Contract report for the Horticultural Development Council

Lettuce and Spinach: preparation of a paper for submission to EFSA supporting the case for lifting levels of permissible nitrate in EU regulations relating to salad

PC 275

April 2007

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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

- Following a request from the European Commission, a paper on Contaminants was prepared by the National Farmers Union on behalf of UK lettuce growers and submitted to the European Food Safety Authority (EFSA) Panel. It evaluates the Risks and Benefits to Consumers from Nitrate in Vegetables.

- The paper argues that the scientific evidence shows that maximum limits for nitrate in lettuce and spinach are not necessary providing the crops are produced under good agricultural practices.

- The continued presence of any maximum limits for nitrate will almost certainly raise doubts in the minds of consumers of the benefits of eating a diet rich in fruit and vegetable

Background and expected deliverables

The UK currently applies a derogation to the limits for nitrate in lettuce and fresh spinach in Commission Regulation (EC) No. 1881/2006 which lasts until 31 December 2008. In practice, this allows UK growers of protected lettuce to market their crops throughout the year regardless of the nitrate level in the crop provided that the grower is following Good Agricultural Practices (GAP). The EU Commission reviews the regulation every three years and warned member states at the 2005 review that it would be difficult to justify any further derogations.

Annual surveys undertaken by the Food Standards Agency have shown that a significant percentage (up to 20%) of UK grown glasshouse lettuce exceed the EU limits particularly in winter. It would therefore not be commercially viable for UK producers to continue growing lettuces in glass houses particularly in winter if these crops had to meet the current or any revised limits for nitrate. Any new limits are likely to be lower than existing limits as the Commission is expected to seek lower limits for all contaminants covered by the proposed new Regulation.

The EU Commission agreed at the 2005 review that it would request the EFSA to provide a scientific risk assessment on nitrate in vegetables which should take into account any relevant considerations on the risks and benefits including, for example, the possible negative impact of nitrate versus the possible positive effects of eating vegetables.

At present, the EFSA Panel on Contaminants is collecting scientific information from Member States and other interested parties on the possible risks and benefits to consumers from nitrate in the diet, which it will need to undertake its review of nitrate.

The purpose of this project is to prepare a paper for submission to the Panel on Contaminants which emphasises the benefits to consumers of eating a healthy diet which includes salads and other vegetables. The paper will also present the evidence which indicates that nitrate in vegetable presents little risk to health and indeed may have some beneficial effects.

Summary of the project and main conclusions

The European Commission has asked the European Food Safety Authority (EFSA) to provide a scientific risk assessment for the longer-term strategy for managing the risk from nitrate in vegetables. In particular, the Commission asked that "the assessment should take into account any relevant information on the risks and benefits, for example of the possible negative impact of nitrate versus the possible positive effects of eating vegetables." EFSA has referred this request to the Scientific Panel on Contaminants in the Food chain (CONTAM).

The need for advice arises from discussions to amend the current controls on nitrate in lettuce, spinach and certain other vegetables set out in Commission Regulation No. 1881/2006 (see Table 1.2 for a summary of the limits for nitrate). The current controls reflect the opinion on nitrate and nitrite given by the Scientific Committee on Food (SCF) on 22 September 1995 which recognised that total intake of nitrate is normally below the acceptable daily intake of 3.7 mg/kg body weight. The SCF also recommended continuation of efforts to reduce exposure to nitrate via food and water while at the same time encouraging the greater consumption of fruit and vegetables. However, further scientific studies mainly on the benefits to human health from nitrate in the diet have been published since the SCF gave its opinion in 1995 which warrants this further review by CONTAM.

The purpose of this paper is to provide evidence to CONTAM which demonstrates that the need for maximum permitted levels of nitrate in lettuce and spinach in particular and other vegetables in general is not justified as recent scientific evidence indicates that nitrate in the diet has many beneficial effects. Further, any move to reduce the existing limits would raise doubts in the minds of consumers about the safety of eating any vegetables which is in direct conflict with current healthy eating campaigns which encourage the increased consumption of fruit and vegetables. Any reductions in the maximum levels could also threaten the commercial viability of glasshouse production of lettuce and other crops in the UK and other member states in northern and eastern Europe.

The paper does not consider the problems which might arise from the presence of nitrate in water which are very different from the issues surrounding nitrate in vegetables.

