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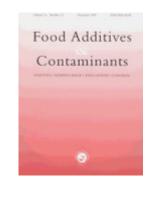
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Food Additives and Contaminants



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NUSSER method. The mean usual daily intake of nitrate was 1.38 mg/kg bodyweight (bw)/day and the usual daily intake at the 97.5 percentile was 2.76 mg/kg bw/day. Exposure of the Belgian population to nitrate at mean intake corresponded to 38% of the ADI (while 76% at the 97.5 percentile). For the average consumer half of the intake was derived from vegetables (especially lettuce) and 20% from water and water-based drinks. Average daily intake of nitrate and nitrite from cheese and meat products was low (respectively 0.2% and 6% of ADI at average intake). Scenario analyses with higher consumption of vegetables or higher nitrate concentration in tap water showed a significant higher intake of nitrate. Whether this is beneficial or harmful must be further assessed.
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Average daily nitrate and nitrite intake in the Belgian population older than 15 years

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15 KEYWORDS

16 Nitrate, nitrite, food consumption survey, exposure assessment, ADI, Belgium

19 ABSTRACT

The aim of this study was to assess the dietary intake of nitrate and nitrite in Belgium. The nitrate content of processed vegetables, cheeses and meat products was analyzed. These data were completed by data from non-targeted official control and from literature. In addition, the nitrite content of meat products was measured. Concentration data for nitrate and nitrite were linked to food consumption data of the Belgian Food Consumption Survey. This study included 3245 respondents, aged 15 years and older. Food intakes were estimated by a repeated 24-h recall using EPIC-SOFT. Only respondents with two completed 24-hour recalls (n=3083) were included in the analysis. For the intake assessment, average concentration data and individual consumption data were combined. Usual intake of nitrate/nitrite was calculated using the NUSSER method. The mean usual daily intake of nitrate was 1.38 mg/kg bodyweight (bw)/day and the usual daily intake at the 97.5 percentile was 2.76 mg/kg bw/day. Exposure of the Belgian population to nitrate at mean intake corresponded to 38% of the ADI (while 76% at the 97.5 percentile). For the average consumer half of the intake was derived from vegetables (especially lettuce) and 20% from water and water-based drinks. Average daily intake of nitrate and nitrite from cheese and meat products was low (respectively 0.2%) and 6% of ADI at average intake). Scenario analyses with higher consumption of vegetables or higher nitrate concentration in tap water showed a significant higher intake of nitrate. Whether this is beneficial or harmful must be further assessed.

38 INTRODUCTION

Recently the role of nitrate and nitrite as healthful dietary components has been reconsidered (Hord et al. 2009; Lundberg et al. 2006; Lundberg 2009; Lundberg et al. 2009; Lundberg et al. 2011; Lundberg and Weitzberg 2009; Lundberg and Weitzberg 2010). Nitrate and nitrite rich food sources may play a physiological role in vascular and immune function. Higher intakes are hypothesized to be associated with lower blood pressure and a better cardiovascular function (Lundberg et al., 2006; Lundberg, 2009; Lundberg et al., 2011).

Till now the risk of nitrate and nitrite has been emphasized. Nitrate toxicity is, for adults, thought to be related to the *in vivo* conversion to nitrite after ingestion (Hartman 1983). Nitrite reacts with amines at the stomach pH to form nitrosamines that are known as carcinogenic compounds (Office fédéral de la santé publique Division science des aliments 2000). The EC Scientific Committee on Food (SCF) considered in their independent expert committee in 1995 the safety of nitrate and recommended an Acceptable Daily Intake (ADI) of 3.65 mg per kg bodyweight per day (equivalent to 220mg per day for an adult of 60 kg) (JECFA Food Additives Series 50, Nitrate and Nitrite). The ADI for nitrite is 0.07 mg per kg bodyweight per day, which is equivalent to 4.2 mg per day for an individual weighing 60 kg.

Nitrate and nitrite are allowed as preservative by European Authorities (European Parliament
and Council Directive No 95/2/EC of 20 February 1995).

It is worthwhile to assess the exposure of the Belgian population to nitrate and nitrite and its
major sources, both because of possible beneficial as well as harmful aspects.

58 The nitrate intake by the Belgian consumer was estimated previously in 1994 (Dejonckheere 59 et al. 1994). Additive intake was not taken into consideration in that study. At that moment 60 actual food intake data were not available, since no national dietary survey had been carried 61 out. Consumption data were estimated from household purchase data.

Food Additives and Contaminants

The objective of the present study was to re-estimate the nitrate and nitrite intake via the diet in Belgium by use of actual concentrations and individual food consumption data. To complement the existing data on occurrence of nitrate in unprepared vegetables and fruits, obtained from Dejonckheere et al (Dejonckheere et al., 1994), analyses were performed in processed vegetables and fruits (canned, jarred or deep frozen, as available on the market).

For some products, for example spinach, including the processing in intake assessment is especially important because of the higher consumption of frozen compared to fresh spinach in Belgium. To our knowledge the effects of processing methods on nitrate concentrations in vegetables available on the Belgium market have not been evaluated before. In addition, food groups (cheese, meat products) were analyzed in which nitrate or nitrite is added as a food additive.

