

## **UK MONITORING PROGRAMME FOR NITRATE IN LETTUCE AND SPINACH 2000-2002**

### **Key Facts**

- Nitrate is a natural component of vegetables although the levels present are affected by growing conditions, fertiliser use and plant species / variety.
- The UK has carried out annual monitoring for nitrate in lettuce and spinach since 1996. Every Member State in the European Union is required to monitor and report levels of nitrate in lettuce and spinach as part of a European Commission Regulation.
- European Commission Regulation 563/2002 sets maximum levels for nitrate in lettuce and spinach, but it also permits UK growers to be exempted from meeting these limits for a transitional period ending on the 1 January 2005 (derogation period).
- The UK is applying the optional derogation in order to allow UK growers to meet the limits through changes in agricultural practice (ie applying the Code of Good Agricultural Practice). Consumers are protected because we still have to ensure, through monitoring, that nitrate levels do not exceed safety limits. Our estimates demonstrate that UK consumers are not exposed to unsafe levels of nitrate through consumption of lettuce and spinach.
- The levels of nitrate detected in lettuce and spinach in the reported surveys do not pose a risk to consumer's health as dietary exposure is assessed to be within the Acceptable Daily Intake (ADI) set by the European Commission's Scientific Committee for Food (SCF). No regulatory or other action has been taken.
- Lettuce and spinach make an important contribution to good health and their consumption, as part of a balanced diet, is encouraged.
- The Agency will continue to monitor the incidence and levels of nitrate in lettuce and spinach as required by EU legislation.

## Summary

The results of the 2000, 2001 and 2002 Monitoring Programmes for nitrate in lettuce and spinach are reported. Samples of UK lettuces and spinach were obtained from 7 growing regions (Southeast England, Humberside, East Anglia, Lancashire, Southwest England, Scotland and Northern Ireland). Local Authority Trading Standards Officers co-ordinated the collection of samples and Public Analyst laboratories performed the analyses for nitrate. Samples of imported lettuce and spinach were also analysed in this survey.

For the year 2000, 181 samples of lettuce were analysed with results in the range 20 - 2689 mg/kg for summer outdoor crops, 202 – 4600 mg/kg for summer indoor crops, 480 - 3300 mg/kg for winter outdoor crops and 1244 – 4700 mg/kg for winter indoor crops. Six UK samples (3 per cent) tested would have been above the regulatory limit if it were applied in the UK. Thirty-nine samples of spinach were analysed with the results in the range 186 - 4290 mg/kg for summer crops and 330 – 3660 mg/kg for winter crops. Four samples (2 from the UK and 2 from the EU) would have been above the regulatory limit if it applied. Details of these are provided in tables 10, 13 and 15.

For the year 2001, 188 samples of lettuce (159 from the UK and 29 from elsewhere in the EU) were analysed with results in the range 136 – 2420 mg/kg for summer outdoor crops, 300 – 3900 mg/kg for summer indoor crops, 270 – 3700 for winter outdoor crops and 472 – 4340 mg/kg for winter indoor crops. Three UK samples (2 per cent) tested would have been above the regulatory limit if it were applied in the UK. No samples of other EU lettuce were above the regulatory limit. Forty-six samples of spinach (36 from the UK and 10 from elsewhere in the EU) were analysed with results in the range <100 – 4010 for summer crops and 1070 – 2980 for winter crops. Two UK samples (4 per cent) would have been above the regulatory limit if it were applied in the UK. Two EU samples (4 per cent) were above the regulatory limit. These samples did not represent a risk to food safety.

For the year 2002, 167 samples of lettuce excluding the iceberg variety (140 from the UK and 27 from other EU countries) were analysed with results in the range 142 – 3064 mg/kg for summer outdoor crops, 1654 – 3527 mg/kg for summer indoor crops, 269 – 3979 for winter outdoor crops and 429 – 5045 mg/kg for winter indoor crops. Eight UK samples (5 per cent) tested would have been above the regulatory limit if it were applied in the UK. One sample of

EU lettuce (4 per cent) was above the regulatory limit. Thirty-three samples of iceberg lettuce (27 from the UK and 6 from the EU) were analysed with results in the range 24 – 2303 for summer crops and 528 – 2691 for winter crops. No UK samples would be above the regulatory limit if it were applied in the UK. Two EU samples were above the regulatory limit. Sixty-two samples of spinach (39 from the UK, 23 from elsewhere in the EU) were analysed with results in the range 122 – 3876 for summer crops and 416 – 4583 for winter crops. Eleven UK samples (18 per cent) would be above the regulatory limit if it were applied in the UK. Eight EU samples (13 per cent) were above the regulatory limit. No samples of frozen, canned or preserved spinach were taken.

Taking into account nitrate intake from the rest of the diet, the mean nitrate levels found in the UK Monitoring Programme are not likely to cause even high-level (97.5th percentile) consumers of lettuce or of spinach to exceed the SCF ADI.

## **Background**

### ***Nitrate in food***

Nitrate is a natural component of vegetables although the levels present are affected by growing conditions, fertiliser use and plant species / variety. Light is the main influence on nitrate concentrations in plants. Plants take up nitrogen in the form of nitrate and convert it into proteins via amino acids (photosynthesis). Poor light (such as in winter or during cloudy spells in summer) can result in a lower rate of photosynthesis, creating an accumulation of nitrate in the tissues.

Vegetables are the main source of nitrate in the diet and contribute 70 to 90 per cent of the total intake.<sup>1</sup> Green leafy vegetables, such as lettuce and spinach, contain higher levels of nitrate than other foods.

Studies of nitrate in food have demonstrated the possibility of both beneficial and detrimental health effects<sup>2-6</sup>. Recent research suggests that dietary nitrate may have beneficial effects based on the actions of nitric oxide in the gut<sup>4-6</sup>. In 1995, the EC Scientific Committee on Food (SCF) agreed to retain its earlier Acceptable Daily Intake (ADI) for the nitrate ion of 3.65 mg/kg body weight (equivalent to 219 mg/day for a 60 kg person)<sup>1</sup>. An ADI is an estimate of the amount of a substance, expressed on a bodyweight basis, that can be

ingested daily over a lifetime without appreciable health risks. In this case, the safety guideline was set to protect against possible harmful effects of nitrate in the diet, such as a form of anaemia that is most likely to occur in young children. Many studies have investigated the possibility of a link between nitrate intake and its conversion to cancer-causing compounds, but so far these have failed to provide convincing evidence that nitrate intake in the UK causes cancer.

