrate levels. Five cases were exposed to water nitrate concentrations of between 48 and 88 mg/L, 36 to between 92 and 220 mg/L and 173 to more than 220 mg/L (Walton G., 1951).

As no cases of methaemoglobinaemia were seen at nitrate concentrations below 10 ppm (expressed as N), i.e. approximately 45 mg/L of nitrates (expressed as NO_3^-)[13], WHO used this value to establish a maximum exposure of 45 mg/L in drinking water.

Conversion of nitrates into nitrites occurs mostly through buccal bacteria and the gastrointestinal tract. The risk of methaemoglobinaemia therefore appears to depend not only on nitrates but also on the number and type of bacteria in the buccal flora. It is estimated that 5% of nitrates can be converted into nitrites in adults.

Our understanding of the effects has changed over the last twenty years; in particular diarrhoea and gastrointestinal disorders have been found to be associated with methaemoglobinaemia in the absence of high levels of nitrates in drinking water or in the diet (Gebara and Goetting, 1994).

As diarrhoea is a predominant symptom in most cases of methaemoglobinaemia associated with drinking water, diarrhoea, infections or inflammation of the gastrointestinal tract would appear to be the major factors involved in the methaemoglobinaemia.

In addition, gastrointestinal problems such as vomiting and diarrhoea are usually not seen in drug-induced, metabolic or genetic methaemoglobinaemias, so inflammation of the gastrointestinal mucosa could explain the methaemoglobin formation. Nitric oxide is produced by tissues in response to infection or inflammation.

An increase in the expression of inducible nitric oxide synthetase mRNA (iNOS mRNA) has been found in young children suffering from inflammatory bowel diseases. The same has been seen in bacterial infections of the colonic epithelial cells (Gupta *et al.*, 1998, Levine *et al.*, 1998).

The nitrite ion is a metabolic product of nitric oxide and increased iNOS expression is associated with increased plasma nitrate and nitrite concentrations. The methaemoglobin reduction system may be unable to cope in young children (note that methaemoglobinaemia is a well documented adverse effect of therapeutic nitric oxide use in respiratory distress syndromes and pulmonary arterial hypertension in the neonate).

This mechanism has also been documented in children suffering from methaemoglobinaemia not exposed to nitrates, who excrete more than 10 times more nitrates than would be predicted from their intake. As nitrites are metabolised into nitrates before being excreted, this increased excretion reflects endogenous nitrite production (Hegesh and Siloah, 1982).

[13] Nitrate and nitrite concentrations in water are often expressed in the literature as “nitrate-nitrogen” and “nitrite-nitrogen”. The equivalents are: 1 mg/L (expressed in N) = 4.43 mg/L (expressed in NO_3), and 1 mg/L (expressed in N) = 3.29 mg/L (expressed in NO_2).

For the same methaemoglobin level, hospital stays are also far longer when they occur in association with gastroenteritis or dehydration rather than exposure to an oxidising medicinal product (Avner, 1990 in Avery 1999).

Epidemiological data suggest that methaemoglobinaemia may be due to infection. In a nested case control study in a cohort of 71 children fed from well water in Romania, Zeman et al. (2002) showed that diarrhoeal diseases played a significant role in methaemoglobinaemia. Several cases were documented in children with urinary tract infections, bacterial enteritis or septicaemia (Jolly et al., 1995).

An Israeli study of 45 cases over 12 years showed seasonal variation, with a peak in January and the summer months, which the authors considered were contemporaneous with viral and bacterial gastroenteritis (Hanukoglu and Danon, 1996).

This finding should be interpreted in parallel with the fall in the incidence of methaemoglobinaemia in the United States despite the fact that at least 40,000 children under 6 months old are exposed to a drinking water content exceeding the regulatory limit (Knobeloch, 2000 in Fewtrell, 2004). It has been suggested that this results in a lesser incidence of infantile diarrhoea although there are no precise statistics available on this subject. It is not formally possible to exclude under-declaration of cases of methaemoglobinaemia.

However, although childhood methaemoglobinaemia is mostly caused by endogenous over-production of nitric oxide, how can the initial observations linking increased incidence of the disease to nitrate contamination be interpreted?

One initial explanation from the above data is that these higher levels of nitrates indicate microbiological contamination. Water intake in the survey was mostly from rural wells. An alternative explanation is more convincing: situations of gastrointestinal tract infection or inflammation predispose to conversion of haemoglobin into methaemoglobin from nitrites produced by the metabolism of the nitric oxide produced.

Under these conditions, exogenous nitrates exacerbate nitrite formation by inhibiting the conversion of nitrites, into ammonia for example. The enzyme nitrite reductase is inhibited by high nitrate concentrations (Roediger and Radcliffe, 1988).

These findings are consistent with Comly's initial hypothesis according to which drinking water nitrates do not represent the main cause of the phenomenon. This mechanism explains the broad variations in methaemoglobinaemia susceptibility seen in children and it is likely that exposure of children to high doses of nitrates plays a role in the severity of the methaemoglobinaemia rather than in its genesis.

Nevertheless, it should be noted that as early as 1951, Walton found that nitrate levels were only known in 214 of 278 cases of childhood methaemoglobinaemia described in the American Public Health Association study. Similarly, some review articles (Fan *et al*. 1987 and Fan and Steinberg, 1996), note that nitrate concentrations are not always reported in cases of methaemoglobinaemia.

Afssa consulted the CAP-TV (French Association of Anti-Poison Centres) in order to list cases of methaemoglobinaemia occurring in France and recorded in the toxicology monitoring and anti-poison centre information system and to identify possible cases associated with nitrate exposure. Its conclusions showed that during the period from July 1999 to January 2008, exposure conditions for the cases were considered under a "nitrate" hypothesis, not related to exposure to phytopharmaceutical products (fertilisers etc.) in only two of the 357 dossiers found and no hypothesis of water contamination was described.

5.3 –Reproductive disorders, developmental abnormalities and other effects

Sodium or potassium nitrate has no teratogenic effect in rats, mice, rabbits or hamsters. Reproduction studies in rats and mice exposed to nitrates and nitrites do not reveal any change in fertility, birth weight, litter size or sex ratio. Only nitrite exposure (sodium nitrite) has been associated with growth retardation and abnormal haemoglobin status in young puppies, without maternal toxicity (Roth *et al.*, 1987). An NOEL of 500 ppm has been calculated for sodium nitrate. Assuming a nitrate to nitrite conversion rate of 10% and consumption of 2 litres per day, a value of 45 mg/L has a large safety coefficient. Behavioural development and learning procedure abnormalities were reported in the offspring of female rats exposed during their gestation to potassium nitrate by Markel *et al.* (1989). These data were used by the NAS to calculate a nitrate effect dose of 317 mg/d in adults based on the observation of neurobehavioural abnormalities at 7.5 mg/kg b.w./d.

Based on a review of work conducted since 1980, Fan and Steinberg (1996) concluded that toxic effects after nitrate exposure only developed at concentrations 100 to 1000 times higher than the quality limit values in water for nitrates and nitrites respectively. The National Toxicology Program (NTP, 1990) reached a similar conclusion after an experimental study in Swiss CD1 mice: sodium nitrite in water had no toxic effect on reproduction up to a dose of 425 mg/kg b.w./d.

Human data are fragmented. Fan *et al.* (1987) found reproductive abnormalities in humans for high doses of nitrites (45 ppm). Epidemiological studies have not to date established any direct relationship between spontaneous miscarriage, teratogenic effects and/or post-natal developmental effects and nitrate concentrations in drinking water. The conclusion by Manassaram *et al.* (2006) recommended further studies.

Overall, these studies do not provide convincing significant information to establish a relationship between nitrate exposure and reproductive disorders. The study by Bove (1992) in New Jersey more specifically examined the relationship between reproductive abnormalities and nitrate concentrations in drinking water from 75 towns and reached the following conclusions: "*the positive association found in this study does not provide sufficient evidence to support the hypothesis that nitrates cause reproductive disorders at the levels usually found in public drinking water supply systems...".* This reflects the general opinion from the studies.

5.4 – Mutagenicity, genotoxicity and carcinogenicity

5.4.1 Mutagenicity and genotoxicity of nitrate and nitrite ions

The nitrate ion is not mutagenic to bacteria or mammal cells *in vitro*. Chromosomal aberrations have been seen in rats after nitrate ingestion although these could have arisen from the endogenous formation of N-nitroso compounds (Speijers, 1989).

Whilst nitrates have not been proven to be mutagenic, the products of nitrosation reactions are, however, mutagenic (Cf. 5.4.2). Sodium nitrite produces cytogenetic lesions *in vivo* in rats, mice and rabbits and *in vitro* in BSC-1 and HeLa cells (Lucas et al, 1987). The results of the *in vivo* studies have been disputed, however (Speijers, 1989; WHO, 2005).

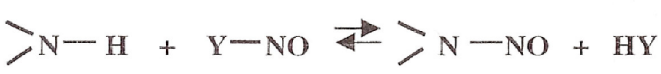
5.4.2 N-nitroso compounds (NOC)

The question of whether nitrate exposure is a risk factor for human cancer is far from being resolved. This has recently been the subject of two symposia which are summarised and discussed in two publications (Ward *et al.*, 2005; Van Grinsven *et al.*, 2006).

NOC are amongst the most potent carcinogens known and low doses of NOC are sufficient to cause tumours in animals (Lijinsky, 1987, 1990, 1992)

Formation of N-nitroso compounds

Reduction of nitrate to nitrite or NO produces nitrosating agents through a reaction with nitrogen-containing substrates, which can result in the production of carcinogenic NOC (Challis and Challis, 1982; Pignatelli *et al.*, 1985; Williams, 1988). The various nitrosating species (NO^+, N_2O_3, N_2O_4, NOX) can be responsible for nitrosation under various conditions and in various media (Pignatelli, 1994; Bartsch *et al.*, 1988) following the general equation:



The main nitrosating agents are listed in table 5.4.2 together with the conditions under which they are formed in aqueous solution or gas or liquid phase.