74 METHODS

75 Study design

For the analysis of nitrate in foods, the focus was on fruits and vegetables, potato (products), cheese and processed meat. Food items for nitrate or nitrite analyses were chosen on the basis of data on nitrate concentrations present in foods available in Belgium: from literature, from the Belgian Federal Agency for the Safety of the Food Chain or available product information. Already existing data on nitrate concentrations in fresh vegetables and fruits from Belgium were used from Dejonckheere et al. (Dejonckheere et al., 1994), as well as more recent data of fresh vegetables from non-targeted official control programmes. In case both consulted sources contained data for a certain vegetable or fruit, the most recent data were used. In Table 1 it can be found for each vegetable or fruit which source for concentration data has been used.

There seemed to be no important evolution in time in concentrations of nitrate in vegetables. Additional analyses were performed that could add to these already existing data. The focus was on the nitrate content of differently processed vegetables and fruits and nitrate used as additive in cheese and processed meats. The average nitrate content of bottled water, as communicated by the industry to the Federal Public Service of Health (2.6 mg/L; average of positive concentrations), was used for mineral and source waters. For other drinks based on water (coffee, tea, soup), the nitrate concentration was assumed to be 21 mg/l, which is the average nitrate concentration as communicated by official distributors of tap water in Brussels and Wallonia. For the analysis of nitrite, focus was on the group of processed meats because of the high allowed levels (residual concentration of 50-175 mg/kg) compared to its presence in other food items (European Parliament and Council Directive No 95/2/EC of 20 February 1995).

Afterwards, the nitrate and nitrite concentrations of the different foods were linked to corresponding foods recorded in the individual food consumption survey after applying correction factors for peeling, washing, and home cooking as done by Dejonckheere (Dejonckheere et al., 1994). The effect of processing was taken into account for vegetables using the corrected concentration value whenever the processing or conservation mode of the food was registered in the food consumption survey. The utilized factors can be found in Table 1.

109 Nitrate and nitrite concentration of selected foods

110 Food sampling

Efforts were made to ensure representative concentration data. Samples of cheese and meat products, however, were targeted towards products for which nitrate or nitrite use was known from the label. Sampling was performed from January till March 2006 in the 5 most frequented supermarket chains (6 for the frozen products).

Different seasons were not taken into account because it is impossible to determine the time of harvest. Moreover nitrate or nitrite addition to meat or cheese is not a seasonal practice. A total of 49 vegetable and fruit, 15 cheese and 43 processed meat pooled samples were analyzed for nitrate and 15 cheese and 43 processed meat pooled samples for nitrite. A pooled sample contained up to 16 different samples, each from a different trade mark and/or a different kind of food within the food group. After sampling and before treatment of samples they were stored in the fridge or in the freezer (-18°C or less) depending on the preservation mode at sampling. If the analyses were not conducted on the same day, the sample was stored deep frozen (-18°C or less) for a maximum time of 1 month. The stability of nitrate during this type of storage has been demonstrated elsewhere (Chung et al. 2004). In case of fresh products, the edible part was analyzed (eg. celeriac was peeled).

7 126 Nitrate a

Nitrate and nitrite quantification method

127 Individual samples from the same kind of foods (type and preservation mode) but from 128 different supermarkets and manufacturers were mixed in equivalent proportions to obtain a 129 pooled sample. 200g of each sample was ground with a Robocoup cutter model 3 000 within 130 a minimum period of time to avoid conversion of nitrate into nitrite (bacterial reduction) or

nitrite into nitrate (air oxidation). All samples of the same types were mixed together in a
pooled sample and two 200g portions were preserved until analysis.

 All reagents solutions were prepared from analytical grade reagents with vacuum filtered water showing less residual resistivity than $18.2M\Omega$ cm⁻¹ (from a Millipore MilliQ Reagent Grade System coupled after a Millipore Elix 100 deionised water production system). 10g of the pooled homogenized sample was mixed at about 15 000rpm in an Ultra-Turrax T25 (IKA, Germany) with 100ml of a 6.55mmol l⁻¹ Borax (Fluka, Switzerland) buffer heated at 60°C just before extraction. The Ultraturax was rinsed 2 times with a total of 40ml extraction solution which is then added to the sample extract. 2ml of a 15% potassium hexacyanoferrate (II) (Merck, Germany) and 2ml of 30% zinc sulfate (Merck, Germany) are added to the extract which is shaken after each addition. After cooling, the extract is brought up to 200.0ml with MilliQ water. The extract was filtered through a 0.22µm PVDF syringe filter after centrifugation at 7 000rpm.

The final extract was injected into an High Performance Liquid Chromatography (HPLC) system formed of a 232XL injector (Gilson, USA), a 9010 HPLC pump (Varian inc., Australia), a column oven (Alltech, USA), a UV detector model 785A (Applied Biosystem -PerkinElmer Inc., USA) operated at 220nm and a signal acquisition system formed of a Star800 interface and Galaxy version 1.7 (Varian Inc, Australia). Further calibration and quality assurance calculations were done in Microsoft Excel version 10 SP3. The analytes were injected isocratically at 1.0ml min⁻¹ on a Lichrospher100 RP18 250mm length, 4.6mm internal diameter and 5µm particles thickness maintained at 35°C. The eluent was constituted by a mix of tetrabutylammonium sulfate 50mmol l⁻¹ (Fluka, Switzerland), purified water and methanol Lichrosolve grade (Merck, Germany) in 10/68/22 proportion.