### ***European Commission legislation on nitrate in lettuce and spinach***

The Contaminants in Food Regulations 1997 (SI [1997] 1499) implement European Commission Regulations, setting maximum levels for nitrate in lettuce and spinach. The limits vary according to season with higher nitrate levels permitted in crops grown in winter compared with those grown in summer and, for lettuce, different limits apply to glasshouse-grown and outdoor crops. The EC Regulation was recast on 8 March 2001 to form part of Regulation (EC) 466/2001, which set maximum limits for a number of contaminants in foodstuffs. The European Commission's review of nitrate in lettuce and spinach, which took place during 2001, resulted in Commission Regulation (EC) No 563/2002, amending Regulation (EC) 466/2001, which was implemented in the UK on 5 April 2002. The amendments are as follows:

- Limits for winter grown outdoor head and leaf lettuce reduced from 4500 to 4000 mg/kg fresh product.
- Limits for outdoor head and leaf lettuce grown between 1 April and 30 September reduced from 3500 to 2500 mg/kg fresh product.
- Separate limits were introduced for indoor iceberg lettuce of 2500 mg/kg and outdoor iceberg lettuce of 2000 mg/kg.
- The optional derogation operated by some Member States (including the UK) shall, in the case of lettuces, cease on 1<sup>st</sup> January 2005. With regard to spinach, the derogation shall be reviewed no later than 1<sup>st</sup> January 2005.

The current limits for nitrate in lettuce and spinach are summarised in Table 1.

### ***Derogation (Exemption from the Regulation)***

Most lettuce and spinach grown in the UK meets the EC levels for nitrates; but some summer spinach and protected lettuces fail to meet these levels when weather during the growth

period, or more particularly prior to harvest, has included cloudy spells. Other Member States also experience these problems.

The current Regulation allows for an optional derogation (exemption) from the limits for nitrate in lettuce and spinach grown and sold in individual Member States for a transitional period.<sup>7</sup> This is provided that nitrate levels in these foods are acceptable on public health grounds and that growers follow Good Agricultural Practice (GAP). GAP is implemented through a product assurance scheme operated through the National Farmers Union (NFU) and the major retailers. All vegetable growers have to be part of the scheme to sell to the major retailers. Details of the Code of GAP are available from the Department for Environment, Food & Rural Affairs (Defra). Lettuce and spinach imported into the UK from other EC Member States and third countries are not exempt from the maximum limits.

### ***UK Monitoring Programme for Nitrate in Lettuce and Spinach***

It is a requirement of the current Regulation that all Member States carry out monitoring for nitrate in lettuce and spinach and report the results annually to the European Commission. Data on nitrate levels in lettuce and spinach obtained from Monitoring Programmes carried out in each Member State were used as part of the review of the EC Regulation in 2001.

The UK Monitoring Programme for Nitrate in Lettuce and Spinach has been carried out since May 1996. Results for previous surveys are reported in MAFF Food Surveillance Information Sheets 121,<sup>9</sup> 154,<sup>10</sup> and Food Standards Agency Food Survey Information Sheet 16/01.<sup>11</sup>

## **Methodology**

### ***Sampling***

The number of samples taken in the 2000, 2001 and 2002 UK Monitoring Programmes was in accordance with European Commission guidelines<sup>12</sup>. These guidelines state that 1 sample is to be taken per 2000 tonnes of production or import with a minimum of 12 samples and a maximum of 120 samples a year for 1 product from each Member State<sup>13</sup>.

Trading Standards Officers collected samples of UK and imported produce. Samples were taken in accordance with Commission Directive 79/700/EEC. At all stages, samples were kept below 10°C and reached the laboratory within 24 hours of harvest or purchase where

they were maintained at or below 10<sup>0</sup>C. They were then analysed within 3 working days of receipt.

Four types of UK produce were sampled according to the EU requirements: glasshouse-grown lettuce, outdoor lettuce and spinach (fresh and canned). For 2000, samples of glasshouse-grown lettuce were taken throughout the year, while samples of outdoor lettuce and fresh spinach were taken between April and November. Samples of canned spinach were collected in November and December. For 2001, samples of glasshouse-grown lettuce and outdoor lettuce were taken throughout the year. Samples of fresh spinach were collected between April and November. Samples of canned spinach were collected between October and March. For 2002, samples of glasshouse-grown lettuce and outdoor lettuce were taken throughout the year, while iceberg lettuce was collected between June and December. Samples of fresh spinach were collected throughout the year. No samples of canned, frozen or preserved spinach were taken in 2002.

### **Analyses**

Samples of lettuce and spinach were prepared and analysed in accordance with European Commission Guideline Document VI/4800/96<sup>13</sup>. Each sample comprised 10 heads of lettuce or packets/boxes of spinach, which were combined and homogenised to produce a representative test sample<sup>13</sup>. The guidelines do not specify particular analytical methods, but set criteria for analytical performance (including recovery and precision) of the methods used.

All samples were analysed by Public Analysts' laboratories. Their methods meet the assessment criteria set out in Article 3 of Council Directive 93/99/EEC<sup>14</sup> relating to official food laboratories. All laboratories participated, and achieved satisfactory performances in the nitrate rounds of the Food Analysis Performance Assessment Scheme (FAPAS).

### **Measurement Uncertainty**

All analytical results have an associated variability termed "measurement uncertainty". For any analytical method the result is reported as the best estimate for the samples and it is always qualified by a measurement uncertainty (MU), e.g. x micrograms/kg ± y microgram/kg. In this case measurement uncertainty was calculated from in-house quality control samples using an accepted approach consistent with Eurochem guidelines. The standard deviation of results

obtained from analysis of in-house quality reference materials (75 analyses) generated data on in-house reproducibility. In accordance with commonly accepted practice, this standard deviation was multiplied by a factor of 2 ("k") to give expanded measurement uncertainty. A single figure for measurement uncertainty applicable throughout the whole range of results was calculated as  $\pm 146$ mg/kg nitrate.