Table 5.4.2: Major nitrosating groups formed from nitrite and nitrogen oxides

Nitrosating species	Reaction equilibrium	Formation conditions
N_2O_3 $(O_2N\text{-}NO)$	$2HNO_2 \rightleftharpoons N_2O_3 + H_2O$ $NO+NO_2 \rightleftharpoons N_2O_3$	Aqueous media/moderate acidity Gas phase, aqueous medium, neutral/basic, lipid phase
NO^+ $(H_2O)NO^+$ $XNO(X=Cl^-, Br^-, I^-, SCN^-)$	$HNO_2 + H^+ NO^+ \rightleftharpoons NO^+ + H_2O$ $HNO_2 + H^+ \rightleftharpoons (H_2O)NO^+$ $H^+ + HNO_2 + X^- \rightleftharpoons XNO + H_2O$	Aqueous medium, strong acidity Aqueous medium, moderate acidity, presence of nucleophile X
N_2O_4 O_2 NO-NO	$2NO_2 \rightleftharpoons N_2O_4$ $2NO+O_2 \rightleftharpoons N_2O_4$	Gas phase, aqueous medium, neutral/ basic, lipid phase

From Pignatelll, 1994; Bartsch et al. 1988

The NO_2^- ion and nitrous acid never react directly with the amine substrate and acidic conditions (pH < 5) are required to generate the nitrosating YNO entity: only the non-protonated form of the amine, which is in equilibrium with its conjugated acid, reacts with YNO. The speed of nitrosamine formation therefore varies depending on the concentration of the amine but, above all, of the nitrite. It also depends on the pH and basicity of the amine. Less basic amines are more rapidly nitrosated and non-protonated forms of amines with very different basicity have similar reactivities to N_2O_3. The reaction kinetics can be altered by the presence of Y^- anions liable to act as catalysts.

Apart from secondary amines and amino acids, many other substrates can produce NOC, including amides and related compounds (ureas, carbamates, guanidines), primary and tertiary amines, quaternary ammonium salts, amine oxides, peptides and proteins.

Weakly basic amine compounds, such as some aromatic amines, amides, ureas, carbamates and guanidines, are not sufficiently reactive to react directly with N_2O_3 although at pH < 2, nitrosation occurs following an alternative pathway involving the neutral amine species and the hydrated or non-hydrated nitrosonium ion (H_2NO^+; NO^+). The reaction, which is slow at pH > 3, becomes faster with increasing acidity. From pH 3 to pH 1 it increases in speed by a factor of approximately 10 for each one unit fall in pH. No optimum pH is seen. The reactivity of nitrosatable substances depends very greatly on their structures.

Nitrogen oxides are formed by chemical or microbiological reduction of nitrites and nitrates. N_2O_3 and N_2O_4 are direct nitrosating agents, NO_2 only in the form of the dimer and NO only develops nitrosating activity after oxidation or in the presence of certain catalysts. NO_2 leads to the formation of NOC by reacting with amine compounds whereas N_2O_4 produces a mixture of NOC and nitrated compounds. These reactions take place in gas phase, in lipid media and in neutral or alkaline aqueous solutions. NOC formation from nitrogen oxides follows the general mechanism and is generally faster and more complete than with aqueous nitrous acid solutions. The speed of the reaction depends mostly on the absence of acidity of the medium, which is associated with a higher concentration of free amine.

Some Y^- anions catalyse nitrosamine formation in aqueous solutions between pH 2 and 5. Of the different anionic catalysts studied, thiocyanate (SCN^-) is the most effective. The sequence shown is in decreasing order of their promoting activity:

$$SCN^- >>> I^- >> Br^- >> Cl^- > Cl0_4^- := H_2PO_4^- := RCO_2^-$$

The SCN^- thiocyanate and I^- ion are important as both are present *in vivo*, the former being present in particularly large amounts in the saliva of smokers.

Compounds that rapidly trap nitrosating agents effectively inhibit N-nitrosation by converting the nitrosating agents into inactive substances. These inhibitors reduce HNO_2 to N_2, N_2O or NO. The latter of these only acts as a nitrosating species in the presence of catalysts such as oxygen. Because of competition between the nitrosatable substrate and inhibitors or nitrosating agents, the level of inhibition depends on the overall and relative concentrations of the nitrosating agents, the inhibitor and the nitrogen-containing substrate and on the relative speeds of the reaction between the nitrosating agent, inhibitor and the nitrogen-containing substrate.

A very large variety of substances, including vitamins C and E, polyphenolic compounds and natural mixtures containing them, can inhibit NOC formation although inhibition is never complete (Cf. Annex 3 – Compounds and mixtures which are natural inhibitors of N-nitrosation). In particular, fruit and vegetables are very rich in compounds that inhibit N-nitrosation.
Although volatile nitrosamines and nitrosamino acids have been studied in depth, non-volatile NOC of unknown structure make up most of the total NOC present in biological fluids, faeces and some foodstuffs (Pignatelli *et al.,* 1994; Massey et *al*., 1990, Rowlang et *al*., 1991; Jakszyn *et al*., 2006). The nature of NOC formed *in vivo* or preformed in the environment is still very poorly understood (Bartsch *et al*., 1988; Pignatelli *et al*., 1985).

Endogenous nitrosation

1) Nitrate reduction (Cf. 5.1 metabolism of nitrates and nitrites)

Nitrates are reduced by a broad range of microorganisms (Shapiro, 1991). Examination of nitrate absorption and metabolism shows that it may be a source of nitrite in different *in vivo* compartments. Buccal and gastrointestinal bacteria can generate NO from nitrates and nitrites (Sobko *et al*., 2005, 2006).

2) Endogenous nitrosation in humans

N-nitrosodimethylamine (NDMA) formation from precursors has been studied in ***in vitro*** models using incubation of precursors with saliva or gastric juice under simulated physiological conditions (Walker, 1991). A few studies have been performed on foodstuffs containing varying amounts of nitrosatable nitrogenous precursors to which artificial saliva or gastric juice has been added (Walters *et al*., 1979; Groenen *et al*., 1982; Sen *et al*., 1985). More recently, a dynamic gastrointestinal model simulating various physiological conditions in the gastric compartment has been used to study NDMA formation after ingesting dimethylamine or cod as a source of amines jointly with nitrite (Krul *et al*., 2004).

In vivo studies have also been conducted in which:

- Analysis of stomach contents after volunteers ate meals containing eggs, milk and tinned pork meat showed the presence of N-nitrosopiperidine and N-nitrosopyrrolidine (Walters et al., 1979).
- A method based on urinary nitrosoproline measurement (NPRO test) after ingestion of protein and nitrate has been developed (Ohshima and Bartsch, 1981). This has been extended to the measurement of urinary N-nitrosoamino acids. Several studies have demonstrated a direct relationship between nitrate ingestion and endogenous NOC formation. Drinking nitrates in water has been associated with an increased capacity for proline nitrosation (Mirvish *et al*., 1992; Moller *et al*., 1989; Vermeer *et al*., 1998). Measurement of urinary N-nitrosamino acid excretion has been used in clinical and epidemiological studies to examine diet, lifestyle, individual factors and the different pathological states which could influence endogenous nitrosation in humans (Mirvish, 1995; Moller *et al*., 1989; Rowland *et al*., 1991). Through this, endogenous NOC synthesis has been found to be greater in people in high risk regions for stomach, oesophageal, oral and bladder cancers. Individual exposure to endogenous NOC was extensively influenced by modulators present in the diet, such as vitamins C and E, phenolic compounds from plants (ferulic, caffeic and chlorogenic acids) and complex mixtures (fruit juices, coffee, tea, betel nut extract). Inflammation, bacterial infection or parasite infestation are associated with a large increase in endogenous NOC (Bartsch *et al*., 1988, 1989; Mirvish, 1995).
- Ingestion of nitrates equivalent to the acceptable daily intake established by JECFA in 2003 (3.7 mg/kg b.w.) led to an increase in NDMA excretion (mean concentration increased by 2.5 to 3 times) in urine when associated with consumption of fish rich in amine precursors (Vermeer *et al*., 1998; van Maanen *et al*., 1998).

In summary, it has been established from the numerous studies that:

a) nitrate exposure can be correlated with capacity for endogenous nitrosation;
b) endogenous nitrosation is modulated by inhibitors such as vitamins C and E, phenolic compounds and complex natural mixtures;
c) urinary nitrosoamino acid concentrations are higher in some populations with a high cancer incidence (gastric cancer in Japan (Bartsch *et al*., 1989), Poland (Zatonski *et al*., 1989),

Columbia (Stillwell *et al.*, 1991) and Costa Rica (Sierra *et al.*, 1993); oesophageal cancer in Northern China (Bartsch *et al.*, 1989; Lu *et al.*, 1986); nasopharyngeal cancer in Southern China (Zeng *et al.*, 1993)).

Although these results do not represent proof of the involvement of endogenous nitrosation in human cancers, they do indicate a possible role for NOC formed *in vivo* in the aetiology of some human cancers. Demonstration of the effectiveness of some vitamins in inhibiting nitrosation provides a plausible interpretation of the results of the epidemiological studies which show fruit and vegetables (source of vitamins and polyphenols) to have protective effects against carcinogenesis, particularly gastric (Bartsch *et al.*, 1988).

Recent studies have demonstrated that the cardia and oesophageal-gastric junction are particularly exposed to nitrosating stress (Moriya *et al.*, 2002; Suzuki *et al.*, 2003 and 2005; Mc Coll *et al.*, 2005 and 2006; Ijima and Shimosagawa, 2006). Marked acidity in healthy volunteers beyond the buffering effect of foodstuffs lasts for several hours in the cardia. In people with gastric reflux, saliva comes into contact with gastric juice acidity in the lower oesophagus. pH is a known nitrosation factor and the incidence of adenocarcinoma at these two anatomical sites has been increasing noticeably for several years (Blot *et al.*, 1991; Okabayashi *et al.*, 2000).

3) Quantification of endogenous nitrosation

Whilst consensus agreement exists about the endogenous formation of NOC, its precise quantification and contribution to total human NOC exposure are still the subject of debate (Gangolli *et al.*, 1994). Mathematical models (Licht and Deen, 1988) and some *in vivo* studies using NPRO have suggested that endogenous exposure may be lower than exogenous exposure (Bartsch and Spiegelhalder, 1996). However, other authors disagree and consider that the contribution of endogenous NOC synthesis may be between 45 and 75% (Chilvers *et al.*, 1984; Moller *et al.*, 1989; Tricker, 1997; Krul *et al.*, 2004). These authors studied the formation of carcinogenic NOC, whereas NPRO is not carcinogenic (Ohshima and Bartsch, 1981; Shapiro *et al.*, 1991): questions have been raised about the use of NPRO to extrapolate the results obtained to the quantification of endogenous synthesis of carcinogenic NOC (Krul *et al.*, 2004). The importance of endogenous NOC production has been supported by the particularly high level of NOC, in the region of 300 µg/day, in faeces, compared to the amount provided in the diet of approximately 13 µg/day (Bingham *et al.*, 1996). The predominance of endogenous compared to exogenous exposure to NOC was reported in a recent study (Jakszyn *et al.*, 2006) conducted by the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST).

The nature and source of nitrate exposure appears to be of major importance. Whereas vegetables also provide protective substances in addition to nitrates (nitrosation inhibitors, e.g. vitamin C, polyphenols), water does not contain these substances. If the nitrate concentration in drinking water exceeds the quality limit, water may contribute substantially to total nitrate intake (Chilvers *et al.*, 1984).