Food Additives and Contaminants

The signal (peak high, in this case) for a particular compound in an unknown sample was compared with the one of the same compound in a standard solution of known concentration. To prove the linearity of the detection system, a minimum of 7 different standard concentrations were used in the range 0.1 to 200mg l^{-1} of NO₃⁻ or NO₂⁻. The calibration solutions were prepared from purified water with resistivity lower then $18.2M\Omega$ cm⁻¹ and NaNO₃ (Riedel-deHaën, Switzerland) and NaNO₂ (Riedel-deHaën, Switzerland) reagent grade or 1000mg l^{-1} solutions. Those solutions were prepared on each series of analysis. The relationship between signal and concentration was linear with a first order polynomial equation between 0.1 and 50 mg l^{-1} and also between 10 and 200 mg l^{-1} . A second order polynomial regression can also be used over the all range. The purity, accuracy and stability (after 18 days) of the standards were checked with a 1/20 dilution of ready certified standard solutions for ion chromatography 1 000mg l^{-1} (Fluka, Switzerland) in NO₃⁻ or NO₂⁻ ions. The recovered amounts were 97.7% nitrate and 101.8% nitrite. Each series of analysis was composed of a calibration curve, solvent blank, sample blank, control sample, samples and control standard.

The method precision was validated by injecting 9 replicates of the same sample over 3 day's of work. This was done at 3 different concentrations chosen in regards of the expected concentration in the type of food targeted. There were 3 different matrices representing meat products, vegetables and cheese (chopped pork meat, tomatoes and Emmental). For nitrate, the precision was greater in meat and vegetable products (less than 5% variation for the highest concentrations tested) than in cheese where very high variation was found at concentration near the quantification limit (nearly 90%). In the case of nitrite, the precision was lower than for nitrate between 25 and 50mg l⁻¹ (maximum variation result of 36% but once again, for concentrations near the quantification limit, the variation observed (in vegetable) was higher (66%).

The quantification limit was 5.0mg kg^{-1} for nitrate and 6.7mg kg^{-1} for nitrite. A selectivity test of the method has been carried out and demonstrated that the separation of nitrate and nitrite was achieved from phosphate, chloride, ascorbic acid and citric acid that can also be present in the sample in plus minus high quantities.

187 Food consumption data

The food consumption data were obtained from the Belgian Food Consumption Survey (FCS) 2004 (De Vriese et al. 2005). The target population covered all Belgian inhabitants of 15 years or older with no upper limit of age. The sample was stratified by province and included 3245 participants randomly selected from the National Register.

Information on dietary intake was collected by a repeated non-consecutive 24h recall in combination with a food frequency questionnaire. The respondents reported the quantity of all foods and beverages consumed during the preceding day. Quantification of foods and recipes was performed using photographs, standard units or ml/g. In order to get information on the within-person variation, two non-consecutive 24h recalls per respondent were collected. The 24h recall was carried out using the standardized EPIC-SOFT program (Slimani and Valsta 2002).

201 Statistical analyses

Only respondents with two complete 24-h recall interviews were included in the analyses (n=
3 083; 1 546 men and 1 537 women). The individual intake of nitrate and nitrite from a
certain food product was estimated by:

$$y_i(mg/kgbw/day) = \frac{c_i \times x_i}{bw_i}$$

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where y_i is the intake of nitrate/nitrite by individual *i* from a particular food (in mg per kg bodyweight and per interview day), x_i is the consumption of a certain food by individual i (kg), c_i is the concentration of nitrate/nitrite in that food (mg per kg; when nitrate concentrations were below the limit of detection (LOD), 0 mg kg^{-1} concentration was applied)(Kroes et al. 2002) and bw_i is the self-reported body weight of individual i (kg). To estimate the total intake of nitrate/nitrite per group of foods and/or per day, individual daily intakes of the contaminant from different foods were summed. The concentration in a food is either a concentration in a pooled sample, or an average concentration from different individual and/or pooled samples.

The *usual* nitrate intake distribution was estimated with the Nusser method (Nusser et al. 1996) using the C-side program (Iowa State University 1996). Several statistical methods are available to estimate *usual* intake distributions with the correct mean, variance and skewness. These statistical procedures adjust for within-person or day-to-day variability. The Nusser method eliminates the within-individual variance and additionally transforms the data to obtain approximately normally distributed data. The method is suitable to estimate usual intake distributions in a population both for normally and non-normally distributed foods and

223 nutrients.



RESULTS

226 Nitrate concentrations of (conserved) foods

The nitrate concentrations in fruits and vegetables used in the exposure assessment are givenin table 1, for cheese and meat products in table 2.

229 Vegetables and fruits

The highest nitrate concentrations were found for lettuce, some other leafy vegetables and celery, with large differences depending on the method of processing. The highest nitrate concentrations for all vegetables studied (except for peas and carrots) were found in fresh vegetables followed by frozen vegetables and canned vegetables or vegetables in glass respectively. Nitrate concentration in peas in glass or can was 3.8 times higher than in fresh peas while nitrate was not detected in frozen peas. Nitrate concentration in fresh carrots was higher than in carrots in glass or can and frozen carrots. Nitrate concentrations in frozen vegetables were on average 43% (19%-62%) and in canned or glass packed vegetables 69%

238 (54%-91%) lower than in fresh vegetables.

In the previous intake calculations of nitrate performed by Dejonckheere et al (Dejonckheere
et al., 1994), only vegetables were considered, for which data were available for the 3
different conservation methods (fresh, frozen and canned or glass).