This key information provided by the paper is summarised as follows:

a) Reasons for considering nitrate differently from other contaminants in food
It is not appropriate to treat nitrate in vegetables (as opposed to nitrate in water or beer) as a contaminant but as a natural component of food. As such, a more holistic approach to the presence of nitrate in vegetables is needed where both the risks and benefits from the presence of nitrate and also the wider benefits of vegetables in the diet are considered together.

b) The role nitrate plays in vegetables and factors affecting its levels

Recent research has improved agricultural practices and emphasised the importance of such factors as growing the best varieties of lettuce, cleaning glass and keeping nitrate in soil below 100 mg/kg. However, growers are still not able to be certain that their crops will be below the maximum levels for nitrate particularly in periods of low light intensity.

c) Levels of nitrate in lettuce and spinach

Growers in the UK and other northern and eastern EU member states face the considerable risk that an unpredictable proportion of their lettuce and spinach crops will exceed the maximum levels for nitrate established by Commission Regulation No. 1881/2006 and therefore will not be marketable particularly in the winter months. These growers may not wish to take the commercial risk of growing these crops which will affect the viability of their entire operation leading to closure. This would have the obvious economic and social consequences for the producing areas. It is therefore essential that any longer-term strategy for managing any risk from nitrate in vegetables takes these wider factors into account.

Table 1.1: Summary of UK results for nitrate in lettuce and spinach from 2004 monitoring programme

(a) Glasshouse lettuce (not Iceberg)

Season	Number of samples	Number above EU limit (%)	EU limit (mg/kg)	Mean nitrate (mg/kg)	Range (mg/kg)
Summer	18	6 (33%)	3500	2999	676 - 4384
Winter	33	5 (15%)	4500	3617	1945 - 5720

(b) Outdoor lettuce (not Iceberg)

Season	Number of samples	Number above EU limit (%)	EU limit (mg/kg)	Mean nitrate (mg/kg)	Range (mg/kg)
Summer	62	1 (1.6%)	2500	1140	181 - 2656
Winter	5	0 (0%)	4000	1997	810 - 3100

Table 1.2: Summary of the maximum levels for nitrate in lettuce and spinach. (European Commission Regulation (EC) No. 1881/2006)

Product	**Harvest period**	**Maximum level (mg nitrate/kg)**
Fresh spinach	Harvested 1 Oct. to 31 March	3000
	Harvested 1 April to 30 Sept.	2500
Preserved frozen spinach		2000
Fresh lettuce (excluding	Harvested 1 October to 31	

Iceberg)	March	
	Grown under cover	4500
	Grown in the open air	4000
	Harvested 1 April to 30 September	
	Grown under cover	3500
	Grown in the open air	2500
Iceberg type	Grown under cover	2500
	Grown in the open air	2000

NB: The UK has a derogation until 31 December 2008 to the above maximum levels for lettuce and spinach grown and marketed in the UK. This derogation recognises that developments in the application of good agriculture practice have not produced the hoped for reductions in nitrate levels and growers have difficulty meeting the existing maximum levels for nitrate particularly in the winter months when light levels are often low as that.

d) Consumer exposure to nitrate from food and other sources

The exposure data emphasise the importance of considering all dietary sources of nitrate and that maintaining or reducing maximum levels for nitrate in only lettuce and spinach, even if they are the vegetables which contain the highest levels of nitrate, will have relatively little impact on total consumer exposure to nitrate as they do not form a significant part of most people's diet. Data from the UK indicate that most consumers do not exceed the Acceptable Daily Intake (ADI) set by the SCF in 1995 although some vegetarians may have exposures close to or exceeding the ADI particularly if exposure from water, beer and endogenous sources are included.

Table 1.3: Dietary exposure to nitrate in the UK (mg/person/day)

Food Group	**Mean exposure**	**97.5 percentile**
Bread	0.9	2.1
Misc. cereals	1.3	3.6
Meat and meat products (incl fish and eggs)	5.0	12.8
Sugars and preserves	0.3	1.0
Green vegetables	16	45
Potatoes	19	44
Other vegetables	8	23
Canned vegetables	0.9	2.8
Fresh fruit	2.2	7.5
Beverages	3.2	7.8
Milk	1.2	3.2
Dairy products	1.6	4.8
Nuts	0.01	0.2
Total	**57**	**105**

e) **Toxicological evidence available since the SCF opinion in 1995**

It is evident from published scientific papers that the nitrate in the diet from vegetables has some beneficial effects which must be balanced against the possible risks identified by, for example, traditional animal feeding studies. However, extensive epidemiological studies on nitrate have failed to demonstrate an association with cancer risk in man while convincing evidence exists showing the consumption of vegetables is associated with a reduced cancer risk in humans**.**

f) **Benefits of vegetable consumption versus any risks from exposure to nitrate**
Current dietary advice strongly promotes the increased consumption of fruit and vegetables which will inevitable lead to an increased intake of nitrate. This emphasises the need for CONTAM to ensure that it gives appropriate weight to the potential benefits of dietary nitrate and avoids any ambiguities in its risk assessment of nitrate**.**

Conclusion

Nitrate is a natural component of vegetables fulfilling an essential role in plants whose levels are not affected to any significant extent by either good agricultural practices or plant breeding. The main factor affecting nitrate levels in lettuce and spinach and other leafy plants is the light level at harvest time. Recent scientific evidence indicates that the beneficial effects of nitrate in the diet are of greater significance than the potential risks from nitrate identified by animal feeding studies although evidence of these risks have not been supported by any epidemiological studies. Further, the recognised benefits of a diet rich in fruit and vegetables (and hence higher nitrate intakes) are supported by epidemiological studies. Overall, the scientific evidence indicates that maximum limits for nitrate in lettuce and spinach are not necessary providing the crops are produced under good agricultural practices. Further, the continued presence of any maximum limits for nitrate will almost certainly raise doubts in the minds of consumers of the benefits of eating a diet rich in fruit and vegetable.