A rise in gastric pH may enable bacterial proliferation and subsequent formation of NO, nitrite and possibly NOC. The prevalence of even asymptomatic, raised gastric pH, (achlorhydria) increases with age. In addition, young children have lower gastric acidity (Speijers *et al.*, 1989). Nitrate exposure may carry greater risk both in these people and in patients who are treated to reduce their gastric acidity or consume nitrosatable medicinal products.

5.4.3 Epidemiological studies

There are almost no relevant epidemiological studies assessing the risk of cancer associated with nitrates in drinking water taking into account individual exposure data and information on nitrosation precursors, catalysts and inhibitors. No definitive conclusion can therefore be drawn.

Most of these studies are ecological ones designed to test a possible correlation between mortality rates or the incidence of certain cancers and nitrate concentrations in drinking water in a town or region of different countries (Annex 4, table and Cantor, 1997). The initial studies examined gastric cancer mortality, referring to nitrate values concomitantly with the period of death. Various results were obtained, some showing a positive association, others no association and one study showing an inverse association (Annex 4, table). In most of these studies, nitrate exposure in water was not assessed for the relevant period involved in induction of the cancer. Recent ecological studies in Slovakia, Spain and Hungary using historical measurements of nitrate concentrations with exposure

levels close to or above the maximum regulatory contamination limit have shown a positive correlation with gastric cancer incidence or mortality (Gulis *et al.*, 2002; Morales-Suarez-Varela *et al.*, 1995; Sandor *et al.*, 1998 and 2001). Two of these studies also involved other cancer sites. The incidences of non-Hodgkin's lymphoma and colorectal cancer were higher in Slovakia in people exposed to nitrate concentrations of between 20 and 50 mg/L in the public water supply (Gulis *et al.*, 2002). No association was found, however, for the incidence of bladder or kidney cancer. A positive correlation was found between nitrate concentrations in drinking water and deaths from prostate cancer in Spain, although no relationship was found with bowel or bladder cancer (Morales-Suarez-Varela *et al.*, 1995). An ecological study in the United Kingdom (Barret *et al.*, 1998) found an association between the risk of brain cancer and exposure to nitrates in drinking water at concentrations over 30 mg/L. An old Canadian study (Thouez *et al.*, 1981) had reported the same conclusion for exposure to nitrate concentrations above the regulatory limit.

Case control and cohort studies have previously examined the historical concentrations of nitrates in public drinking water supplies, usually ≤ 45 mg/L, and the risk of several cancers (Annex 4, table). Some included factors influencing nitrosation, such as vitamin C concentration. A cohort study conducted in the Netherlands (Van Loon *et al.*, 1998) did not find any association between the risk of gastric cancer and nitrate concentration in drinking water below the regulatory limit. A cohort study conducted in Germany (Volkmer *et al.*, 2005) reported an association between the incidence of bladder cancer and nitrate levels in water. In the same study no association was found for kidney or prostate cancer. A prospective cohort study in the United States on a female population showed increases of 2.8 and 1.8 respectively in the risk of bladder and ovarian cancer associated with long-term exposure to nitrate concentrations over 11 mg/L (Weyer *et al.*, 2001). An inverse association was found for uterine and rectal cancer in the same study and no significant association for non-Hodgkin's lymphoma, leukaemia, melanoma, bowel, pancreatic, renal or lung cancer. American case control studies on bladder (Ward *et al.*, 2003), brain (Ward *et al.*, 2004), pancreas (Coss *et al.*, 2004), and colorectal (De Roos *et al.*, 2003) cancer found no association between the risk of cancer and mean nitrate concentrations. These studies took into account some nitrosation inhibitors and precursors. The risk of bowel cancer was found to be significantly higher in sub-groups exposed long-term to water nitrate concentrations of over 22 mg/L and low vitamin C or high meat consumption, both of which predispose to nitrosation (De Roos *et al.*, 2003).

Two case control studies conducted by the same North American team on non-Hodgkin's lymphoma produced contradictory results. In the first, a significant positive association was found between mean nitrate concentration in drinking water and risk, which was increased by a factor of two when nitrate concentration was over 18 mg/L (Ward *et al.*, 1996). In the second study, no association was found for similar exposure levels (Ward *et al.*, 2006). A third American study did not show any association between the risk of non-Hodgkin's lymphoma and nitrate exposure in water up to a mean nitrate concentration of 10.6 mg/L (Freemann *et al.*, 2000). A case control study conducted in Italy found the incidence of non-Hodgkin's lymphoma to be associated with nitrate concentration in water but only in males (Cocco *et al.*, 2003).

Two case control studies conducted in the USA (Ward *et al.*, 2005) and in Germany (Steindorf *et al.*, 1994) did not find any association between long-term nitrate exposure in drinking water and brain cancer in adults. Incorporating vitamin C consumption into the American study did not alter the results. These two studies contradict the two ecological studies described above (Barret *et al.*, 1998; Thouez *et al.*, 1981). Specific NOC are seen in transplacental neurocarcinogenesis in animals and an American study concerning the risk of brain cancer in children according to nitrate exposure during pregnancy has been performed (Mueller *et al.*, 2001). Nitrate and nitrite concentrations in drinking water were measured, often several years after the pregnancy. Overall, these were not associated with risk although the children of women from one of the regions studied (West Washington state) who used well water as their drinking water source during their pregnancy were at increased risk of brain cancer.

The persistent discrepancies between these epidemiological studies are due, in particular, to difficulties measuring nitrate exposure. There are major uncertainties with respect to the exact assessment of individual and cumulative exposure. Population movements and the latent period between exposure and the diagnosis of the disease are often difficult factors to take sufficiently into account. The potential carcinogenic effects of nitrates in drinking water result from complex interactions between ingested nitrates and many factors involved in the endogenous formation of NOC: a) nitrate metabolism and in particular the presence, species and nitrate-reducing activity of the *in vivo* bacterial flora and catalytic potential of nitrosation at neutral pH; b) the type and concentrations of nitrosatable substances; c) the presence of catalysts or inhibitors; d) physiological states

predisposing to nitrosation (gastric achlorhydria, infection, inflammation) and other individual factors, such as the pH of various possible nitrosation sites, saliva production, age, ability to metabolise carcinogens and repair gene damage.

5.4.4 Classification of nitrates and nitrites by IARC

The International Cancer Research Centre reassessed the carcinogenicity of ingested nitrates and nitrites and has proposed a new classification for these substances (IARC, 2006):

- *"There is limited evidence in humans for the carcinogenicity of nitrite in food. Nitrite in food is associated with an increased incidence of stomach cancer.*
- *There is inadequate evidence in humans for the carcinogenicity of nitrate in food.*
- *There is inadequate evidence in humans for the carcinogenicity of nitrate in drinking water.*
- *There is sufficient evidence in experimental animals for the carcinogenicity of nitrite in combination with amines or amides.*
- *There is limited evidence in experimental animals for the carcinogenicity of nitrite per se.*
- *There is inadequate evidence in experimental animals for the carcinogenicity of nitrate.*

Ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A).
The underlying mechanism is endogenous nitrosation, which in the case of nitrate must be preceded by reduction to nitrite. Nitrate and nitrite are interconvertible in vivo. Nitrosating agents that arise from nitrite under acidic gastric conditions react readily with nitrosatable compounds, especially secondary amines and alkyl amides, to generate N-nitroso compounds. Many N-nitroso compounds are carcinogenic".

6 - Reference values

6.1 - Toxicological reference values (TRVs)

6.1.1 - Nitrates

6.1.1.1 History of JECFA scientific assessments

First JECFA assessment 1961 / 6th meeting
The first official risk assessment on ingestion of nitrates and nitrites was conducted by JECFA in 1961. This initial assessment was performed only on the use of nitrates and nitrites as food additives.
The NOAEL was derived from a long-term toxicity study in rats and a short-term toxicity study in dogs. The critical effect was delayed growth. On the basis of an NOAEL of 500 mg/kg b.w./d for nitrates and a safety factor of 100, JECFA proposed an ADI of 0-5 mg/kg b.w./d expressed as sodium nitrate. JECFA then stated that this assessment was not relevant to children under 6 months old.

Second and third JECFA assessments 1965 (8th Meeting) and 1974 (17th Meeting)
JECFA restated the ADI for nitrates

Fourth JECFA assessment 1994 / 44th meeting 1994
This assessment was conducted in light of new toxicological and epidemiological studies on both nitrates and nitrites.
For nitrates, JECFA performed an assessment taking into account the endogenous conversion of nitrates to nitrites. This stated as a result that the rat was not a suitable model to assess nitrate toxicity although, as there were inadequate data from other animal species, the results of rat experiments were still taken into account, applying the nitrate/nitrite conversion factor to these results.
Based on a long-term study in rats (Lehman, 1958), JECFA established an NOAEL of 370 mg/kg b.w./d and derived an ADI from this of 3.7 mg/kg b.w./d (expressed as nitrate).
JECFA also calculated a transposed NOAEL (NOAELt) from the NOAEL for nitrites (6.7 mg/kg b.w./d) using a nitrate to nitrite conversion factor of between 5 and 20%. Based on this, the NOAELt was between 40 and 160 mg/kg b.w./d and the ADI derived from this was 0-3.2 mg/kg b.w./d. JECFA considered that these two values (NOAEL and NOAELt) were of the same order and adopted the ADI of 3.7 mg/kg b.w./d.
The nitrate ion is not genotoxic or carcinogenic except at very high doses of nitrates and precursors of N-nitroso compounds.
Because of the possible conversion of nitrates into nitrites and the specific susceptibility of infants under three months old, JECFA stated that this ADI did not apply to this sensitive population.

Fifth JECFA assessment 2003 / 59th meeting
JECFA emphasised that nitrates should be assessed based on their *in vivo* conversion to nitrites but stated that new data on nitrites did not cast doubt on the nitrate assessment. JECFA therefore reaffirmed the ADI set previously.

Table 6.1.1.1: Change in TRV proposed by JECFA for nitrate ingestion

Date	Critical effect	NOAEL mg/kg b.w./d	SF	ADI mg/kg b.w./d
1961	Gain in body weight (long- term study, rats and short-term study, dogs)	500 (expressed as $NaNO_3$)	100	0-5 (expressed as $NaNO_3$)
1994	(rats, long-term study)	370 (expressed as NO_3)	100	0-3.7 (expressed as NO_3)
	Transposition of the nitrite NOEL	160 (expressed as NO_3)	50	0-3.2 (expressed as NO_3)
2003	(rats, long-term study)	370 (expressed as NO_3)	100	0-3.7 (expressed as NO_3)

6.1.1.2 History of the European scientific assessments

European Commission Scientific Committee on Food

This committee concluded that long-term animal studies did not show that nitrates or nitrites are directly carcinogenic and that there were no quantitative data on endogenous formation of carcinogenic N-nitroso compounds after exposure to realistic levels of nitrates and N-nitrosatable precursors. In addition the committee stated that the epidemiological studies available did not enable a conclusion to be reached as to the carcinogenicity of nitrates in humans. The committee therefore confirmed the ADI proposed in 1990 of 3.7 mg NO_3/kg b.w./d (SCF, 1995).
The SCF confirmed that this ADI applied to all sources of dietary exposure.
In addition, **the European Food Safety Authority (EFSA)** provided an opinion to the European Commission in April 2008 on the assessment of risks and benefits related to the presence of nitrates in vegetables on a European level. In order to establish the health risk, EFSA used the nitrate ADI proposed by JECFA in 2003.