⁸ 242

In order to estimate the variation in concentrations obtained in pooled samples, the analysis was done within samples of the same processing method for some vegetables, namely endive, beans with pods and celery. Nitrate (NO_3^-) concentrations in frozen endive (n=2) were 1264±119 mg/kg (CV of 9.4%), in frozen endive with added cream (n=9) 758±95 mg/kg (CV of 13%) and in white celery, in can or glass (n=5) 757±173 mg/kg (CV of 23%).

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The largest variations were observed for frozen beans (n=12) with a nitrate concentration of 429±250 mg/kg (CV of 59%) and beans in glass jar (n=10) 264±79 mg/kg (CV of 30%). The average concentrations measured in these additional analyses, however, were similar to the pooled sample analyses as reported in table 1, except for white celery.

Nitrate and nitrite additive use in cheese and meat products

Some cheeses were analyzed and the concentrations found in pooled samples are shown in table 2. Some cheeses were not pooled because they are special foods ("appellations") of controlled origin. The average concentration of 9 pooled Gouda half- and old cheeses was about the same as the average concentration of 11 young ones. This was an unexpected result because half and old Gouda are more dry than the young one, so the concentration was expected to be higher. More investigations should be done on individual analysis basis.

Meat products were analyzed for nitrate after pooling of individual samples. The results are given in table 2. Due to the huge number of meat products available on the market, it was decided in some cases to make more than one pooled sample in order to represent major types of processed meat products. Not more than 15 individual samples were pooled per sample.

The standard deviation of the mean nitrate concentration found in samples of the same meat product (samples within a pooled sample) varied from 11% of the mean concentration in the case of creamy pie to up to 81% of the mean concentration in the case of raw meat sausages. Those results suggested that depending on the manufacturer the concentration can vary substantially. The obtained results of nitrite concentrations in meat products are also given in table 2. The measured concentrations were between 5 and 34 mg kg⁻¹.

This result was unexpected because of the relatively high allowed residual concentrations (50-175 mg/kg). Even for bacon, the concentration was very low (5 mg kg⁻¹).

273 Nitrate intake

The average age of the population was 47±19 yr: 46±18 yr for men and 48±19 yr for women.
The average self-reported weight of the population was (71±14) kg; (78±13) kg for men and
(65±12) kg for women (De Vriese et al. 2006)

The estimated average usual daily intake of selected foods (g/day) is given in table 3. The combination of individual consumption data and average concentration data for the different food items regrouped in categories is given in table 4. The total estimated average daily intake of nitrate from these foods was 1.38 mg/kg bw/day (or 96 mg/day) when beverages (nonalcoholic and soup) were included and 0.885 mg/kg bw/day (or 61 mg/day) on average when only potatoes, vegetables and fruits were included. When all foods were considered, half of the mean usual nitrate intake was from vegetables and 20% from non-alcoholic beverages.

Potatoes and soups (soups were supposed to consist of 30% vegetable mix and 70% tap water, resulting in a calculated nitrate concentration for soups of 105 mg/kg) contributed each for around 10% to the daily nitrate intake. Of the vegetables, lettuce was the main contributor to total nitrate intake, both because of its high nitrate concentration and high consumption in the Belgian population. Carrots had a relatively lower nitrate concentration but a high consumption; on the other hand spinach has a high nitrate content but was consumed only by a small part of the population. The category 'other (mixtures) of vegetables' consisted of vegetables, which were not specified by the respondent, during the 24-h recalls, or mixtures of vegetables.

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Fruits contributed only little to nitrate intake. Of the drinks almost half of the nitrate is from the tap water used to brew coffee and tea. The estimated daily food additive intake of nitrate, from meat products and cheese, was 1.16 mg (or 0.013 mg/kg bw/day), which was only 0.9% of the total average daily intake. The ADI was not exceeded even in the high percentiles of intake, although it should be noted that average and not maximum concentrations of nitrate were used in the different foods At total average intake, the intake represented 38% of ADI and at the 97.5 percentile of intake it corresponded to 76% of ADI.

303 Nitrite intake from processed meat

Table 5 shows the estimated usual food additive intake of nitrite from meat products, which was on average 0.24mg/day or 0.003mg/kg bw/day, representing 6% of the ADI. Although boiled ham had a nitrite concentration near to the LOD, because of the high consumption of this meat product, it was the main source of intake (24%).

DISCUSSION

Nitrate concentrations of foods

Concentrations of nitrate were the highest for fresh rucola (rocket salad), lamb's lettuce, lettuce, radish, celery, spinach and endive (Table 1). Nitrate concentrations of most vegetables analyzed during this study fall within the wide range of values reported before by Dejonckheere et al, 1996) However, other vegetables contained higher nitrate concentrations (e.g. turnip) and for others no concentrations have been reported so far (rhubarb, courgette) (Belitz H-D et al. 1999; Chung et al. 2003; Penttilä PL 1995; Petersen and Stoltze 1999; Tamme et al. 2006).

Concentrations of nitrate in vegetables vary, in addition, according to the processing method with the highest concentrations in fresh, then frozen and the lowest in vegetables preserved in can or glass. This was confirmed in this study, except for peas and carrots. The nitrate content in processed carrots (frozen, canned or jarred) was lower than in fresh carrots, but the level in frozen carrots was not higher than the level in canned or jarred carrots. A likely explanation is that nitrate is lost during blanching of frozen vegetables and into the water in the jar, as far as the nitrate content in the vegetable is higher than in water. For vegetables with low nitrate content such as peas and carrots, a transfer of nitrate to the vegetables might be possible from the added water used for preparation (if the nitrate concentration in water is higher than that in the vegetables). Variation of concentrations even within the same vegetable and processing method is high; within the group of processed vegetables studied, the highest variation of 58% was noticed for frozen beans with pods. This spread may be explained by different varieties, growing and processing conditions. Food additive nitrate concentrations of cheese and meat products are low compared to the possible contamination observed in vegetables and tap water.