## **Results**

### ***UK glasshouse-grown lettuce 2000 crop***

A total of 61 samples of glasshouse-grown lettuce were taken from Southeast England, Southwest England, Humberside, East Anglia, Lancashire, Scotland, and Northern Ireland. The results of the analyses of these samples are summarised in Table 2. Nitrate concentrations were higher during the winter months (i.e. October to March) as expected, with a mean level of 3017 mg/kg, than in the summer months (i.e. April to September) when the mean nitrate level was 2173 mg/kg. Three samples of the 24 indoor lettuces obtained in the summer and 2 of the 37 lettuce samples obtained in the winter would have exceeded the maximum level specified in the EC Regulation if it had been applied.

### ***UK glasshouse-grown lettuce 2001 crop***

A total of 60 samples of glasshouse-grown lettuce were taken from Southeast England, Southwest England, Humberside, East Anglia, Lancashire, Scotland, and Northern Ireland. The results of the analyses of these samples are summarised in Table 3. Nitrate concentrations were higher during the winter months (i.e. October to March) with a mean level of 2998 mg/kg, than in the summer months (i.e. April to September) when the mean nitrate level was 2322 mg/kg. Three samples of the 36 indoor lettuces obtained in the summer would have exceeded the maximum level specified in the Regulation if applied. None of the 24 lettuce samples obtained in the winter exceeded the maximum level specified in the Regulation if it had been applied.

### ***UK glasshouse-grown lettuce 2002 crop***

A total of 40 samples of glasshouse-grown lettuce were taken from South East England, South West England, Humberside, East Anglia, Lancashire, Scotland, and Northern Ireland. The results of the analyses of these samples are summarised in Table 4. Nitrate concentrations were higher during the winter months (i.e. October to March) with a mean level

of 3238 mg/kg, than in the summer months (i.e. April to September) when the mean nitrate level was 2613 mg/kg. Three samples (2 winter, 1 summer) would have exceeded the maximum level specified in the Regulation if applied.

The nitrate levels from the Monitoring Programme from 2000 - 2002 for UK-grown glasshouse lettuce are similar to those that have been reported in previous years. The mean nitrate levels in the 1999 programme were 3158 mg/kg and 2247 mg/kg, and in 1997/1998 were 3432mg/kg and 2545mg/kg<sup>9</sup>, for the winter and summer seasons respectively.

### ***UK outdoor lettuce 2000 crop***

A total of 92 samples of outdoor lettuce were taken from South East England, East Anglia, Lancashire, Humberside, South West England, Scotland, and Northern Ireland. Only 1 of the 54 samples taken in the mid-summer period would have been above the EC maximum levels if they applied, as shown in Table 5. No samples were over the maximum levels set down in the Regulation in the early / late-summer periods or winter. The results of the 2000 Monitoring Programme are compared with those found in previous years. Mean nitrate levels for the period May to August in 2000 were 709 mg/kg, and for the same period in 1996, 1997/1998 and 1999 they were 1089 mg/kg, 937 mg/mg and 1045 mg/kg respectively. For samples taken between 1 September and 30 September 2000, the mean nitrate level was 1143 mg/kg. The mean nitrate levels in 1997 / 1998 and 1999 for the same period were 850 mg/kg and 1090mg/kg.

### ***UK outdoor lettuce 2001 crop***

A total of 99 samples of outdoor lettuce were taken from South East England, East Anglia, Lancashire, Humberside, South West England, Scotland, and Northern Ireland. None of the samples taken in the summer period (88 samples) nor any of the winter samples (11) would have been above the EC maximum levels if they applied, as shown in Table 6.

### ***UK outdoor lettuce 2002 crop***

A total of 100 samples of lettuce grown outdoors were taken from South East England, East Anglia, Lancashire, Humberside, South West England, Scotland, and Northern Ireland. The results of these analyses are summarised in Table 7. As in previous years, higher nitrate concentrations were observed over the winter (mean of 1785 mg/kg) than over summer

(mean of 1219 mg/kg). Of the 75 samples taken over the summer, 5 (7%) would exceed the maximum permitted level if it applied to the UK.

The results of the Monitoring Programme from 2000-2002 for UK-grown outdoor lettuce are comparable to those found in previous years. Mean nitrate levels for the summer period (April-September) in 2001 were 1042 mg/kg, and for the same period in 1996, 1997/1998 and 1999 they were 1089 mg/kg, 937 mg/mg and 1045 mg/kg respectively. Mean nitrate levels for the winter period (January-March and October-December) in 2001 were 1350 mg/kg. The mean nitrate levels in 1997 / 1998 and 1999 for the same period were 850 mg/kg and 1090mg/kg.

#### ***UK iceberg lettuce 2002 crop***

Analyses of nitrate in iceberg lettuce are presented separately from 2002 due to the inclusion of a separate maximum permitted level for this variety of lettuce. Twenty-seven samples of UK-grown iceberg lettuce were analysed for nitrate concentration. The results of the analyses are summarised in Table 8. The 20 summer samples ranged from 164-1952 mg/kg and averaged 811 mg/kg. None of the samples analysed were above the maximum permitted level.

#### ***Imported iceberg lettuce 2002 crop***

Six samples of imported iceberg lettuce were analysed for nitrate concentration. The results of the analyses are summarised in Table 9. The 4 summer samples ranged in concentration from 24-2303 mg/kg and averaged 669 mg/kg. One sample from the summer crop was above the maximum permitted level. Two samples were taken from the winter crop, measuring 1348 and 2691 mg/kg respectively. The second value exceeded the maximum permitted level.