6.1.1.3 TRV proposed by the US EPA

Based on the work by Bosch (1950) and Walton (1951) using the initial clinical effects of methaemoglobinaemia in children under 3 months old as the effect, an NOAEL of 1.6 mg/kg b.w./d (expressed as N) was set, i.e. 7 mg/kg b.w./d (expressed as NO_3), equivalent to a nitrate concentration of 44 mg/L and daily ingestion of 0.64 L of water, i.e. 0.16 L/kg b.w./d. This was also the reference dose without applying an additional uncertainty factor as the US EPA considered that this was the "critical" toxic effect in the most sensitive population (IRIS, 1991).
A LOAEL of 1.8-3.2 mg/kg b.w./d (expressed as N) was established. The safety margin between the NOAEL and the LOAEL is small.

6.1.1.4 Proposed TRV for nitrates

Table 6.1.1.4 summarises the TRV proposed by the different bodies.

Table 6.1.1.4: Summary of studies used to construct the TRV

Source	TRV	Value	Study	Population	Effect
EFSA (2008)	Ditto JECFA (2003)				
JECFA (2003)		3.7 mg/kg b.w./d	Lehman, 1958	rats	Inhibition of growth
SCF (1995)		3.7 mg/kg b.w./d	Maekawa, 1982	rats	Reduction in weight gain
IRIS EPA (1991)	RfD reference dose	7 mg/kg b.w./d	Bosch (1950) and Walton (1951)	Infants	Methaemoglobinaemia

Conclusion concerning the TRVs proposed by these bodies

The nitrate ADI proposed by JECFA and SCF was obtained on the basis of chronic toxicity studies in rats. WHO stated in 2003 that rat experiments should not be taken into account as this toxicological model is unsuitable for humans because of the absence of entero-salivary recirculation and therefore lesser reduction of nitrates into nitrites. However, this ADI was reaffirmed by JECFA in 2003. Although JECFA had stated in 1994 that the ADI should not be applied to infants, this restriction does not appear in its subsequent assessments. In order to determine the risk from ingestion of nitrates in vegetables, in 2008, EFSA adopted the nitrate ADI proposed by JECFA in 2003.
Afssa nevertheless considers that the data published on the pivot study by Lehman A.J. (1958) can be criticised methodologically when used to establish a toxicological reference value for nitrates.

6.1.2 - Nitrites

6.1.2.1 History of JECFA scientific assessments

First JECFA assessment 1961 / 6th meeting

The first official risk assessment on ingestion of nitrates and nitrites was conducted by JECFA in 1961. This initial assessment was performed only on the use of nitrates and nitrites as food additives.
The NOAEL was derived from a long-term toxicity study in rats. On the basis of an estimated NOAEL of less than 100 mg/kg b.w./d for nitrites, JECFA proposed an ADI of 0-0.4 mg/kg b.w./d expressed as sodium nitrite.

Second JECFA assessment 1965 / 8th meeting

This study reduced the nitrite ADI to 0-0.2 mg/kg b.w./d. JECFA then used an unusual safety factor of 500 as the critical effect was a marginal one and highlighted the possible endogenous formation of N-nitroso compounds from nitrites without being able to establish the NOAEL for these compounds.

Third JECFA assessment 1974 / 17th meeting

Based on a WHO report and an IARC report on N-nitroso compounds, JECFA concluded that there was insufficient evidence to revise the temporary status of the nitrite ADI.

Fourth JECFA assessment 1994 / 44th meeting 1994

This assessment was conducted in light of new toxicological and epidemiological studies on both nitrates and nitrites.
These new findings demonstrated that nitrites were genotoxic *in vitro* whether or not accompanied by precursors of N-nitroso compounds, although all but one of the *in vivo* test results were negative. The results of the carcinogenicity studies were also negative, except for the test performed using high concentrations of nitrates and NOC.
During this meeting, JECFA noted the formation of N-nitroso compounds from nitrites and precursors of N-nitroso compounds, when ingested in large amounts, although quantitative data concerning the formation of N-nitroso compounds are only available for those compounds which are not carcinogenic (N-nitrosoproline) and do not enable a quantitative assessment of the risks of the endogenous formation of N-nitroso compounds from ingesting nitrites *per se* to be performed.
JECFA also based its assessment on two toxicological studies:

- a 90-day study in rats and a critical effect related to hypertrophy of the adrenal zona glomerulosa, which allowed an NOAEL of 5.4 mg/kg b.w./d (expressed as N) to be established,
- a 2-year study in rats and a critical effect on the heart and lungs, enabling an NOAEL of 6.7 mg/kg b.w./d (expressed as N) to be established.

Applying a safety factor of 100, JECFA calculated an ADI of 0-0.06 mg/kg b.w./d (expressed as N).

Fifth JECFA assessment 2003 / 59th meeting

JECFA considered that hypertrophy of the adrenal zona glomerulosa was not a critical effect reflecting a direct toxic action, as a result of which the NOAEL of 5.4 mg/kg b.w./d was considered not to be pertinent. Other critical effects must be used for the assessment. The NOAEL of 6.7 mg/kg b.w./d, adopted previously and based on effects on the heart and lung was therefore used to derive an ADI of 0-0.07 mg/kg b.w./d. JECFA also concluded that it was necessary to estimate a reference dose for the acute effects of nitrites.

Table 6.1.2.1: Change in TRVs proposed by JECFA for nitrite ingestion

Date	Critical effect	NOAEL mg/kg b.w./d (expressed as NO_2)	SF	ADI mg/kg b.w./d
1961	Marginal reduction in body weight gain (rats, 2 years)	100	1000	0-0.4 (expressed as $NaNO_2$)
1965	Marginal reduction in body weight gain (rats, 2 years)	100	500	0-0.2 (expressed as NO_2)
1974	Marginal reduction in body weight gain (rats, 2 years)	100	500	0-0.2 (expressed as NO_2)
1994	Adrenal gland hypertrophy (rats, 90 d)	5.4	100	0-0.06 (expressed as NO_2)
2003	Effect on heart and lungs (rats, 2 years)	6.7	100	0-0.07 (expressed as NO_2)

6.1.2.2 History of European Scientific evaluations

European Commission Scientific Committee on Food

This committee concluded that long-term animal studies did not show that nitrates or nitrites are directly carcinogenic and that there was no quantified evidence of the endogenous formation of carcinogenic N-nitroso compounds after exposure to realistic levels of nitrates and N-nitrosatable precursors.

In 1995, the SCF examined the 2-year chronic study conducted in rats. An NOEL of 10 mg sodium nitrite/kg b.w. (equivalent to 6.7 mg NO_2^-/kg b.w.) was established based on histological changes in the lung and heart. In 1995, after examining the available toxicological data, the SCF concluded that the most sensitive toxicological effect was hypertrophy of the adrenal zona glomerulosa in the rat. The estimated NOAL was 10 mg of potassium nitrite / kg b.w. (equivalent to 5.4 mg NO_2^- / kg b.w.).

The SCF deemed that in view of these two target effects an ADI could be derived for nitrite ions *per se* from data on the potassium and sodium salts. Applying a safety factor of 100, the SCF established an ADI for nitrite ions of 0 – 0.06 mg/kg b.w./d.

The SCF confirmed that this ADI was applicable to all sources of dietary exposure.

EFSA adopted the ADI of 0.07 mg/kg b.w./d established by JECFA in 2003 for nitrites in its 2008 report.

6.1.2.3 Chronic TRVs proposed by the US EPA

Based on the work by Bosch (1950) and Walton (1951) using the initial clinical manifestations of methaemoglobinaemia in children under 3 months old as the effect, a NOAEL of 1.6 mg/kg b.w./d (expressed as N) was set, i.e. 7 mg/kg b.w./d (expressed as NO_3), equivalent to a nitrate concentration of 44 mg/L and daily ingestion of 0.64 L of water, i.e. 0.16 L/kg b.w./d. This was also the reference dose without applying an additional uncertainty factor as the US EPA considered that this was the “critical” toxic effect in the most sensitive population (IRIS, 1991).

A LOAEL of 1.8-3.2 mg/kg b.w./d (expressed as N) was established. The safety margin between the NOAEL and the LOAEL is small.

Assuming a nitrate to nitrite reduction factor of 10% in children due to the effect of gastrointestinal tract bacteria, the NOAEL for nitrites was found to be 3.3 mg/L and the Reference Dose 0.16 mg/kg b.w./d.

6.1.2.4 Chronic TRV proposed for nitrites

Table 6.1.2.4 summarises the Chronic TRV proposed by different bodies.

Table 6.1.2.4: Summary of studies used to establish the TRVs

Source	TRV	Value	Study	Population	Effect
EFSA (2008)	Ditto JECFA (2003)				
JECFA (2003)		0.07 mg/kg b.w./d	Shuval and Gruener, 1972	rats	Effects on heart and lung
SCF (1995)		0.06 mg/kg b.w./d	Till et al, 1988, 1990	rats	Hypertrophy of the adrenal zona glomerulosa
IRIS EPA (1991)	RfD reference dose	0.16 mg/kg b.w./d	Bosch (1950) and Walton (1951)	infants	Methaemoglobinaemia

6.1.3 N-nitroso compounds

Several bodies have proposed chronic TRVs for the N-nitroso compounds. Details of how the TRVs were established for NDMA are shown in annex 5. NDMA, which is representative of the volatile nitrosamines class, is the most widely studied.

The TRVs proposed for these N-nitroso compounds are not detailed here since, in view of the uncertainties concerning the quantification of endogenous nitrosation from a given exposure to exogenous nitrate, the working group is not able to perform a quantitative assessment of health risks which takes into account a potential genotoxic carcinogenic risk associated with nitrate and nitrite ingestion.