333 Nitrate intake

In the current intake assessment, using individual consumption data and average concentration data, the nitrate intake was estimated to be 96 mg per day or 1.38 mg/kg bw/day (38% of the ADI). The intake assessment took into account potatoes, vegetables and fruits, processed meat, cheese, water and drinks based on water. Half of the intake was from vegetables, of which 20% from lettuce. When comparing these figures with previous ones, different aspects have to be taken into account, like the type of food consumption data and the food groups included in the analyses.

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Comparing our results with the results of the previous Belgian intake estimate (Dejonckheere et al., 1994), a higher intake of 2.11 mg/kg bw/day was recorded in the previous Belgian study. The data used in the latter study were derived from household budget surveys and food balance sheets for Belgium. These types of food consumption data are collected at group level with no information on the actual amounts consumed by individuals, including waste, resulting thus in a higher estimate of the daily intake. In addition, dietary patterns might have changed during the past years. In the previous study for instance, the intake of vegetables and potatoes was 202 and 250g per person per day respectively (Dejonckheere et al., 1994), while the consumed quantities currently measured were only 132 and 98 g per person per day, respectively.

The recommended intake of vegetables (Vlaams Instituut voor Gezondheidspromotie 2007) is much higher than the amounts actually consumed. In a scenario where vegetable intake would comply with the recommended amount of 350g/day instead of 132g/day, the mean intake of nitrate would be 2.49mg/kg bw/day (68% of ADI) instead of 1.38mg/kg bw/day.

As the ADI of nitrate will not be exceeded by complying with the recommendation for vegetable consumption, the beneficial effects of increasing vegetable intake outweigh the possible adverse effects. In the current food consumption survey only 1% of the respondents reached the recommendations for vegetables (Vandevijvere et al. 2008). In addition, certain nutritional habits, such as changing especially in the winter season from green house head lettuce to iceberg lettuce with lower nitrate concentration could reduce intake. Among beneficial effects might be the effects of nitrate and nitrite themselves. Recent prospective epidemiologic studies have shown that green leafy vegetables are among the foods most protective against coronary heart disease and ischemic stroke risk (Joshipura et al. 1999). The authors hypothesize that: the content of inorganic nitrate in certain vegetables and fruit can provide a physiologic substrate for reduction to nitrite, nitric oxide, and other metabolic

367 products (NOx) that produce vasodilatation, decrease blood pressure, and support 368 cardiovascular function (McKnight et al. 1999).These studies call into question the rationale 369 for recommendations to limit nitrate and nitrite intake from plant foods. EFSA's 370 Contaminants Panel (CONTAM) has assessed the risks and benefits to consumers from 371 nitrates in vegetables and concluded that the beneficial effects of eating vegetables and fruit 372 outweigh potential risk to human health from exposure to nitrate through vegetables.

In studies with similar individual dietary assessments as in the current study (24h recall, food records), intake is largely influenced by whether water and water based drinks are taken into account. Taking into account only potatoes, vegetables and fruits and some drinks, results are similar to a Finnish study that reported an intake of 55mg/day (or 0.92mg/kg bw/day) (Penttilä et al. 1990). Penttilä et al (Penttilä et al., 1990) probably underestimated the intake of nitrate by drinks. Calculating back the intake of coffee and soft drinks is on average 50ml/day. In the present study the intake of coffee was 270ml/day. Another important difference is that the nitrate content of water was estimated to be 10mg/kg whereas in the current study an average content of 21mg/L was used. Individual food consumption data (dietary history) estimated a total intake of 77mg/day from foods for Finnish adults (Dich et al. 1996). Nitrate from drinking water was not taken into account. Of this estimate 90% was derived from vegetables (including potatoes). Our results are very similar; the average estimated nitrate intake is 76 mg when the non alcoholic beverages are not taken into account, 60% of this intake is from vegetables.

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In a recent French study among adults (Menard et al. 2008) the average nitrate intake was 3.7
In a recent French study among adults (Menard et al. 2008) the average nitrate intake was 3.7
mg per kg bw/day or 40% of the ADI.. The major contributors were, vegetables (24% of ADI)
ADI), potatoes (5% of ADI), and water (5% of ADI). Intake of nitrite was 0.06 mg per kg

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392 bw/day (33-67% of the ADI), with major contributions of additive food vectors. Another
393 dietary assessment method was used (7-day food record) than in our study.

> To estimate the intake from water and water based drinks it was assumed that the concentration of nitrate in water was on average 21mg/L. This average is based on concentrations communicated for tap water in the Wallonia and Brussels regions. For different food groups (soft drinks, juices reconstituted or not) it was assumed that they consisted for 100% of drinking water, which might be an overestimation. A maximal level of 50mg/kg is allowed for drinking water. In case of this upper level, intakes will be higher (average of 1.76mg/kg bw/day instead of 1.38mg/kg bw/day or 48% of ADI). Drinking water in Belgium, however, rarely exceeds this limit although regional differences might exist (van Grinsven et al. 2006).