Financial benefits

The introduction of EU limits for nitrate in lettuce, spinach and possibly other salad crops at or below the levels in current Commission Regulation No. 466/2001, without the option for member states to apply any derogations, would seriously affect the commercial viability of the UK lettuce and spinach sectors (and possibly at a later stage rocket and celery sectors). Evidence from surveys undertaken by the FSA and commercial data show that a significant proportion of the lettuces grown in greenhouses during the winter months exceed the current limits for nitrate. The UK protected crops industry must convince the EFSA Panel that benefits to consumers of eating a healthy diet which includes salads and other vegetables outweigh any risks to health from the presence of nitrate in these foods.

Action Points for Growers

A well argued submission to the EFSA on permissible nitrate levels in lettuce and spinach which is supported by other activities may ensure that the UK growers are able to continue producing lettuce and spinach and other protected crops throughout the year.

Science Section

Introduction

1. The European Commission has asked the European Food Safety Authority (EFSA) to provide a scientific risk assessment for the longer-term strategy for managing the risk from nitrate in vegetables. In particular, the Commission asked that "the assessment should take into account any relevant information on the risks and benefits, for example of the possible negative impact of nitrate versus the possible positive effects of eating vegetables." EFSA has referred this request to the Scientific Panel on contaminants in the food chain (CONTAM).

2. The need for advice arises from discussions to amend the current controls on nitrate in lettuce, spinach and certain other vegetables set out in Commission Regulation No. 1881/2006 (see Annex 1 for a summary of the limits for nitrate). The current controls reflect the opinion on nitrate and nitrite given by the Scientific Committee on Food (SCF) on 22 September 1995 which recognised that total intake of nitrate is normally below the acceptable daily intake of 3.7 mg/kg body weight. The SCF also recommended continuation of efforts to reduce exposure to nitrate via food and water while at the same time encouraging the greater consumption of fruit and vegetables. However, further scientific studies mainly on the benefits to human health from nitrate in the diet have been published since the SCF gave its opinion in 1995 which warrants this further review by CONTAM.

3. The purpose of this paper is to provide evidence to CONTAM which demonstrates that the need for maximum permitted levels of nitrate in lettuce and spinach in particular and other vegetables in general is not justified as recent scientific evidence indicates that nitrate in the diet has many beneficial effects. Further, any move to reduce the existing limits would raise doubts in the minds of consumers about the safety of eating any vegetables which is in direct conflict with current healthy eating campaigns which encourage the increased consumption of fruit and vegetables. Any reductions in the maximum levels could also threaten the commercial viability of glasshouse production of lettuce and other crops in the UK and other member states in northern and eastern Europe.

4. The paper does not consider the problems which might arise from the presence of nitrate in water which are very different from the issues surrounding nitrate in vegetables.

5. This paper summarises the key information available on the following issues:

(a) Reasons for considering nitrate differently from other contaminants in food
(b) The role nitrate plays in vegetables and factors affecting its levels.
(c) Levels of nitrate in lettuce and spinach
(d) Consumer exposure to nitrate from food and other sources.
(e) Toxicological evidence available since the SCF opinion in 1995
(f) Benefits of vegetable consumption versus any risks from exposure to nitrate.

(a) Nitrate as a component of food

6. Commission Regulation No. 1881/2006 establishes maximum levels for certain contaminants in food including nitrate, mycotoxins, heavy metals, dioxins and PCBs and polycyclic aromatic hydrocarbons (PAH). Each contaminant often has its own specific routes of entry into the food chain and therefore requires individual approaches to minimising the levels present in food. For example, following good agricultural practices during the growing and storage of crops will minimise the level of mycotoxins in raw materials. Further, it is also often possible to inspect these raw materials prior to their use in food manufacture to remove any defective material further reducing the level of mycotoxin (e.g. colour inspection of peanuts). Similarly, the levels of heavy metals, dioxins and PCBs and PAH in foods can be minimised by using appropriate agricultural practices and food manufacturing processes (e.g. minimising use of brass in food manufacturing plant, treating edible oils with activated carbon).

7. In contrast, nitrate is present in vegetables as a natural and essential component for growth of the vegetables (see below for a more detailed explanation) with no way known for its removal. The level of nitrate present in plant material can be reduced to some extent by producers following good agricultural practice but it is largely controlled by the level of light and other factors which producers are not able to control. In addition, plant breeding has failed to develop low nitrate cultivars of lettuce and other crops which emphasises the important metabolic role played by nitrate in plants. Further, many vegetables including lettuce are usually eaten raw and receive no processing (other than washing) prior to consumption so no opportunity exists to reduce nitrate levels. Those vegetables which are processed prior to consumption by peeling, washing, blanching or cooking such as spinach will have a reduced nitrate level but most nitrate remains in the vegetable.