#### ***UK fresh spinach 2000 crop***

Twenty-four samples of UK produced fresh spinach were obtained, all in the summer period. Of the 24 samples taken 2 would be over the EC limit if it had applied (8 per cent). The mean level was 1554 mg/kg (Table 10). In 1997/1998 and 1999, the mean levels of summer samples were 2498 mg/kg and 1487 mg/kg, respectively.

### ***UK fresh spinach 2001 crop***

Twenty-six samples of UK produced fresh spinach were obtained, all in the summer period (April-September). Of the 26 samples taken 2 would be over the EC limit if it had applied (8 per cent). The mean level was 1270 mg/kg (Table 11).

### ***UK fresh spinach 2002 crop***

Thirty-nine samples of fresh UK-grown spinach were sampled over summer and winter. The samples were analysed for nitrate concentration and the results of these analyses are summarised in Table 12. The 27 summer samples averaged 2207 mg/kg while the 12 winter samples averaged 2349 mg/kg. Nine summer samples (33%) would have been over the maximum permitted level if it had applied to the UK, whereas 2 winter samples (17%) would have exceeded the limit.

### ***UK canned, frozen or preserved spinach 2000 crop***

Six samples of canned spinach were obtained throughout the year. The nitrate levels in these samples were all below the maximum of 2000mg/kg. The mean level was 678mg/kg (Table 13). Samples in 1997/1998 and 1999 were also below the maximum limit at a mean level of 787 mg/kg and 545 mg/kg respectively.

### ***UK canned, frozen or preserved spinach 2001 crop***

Ten samples of canned spinach were obtained during the winter period (January-March and October-December 2001). The nitrate levels in these samples were all below the maximum of 2000mg/kg. The mean level was 1338mg/kg (Table 14).

### ***UK canned, frozen or preserved spinach 2002 crop***

No samples of canned spinach were recorded during 2002 sampling. In order to perform estimates of exposure to nitrates, a value was taken from spinach sampled during a survey of retail salads conducted during 2002.

### ***Imported fresh spinach 2000 crop***

Nine samples of imported fresh spinach were obtained. Six of these were obtained in summer, between April-October and 3 in winter between November and December. The

mean nitrate level for the 6 summer samples and 3 winter samples were 1121mg/kg and 2137mg/kg respectively. One winter sample and one summer sample, both imported from Italy, exceeded the EC maximum limits (Table 15). However, once measurement uncertainty had been taken into account, the levels were below the EC limits and no action was deemed necessary.

#### ***Imported fresh spinach 2001 crop***

Ten samples of imported fresh spinach were obtained. Four of these were obtained in summer between April-September and 6 during the winter between January-March and October-December 2001. None of the winter samples exceeded the EC maximum limits. Two of the summer samples exceeded the EC maximum limits (Table 16). There is no information on the country of origin of these samples. The mean nitrate level for the 4 summer samples and 6 winter samples were 2559mg/kg and 2356mg/kg respectively.

#### ***Imported fresh spinach 2002 crop***

Of the 23 samples of imported fresh spinach obtained, 10 were from summer crops and the remaining 13 from winter crops. The results of the analyses are summarised in Table 17. For summer crops, the mean concentration was 1007 mg/kg, although 2 samples (20%) exceeded the maximum permitted levels limits. There is no information on the country of origin of these samples. For winter crops, the mean concentration was 2380 mg/kg, with 6 samples (46%) over the maximum permitted level.

#### ***Imported lettuce 2000 crop***

Twenty-eight samples of lettuce imported from other Member States were included in the UK Monitoring Programme (Table 18). None of these samples were over the EC specified levels.

#### ***Imported lettuce 2001 crop***

Twenty nine samples of lettuce imported from other Member States were included in the UK Monitoring Programme (Table 19). None of these samples were over the EC specified levels. The mean nitrate level for summer produce (April-September) was 987 mg/kg. The mean nitrate levels for winter produce (January-March and October-December 2001) was 1163 mg/kg.

### ***Imported lettuce 2002 crop***

Twenty-seven samples of imported lettuce were taken and analysed and the results are summarised on Table 20. The average concentration for the winter samples was 1571 mg/kg, while the average for the summer samples was 1753 mg/kg. None of the winter samples were over the maximum permitted level. It was not possible to calculate how many of the summer samples would have been over the maximum permitted level. This was because it was not possible for the sampling contractor to ascertain the growing conditions of the imported lettuce.

### ***Dietary exposure estimates***

Dietary exposures of nitrate have been estimated for adults, toddlers and school children (4-18 years) who eat average amounts of lettuce, spinach and foods containing them (mean consumers) and for those who eat significantly more than average (high level consumers). These estimates can then be compared with the ADI for nitrate set by the SCF. The ADI can be used as a guideline to assess whether nitrate exposure through lettuce and spinach consumption is a risk to consumer health. Within the calculations no account has been taken for the reduction of nitrate concentrations during cooking. A previous study has shown that nitrate concentrations in spinach are reduced by approximately 75 per cent by cooking (providing the cooking water is discarded and not used to make gravy or sauces)<sup>20</sup>.

The Food Standard Agency's INTAKE computer programme was used to combine the distribution of food consumption by adult<sup>15</sup>, young person (4-18 years)<sup>16</sup>, and toddler<sup>17</sup> consumers with the level of nitrate in foods in the diet. A recipe database provided consumption data on processed foods containing lettuce or spinach as an ingredient. Data on nitrate levels in lettuce and spinach were obtained from the 2000 – 2002 Monitoring Programmes. The 1997 Total Diet Study (TDS) on Nitrate and Nitrite<sup>18</sup> provided information on other sources of nitrate in foods such as potatoes and cured meat products. Estimates of the intake from water and beer were also included.

The mean and high level intakes (97.5<sup>th</sup> percentile) of nitrate in lettuce and spinach were calculated for the 2000, 2001 and 2002 crops (Table 21 and 22) for adults, toddlers and school children. The mean intake of nitrate from the total diet (based on the 1997 Total Diet Study<sup>18</sup>) was calculated for comparative purposes. These calculations of nitrate intake from

the Total Diet Study 1997<sup>16</sup> include some contributions from green vegetables (including lettuce and spinach) and from canned vegetables (including spinach). The estimates indicate that intake of nitrate from lettuce and spinach is likely to contribute a significant proportion to the total dietary intake of nitrate in most individuals. Therefore, estimates of total nitrate intakes, obtained by adding the values calculated from the results of this survey to those from the TDS, would result in an overestimation of total exposure to nitrate. Allowing for this overestimation, it is unlikely that consumers of any age would exceed the ADI for nitrate as a result of the concentrations in lettuce and spinach.