In a combined situation of both an assumed median vegetable intake which equals the recommended vegetable intake and water with maximum nitrate concentrations, the average nitrate intake will be 2.59 mg/kg bw/day (75.7% of ADI) instead of 1.38 mg/kg bw/day. It is thus clear that the recommendation for vegetable consumption is safe with regard to nitrate intake. In such unlikely scenario in which all consumers increase their vegetable intake with the same percentage as the median consumer would do to reach the recommended vegetable intake, and in which all tap water (also used to make coffee, tea, to reconstitute fruit juice and to make soft drinks) reaches the maximum nitrate level, almost 25% of the adult population in Belgium would exceed the acceptable daily intake. Whether this is beneficial or harmful must be further assessed.

- 0 416

417 Nitrite

Intake of the additive nitrite from processed meats was 0.24mg/day. This is only 6% of the ADI. For nitrite the main issue is the use as additive. Further evaluation must be focused on more vulnerable groups such as children, with possibly high consumption of certain products like ham sausages.

423 Methodological issues

In this study, the intake of nitrate and nitrite in the adult Belgian was estimated as accurately as possible. The nitrate concentration data for potatoes, vegetables and fruits took into account, if available, waste and preparation method. Consumption data are reported as consumed. In many cases, however, information on correction factors is not available, resulting in an overestimation of the nitrate concentration.

In only a very limited number of foods analyzed, only some cheeses, nitrate concentrations
were below the limit of detection and in these cases a concentration of 0 mg/kg was applied.
This could have lead to an underestimation of the intake but the foods concerned were not
consumed at a high level and not frequently. Additionally, the mean concentration values
were used in the intake assessment; no maximum concentrations.

The variation in concentration among the different variety or brands of one particular food could expose some consumers to higher nitrate concentrations in time than the mean concentration value if brands or varieties are not regularly varied and if consumers choose regularly those ones with the higher nitrate concentrations.

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Food Additives and Contaminants

A common problem in dietary assessment in general (Zhang et al. 1999; Zhang et al. 2000) and also in the present study is the possibility of underreporting of foods. It was estimated, based on energy requirements, that 12% of the respondents under recorded their real food intake. This might affect nitrate intake assessment. On the other hand large efforts were made in this study to include a representative sample of the Belgian population older than 15 years of age by choosing respondents among all categories of age, regions and social classes of Belgium. In addition, this study is reliable because it takes into account the most important foods consumed in Belgium and food concentrations were reported not only in the case of an additive usage but also when it could be assumed to be present on a natural way ("contaminant") in the food.

CONCLUSION

Usual exposure to nitrate in Belgian adults was on average 96 mg per day or 1.38 mg/kg bw/day and represented 38% of the ADI. Half of the intake was derived from vegetables (especially lettuce) and 20% of water and drinks based on water. Daily intake of the food additives nitrate (0.2% of ADI) and nitrite (6% of ADI) from cheese and meat products was low. Scenario analyses showed the possible effect of a higher vegetable intake and higher nitrate content of water on estimated nitrate intake.

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<text>

	Number	Number			
	of	of	Preparation or	$[NO_3]$	Source
Food item	samples	samples	Preparation correction	(mg/kg) after	
		per		correction	
		pooled		concetton	
		sample			
VEGETABLES	($W_{1} = 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1$	2400	
Salad Rocket	6		Washed without exterior leaves (-13.9%)	3488	FASFC(2006)
Lettuce, lambs	41		Washed (-9.4%)	2736	· · · · ·
Lettuce	305		Washed without exterior leaves (-13.9%)		FASFC (2006)
Lettuce, Iceberg	12		Washed without exterior leaves (-13.9%)		FASFC (2006)
Celery (green, white)	73				FASFC (2006)
Celery white, frozen		l		1624	IPH (2006)
Celery white, can/glass		5	~	964	IPH (2006)
Spinach	44		Stewed without stems and midribs (-30.6%)		FASFC (2006)
Spinach, frozen		8		846	IPH (2006)
Spinach with cream,					
frozen		8		702	IPH (2006)
Spinach, can/glass		5		496	IPH (2006)
Endive	36		Washed (-11.4%)		FASFC (2006)
Endive, frozen		1		1320	IPH (2006)
Endive with		4			IPH (2006)
cream,frozen				684	
Celeriac		3	Peeled	901	IPH (2006)
Celeriac, frozen		1		334	IPH (2006)
Celeriac, can/glass		3		83	IPH (2006)
Leek				841	*
Leek green, frozen		3		348	IPH (2006)
Leek white, frozen		3	<u>N</u>	286	IPH (2006)
Beans with pods				585	*
Beans with pods,					
frozen		9		432	IPH (2006)
Beans with pods,					
can/glass		16		244	IPH (2006)
Carrot			Stewed (-25%)	348	*
Carrot, can/glass		9		176	IPH (2006)
Carrot, frozen		6		132	IPH (2006)
Brussels sprouts,					
can/glass		1		874	IPH (2006)
Cabbage, white, frozen		1		127	IPH (2006)
Cabbage, red, frozen		5		115	IPH (2006)
Cabbage, savoy, frozen		1		89	IPH (2006)
Cauliflower, frozen		5		77	IPH (2006)
Sauerkraut, can/glass		8		65	IPH (2006)
Cabbage, red, can/glass		9		20	IPH (2006)
Chinese cabbage, fresh		1	Without exterior leaves and hart	0	IPH (2006)
Brussels sprouts, frozen		5		0	IPH (2006)
Broccoli, frozen		5		0	IPH (2006)
Mushrooms				58	*
Mushrooms, frozen		4		47	IPH (2006)
Mushrooms, can/ glass		13		10	IPH (2006)