6.2 Reference values in drinking water

For nitrates, WHO states that the guideline value of 50 mg/L is based on epidemiological data highlighting cases of methaemoglobinaemia in infants. This is a protective guideline value for infants in the event of acute exposure but also for the entire population. WHO stresses the role of microbiological contamination of water resulting in gastrointestinal tract infection, which can significantly increase the risk to infants. Because of this, WHO proposes administrative intervention for non-compliance with the guideline value for nitrates, at a concentration of between 50 and 100 mg/L. Through this, and subject to the dual conditions, firstly, of microbiological water purity and, secondly, increased medical vigilance of the sensitive population exposed (particularly infants), water containing a nitrate concentration of between 50 and 100 mg/L may be temporarily supplied (WHO, 2007).

For nitrites, WHO used human data showing that the doses of nitrites which cause methaemoglobinaemia in infants are between 0.4 and over 200 mg/kg b.w. Using the lower dose value (0.4 mg/kg b.w.), ingestion of 0.75 litres of water by an infant weighing 5 kg, a guideline value of 3 mg/L (rounded up value) is proposed for drinking water.

These two ions may be present jointly in water and WHO therefore recommends that the sum of the ratios of the concentration over the guideline value for each should not exceed 1:

$C_{NO3} / 50 + C_{NO2} / 3 < 1$

For chronic exposure to these two ions, WHO recalls that JECFA proposes acceptable daily intakes (ADI) and considers that the ADI proposed for nitrates by JECFA is inappropriate to assess health risks, in view of metabolic differences between humans and rodents. It does, however, consider it prudent to propose a guideline value for nitrites associated with chronic exposure and for this uses the ADI proposed by JECFA (2003) of 0.07 mg/kg b.w./d, assuming that an individual weighing 60 kg ingests 2 L of water per day and allocating 10% of the ADI to water. The provisional guideline value proposed is 0.2 mg/L. This is a provisional value in view of the uncertainties concerning the susceptibility of humans compared to animals.

Table 6.2-1: History of the guideline values established for nitrates and nitrites.

Date	Document	Nitrate	Nitrite
1958	International Standards for drinking water	Ingestion of water containing more than 50-100 mg NO_3/L carries a risk of causing methaemoglobinaemia in infants under one year old	
1963	International Standards	Recommended value 45 mg NO_3/L	
1971	International Standards	Recommended value 45 mg NO_3/L + statement of possible endogenous formation of N-nitroso compounds	
1984	1st edition Guidelines for drinking water quality	Guideline value: 10 mg N-Nitrate /L	N-nitrite concentrations must be far below 1 mg /L when the water is correctly treated.
1994	2nd edition Guidelines for drinking water quality	Guideline value: 50 mg NO_3 /L	Provisional guideline value: 3 mg /L
		C_{NO3} / 50 + C_{NO2} / 3 < 1	
1998	2nd edition Guidelines for drinking water quality addendum	Guideline value: 50 mg NO_3 /L	Guideline value: 3 mg /L (infantile methaemoglobinaemia) Provisional chronic guideline value: 0.2 mg /L
		C_{NO3} / 50 + C_{NO2} / 3 < 1	
2007	3rd edition Guidelines for drinking water quality	No new information to review the guideline value of 50 mg/L for nitrates. However, WHO emphasises the role of microbiological contamination of water resulting in gastrointestinal tract infection, which can significantly increase the risk to infants.	

Table 6.2-2: Reference values proposed for nitrates by different organisations

Value from directive 98/83/EC Annex IB	WHO 2005 Guideline value	Health Canada	US EPA
50 mg/L	50 mg/L	45 mg/L	45 mg/L

Table 6.2-3: Reference values proposed for nitrites by different organisations

Value from directive 98/83/EC Annex IB	WHO 2005 Guideline value	Health Canada	US EPA
0.5 mg/L	3 mg/L (acute) 0.2 mg/L (chronic)	3.2 mg/L	3.3 mg/L

7 - Summary

7.1. Infantile methaemoglobinaemia and guideline value for nitrates

The guideline value of 50 mg/L proposed by WHO for the nitrate ion is derived from an old study (Walton, 1951). As no cases of methaemoglobinaemia have been seen when the nitrate concentration in water was below approximately 45 mg/L, WHO has reviewed this value and set a maximum exposure level of 50 mg/L in drinking water.

However, as highlighted by the publication by Ward *et al.*, 2005, and following a recent forum, the role of exposure to nitrates alone in the development of methaemoglobinaemia has been questioned and a greater understanding of the interactions between factors resulting in methaemoglobinaemia is required in order to assess the relative importance of each factor and to identify exposure conditions to nitrates in drinking water which would carry risks of methaemoglobinaemia.

Of these factors, epidemiological findings suggest that methaemoglobinaemia is often associated with infection.

7.2. Other effects and toxicological reference values for nitrates and nitrites

7.2.1. Nitrates, nitrites and cancer

The potential carcinogenic effects of nitrates in drinking water and in the diet result from complex interactions between exogenous nitrate and the many factors involved in the endogenous formation of NOC described in point 5.4.

Based on the currently available scientific knowledge, it is not possible to quantify endogenous formation of N-nitroso compounds from ingested nitrates or to assess the carcinogenic risk.

7.2.2 Toxicological reference values for nitrates

The ADI proposed for nitrates by JECFA and SCF has been established from chronic toxicity studies in rats. It is generally accepted that the rat is not a suitable model for humans, particularly because of the absence of entero-salivary nitrate recirculation.
In 1961, JECFA stated that the ADI could not be applied to children under 6 months old although this restriction was not subsequently restated. However, this ADI was revised by JECFA in 1994 and a value set at 3.7 mg/kg b.w./d, which does not apply to infants under three months old. JECFA confirmed this ADI in 2003.
In 2008, EFSA adopted the same ADI in its health risk assessment relative to nitrates in vegetables.
In its quality directives for drinking water, WHO considered that the ADI proposed for nitrates by JECFA was inappropriate to assess health risks because of metabolic differences between humans and rodents (WHO, 2007).
Afssa also considered that the pivot study by Lehman A.J. (1958) used in 2003 by the JECFA could be criticised methodologically, did not allow a toxicological reference value to be established for nitrates and that it was therefore desirable to have access to toxicological studies in relevant animal models.

7.2.3. Toxicological reference values for nitrites

WHO uses the ADI of 0.07 mg/kg b.w./d from JECFA (2003) to set a provisional guideline value associated with chronic sodium nitrite exposure.

The study conducted by Afssa (Afssa – Paser, 2007a), provides an estimate of cumulative nitrite exposure in drinking water for different scenarios (Table 4.2.3) using a conservative approach. These values show that for all of the estimates produced (even in the absence of nitrites in water), the daily intakes in the highest adult and child consumers are above the ADI proposed by JECFA.

7.3. Conclusion

In its assessment of the health risks relative to nitrates in vegetables in 2008, EFSA considered different nitrate exposure scenarios, restated the ADI from JECFA (2003) and showed that this is liable to be exceeded in adults consuming 400 g of a mixture of vegetables and that daily exposure from non-vegetable sources was estimated to be 44 mg/person/day, of which 20 mg/person/day could be attributed to water. The nitrate exposure calculations are conservative, leading EFSA to conclude that nitrate exposure in vegetables should not result in significant health risks.

Because of scientific uncertainties about the role of nitrates alone in the development of infantile methaemoglobinaemia and in the absence of a sufficiently robust ADI, Afssa is not able to assess the risk to the population, particularly to infants, when the quality limit of 50 mg/L is exceeded for nitrates and, because of this, is not able to propose a derogation value.

Afssa emphasises that in light of these undoubtedly conservative estimates of cumulative nitrite intake (foodstuff and water), daily intakes exceed the JECFA (2003) ADI for both children and adult high consumers. As a result, no non-compliance with the quality limit for nitrites can be accepted in drinking water.

As knowledge currently stands and in the context of uncertainties about the probable carcinogenic effects of nitrates and nitrites, particularly under certain conditions which result in endogenous nitrosation, it is not possible to quantify the endogenous formation of N-nitroso compounds from exogenous nitrate intake or to assess the carcinogenic risk.

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Annex 1 – Fact sheet 19: Description of methodology used to estimate dietary nitrite and nitrate intake

The study estimating nitrate and nitrite intake combines individual food consumption data from the INCA1 survey and concentration data from the DGCCRF control and surveillance programmes (2000-2006). Data were also extracted from the SISE-Eaux database (DGS (Directorate General for Health), 2005) for nitrates in tap water.

1- *Consumption data for adults and children over 3 years old*

The data were obtained from the INCA national individual consumption survey. This survey was conducted in mainland France from August 1998 to June 1999, and therefore includes seasonal effects, in 3003 children and adults representative of the French population. National representivity was ensured by stratification (by age, sex, individual socio-professional category and size of household). The calculations only relate to "non-underreporters", i.e. 1,474 adults over 15 years old and 1,018 children between 3 and 14 years old. This survey enabled all portions of food taken by people to be recorded for an entire week using food diaries[14].

2- *Data from the DGCCRF surveillance programmes*

Description of data

The data from the DGCCRF surveillance programmes used in this study are the results of annual surveys concerning foodstuffs liable to contain nitrates and nitrites from 2000 to 2006 obtained from 8 different laboratories: Bordeaux, Montpellier, Rennes, Lille, Massy, Strasbourg, Lyons and Réunion island. The analytical capacities of the 8 laboratories were provided by DGCCRF.

The results of these surveys included 15,230 nitrite and nitrate analyses. However, corrections were made in order to link these data to the consumption data obtained from the INCA1 survey (nomenclature correspondence).

- Removal of some foodstuffs (n = 943 analyses, i.e. 6% of all analyses), in particular:
  - specific infant foodstuffs which had no equivalent in the INCA1 consumer survey which did not record consumption by children under 3 years old,
  - foodstuffs that are very rarely consumed by the French population and for which the INCA1 survey has no consumption data.
- Removal of analyses from the Réunion island laboratory as the INCA1 survey was representative of mainland French consumers (n= 486 analyses, i.e. 3% of all analyses).
- Removal of some unquantified analyses for which analytical data were not available from the corresponding laboratories (n= 144 analyses, i.e. 1% of all analyses).

A total of 13,657 nitrite and nitrate concentration data points concerning 138 foodstuffs for nitrates and 109 foodstuffs for nitrites were used.

Foodstuff correspondence

The 13,657 foodstuffs analysed were linked to the foodstuffs contained in the INCA1 survey although it must be noted that some foodstuffs analysed were difficult to identify as the concentration database provided by DGCCRF contained:

- inaccurate names,
- unusable codes,
- groupings which were incompatible with Afssa's requirements.

It should also be stressed that the data from these surveys are very often biased towards products in which the use of nitrites is permitted. Some concentration data also related to processed meat products that are very rarely consumed by the French population. In order not to remove these many data points on processed products in which nitrite use is permitted, a new class of food was created: "other cured or tinned products" including:

[14] Volatier J-L. (2000). Enquête INCA individuelle et nationale sur les consommations alimentaires. Edition TEC&DOC.