8. For the above reasons, it is therefore not appropriate to treat nitrate in vegetables (as opposed to nitrate in water or beer) as a contaminant but as a natural component of food. As such, a more holistic approach to the presence of nitrate in vegetables is needed where both the risks and benefits from the presence of nitrate and also the wider benefits of vegetables in the diet are considered together.

(b) Role of nitrate in vegetables

9. Despite adherence to the code of good agricultural practice (GAP), the UK lettuce industry currently has no proven system that can guarantee nitrate levels in the harvested lettuce crops will meet the maximum levels established by Commission Regulation (EC) No. 1881/2006. For this reason, the Regulation provides the UK and certain other member states with a temporary derogation from these limits until 30 December 2008. This section reviews some of the research undertaken to investigate the factors which affect nitrate levels in lettuce.

10. The two main plant tissues involved in the transport of nutrients in plants are the xylem and phloem. The xylem carries water, nitrate and other nutrients from the roots where they have been absorbed to the leaves at a rate dependant on the extent to which the plant is transpiring. The phloem carries the products of photosynthesis from the leaves to the growing points of the plant where they are used to create new plant material. Early in the life of the plant, the main growth points are the tips of leaves, shoots and roots but storage organs such as seeds and tubers later become the main growth points and receive the bulk of the flow of the phloem. A key difference between

the xylem and the phloem is that nitrate is readily carried in the xylem but very little is carried in the phloem. This means that leaf crops such as lettuce and spinach tend to have fairly high nitrate concentrations whereas storage organs such as grain and beans have very low levels. Similar considerations also explain the differences in the nitrate levels between the stem and leafy tissue of vegetables.

11. Another consequence of the transport system is that young leaves which are the growing point of plants such as those in the centre of lettuce and fed through the phloem have relatively low levels of nitrate compared with older more strongly transpiring and outer leaves which are mainly fed by the xylem.

12. Nitrate has some uses in plants such as helping to maintain the plant's balance between positively and negatively charged ions. However, its main function is as the source of nitrogen needed by the plant for the synthesis of proteins and other nitrogenous compounds. The nitrate is reduced to ammonium which is changed to glutamine and other amines useful to the plant. This key process of nitrate assimilation is performed by the enzymes nitrate reductase and nitrite reductase. These enzymes operate in sequence with the first reducing nitrate to nitrite and the second reducing nitrite to ammonium. In leaves, the second step is located in the chloroplast, where photosynthesis takes place, and is coupled to the photosynthetic electron transport system. Nitrogen assimilation therefore depends on photosynthesis and hence light with greater light intensities leading to lower nitrate levels.

13. As explained above, nitrate in lettuces levels above the EU limits mainly occur after periods of low light levels, particularly in the winter. Low rates of photosynthesis in these instances result in slower plant growth that is not matched by a decrease in nitrate uptake from the soil. Nitrate uptake into the xylem of plants is a process that requires energy and it has also been shown that nitrate itself can stimulate its own uptake(5). Genetic or environmental factors that decrease nitrate reductase and nitrite reductase activity will increase the levels of nitrate accumulated in leaves.

14. Research in the UK has highlighted that a great deal of variation exists within heads of the same cultivar but no obvious differences between cultivars tested. The timing of harvest during the day did not significantly affect nitrate levels even on sunny days. However, there is a tendency for lower nitrate residues in lettuce after bright days than after dull weather. Other research (6) looked at the distribution of nitrate within the plant and confirmed that the lettuce heart contained the least nitrate (2880 mg/kg), surrounding leaves 4703 mg/kg and outer leaves 6000 mg/kg. This agrees with work in other areas and highlights the value of removing some of the older leaves as a means of decreasing nitrate in the product at point of sale.

15. Projects funded by the UK's Horticultural Development Council (HDC) have also addressed the issue of nitrate levels in lettuce. These studies found a degree of variability in nitrate residues in heads as well as variation between cultivars. Agronomic strategies were pursued that led to a decrease in nitrate in the leaves (delayed harvest, time of harvest, fertiliser use) but these were often shown to have negative implications for crop quality. Further work looked more closely at strategies for using fertilisers and nitrate uptake characteristics in lettuce as a means of achieving a reduction in levels in leaves. This work also confirmed that it is the outer (older) leaves that have the highest levels of nitrate, with values of over 6000 ppm being recorded (6). Removal of these

leaves is an option that was recommended, provided sufficient younger foliage is present to achieve target head weights.