	Number	Number		$[NO_3^{-}]$	
	of	of	Preparation or	(mg/kg)	Source
Food item	samples	samples	Preparation correction	after	
		per	Freparation correction	correction	
		pooled		correction	
		sample			
Tomato, paste		10		53	IPH (2006)
Tomato, fresh				36	*
Tomato, can/glass		14		9	IPH (2006)
Salsify, frozen		4		104	IPH (2006)
Salsify, can/glass		11		34	IPH (2006)
Peas, can/glass		13		57	IPH (2006)
Peas				15	*
Peas, frozen		7		0	IPH (2006)
Other vegetables fresh					
Radish				2136	*
Turnip		3	Without exterior leaves	1018	IPH (2006)
Courgette		5	Not peeled	897	IPH (2006)
Rhubarbe		1	With peel	572	IPH (2006)
Cucumber				344	*
Aubergine		5	Not peeled	302	IPH (2006)
Sweet pepper				93	*
Chicory				77	;
Onion				59	2
Other vegetables					
can/glass					
Beetroot, can/glass		3		999	IPH (2006)
Gherkin, can/glass		12		55	IPH (2006)
Legumes, can/glass		8		40	IPH (2006)
Asparagus, can/glass		7		0	IPH (2006)
Other vegetables,					
frozen					
Persil, frozen		3		1173	IPH (2006)
Mix of vegetables					
(other than peas and		15			IPH (2006)
carrots)				300	
POTATOES					
Fresh potatoes			Peeled (-33.8%)	102	*
Potatoe crips		13		223	IPH (2006)
Potato croquettes,		11	r.	70	
frozen		11	Frozen	72	IPH (2006)
French fries, frozen		13	Frozen	40	IPH (2006)
FRUITS				221	
Melon			Peeled (-41.1%)	221	*
Strawberry			D = 1 + 1 + (-1 + 0.07)	156	
Banana			Peeled (-61.9%)	153	k
Grape				46	;
Pear				14	:
Orange				13	:
Nectarine				12	:
Apple				11	2
Peach				10	*
Kiwi		7	Peeled	0	IPH (2006)

Food item	Number Number of of samples samples per pooled sample	[NO ₃ ⁻] (mg/kg) after correction	Sourc
Pineapple, can/glass	10	7	IPH (2006
FASFC 2006: Non-targ Agency for Safety of th	geted official control data from 2004-2005 received fr le Food Chain, unpublished data, personal communica	ation.	

Number Number of of samples pooled Food item in pools samples $[NO_3]$ (mg/kg) $[NO_2^-]$ (mg/kg) **Meat products** Sausages, ham 11 1 31.6 34.4 24 Sausages, boiled 3 35.3±5.9 21.6±11.8 81 8 40.4±32.9 13.2±7.7 Sausage, raw meat Sausages boiled in can 45 6 23.5 ± 6.7 12.0 ± 3.1 33 Poultry meat 4 32.0±11.6 19.5±7.8 6 1 187.0 18.2 Horse meat Beef 59.1 17.8 6 1 43 6 149.0 ± 76.4 8.5 ± 2.5 Ham, raw 22 3 18.0 ± 4.2 Ham, boiled 6.6 ± 0.8 8 14,7 <LOQ** Ham, boiled Magistral 1 18.4 Hure 8.8 13 1 24 Liver paste 3 57.3±6.5 6.6±1.3 Lard 21 3 34.4±10.9 5.3±0.3 Bacon 8 1 85.9 4.8 Meat loaf 10 1 50.9 <LOQ** Cheese Milner 5 1 25.6 <LOQ** Gouda, semi-matured 15.8 <LOQ** 11 1 1 <LOQ** Gouda, matured 9 16.1 Emmentaler 9 1 <LOQ* <LOQ** Edam, Leerdam, Maasdam 7.0 <LOQ** 6 1 Gruyères 5 1 <LOQ* <LOQ** 5 <LOQ* <LOQ** Parmesan 1 9 16.0 <LOQ** Melted cheeses 1 9.3 <LOQ** Passendale 1 1

Table 2. Concentration of nitrate $(NO_3^- (mg/kg) \text{ and nitrite } (NO_2^-] (mg/kg))$ in meat products and cheese ^a.

56 57

58

2				
3 4	Père Joseph	1 1	28.8	<loq**< td=""></loq**<>
5	Vieux-Bruges	1 1	23.8	<loq**< td=""></loq**<>
6 7	Vieux-Bruxelles	1 1	8.5	<loq**< td=""></loq**<>
8 9	Nazareth Classic	1 1	22.8	<loq**< td=""></loq**<>
10	Mi-vieux de Bruges	1 1	<loq*< td=""><td><loq**< td=""></loq**<></td></loq*<>	<loq**< td=""></loq**<>
11 12	Gouda with mustard seeds	1 1	<loq*< td=""><td><loq**< td=""></loq**<></td></loq*<>	<loq**< td=""></loq**<>
13				

 $LOQ^* = 5.0 \text{ mg } NO_3/kg$

 $LOQ^{**} = 6.67 \text{ mg } NO_2/\text{kg}$

^aAll analyses performed by the Scientific Institute of Public Health (2006).