- meat products processed or treated with nitrites (particularly chicken, turkey, pork, beef),
- tinned meat products.

The INCA1 individual consumer survey contains very little data on these processed meat products. In order to take into account the "other cured or tinned products" when estimating nitrate and nitrite exposure in the French population, consumption of these different processed meat products was linked to the consumption of the corresponding meats giving a maximum case scenario.

Adjustments for concentration data

The results of the surveillance programme present the results of the food analyses which can be:

- Quantified,
- Detected but not quantified,
- Undetected,
- Below a specific threshold.

In order to process the censored data and allocate a value to these, several hypotheses were advanced following the international guidelines[15] (Table 1):

- Hypothesis 1: taking into account
  - quantified values
  - values which were detected but not quantified and assumed to be equal to the LOD
  - values which were not detected and were assumed to be equal to 0
  - values which were below a threshold, assumed to be equal to 0
- Hypothesis 2: taking into account
  - quantified values
  - values which were detected but not quantified and assumed to be equal to (LOD+LOQ)/2
  - values which were not detected and were assumed to be equal to LOD/2
  - values which were below a threshold, assumed to be equal to INF/2
- Hypothesis 3: taking into account
  - quantified values
  - values which were detected but not quantified and assumed to be equal to the LOQ
  - values which were not detected and were assumed to be equal to the LOD
  - values which were below a threshold assumed to be equal to INF

Table 1: Different scenarios for the concentration values

Type of results	hypothesis 1	hypothesis 2	hypothesis 3
Quantified through a value	value	value	value
Detected but not quantified	LOD	(LOD+LOQ)/2	LOQ
Undetected	0	LOD/2	LOD
Below a threshold value (INF)	0	INF/2	INF

Calculation of mean nitrate and nitrite concentration

According to the international guidelines[16], the use of a mean concentration in calculating intake is a realistic and appropriate estimate of long-term exposure. Once concentrations have been determined, the means must be calculated as a function of the censored data (Table 2) using:

- an upper estimate,
- a lower estimate.

[15] International Program on Chemical Safety/Gems/Food Euro Workshop on reliable evaluation of low level contamination of food, Kulmbach, Federal Republic Germany. May 1995.
[16] FAO/WHO, 1985, Guidelines for the study of dietary intakes of chemical contaminants Geneva: WHO, offset publication No. 87.

Table 2: Different scenarios for mean concentrations

Mean concentration	censor <60%	60 <censor ≤ 100
Upper estimate	mean of hypothesis 2	mean hypothesis 3
Lower estimate	mean of hypothesis 2	mean hypothesis 1

3. Conclusion

Some calculation hypotheses have been adopted for the upper estimate of total daily nitrite intake used in this file.

- the consumption data for foodstuffs defined in the “other cured or tinned products” not present in the INCA 1 database were populated using consumption data for the corresponding meats.
- as the upper estimate was used in the fact sheet and many of the unquantified results were used to give information about the food nitrite contamination levels, particularly for vegetables, the hypotheses for calculations 2 or 3 were used. These estimates imply that no analytical value is taken to be 0, even in the case of undetected values.

These hypotheses result in an over-estimate of total nitrite exposure.

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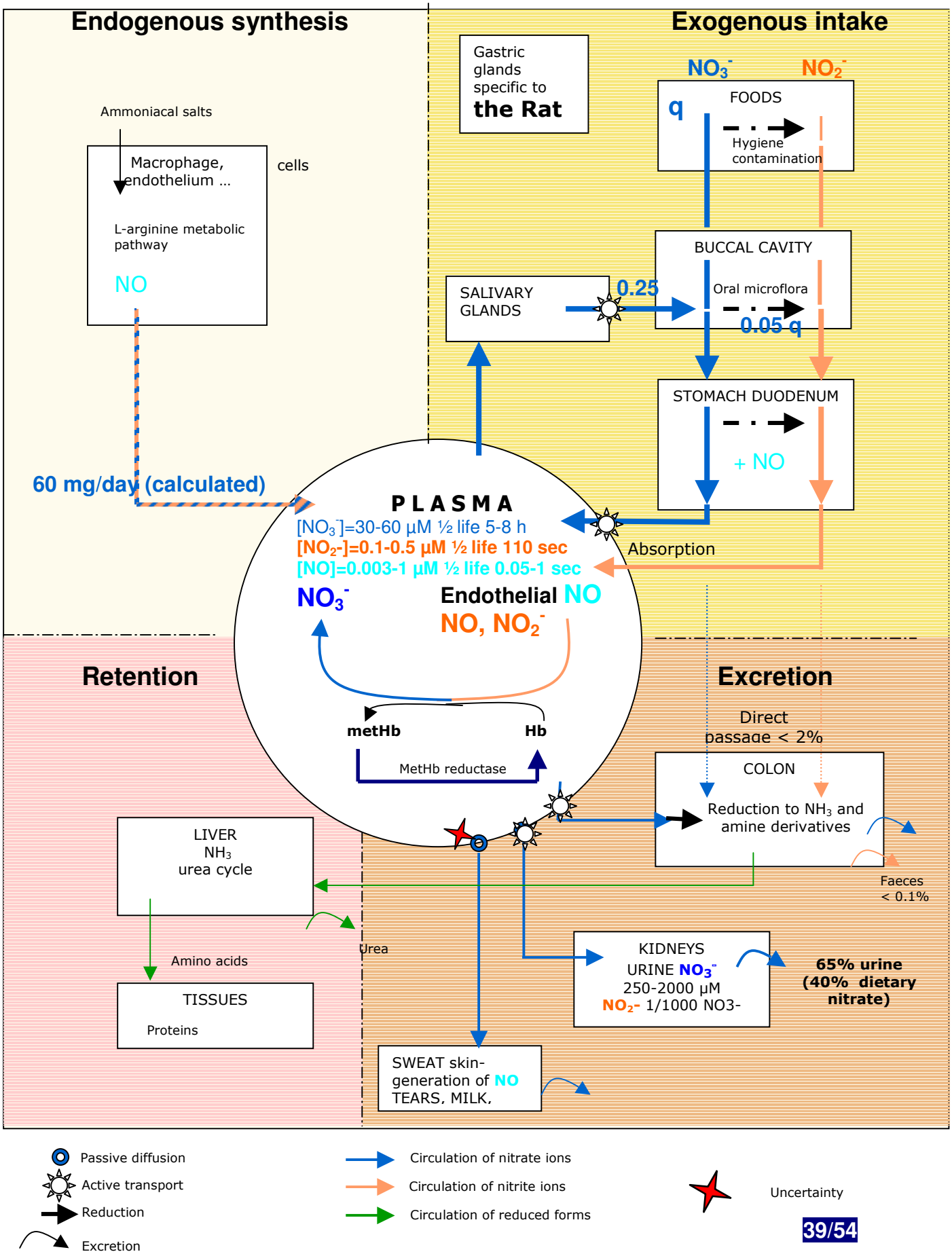
Annex 2 – Fact sheet 19: Nitrate metabolism (extracellular)



Annex 3 – Fact sheet 19: Compounds and natural mixtures which are inhibitors of N- nitrosation

Vitamins E (ascorbic acid) E (α-tocopherol) **Phenolic compounds** Catechol Cinnamic, chlorogenic and gallic acids Hydroquinones Phenolic acids PyrogallolTannic acid and tannins Thymol **Complex mixtures** Betel nut extracts Coffee Fruit juice Milk and dairy products Soya products Tea	**Sulphur-containing compounds** Cysteine Glutathione Methionine **Various compounds** Alcohols Caffeine Carbohydrates Unsaturated fatty acids Urea

From Bartsch *et al.,* 1988; Pignatelli *et al.,* 1985

Annex 4 – Fact sheet 19: Epidemiological studies on the relationship between the cancer risk and exposure to nitrates in drinking water.

Reference Country	Design of study	Exposure factors	Sites of cancer	Main results	Exposure: comparison with max legal value 50 mg/l
Hill *et al.* 1973 United Kingdom	Ecological	Deaths Nitrate concentration 90 mg/l versus 10 mg/l	Stomach	Association between cancer deaths and high nitrate concentration in water	Above
Armiio et Coulson. 1975 Chile	Ecological	Use of fertilisers	Stomach	Association	Above
Cuello *et al.* 1976 Columbia (Narino)	Case control	Cancer n = 276; Controls n = 276 High nitrate concentration in well water up to 300 mg/l	Stomach	Association between the risk of cancer and high nitrate concentration in well water	Above
Gelperin *et al.*, 1976 USA (Illinois)	Ecological	Deaths from cancer	Stomach Oesophagus	No association between cancer deaths and nitrate level in drinking water	Below
Zaldivar and Vetterstrand, 1978 Chile (25 provinces)	Ecological	Deaths from cancer Nitrate concentration < 50 mg/l mean = 6.4 mg/l	Stomach	Trend towards positive correlation between cancer deaths and nitrate level in water, but not statistically significant	Below
Juhàsz *et al.* 1980 Hungary	Ecological	Incidence of cancer	Stomach	Association between incidence of cancer and nitrate level in water	Above
Zemla. 1980 Poland	Ecological	Incidence of cancer (1965-1975) Two districts with variations in water quality, including nitrate concentration	Stomach	Association between incidence of cancer and nitrate concentration	Above
Thouez *et al.*, 1981 Canada (Province of Quebec)	Ecological	Deaths	Brain and central nervous system Prostate	Association between risk of cancer and exposure to nitrate in drinking water Association between risk of cancer and exposure to nitrate in drinking water.	Above

Reference Country	Design of study	Exposure factors	Sites of cancer	Main results	Exposure: comparison with max legal value 50 mg/l
Jensen *et al.*, 1982 Denmark	Ecological	Incidence of cancer	Stomach	Association between incidence of cancer and exposure to nitrate when concentration > 30 mg/l	
Vincent *et al.*, 1983 France (Nord)	Ecological	Deaths from cancer (1968-1975) Nitrate measurement (1974-1976) 93% < 43 mg/l	Stomach	No association between cancer deaths and nitrate level in drinking water	Below
Gilli *et al.*, 1984 Italy (Piedmont)	Ecological	Incidence of cancer Nitrate concentration > 20 mg/l versus low	Stomach	Association between incidence of cancer and nitrate concentrations in drinking water	
Beresford, 1985 United Kingdom (253 urban areas)	Ecological	Deaths from cancer (1969-1973) Nitrate concentration < 100 mg/l	Stomach	Inverse association between deaths from cancer and nitrate concentration in drinking water	Usually Below
Zaldivar *et al.*, 1987 Germany	Ecological	Deaths	Stomach	No association between deaths from cancer and nitrate level in water	Below
Sanz Anquela *et al.*, 1989 Spain (Province of Soria)	Ecological	Deaths from and incidence of cancer Nitrate concentration< 50 mg/l usually	Stomach	Association between cancer deaths and incidence and nitrate concentration in drinking water	Below
Nousbaum, 1989 France	Case control	Cancer n = 143 Controls n = 579	Stomach	Positive association between cancer and nitrate level only for women	
Cauvin *et al.* 1990 France		Nitrate concentration > 75 mg/l versus low	Stomach	Positive association between cancer and nitrate level	Above
Boeing *et al.*, 1991 Germany	Case control	Concentration of high and low cancer risk regions Cases n = 143 Controls n = 579 Comparison of well water and public supply.	Stomach	Association between risk of cancer and consumption of well water	