16. Previous scientific investigations have focussed on nitrogen nutrition in lettuce cultivars with the intention of understanding how this input can be used optimally (7,8). Some interesting relationships with regard to light levels and nitrogen level have been established which suggest that, during periods of low light level, maximum radiation use efficiency (rate of biomass accumulation per unit of photosynthetically active radiation) is achieved at lower nitrogen levels than at higher irradiance (7). This work also suggested a trade-off for nitrogen between the maintenance of a certain organic nitrogen (per unit leaf area) concentration for photosynthesis, and expansion of the canopy for light interception.

17. Other research has addressed the relationships between crop growth, total-nitrogen and nitrate concentration by both experimentation and dynamic model simulation (8). Lettuces were maintained using a nutrient film technique system for 74 days, with three treatments: control (continual nutrient supply), T1 (nutrient supply withdrawn after 35 days) and T2 (nutrient supply withdrawn after 54 days). It was found that nitrate and organic-N gradually declined in control plants up to 800 effective day-degrees (function of accumulated light and temperature) after which point the nitrate rose rapidly. Interestingly, in T1 and T2 treatments rapid and almost complete loss of nitrate occurred. However, this nutrient withdrawal also led to a significantly large decrease in plant growth.

18. Two models for the control of nitrate in leaf tissue have been proposed. In the simpler model, plant nitrate content is viewed as an imbalance between net absorption and assimilation, these factors being affected by both endogenous (genetic) and exogenous (nutrition, climate) factors (9). Thus, in some way, nitrate is responsible for its own regulation by some form of feedback control. Another, more complex model termed the ‘turgor maintenance concept’ is suggested (9). This is based on the observation that nitrate level is negatively correlated with the level of non-structural carbon compounds and that these have a combined role in turgor maintenance. Whichever model holds true, the water relations, or manipulation thereof, of the plant will be fundamental in either the delivery of nitrate to the shoots or maintenance of cell turgor.

19. It is considered that allowing the soil to dry could reduce nitrate delivery to the plants in several ways. As the soil dries, nitrate delivery to shoots can be limited by a) reduced supply through the soil to the root surface, b) reduced activity of nitrate transporters in the root membranes and c) reduced flux of water into and through the plant. Over the past 12 years, HDC projects have been undertaken to gain a better understanding of the influence of a range of agronomic factors on nitrate accumulation. These factors have usually been associated with aspects of the Good Agricultural Practice and have included:

1. Studying the effect of shading
2. Studying the effect of soil nitrogen fertiliser rate
3. Comparing trickle irrigation and overhead irrigation
4. Studying the effect of partial root drying#
5. Studying the interaction between the rate of nitrogen fertiliser and irrigation method

6. Comparing the effect of reduced light levels in the month prior to harvest

Partial root drying is a mechanism that exposes half of a root system to drying conditions, whilst maintaining the other half in a well-watered state. This situation is reversed after a defined period of time such that the root system is subject only to a mild drought stress before full irrigation is resumed. Whilst the well-watered side of the roots enables plant water relations to be maintained, a hormone signal is generated in the drying side that can influence shoot growth and nutrient dynamics.

20. The research described above has been used to inform the development of Good Agricultural Practices which are intended to minimise the levels of nitrate in lettuces although these do not guarantee that crops will meet the current maximum levels for nitrate. For example, the crop protocol for protected lettuce developed by Assured Produce in the UK sets out the best existing production practices including a section which lists the procedures for minimising nitrate levels while at the same time producing a crop which meets the exacting standards sets by supermarkets and other purchasers of lettuce. The procedures cover the following key points:

- light maximisation by using the best light-transmitting houses and regular glass cleaning
- fertiliser usage including the requirement for regular nitrate/nitrogen analysis
- crop spacing to allow some trimming of outer leaves
- monitoring the nitrate of lettuce including sampling and analysis
- record keeping of soil analyses, fertiliser applications, planting dates and analytical results.

Growers who follow these standards are monitored by independent assessors to ensure that they observe the crop protocols. Full details of the Assured Produce protocols are on its website; www.assuredproduce.co.uk

21. In summary, recent research has improved agricultural practices and emphasised the importance of such factors as growing the best varieties of lettuce, cleaning glass and keeping nitrate in soil below 100 mg/kg. However, growers are still not able to be certain that their crops will be below the maximum levels for nitrate particularly in periods of low light intensity.

(c) Levels of nitrate in lettuce and spinach

22. Commission Regulation (EC) No 1881/2006 required all member states to carry out monitoring of nitrate in lettuce and spinach and report the results annually to the European Commission. The last results reported for the UK were for 2004 (4) which reported the analytical results for 118 samples of lettuce and 82 samples of spinach – see Table 2.1. Twelve samples (10%) of lettuce and five samples of spinach exceeded the EU limits although the UK has implemented the derogation allowed under the above regulation. The results were similar to those found in earlier years.