### ***Interpretation***

All the estimated nitrate dietary exposures for mean and high level consumers of lettuce and spinach were below the SCF's ADI for nitrate, 3.65 mg/kg bodyweight, which is equivalent to 219mg/day for a 60kg adult (Table 21 and 23). The optional derogation from the Regulation, which the UK is applying, allows lettuce and spinach grown and sold in the UK to be exempt from the maximum levels, provided that their nitrate content is not so high as to present a risk to health. Taking into account nitrate intake from the rest of the diet, the mean nitrate levels found in the UK Monitoring Programme are not likely to cause even high-level (97.5th percentile) consumers of lettuce or of spinach to exceed the SCF ADI.

### **Agency actions**

#### ***Analysis***

The laboratories chosen to analyse samples for this study operate a variety of nitrate extraction methods and analysis techniques within the criteria set by the European Commission<sup>13</sup>. It is important that the Agency has confidence in its data so a study was commissioned in 2001 to assess the comparability of the commonly used extraction and analytical methods and to recommend the most suitable method. The study indicated that laboratories should adopt a hot water extraction method. Method BS EN 12014-2:1997 has been shown to be robust and reliable and was verified by full collaborative trial. It is described in full in Food Standards Agency Bulletin on Methods of Analysis and Sampling of Foodstuffs No 16 (January 2001). Laboratories have been alerted to the importance of thorough homogenisation of samples prior to analysis. A report of this study can be obtained from the Agency library<sup>20</sup>.

## ***Sampling***

Studies have demonstrated that nitrate levels in lettuce and spinach are seldom uniformly distributed throughout the crop. For this reason, sampling procedures are required which allow the most accurate estimation to be made of nitrate contamination in lettuce and spinach in the field or at retail level. The EU sampling plan currently in use<sup>12</sup> was written for pesticide monitoring and requires that a sample consists of 10 heads of lettuce or boxes of spinach collected from the field in a predetermined pattern or 10 packs of lettuce or spinach taken from the same batch. The European Commission has expressed interest in developing a sampling plan specifically for lettuce and spinach and in response, the Agency has set up a study to characterise sampling variability caused by the non-uniform distribution of nitrate throughout lettuce and spinach crops. The results of this work will be made generally available when the project has been completed.

## ***Results***

The UK has carried out annual monitoring for nitrate in lettuce and spinach since 1996. Every Member State in the European Union is required to monitor and report levels of nitrate in lettuce and spinach as part of a European Commission Regulation. Data on nitrate levels in lettuce and spinach obtained from Monitoring Programmes carried out in each Member State have been used as part of the review of the EC Regulation in 2001.

The mean concentration of nitrate observed in lettuce remained fairly consistent from 2000 to 2002, although a slight rise was evident in some kinds of lettuce, specifically UK summer lettuce grown outdoors and UK summer lettuce grown indoors.

## **Summary of Agency actions**

None of the UK samples tested pose a risk to consumer's health as dietary exposure is likely to be within the ADI set by the SCF. No further action has therefore been taken, apart from the sending out of a notice under the Rapid Alert System for Food and Feed (RASFF). This notice was sent out from the UK in 2001 regarding a sample of fresh spinach from Portugal. The results of the UK Monitoring Programme have been submitted to the European Commission as have those from other Member States. The 2003/2004 UK Monitoring Programme is currently being undertaken. The Agency has an on-going nitrate research and development programme, including a comprehensive review on the toxicity of nitrate and a

study of sampling variability in lettuce and spinach. A survey of nitrate levels in retail lettuce, spinach and mixed salad products took place in 2002 and will soon be reported.

## **Conclusions**

Nitrate is a natural component of vegetables although the levels present are affected by growing conditions, fertiliser use and plant species / variety. The UK has carried out annual monitoring for nitrate in lettuce and spinach since 1996. Every Member State is required to monitor and report levels of nitrate in lettuce and spinach as part of European Commission Regulation (EC) 466/2001. The European Commission's review of nitrate in lettuce and spinach, which took place during 2001, resulted in Commission Regulation (EC) No 563/2002, amending Regulation (EC) 466/2001, which was implemented in the UK on the 5 April 2002. The Regulation sets maximum levels for nitrate in lettuce and spinach. However UK growers are currently exempted from meeting these limits for a transitional period. The levels of nitrate in lettuce and spinach sampled in this survey indicate that UK growers can generally meet maximum levels set by the Regulation although some results exceed these despite application of the Code of Good Agricultural Practice. None of the samples pose a risk to consumer health as dietary exposure is likely to be within the ADI set by the SCF. No further action has been taken. Consumers are advised that lettuce and spinach make an important contribution to good health and their consumption, as part of a balanced diet, is encouraged.