Reference Country	Design of study	Exposure factors	Sites of cancer	Main results	Exposure: comparison with max legal value 50 mg/l
Leclerc *et al.*, 1991 France	Ecological	Deaths from and incidence of cancer Nitrate concentration < 50 mg/l	Stomach Urinary system	No association between deaths or incidence of cancer and nitrate level in drinking water	Below
Weisenburger, 1991 USA (Nebraska)	Ecological	Incidence of cancer Nitrate concentration in water from 20% of wells > 40 mg/l	Non-Hodgkin's lymphoma	Association between incidence of cancer and nitrate level > 40 mg/l	
Rademacher *et al.*, 1992 USA	Case control	Deaths cases n = 1268 Mean highest nitrate concentration = 29.8 mg/l	Stomach	No association between deaths from cancer and nitrate level	Below
Xu *et al.*, 1992 China (Shandong Peninsula)	Cross-over	Neoplastic mucosal changes cancer + dysplasia n= 30; metaplasia n = 32; normal n = 30 Nitrate: mean concentration: 60-138 mg/l	Stomach	Association between risk of cancer and nitrate in water	Above
Morales Suarez-Valera *et al.*, 1993 Spain (Valencia province)	Ecological	Nitrate levels particularly high in Valencia province. Incidence of cancer for different levels of nitrate exposure in Valencia province.	Bladder	Relative risk of cancer > 1 in Valencia province associated with consumption of water containing a mean nitrate concentration of > 50 mg/l	Above
Steindorf *et al.*, 1994 Germany	Case control	Cancer (1987-1988) n = 173 Population divided into quartiles according to nitrate levels in public supply water after 1970 (Highest quartile: >25.2 mg/l	Brain and central nervous system	No association between risk of cancer and nitrate level in drinking water	Below
Morales-Suarez-Valera *et al.*, 1995 Spain (Valencia province, 258 municipalities)	Ecological	Deaths from cancer Nitrate concentration > 50 mg/l versus low	Stomach Colon Bladder Prostate	Association between deaths from cancer and nitrate level in drinking water If concentration> 50 mg/l RR = 1.9 (M); 1.8 (F) No association No association Association between deaths from cancer and nitrate level in drinking water	Above

Reference Country	Design of study	Exposure factors	Sites of cancer	Main results	Exposure: comparison with max legal value 50 mg/l
Han *et al.*, 1995 China	Ecological	Deaths from cancer Two population groups drinking water with different nitrate and nitrite concentrations	Stomach	Association between deaths from cancer and nitrate level in drinking water	Above
Van Maanen *et al.*, 1996	cross-over	Genotoxicity: frequency of hypoxanthine-guanine phosphoribosyl transferase variants in peripheral blood lymphocytes Four groups with low (l) or high (h) nitrate concentrations: Public supply water 0.02 mg/l, n = 14; h, 18 mg/l, n = 21 well water low, 25 mg/l, n = 6; h, 140 mg/l, n = 9	Genotoxicity	Association between risk of genotoxicity and nitrate level in water, particularly well water	Above for 9/41
Ward *et al.*, 1996 USA (Nebraska)	Case control	Incidence of cancer (1983-1986), Cases n = 156; Controls n = 527 Population divided into quartiles depending on the nitrate level in public supply water from 1945-start 1980 (lowest 7.1, highest 17.7 mg/l	Non-Hodgkin's lymphoma	Association between incidence of cancer and nitrate level in drinking water > 18 mg/l Stronger association for lower consumers of vitamin C and carotene	Below
Moller *et al.*, 1997 Denmark	Case control	Cancer in offspring Farming parents, childhood home (country, area with high nitrate concentration in water)	Testes	Probably indirect association in risk with living in an area with high water nitrate concentration in childhood. Excess risk limited to men who had not grown up in a farm or in the country	

Reference Country	Design of study	Exposure factors	Sites of cancer	Main results	Exposure: comparison with max legal value 50 mg/l
Barret *et al.*, 1998 United Kingdom (Yorkshire)	Ecological	Incidence of cancer (1975-1994) Nitrate (1990-1995) Mean concentration lowest: 2.4 mg/l; highest= 29.8 mg/l	Stomach Oesophagus Brain and central nervous system	No association between risk of cancer and nitrate level in drinking water Association between risk of cancer and nitrate level in drinking water (for mean concentration > 29.8 mg/L) RR = 1.2	Below
van Loon *et al.*, 1997 et 1998 Netherlands	Prospective cohort n = 120852	Incidence (1986-1992) Cases M n= 319; F n = 63 Sub-cohort M n = 1688; F n = 1812 Nitrate intake from mains supply water. Highest nitrate concentration = 40 mg/l; mean nitrogen consumption from water: 3.7 mg/day	Stomach	No association between incidence of cancer and consumption of nitrate in drinking water.	Below
Sandor *et al.*, 1998	Ecological	Deaths from cancer Mean nitrate: 98 mg/l Factors taken into account: smoking, ethnicity	Stomach	Association between cancer deaths and nitrate concentration in drinking water; Reference value for significant increase in risk of cancer: nitrate level > 95 mg/l	Above
Yang *et al.*, 1998 Taiwan.	Case control	Deaths from cancer (1987-1991) nitrate concentration << 50 mg/l 3 groups with different nitrate concentrations	Stomach	Association between deaths from cancer and exposure to nitrate in drinking water	Below
Law *et al.*, 1999 United Kingdom	Ecological Population n = 20 702	Incidence of cancer (1984-1993) according to three levels of nitrate concentration in drinking water (< 3.24; 3.24-14.85 and > 14.85 mg/L; nitrate concentration measured in 1990-1995); mean 12 mg/L; highest 40 mg/L	Non-Hodgkin's lymphoma	No association between the incidence of cancer and nitrate level in drinking water. However, an association was found over the period 1984-1988.	Below

Reference Country	Design of study	Exposure factors	Sites of cancer	Main results	Exposure: comparison with max legal value 50 mg/l
Van Leeuven *et al.*, 1999 Canada (Ontario)	Ecological	Incidence of cancers, Atrazine (50-649 ng/l) and nitrate (0.22-34.5 mg/l levels: mean 8 mg/l) in water and agricultural practices (1987-1991)	Stomach Bowel	Inverse association between incidence of cancer and nitrate concentration Association between incidence of cancer and atrazine concentration No association between incidence of cancer and nitrate concentration Inverse association between incidence of cancer and atrazine concentration Association between nitrate and atrazine concentrations	Below
Freemann *et al.*, 2000 USA (Minnesota, excluding the 4 largest towns)	Case control	Population M, Cancer Cases n = 73 (1980-1982); Controls n = 147 Population divided according to an estimate by place of residence and analytical records of three nitrate levels in public mains water supply (1947-1975) median: 10.6 mg/l; 0.44-31.9 mg/l	Non-Hodgkin's lymphoma	No association between risk of cancer and exposure to nitrate in water (up to mean exposure of 10.6 mg/l	Below
Mueller *et al.*, 2001 USA (Los Angeles County, San Francisco Bay region, California, West Washington Region) ,	Case control	Occurrence of tumours in offspring (1984-1990) Cases n = 540; Controls n = 801 Distinction of drinking water source, either private wells or public water supply during pregnancy. Measurement of nitrite and nitrate levels in water for people with same residence as during their pregnancy.	Brain (in children)	Overall, no association between the risk of cancer in descendants and source of drinking water. OR = 1.2 CI 95% 0.8-2.2 Use of wells in the West of Washington State is associated with increased risk in descendants. OR = 2.6 CI 1.3-5.2 Inverse association between risk of cancer in descendants and source of drinking water in the county of Los Angeles.	99% Below

Reference Country	Design of study	Exposure factors	Sites of cancer	Main results	Exposure: comparison with max legal value 50 mg/l
Sandor *et al.*, 2001 Hungary	Ecological	Population n = 108000; Deaths from cancer (1984-1993) n = 407 Measurement of nitrate concentrations (1974-1993); median: 72 mg/l; 95%: 290.7 mg/l	Stomach	Association between cancer deaths and nitrate concentration (log mean); linear regression analysis Exposure > 88 mg/l results in significant increase in risk	Above (usually)
Ward *et al.*, 2001			Non-Hodgkin's lymphoma	No association	
Weyer *et al.*, 2001 USA (Iowa)	Prospective cohort	Women n = 21977 using the same water supply for more than 10 years; public supply n = 16541; wells = 5436 Cancers (1986-1998), cases n = 3150 Estimation of nitrate exposure (1955-1988), cut off points by quartiles: 1.6 4.5 and 10.9 mg/l	Bladder Ovary Rectum Uterus Non-Hodgkin's lymphoma Leukaemia, Melanoma Bowel, Breast, Lung, Pancreas	Association between incidence of cancer and nitrate concentration Inverse association between incidence of cancer and nitrate concentration No association or non-significant trend between incidence of cancer and nitrate concentration	Below

Reference Country	Design of study	Exposure factors	Sites of cancer	Main results	Exposure: comparison with max legal value 50 mg/l
Gulis *et al.*, 2002 Slovak Republic (Trnava District, farming)	Ecological n = 189 600	Cases of cancer (1986-1995) Population divided according to mean nitrate levels in drinking water from 1975-1995 in mg/l: low = 0-10, medium = 10.1-20 and high = 20.1-50	Stomach Colorectal Non-Hodgkin's lymphoma Bladder Kidney	Trend towards association between cancer and nitrate level in water only for female subjects SIR (95% CI): 0.81 (0.48-1.34); 0.94 (0.67-1.33); 1.24 (0.91-1.70); p<0.10 Cancer associated with nitrate level in water SIR (95% CI) for the three nitrate levels: M/F: 0.71 (0.57-0.88); 1.05 (0.92-1.20); 1.18 (1.04-1.34) respectively: p< 0.001 Cancer associated with nitrate level in water SIR (95% CI) for the three nitrate levels: M/F: 0.36 (0.11-1.11); 1.26 (0.82-1.93); 1.22 (0.76-1.96); p< 0.02 No association No association	Below
Cocco *et al.*, 2003 Italy (Sardinia) 153 communes	Case control	Population n = 703000; Incidence of cancer (1974-1993), n = 737 Nitrate concentrations in water (1971-1994), mean: 4.57 mg/l; ≤ 2-26.64 mg/l	Non-Hodgkin's lymphoma	Association between incidence of cancer and nitrate concentrations in communal water supply but only for male subjects (limited evidence)	Below