Table 2.1: Summary of UK results for nitrate in lettuce and spinach from 2004 monitoring programme

(a) Glasshouse lettuce (not Iceberg)

Season	Number of samples	Number above EU limit (%)	EU limit (mg/kg)	Mean nitrate (mg/kg)	Range (mg/kg)
Summer	18	6 (33%)	3500	2999	676 - 4384
Winter	33	5 (15%)	4500	3617	1945 - 5720

(b) Outdoor lettuce (not Iceberg)

Season	Number of samples	Number above EU limit (%)	EU limit (mg/kg)	Mean nitrate (mg/kg)	Range (mg/kg)
Summer	62	1 (1.6%)	2500	1140	181 - 2656
Winter	5	0 (0%)	4000	1997	810 - 3100

23. Other member states have reported results from similar monitoring programmes. For example, a recent paper has reported the monitoring results from Sweden for 1996 to 2005 (4). The results of this programme were similar to those of the UK with nitrate levels in spinach (63 samples) averaging 1747 mg nitrate/kg and 19% of samples exceeding the EU maximum levels. Average nitrate levels for lettuce and Iceberg lettuce were respectively 2684 mg nitrate/kg and 931 mg nitrate/kg with 4% and 1% of samples exceeding the EU maximum levels. However, it worth noting that all glass houses in winter in Sweden have lighting which would promote lower nitrate levels but this is only made possible by a degree of market protection with lettuce being over four times as expensive in Sweden than the UK.

24. It is evident from these surveys that a significant percentage of lettuce and spinach grown in northern and eastern Europe will exceed the current EU maximum levels particularly if the weather is overcast. Growers have adopted good agriculture practices which have undoubtedly had some effect at reducing nitrate levels. However, the low light levels often prevalent in winter in northern and eastern Europe are beyond the control of producers and may lead to an increase in nitrate levels to above the EU maximum. The use of artificial lighting is not commercially viable in the open markets found in the UK and most other member states.

25. Growers in the UK and other northern and eastern EU member states face the considerable risk that an unpredictable proportion of their lettuce and spinach crops will exceed the maximum levels for nitrate established by Commission Regulation No. 1881/2006 and therefore will not be marketable particularly in the winter months. These growers may not wish to take the commercial risk of growing these crops which will affect the viability of their entire operation leading to closure. This would have the obvious economic and social consequences for the producing areas. It is therefore essential that any longer-term strategy for managing any risk from nitrate in vegetables takes these wider factors into account.

(d) Consumer exposure to nitrate

26 Consumer exposure to nitrate arises from three major sources:

- food
- tap water and beer
- endogenous formation mainly from L-arginine

The actual exposure experienced by individual consumers will vary depending on their dietary habits although exposure to the bulk of the population will fall within a relatively small range.

27. Food is the major source of exposure to nitrate for most consumers. Table 2.2 shows the exposure to nitrate from food for UK consumers based on the 1997 Total Diet Study (1). The mean and 97.5 percentile exposures were estimated as 57mg nitrate/day and 105 mg nitrate/day. A duplicate diet study of vegetarians in the UK found that exposures from food ranged from a low of 52 mg nitrate/day to a high of 178 mg nitrate/day (2). These exposures are below the ADI for the nitrate ion of 3.7 mg/kg body weight (equivalent of 222 mg/day for a 60 kg person) established by the SCF in 1995. Consumer exposure reported for other member states in the SCF report on nitrate and nitrite published in 1995 (3) showed similar levels of exposure to those reported above for the UK. It is evident that from the data in Table 2.2 that vegetables account for about 75% of the total exposure with potatoes making the single largest contribution.

28. Tap water and beer can make a significant contribution to total nitrate exposure. If it is assumed that the average consumption of tap water is 1 litre/day and the average nitrate concentration in tap water is within the range of 10 mg/litre to 20 mg/litre, then the additional contribution to total nitrate exposure will be 10 mg/day to 20 mg/day. A MAFF survey of nitrate in beer carried out in 1988/89 found a mean nitrate concentration of 16 mg/kg. If it is assumed that the average consumption of beer is 0.7 l/day, the nitrate exposure from this source will be 11mg/day. Thus, the consumption of tap water and beer could give average consumer an additional exposure to nitrate of around 25 mg/day.

Table 2.2: Dietary exposure to nitrate in the UK (mg/person/day)
(See reference 1 for full explanation of data)

Food Group	**Mean exposure**	**97.5 percentile**
Bread	0.9	2.1
Misc. cereals	1.3	3.6
Meat and meat products (incl fish and eggs)	5.0	12.8
Sugars and preserves	0.3	1.0
Green vegetables	16	45
Potatoes	19	44
Other vegetables	8	23
Canned vegetables	0.9	2.8
Fresh fruit	2.2	7.5
Beverages	3.2	7.8
Milk	1.2	3.2
Dairy products	1.6	4.8
Nuts	0.01	0.2
Total	**57**	**105**

29 The main source of endogenous nitrate in mammals is the L-arginine-NO pathway which is constitutively active in numerous cell types throughout the body. Healthy adults are estimated to synthesise between 45 and 70 mg of nitrate/day which is comparable to the exposure from food.