## **Further Information**

Dr Wendy Matthews

Food Standards Agency

Contaminants Division

Room 702c, Aviation House

125 Kingsway

WC2B 6NH

Tel: +44 (0) 20 7276 8707

Fax: +44 (0) 20 7276 8717

## References

1. Commission of the European Communities Scientific Committee for Food. Opinion on Nitrate and Nitrite, expressed on 22 September 1995. Annex 4 to Document **III/5611/95**, 1995.
2. Gangolli, S.D., van den Brandt, P.A., Feron, V.J., Janzowski, C., Koeman, J.H., Speijers, G.J.A., Spiegelhalder, B., Walker, R. and Wishnock, J.S. Assessment: nitrate, nitrite and N-nitrosocompounds. *European Journal of Pharmacology, Environmental Toxicity and Pharmacology*. 1994, Section **292**, 1-38.
3. Sugimura, T., Fujimura, S. and Baba, T. Tumour production in the glandular stomach of the rat by N-methyl-N<sup>1</sup>-nitro-N-nitroguanidine. *Cancer Research*. 1970, **30**, 455-465.
4. Duncan, C., Dougall, H., Johnston, P., Green, S., Brogan, R., Leifert, C., Smith, L., Golden, M. and Benjamin, N. Chemical generation of nitric oxide in the mouth from the enterosalivary circulation of dietary nitrate. *Nature Medicine*. 1995, **1**, 546-551.
5. Dykhuizen, R.S., Frazer, R., Duncan, C., Smith, C.C., Golden, M., Benjamin, N. and Leifert, C. Antimicrobial effect of acidified nitrite on gut pathogens: importance of dietary nitrate in host defence. *Antimicrobial Agents and Chemotherapy*. 1996, **40**, 1422-1425.
6. McKnight, G.M., Smith, L.M., Drummond, R.S., Duncan, C.W., Golden, M. and Benjamin, N. Chemical synthesis of nitric oxide in the stomach from dietary nitrate in humans. *Gut*. 1997, **40**, 211-214.
7. European Commission. Commission Regulation (EC) No 194/97 of 31 January 1997. *Official Journal of the European Communities No. L31/48*, 1997.
8. European Commission. Commission Regulation (EC) No. 563/2002 of April 2002. *Official Journal of the European Communities No. L86/45*, 2002.
9. Ministry of Agriculture, Fisheries and Food. 1996/97 UK Monitoring Programme for Nitrate in Lettuce and Spinach. *Food Surveillance Information Sheet No. 121*, 1997.
10. Ministry of Agriculture, Fisheries and Food. 1997/1998 UK Monitoring Programme for Nitrate in Lettuce and Spinach. *Food Surveillance Information Sheet No. 154*, 1998.
11. Food Standards Agency. 1999 Monitoring Programme for Nitrate in Lettuce and Spinach. Food Survey information sheet **16/01**, 2001
12. European Commission. Monitoring Programme Nitrates: Number of Samples. Document **VI/1655/97**, 1997.

13. European Commission. Guidelines for Laboratories Carrying Out The Determination of Nitrate in Lettuce and Spinach: EC Monitoring Programme. Document **VI/4800/96**, 1997.
14. Council of the European Communities. Council Directive 93/99/EEC of 29 October 1993 on the subject of additional measures concerning the official control of foodstuffs. *Official Journal of the European Communities No. L 290/14*, 1993.
15. Gregory, J., Foster, K., Tyler, H. and Wiseman, M. The Dietary and Nutritional Survey of British Adults. London: The Stationery Office, 1990.
16. Gregory, J., Lowe, S., Bates, C.J., Prentice, A., Jackson, L.V., Smithers, G., Wenlock, R., Farron, M. National Diet and Nutrition Survey: Young people aged 4 to 18 years. Volume 1: Report of the diet and nutrition survey. The Stationary Office, 2000.
17. Gregory, J.R., Collins, D.L., Davies, P.S.W., Hughes, J.M., Clarke, P.C. National Diet and Nutrition Survey; Children aged 1½ to 4½ years. Volume 1. Report of the Diet and Nutrition Survey. London: HMSO 1995.
18. Ministry of Agriculture, Fisheries and Food. 1997 Total Diet Study - Nitrate and Nitrite. Food Surveillance Information Sheet No. **163**, 1997.
19. Ministry of Agriculture, Fisheries and Food. Nitrate in Vegetables. Food Surveillance Information Sheet No. **158**, 1998.
20. Food Standards Agency. A comparison of the accuracy of extraction methods used in the nitrate residues monitoring programme. Final report, February 2002.

**Table 1**

**Summary of maximum levels in European Commission Regulation (EC) No. 563/2002, amending Regulation (EC) No. 466/2001**

Product	Harvest period	Max. nitrate levels (mg/kg fresh product)
Spinach (fresh)	1 November to 31 March	3000
	1 April to 31 October	2500
Preserved, deep-frozen or frozen spinach		2000
Lettuce (protected and open-grown lettuce)	1 October to 31 March	4500
	1 April to 30 September	3500
	with the exception of open grown lettuce harvested from 1 May to 31 Aug.	2500

**Table 2**

**Summary of results of nitrate analyses of UK glasshouse lettuce in 2000**

Season	Sampling dates	EC Max. level (mg/kg)	No. samples	Nitrate levels (mg/kg fresh weight)		Number exceeding max. level
				range	Mean	
Summer	1 Apr 2000 - 30 Sept 2000	3500	24	202-4600	2173	3
Winter	1 Jan 2000 – 31 Mar 2000 1 Oct 2000 – 31 Dec 2000	4500	37	1244-4700	3017	2

**Table 3**

**Summary of results of nitrate analyses of UK glasshouse lettuce in 2001**

Season	Sampling dates	EC Max. level (mg/kg)	No. samples	Nitrate levels (mg/kg fresh weight)		Number exceeding max. level
				range	Mean	
Summer	1 Apr 2001 - 30 Sept 2001	3500	36	300-3900	2322	3
Winter	1 Jan 2001 – 31 Mar 2001 1 Oct 2001 – 31 Dec 2001	4500	24	1300-4340	2998	0

**Table 4**

**Summary of results of nitrate analyses of UK glasshouse lettuce (excluding iceberg) in 2002. Note: 2002 limits from EC Reg 563/2002 apply.**

Season	Sampling dates	EC Max. level (mg/kg)	No. samples	Nitrate levels (mg/kg fresh weight)		Number exceeding max. level
				range	Mean	
Summer	1 Apr 2002 – 30 Sept 2002	3500	15	1654-3527	2613	1
Winter	1 Jan 2002 – 31 Mar 2002 1 Oct 2002 – 31 Dec 2002	4500	25	429-5045	3238	2