Reference Country	Design of study	Exposure factors	Sites of cancer	Main results	Exposure: comparison with max legal value 50 mg/l
Ward *et al.*, 2003 USA (Iowa)	Case control	Bladder cancer (1986-1989) cases n = 808; Controls n = 1259 Population (male subjects M; female subjects F) divided according to mean nitrate levels in public water supply from 1960-1987. Highest quartile: M, 13.7 mg/l; F, 10.6 mg/l Small proportion of population exposed to more than 44.3 mg/l of nitrate Number of years exposure > 44.3 mg/l	Bladder	No association between risk of cancer and increasing quartiles of nitrate level in drinking water for females. Inverse association for males. Same results when number of years exposure to nitrate are considered > 44.3 mg/l	Below
De Roos *et al.*, 2003 USA (Iowa)	Case control	Incidence of cancer (1986-1989), Cases n = 376 (colon); Cases n = 338 (rectum) Controls n = 1244 Population divided into quartiles according to mean nitrate levels in public mains water supply from 1960-1987(lowest 4.4, highest 22.1 mg/l) and number of years exposure > 22.1 and >44.3 mg/l	Bowel Rectum	Risk of bowel cancer only slightly raised in sub-groups exposed to a nitrate concentration of >22.1 mg/ in water for 10 years or more and low consumption of vitamin C or high meat consumption. No association between cancer and more than 5 years of exposure to nitrate > 44.3 mg/l No association	Below
Zhang *et al.*, 2003 China (Province of Hebei)	Ecological	Cancer (1991-1995) in 2 regions: Cixian, high incidence of cancer; Chichen, lower incidence of cancer. Nitrate in well water, mean =38.8 mg/l, Cixian mean = 17 mg/l, Chichen	Oesophagus	Positive correlation (coeff 0.5992) between nitrate level in well water and deaths from cancer. Pollution due to use of nitrogen-containing fertilisers	

Reference Country	Design of study	Exposure factors	Sites of cancer	Main results	Exposure: comparison with max legal value 50 mg/l
Coss *et al.*, 2004 USA (Iowa)	Case control	Inclusion: Pancreatic cancer (1985-1987) n = 189; Controls n= 1244 Population divided into quartiles according to mean nitrate levels in public water supply from 1960-1987: mean = 5.6 mg/l; interquartile range, 2.6 to 12.4 mg/l and number of years of exposure to levels of > 33.2 and 44.3 mg/l affecting less than 25% of cases and controls, 50% of cases 49% of controls not included mostly because they used well water.	Pancreas	No association between risk of cancer and increasing quartiles of nitrate level in drinking water or number of years of exposure to nitrate concentrations > 32.2 and 44.3 mg/l Nitrate intake for 75% of the population was < 10%. Most nitrate intake from diet and particularly vegetables Author's comment: analytical limit for nitrate levels far below 22 mg/L	Below
Mueller *et al.*, 2004	Multicentre case control 5 countries, 7 regions	Cases n = 836 Controls n = 1485 Information about water source (wells or public water supply), place of residence during pregnancy from mothers Measurement of nitrate levels in tap water; < 10 mg/l (63% of cases; 56% of controls); < 50 mg/l (92% of cases and controls)	Brain (in children)	Association between risk of cancer and dependency on use of well water during pregnancy compared to public water supply in two of the seven regions: Canada OR = 5.3 CI 1.2-23.1; Seattle OR = 2.6 CI 1.3-5.4 Inverse association for Los Angeles region: OR = 0.2 CI 0.1-0.8 No association between risk and nitrate level although only measured for some of the subjects and years after pregnancy. Possible sources of bias discussed	Below for 92% of cases of cancer and controls
Ward *et al.*, 2004 USA (Nebraska)	Case control	Cancer (1988-1993) Population divided into quartiles according to mean nitrate levels in public water supply from 1960-1986	Brain (glioma)	No association between the risk of cancer and increasing quartiles of nitrate level in drinking water.	

Reference Country	Design of study	Exposure factors	Sites of cancer	Main results	Exposure: comparison with max legal value 50 mg/l
Volkmer *et al.*, 2005 Germany	Community cohort n = 67290	Urological system cancers (1986-1997), n = 527; Incidence per 10,000 people/year For 28 years (1957-1986), distribution of water with different nitrate concentrations: group A, 60 mg/l, n = 57253 group B, 10 mg/l, n = 10037	Urothelial cancer Kidney, prostate, penis Testes	Association with incidence of cancer No association with incidence of cancer Inverse correlation with incidence of cancer	Above for cases of cancer
Ward *et al.*, 2005 USA (Nebraska)	Case control	Cancer Historical measurements of nitrate in water sources for place of residence; mean calculated over 20 years	Brain (glioma)	No association between risk of glioma and mean nitrate level in drinking water	
Ward *et al.*, 2006 USA (Iowa)	Case control	Cancer (1998-2000) Cases n = 181; Controls n = 142 Measurement of nitrate in public water supply from 1960 (mean 13.3 mg/l)	Non-Hodgkin's lymphoma	No association between risk of cancer and nitrate concentration in drinking water.	Below
Zeegers *et al.*, 2006 Netherlands	Cohort n = 120852	Incidence of cancer (1986-1995) n = 871 Sub-cohort controls n = 4359 Estimate of nitrate concentration in water from data available in 1986 and calculation of amount of water consumed by questionnaire mean concentration 5.3 ± 6.2 mg/l, cases 4.9 ± 6.2 mg/l,	Bladder	No association between risk of cancer and nitrate level in drinking water but nitrate levels particularly low	Far below.
Kuo *et al.*, 2007 (Taiwan)	Case control	Deaths from cancer 1999-2003 Nitrate concentration in water for place of residence	Rectum	Upper tertile OR = 1.36 (1.08-1.70) Lower tertile OR = 1.22 (0.98-1.52) Study inconclusive; other investigations required	

Reference Country	Design of study	Exposure factors	Sites of cancer	Main results	Exposure: comparison with max legal value 50 mg/l
Chiu *et al.*, 2007 Taiwan	Case control	Deaths from cancer 1999-2003 Nitrate concentration in water for place of residence	Bladder	upper tertile OR = 1.96 (1.41-2.72) lower tertile OR = 1.76 (1.28-2.42) Association between deaths from cancer and nitrate level in drinking water	
Yang *et al.*, 2007 Taiwan	Case control	Deaths from cancer 1999-2003 Nitrate concentration in water for place of residence.	Bowel	upper tertile OR = 10.98 (0.84-1.14) lower tertile OR = 0.98 (0.83-1.16) No association between deaths from cancer and nitrate level in drinking water	

Abbreviations:

F: Female	CI: Confidence interval
M: Men	RR: relative risk
OR: Odds Ratio	SIR: standardised incidence rate

Annex 5 – Fact sheet 19: Chronic toxicological reference values (TRVs) proposed for NDMA

Chronic TRVs are proposed by different bodies for NDMA which is one of the most widely studied nitrosamines.

OEHHA (Office of Environmental Health Hazard Assessment of California)
This body set a Public Health Goal (PHG) of 0.003 µg/L for water intended for human consumption. This concentration in water is associated with a 10^{-6} excess risk of cancer when the water is drunk over a whole lifetime.

Scientific data on the genotoxicity and mechanism of carcinogenesis of NDMA, carcinogenicity in animals and the high probability of carcinogenicity in humans led this body to conclude that NDMA in drinking water carries a carcinogenic risk.

Using data from Peto *et al.* (1991a) a dose-response relationship was established based on the incidence of biliary tract tumours in female rats and using a multi-step linearised model. The model was used to estimate the dose associated with a 10% tumour incidence. As the relationship was linear, the results could be extrapolated to low doses and a level associated with the risk of 10^{-6} cases of additional cancer could be established. A dose-equivalent for humans was determined. This body considered that inhalation or cutaneous exposure contributed only very little to overall exposure.

US EPA (United States Environmental Protection Agency)
The US EPA classifies this substance in category B2: probably carcinogenic to humans: data available only in animals. The body based its findings on the study by Peto *et al.* (1984). A dose-response relationship was constructed based on cases of liver tumours in female rats. An "Oral Slope Factor" was calculated from these findings [51 $(mg/kg/d)^{-1}$]. This body proposes a Water Unit Risk of $1.4.10^{-3}$ $(\mu g/L)^{-1}$, i.e. a concentration of 0.0007 µg/L (0.7 ng/L) in drinking water associated with a 10^{-6} excess risk of cancer.

Germany
The German Federal Agency for Environmental Protection set an NDMA guide value in water of 10 ng/L. The Germans based their process on the studies by Lutz (1999) and Tricker et al. (1991).

- The study by Tricker et al. (1991) is a dietary exposure study which shows that of the 1000 ng/d of nitrosamines ingested, 200 ng are NDMA.
- The study by Lutz (1999) concluded that there was an excess of risk of cancer of 8.10^{-6} associated with exposure to all of the nitrosamines present in foodstuffs. This excess risk was believed to be associated with NDMA (200 ng/d), i.e. an excess risk of cancer of 1.10^{-6} due to ingestion of 25 ng/d of NDMA. A daily intake of 10 ng/L of NDMA in water would therefore be associated with an excess risk of 1.10^{-6}.

Canada
Health Canada proposed a risk assessment linked to NDMA (Environment Canada, 2000) based on the two studies which are considered to be the most relevant to propose a dose-response relationship for NDMA: Brantom (1983) and Peto et al. (1991) who exposed male and female rats to NDMA in drinking water. A TD_{05} of 34 µg/kg b.w./d (TD_{05}: dose causing a 5% increase in the incidence of tumours compared to controls) was calculated from a linearised multi-stage model.

Ontario (Canada) proposed a maximum acceptable concentration of 9 ng/L in drinking water (Cheng et al, 2006).

Table – annex 6: Toxicological reference values proposed for NDMA by the different bodies

Source	Reference value in drinking water		Study	Species	Effect
OEHHA (2006)	PHG Public Health Goal	0.003 µg/L Excess risk of cancer (10^{-6})	Peto *et al.* (1991a)	Rat	Liver tumour
IRIS US EPA (1986) 1993 update	Oral Slope Factor Drinking Water Unit Risk	51 $(mg/kg/j)^{-1}$ 1.4×10^{-3} per (µg/L) or 0.0007 µg/L Excess risk of cancer (10^{-6})	Peto *et al.*, 1984	Rat	Liver tumour
Germany	Guideline Value	0.01 µg/L Excess risk of cancer (10^{-6})	(Lutz 1999) (Tricker *et al.*,1991)		