30. The exposure data emphasise the importance of considering all dietary sources of nitrate and that maintaining or reducing maximum levels for nitrate in only lettuce and spinach, even if they are the vegetables which contain the highest levels of nitrate, will have relatively little impact on total consumer exposure to nitrate as they do not form a significant part of most people's diet. Data from the UK indicate that most consumers do not exceed the ADI set by the SCF in 1995 although some vegetarians may have exposures close to or exceeding the ADI particularly if exposure from water, beer and endogenous sources are included.

(e) Toxicological evidence available since the SCF opinion in 1995

31. Safety concerns surrounding nitrate have been extensively researched and originally focused on methaemoglobinaemia and possible carcinogenic risks particularly associated with the endogenous formation of nitrosoamines. More recently, research has concerned the possible beneficial role of nitrate and more specifically the physiological role of nitric oxide - an area which received little consideration in the SCF opinion of 1995.

32. The key points which have emerged in recent years include:

(a) Concern about the safety of nitrate first arose when it was associated with cases of methaemoglobineamia in infants in rural areas fed reconstituted baby food made with water from wells. Methaemoglobineamia was originally attributed simply to the presence of nitrate in the water from these wells. However, it is now recognised that both nitrate and a high level of bacterial contamination of the well water are needed to cause methaemoglobineamia. The proposed mechanism for infant well-water methaemoglobinaemia is that the nitrate in the water is reduced to nitrite in the feeding bottle by bacterial nitrate-reductase enzymes when high levels of bacteria are present. After consumption by the infant, the nitrite is absorbed and passes from the stomach and the upper small intestine into the bloodstream where the infant's haemoglobin is partly oxidised to methaemoglobin. It is likely that very young infants under 6 months old are more susceptible to methaemoglobineamia than older infants and adults as their methaemoglobin reductase enzyme in their red blood cells has not yet reached its full activity.

(b) Many studies have investigated the possible endogenous formation of carcinogenic N-nitroso compounds following the ingestion of nitrate. However, no quantitative evidence exists that this is a significant risk. In contrast, convincing evidence exists to show that the consumption of vegetables (i.e. high intake of nitrate) is associated with a reduced cancer risk in humans.

(c) Nitrite and other reactive nitrogen intermediates formed from nitrate have been shown to have antimicrobacterial effects through the synergistic enhancement of gastric acid's antibacterial activity

(d) Nitric oxide (derived from nitrate) is a potent regulator of cell function and tissue viability with a range of potential beneficial effects from reducing blood pressure to the inhibition of platelet aggregation which may account for the evident cardioprotective effects of diets with a high content of fruit and vegetables.

33. These issues are reviewed in several recent publications (11,12) and a more detailed literature search which CONTAM and its Working Group on nitrates in vegetables will undoubtedly undertake will provide an extensive list of further references. Other organisations including the UK's Food Standards Agency have already provided lists of relevant references for CONTAM to consider.

34. It is evident from published scientific papers that the nitrate in the diet from vegetables has some beneficial effects which must be balanced against the possible risks identified by, for example, traditional animal feeding studies. However, extensive epidemiological studies on nitrate have failed to demonstrate an association with cancer risk in man while convincing evidence exists showing the consumption of vegetables is associated with a reduced cancer risk in humans.

(f) Benefits of vegetable consumption versus any risks from exposure to nitrate.

35. Most advice from Government departments such as the UK's Food Standards Agency recommends that people should eat at least five portions of fruit and vegetables each day - potatoes are excluded from this advice as they are considered essentially a starchy food. The scientific basis for this advice is that fruit and vegetables are good sources of many essential vitamins and other beneficial chemicals including minerals, fibre and anti-oxidants. In addition, there is mounting evidence that that people who eat lots of fruit and vegetables are less likely to develop chronic diseases such as coronary heart disease and most types of cancer.

36. The SCF in its Opinion of 1995 noted these findings of the beneficial effects of diets rich in fruit and vegetables and further stated that “concern over the presence of nitrate should not, however, discourage the consumption of vegetables”. However, this is effectively contradicted by a further recommendation of the SCF which “recommends the continuation of efforts to reduce exposure to nitrate via food and water” because green vegetables and potatoes account for between 70% and 90% of total dietary exposure to nitrate and any increase in the consumption of vegetables will inevitably increase consumer exposure to nitrate. Research to reduce nitrate levels in lettuce through both plant breeding and improved agricultural practices has met with relatively little success as the major determinant of nitrate levels is the light level immediately prior to harvest. Similar results are likely for other green vegetables as nitrate plays a similar role in these vegetables while potatoes and other root crops contain relatively stable amounts of nitrate.