**Table 5**

**Summary of results of nitrate analyses of UK outdoor lettuce in 2000**

Season	Sampling dates	EC Max. level (mg/kg)	No. samples	Nitrate levels (mg/kg fresh weight)		Number exceeding max. level
				range	Mean	
Summer	1 April 2000- 30 April 2000	3500	2	703 -832	351.5	0
	1 May 2000- 31 Aug 2000	2500	54	20 -2689	709	1
	1 Sept 2000- 30 Sept 2000	3500	10	300 -2400	1143	0
Winter	1 Oct 2000 - 31 Dec 2000	4500	26	480 - 2200	1238	0

**Table 6**

**Summary of results of nitrate analyses of UK outdoor lettuce in 2001**

Season	Sampling dates	EC Max. level (mg/kg)	No. samples	Nitrate levels (mg/kg fresh weight)		Number exceeding max. level
				range	Mean	
Summer	1 April 2001-30 Sept 2001	3500	88	136-2420	1042	0
Winter	1 Jan – 31 Mar 2001 1 Oct – 31 Dec 2001	4500	11	270-2010	1350	0

**Table 7**

**Summary of results of nitrate analyses of UK outdoor lettuce (excluding iceberg) in 2002. Note: 2002 limits from EC Reg 563/2002 apply.**

Season	Sampling dates	EC Max. level (mg/kg)	No. samples	Nitrate levels (mg/kg fresh weight)		Number exceeding max. level
				range	Mean	
Summer	1 Apr 2002 - 30 Sept 2002	2500	75	142-3064	1219	5
Winter	1 Jan – 31 Mar 2002 1 Oct – 31 Dec 2002	4000	25	269-3979	1785	0

**Table 8**

**Summary of results of nitrate analyses of UK-grown iceberg lettuce in 2002. Note: all samples collected were grown outdoors.**

Season	Sampling dates	EC Max. level (mg/kg)	No. samples	Nitrate levels (mg/kg fresh weight)		Number exceeding max. level
				range	Mean	
Summer	1 Apr 2002 - 30 Sept 2002	2000	20	164-1952	811	0
Winter	1 Jan – 31 Mar 2002 1 Oct – 31 Dec 2002	2000	7	528-1097	822	0

**Table 9**

**Summary of results of nitrate analyses of imported iceberg lettuce in 2002. Note: all samples collected were grown outdoors.**

Season	Sampling dates	EC Max. level (mg/kg)	No. samples	Nitrate levels (mg/kg fresh weight)		Number exceeding max. level
				range	Mean	
Summer	1 Apr 2002 - 30 Sept 2002	2000	4	24-2303	669	1
Winter	1 Jan – 31 Mar 2002 1 Oct – 31 Dec 2002	2000	2	1348-2691	2020	1

**Table 10**

**Summary of results of nitrate analysis of UK fresh spinach in 2000**

Season	Sampling dates	EC Max. level (mg/kg)	No. samples	Nitrate levels (mg/kg fresh weight)		Number exceeding max. level
				range	Mean	

<b>Summer</b>	1 Apr – 31 Oct 2000	2500	24	186 - 4290	1554	2
---------------	---------------------	------	----	------------	------	---

**Table 11**

**Summary of results of nitrate analysis of UK fresh spinach in 2001**

Season	Sampling dates	EC Max. level (mg/kg)	No. samples	Nitrate levels (mg/kg fresh weight)		Number exceeding max. level
				range	Mean	
<b>Summer</b>	1 Apr – 31 Oct 2001	2500	26	<100-2800	1270	2

**Table 12**

**Summary of results of nitrate analysis of UK fresh spinach in 2002. Note: 2002 limits from EC Reg 563/2002 apply.**

Season	Sampling dates	EC Max. level (mg/kg)	No. samples	Nitrate levels (mg/kg fresh weight)		Number exceeding max. level
				range	mean	
<b>Summer</b>	1 Apr – 31 Oct 2001	2500	27	680-3876	2207	9
<b>Winter</b>	1 Jan 2002 – 31 Mar 2002 1 Oct 2002 – 31 Dec 2002	3000	12	1516-3778	2349	2

**Table 13**

**Summary of results of nitrate in UK canned, frozen or preserved spinach in 2000**

Season	Sampling dates	EC Max. level (mg/kg)	No. samples	Nitrate levels (mg/kg fresh weight)		Number exceeding max. level
				range	mean	
<b>Winter</b>	1 Nov - 31 Dec 2000	2000	6	330 - 1280	678	0

**Table 14**

**Summary of results of nitrate in UK canned, frozen or preserved spinach in 2001**

Season	Sampling dates	EC Max. level (mg/kg)	No. samples	Nitrate levels (mg/kg fresh weight)		Number exceeding max. level
				range	mean	
<b>Winter</b>	1 Jan – 31 Mar 2001 1 Oct – 31 Dec 2001	2000	10	1070-1670	1338	0

**Table 15****Summary of results of nitrate in imported fresh spinach in 2000**

Season	Sampling dates	EC Max. level (mg/kg)	No. samples	Nitrate levels (mg/kg fresh weight)		Number exceeding max. level
				range	mean	
Summer	1 April – 31 Oct 2000	2500	6	56 – 2800	1121	1
Winter	1 Nov – 31 Dec 2000	3000	3	650 - 3660	2137	1

**Table 16****Summary of results of nitrate in imported fresh spinach in 2001**

Season	Sampling dates	EC Max. level (mg/kg)	No. samples	Nitrate levels (mg/kg fresh weight)		Number exceeding max. level
				range	mean	
Summer	1 April – 31 Sept 2001	2500	4	697-4010	2559	2
Winter	1 Jan – 31 Mar 2001 1 Oct – 31 Dec 2001	3000	6	1570-2980	2356	0

**Table 17****Summary of results of nitrate in imported fresh spinach in 2002. Note: 2002 limits from EC Reg 563/2002 apply.**

Season	Sampling dates	EC Max. level (mg/kg)	No. samples	Nitrate levels (mg/kg fresh weight)		Number exceeding max. level
				range	mean	
Summer	1 April – 31 Sept 2001	2500	10	122-3188	1007	2
Winter	1 Jan – 31 Mar 2001 1 Oct – 31 Dec 2001	3000	13	416-4583	2380	6