37. Current dietary advice strongly promotes the increased consumption of fruit and vegetables which will inevitable lead to an increased intake of nitrate. This emphasises the need for CONTAM to ensure that it gives appropriate weight to the potential benefits of dietary nitrate and avoids any ambiguities in its risk assessment of nitrate.

Conclusion

38. Nitrate is a natural component of vegetables fulfilling an essential role in plants whose levels are not affected to any significant extent by either good agricultural practices or plant breeding. The main factor affecting nitrate levels in lettuce and spinach and other leafy plants is the light level at harvest time. Recent scientific evidence indicates that the beneficial effects of nitrate in the diet are of greater significance than the potential risks from nitrate identified by animal feeding studies although evidence of these risks have not been supported by any epidemiological studies. Further, the recognised benefits of a diet rich in fruit and vegetables (and hence higher nitrate intakes) are supported by epidemiological studies. Overall, the scientific evidence indicates that maximum limits for nitrate in lettuce and spinach are not necessary providing the crops are produced under good agricultural practices. Further, the continued presence of any maximum limits for nitrate will almost certainly raise doubts in the minds of consumers of the benefits of eating a diet rich in fruit and vegetable.

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References

1. **UK Ministry of Agriculture, Fisheries and Food (1998)** 1997 Total Diet Study – Nitrate and Nitrite, Food Surveillance Paper No.163.

2. **UK Ministry of Agriculture, Fisheries and Food (**1998) MAFF UK – Duplicate diet study of vegetarians – nitrate analysis. Food Surveillance Paper No. 165

3. **Commission of the European Communities (1995)** Report of the Scientific Committee for Food Opinion on Nitrate and Nitrite (Thirty-eighth Series). [Opinion expressed on 22 September 1995]

4. **Merino, L., Darnerud, P.O.,Edbueg, U., Aman, P., and Castillo, M.D.P**. *Food Additives and Contaminants*, 2006, **23,** 1283-1289.

5. **Byrne, C., Maher, M.J. and Hennerty, M. J.** (2001). Reducing the nitrogen content of protected lettuce crops. *Irish Journal of Agricultural & Food Research* **39** 3 491

5 **Taiz, L. and Ziezer, E. (2006) Plant Physiology, 3rd Edition, Sunderland:Sinaur Associates**

6 **Davies, W.J., Bacon, M.A., Thompson, D.S., Sobeih, W., Gonzalez-Rodriguez, L.** (2000). Regulation of leaf and fruit growth in plants growing in drying soil: Exploitation of the plants' chemical signalling system and hydraulic architecture to increase the efficiency of water use in agriculture. *Journal of Experimental Botany* **51** 350 1617-1626

7. **Henriques, A.R.P. & Marcelis, L.F.M** (2000). Regulation of growth at steady-state nitrogen nutrition in Lettuce: Interactive effects of nitrogen and irradiance. *Annals of Botany* **86** 1073-1080

8. **Broadley, M.R., Seginer, I., Burns, A., Escobar-Gutierrez, A.J., Burns, I.G., White, P.J.** (2003). The nitrogen and nitrate economy of butterhead lettuce. *Journal of Experimental Botany* **54** 390 2081-2090

9**. Cardenas-Navarro, R., Adamowicz, S., Robin, P.** (1999). Nitrate accumulation in plants: a role for water. *Journal of Experimental Botany* **50** 334 613-624

10. **Buwalda, F. & Warmenhoven, M.** (1999). Growth-limiting phosphate nutrition suppresses nitrate accumulation in greenhouse lettuce. *Journal of Experimental Botany* **50** 335 813-821

11. **McKnight, G.M., Duncan, C.W., Leifert, C. and Golden, M.H.** (1999) Dietary nitrtate in man: friend or foe? *British Journal of Nutrition* **81,** 349-358

12. **Lundberg, J.O., Weitzberg,E., Cole, J.A.and Benjamin,N.** (2004) Nitrate, bacteria and human health. *Nature Reviews/Microbiogy* **2** 593-602

Annex 1- Summary of the maximum levels for nitrate in lettuce and spinach. (European Commission Regulation (EC) No. 1881/2006)

Product	**Harvest period**	**Maximum level (mg nitrate/kg)**
Fresh spinach	Harvested 1 Oct. to 31 March	3000
	Harvested 1 April to 30 Sept.	2500
Preserved frozen spinach		2000
Fresh lettuce (excluding Iceberg)	Harvested 1 October to 31 March	
	Grown under cover	4500
	Grown in the open air	4000
	Harvested 1 April to 30 September	
	Grown under cover	3500
	Grown in the open air	2500
Iceberg type	Grown under cover	2500
	Grown in the open air	2000

The UK has a derogation until 31 December 2008 to the above maximum levels for lettuce and spinach grown and marketed in the UK. This derogation recognises that developments in the application of good agriculture practice have not produced the hoped for reductions in nitrate levels and growers have difficulty meeting the existing maximum levels for nitrate particularly in the winter months when light levels are often low as that.