**Table 18****Summary of results of nitrate in imported lettuce in 2000**

Season	Sampling dates	EC Max. level (mg/kg)	No. samples	Nitrate levels (mg/kg fresh weight)		Number exceeding max. level
				range	mean	
Summer	1 May – 31 August 2000	2500	4	466 - 967	629	0
Winter	1 Oct – 31 Mar 2000	4500	24	438 - 3300	1016	0

**Table 19**

**Summary of results of nitrate in imported lettuce in 2001**

Season	Sampling dates	EC Max. level (mg/kg)	No. samples	Nitrate levels (mg/kg fresh weight)		Number exceeding max. level
				range	mean	
Summer	1 Apr – 30 Sept 2001	2500	8	389-2020	987	0
Winter	1 Jan – 31 Mar 2001 1 Oct – 31 Dec 2001	4500	21	472-3700	1163	0

**Table 20**

**Summary of results of nitrate in imported lettuce in 2002. Note: 2002 limits from EC Reg 563/2002 apply. Also, because of a lack of information on growing conditions, it is not possible to ascertain how many of the summer samples would be over the maximum permitted level.**

Season	Sampling dates	EC Max. level (mg/kg)	No. samples	Nitrate levels (mg/kg fresh weight)		Number exceeding max. level
				range	mean	
Summer	1 Apr – 30 Sept 2001	2500/3500	8	297-3954	1753	N/A
Winter	1 Jan – 31 Mar 2001 1 Oct – 31 Dec 2001	4500	19	519-3363	1571	0

**Table 21**

**Dietary intake of nitrate by UK consumers from lettuce and spinach (2000 Monitoring Programme) and the 1997 Total Diet Study.**

	Food consumption (g/person/day)		Intake of Nitrate from Total Diet Study 1997 (mg/kg bw/day)	Intake of Nitrate from Lettuce and Spinach 2000 crop (mg/kg bw/day)	
	Mean	97.5%ile	Mean	Mean	97.5%ile
<b>Adult</b>	2545.7	4589.2	0.72	0.30	1.08
<b>Toddler</b>	1258.6	2042.8	1.85	0.49	2.30
<b>School Children</b>					
<b>4-6 years</b>	1358.1	2035.2	1.51	0.39	1.29
<b>7-10 years</b>	1536.7	2303.8	1.23	0.30	1.33
<b>11-14 years</b>	1615.7	2626.1	0.90	0.22	0.79
<b>15-18 years</b>	1945.9	3459.4	0.77	0.22	0.80
<b>4-18 years</b>	1626.4	3028.8	1.08	0.26	1.12

**Table 22**

**Dietary intake of nitrate by UK consumers from lettuce and spinach (2001 Monitoring Programme) and the 1997 Total Diet Study.**

	Food consumption (g/person/day )		Intake of Nitrate from Total Diet Study 1997 (mg/kg bw/day)	Intake of Nitrate from Lettuce and Spinach 2001 crop (mg/kg bw/day)	
	Mean	97.5%ile	Mean	Mean	97.5%ile
<b>Adult</b>	2545.7	4589.2	0.72	0.31	1.13
<b>Toddler</b>	1258.6	2042.8	1.85	0.52	2.40
<b>School Children</b>					
<b>4-6 years</b>	1358.1	2035.2	1.51	0.40	1.34
<b>7-10 years</b>	1536.7	2303.8	1.23	0.32	1.38
<b>11-14 years</b>	1615.7	2626.1	0.90	0.23	0.82
<b>15-18 years</b>	1945.9	3459.4	0.77	0.23	0.83
<b>4-18 years</b>	1626.4	3028.8	1.08	0.27	1.16

**Table 23**

**Dietary intake of nitrate by UK consumers from lettuce and spinach (2002 Monitoring Programme) and the 1997 Total Diet Study.**

	Food consumption (g/person/day )		Intake of Nitrate from Total Diet Study 1997 (mg/kg bw/day)	Intake of Nitrate from Lettuce and Spinach 2002 crop (mg/kg bw/day)	
	Mean	97.5%ile	Mean	Mean	97.5%ile
<b>Adult</b>	2545.7	4589.2	0.72	0.34	1.28
<b>Toddler</b>	1258.6	2042.8	1.85	0.66	3.09
<b>School Children</b>					
<b>4-6 years</b>	1358.1	2035.2	1.51	0.48	1.71
<b>7-10 years</b>	1536.7	2303.8	1.23	0.36	1.50
<b>11-14 years</b>	1615.7	2626.1	0.90	0.27	1.01
<b>15-18 years</b>	1945.9	3459.4	0.77	0.25	0.94
<b>4-18 years</b>	1626.4	3028.8	1.08	0.31	1.34

**Notes:**

- Food consumption (g/person/day) is based on the total diet (including lettuce and spinach).
- The 1997 Total Diet Study includes a contribution from drinking water and beer. It also includes a contribution from green leafy vegetables, therefore the estimates shown here will be an overestimate due to some double counting.
- ADI: Scientific Committee of Food's Acceptable Daily Intake for nitrate is equivalent to 3.65 mg/kg bodyweight (219 mg/day for a 60 kg person).
- The following figures for nitrate concentration of lettuce and spinach were used for calculations of exposure:

- 1528 mg/kg is the mean nitrate level for fresh spinach from the 2000 Monitoring Programme.
- 1594 mg/kg is the mean nitrate level for fresh spinach from the 2001 Monitoring Programme.
- 2250 mg/kg is the mean nitrate level for fresh spinach from the 2002 Monitoring Programme.
- 678 mg/kg is the mean nitrate level for preserved spinach from the 2000 Monitoring Programme.
- 1338 mg/kg is the mean nitrate level for preserved spinach from the 2001 Monitoring Programme.
- 1458 mg/kg is the mean nitrate level for preserved spinach taken from a 2002 survey of retail salads that will shortly be published.
- 1510 mg/kg is the mean nitrate level for lettuce from the 2000 Monitoring Programme.
- 1566 mg/kg is the mean nitrate level for lettuce from the 2001 Monitoring Programme.
- 1666 mg/kg is the mean nitrate level for lettuce from the 2002 Monitoring Programme.