		Habitual	consumptic	on (g/day)	Consumption d
	Mean	SD	P50	P97,5	%
Potatoes	<u>97.9</u>	46.4	<u>90.0</u>	211.0	
Soup	<u>93.3</u>	<u>89.0</u>	<u>77.0</u>	<u>296.0</u>	
Vegetables	<u>131.8</u>	<u>49.7</u>	126.0	<u>246.0</u>	
Tomato	33.4	21.2	30.0	84.0	
Carrot	12.8	11.1	9.8	42.6	
Chicory	9.8	13.8	1.6	46.2	
Lettuce (incl iceberg lettuce)	8.3	6.9	6.5	26.6	
Broccoli and cauliflower	8.2	7.6	6.6	27.4	
Onion	6.6	4.5	5.7	17.9	
Beans with pods	5.9	4.9	4.6	18.6	
Head cabbage	4.2	2.9	3.6	11.7	
Mushrooms	4.2	3.9	3.1	14.9	
Cucumber	3.5	5.8	0.0	19.1	
Spinach*	3.1	20.3	0.0	52.0	
Sweet pepper	2.8	2.8	2.1	10.2	
Leek	2.3	2.9	1.4	10.3	
Courgette	1.8	2.1	1.2	7.5	
Other (mixtures of) vegetables	24.6	17.9	20.2	71.3	
Fruits	<u>118.2</u>	84.3	105.0	251.0	
Apple	39.0	39.8	30.0	135.0	
Citrus fruit	18.8	28.8	3.0	98.0	
Banana	14.5	22.1	0.0	73.0	
Grapes	6.0	13.7	0.0	46.4	
Melon (excl water melon)	4.9	10.4	0.0	35.7	
Strawberry	3.7	11.5	0.0	40.1	
Mixed fruits	2.5	9.7	0.0	36.0	
Other fruits	30.2	38.0	18.0	131.0	
Cheese	<u>30.1</u>	<u>18.0</u>	27.0	74.0	
Meat products	<u>32.2</u>	<u>18.9</u>	<u>29.0</u>	<u>78.0</u>	
Nonalcoholic beverages	<u>1420.6</u>	<u>588.8</u>	1336.0	2823.0	
Mineral and source water	541.1	448.2	451.0	1636.0	
Other nonalcoholic beverages					
Coffee and tea	445.8	351.0	385.0	1331.0	
Soft drinks	212.3	260.9	134.0	911.0	
Tap water	97.1	206.9	0.0	716.0	
Juices	62.5	83.2	32.0	286.0	
Beer	122.1	228.6	0.0	778.0	

Table 3. Estimated habitual consumption of foods (g/day) in the Belgian population older than 15 years

sex distribution of the Belgian population 2004 and adjusted for interview day and season.

Total number of consumption days is 6166

* Habitual intake could not be estimated by the Nusser method because of too few replicate intakes.

1	2
1	3
1	4
1	5
1	6
1	2
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1	9
2	0
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2	2
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2	4
2	5
2	6
2	7
2	1
2	8
2	9
3	0
3	1
3	2
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3 3	4
2	5
2	6
ა ი	0
5	1
3	7 8
3 3	7 8 9
3 3 4	8 9 0
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3 3 4 4 4	/ 8 9 0 1 2
3 3 4 4 4 4	7 8 9 0 1 2 3
3 3 4 4 4 4 4 4	7 8 9 0 1 2 3 4
3 3 4 4 4 4 4 4 4	789012345
3 3 4 4 4 4 4 4 4 4	7890123456
3 3 4 4 4 4 4 4 4 4 4 4	78901234567
3 3 4 4 4 4 4 4 4 4 4 4 4 4	789012345670
3 3 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	7890123456780
3 3 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	7890123456789
3 3 4 4 4 4 4 4 4 4 5	23456789012345678901234567890
5	1
5 5	1 2
5 5	1 2
5 5	1
5 5 5 5	1 2 3 4
5 5 5 5 5	1 2 3 4 5
5 5 5 5 5 5 5 5 5	1 2 3 4 5 6
55555555	1 2 3 4 5 6 7
555555555	1 2 3 4 5 6 7 8
55555555555	123456789
55555555555	1 2 3 4 5 6 7 8
55555555555	123456789

Table 4. Estimated habitual nitrate intake (mg/kg bodyweight/day) in the Belgian population older than 15 years

			-		ight/day) % o	л АІ
_		Mean	SD	P50	P97,5	
Potatoes		<u>0.132</u>	<u>0.065</u>	<u>0.121</u>	<u>0.290</u>	
Soup		<u>0.156</u>	<u>0.254</u>	<u>0.112</u>	<u>0.487</u>	
Vegetables		0.675	<u>0.382</u>	<u>0.600</u>	<u>1.630</u>	
	Lettuce (incl iceberg lettuce)	0.269	0.232	0.207	0.886	
	Carrot	0.063	0.057	0.046	0.216	
	Spinach*	0.057	0.331	0.000	0.629	
	Beans with pods	0.042	0.038	0.032	0.145	
	Leek	0.026	0.034	0.015	0.120	
	Courgette	0.023	0.029	0.004	0.014	
	Cucumber	0.014	0.084			
	Tomato	0.014	0.010	0.011	0.040	
	Broccoli and cauliflower	0.006	0.006	0.004	0.020	
	Chicory	0.005	0.004	0.005	0.014	
	Head cabbage	0.005	0.004	0.004	0.014	
	Sweet pepper	0.004	0.004	0.003	0.014	
	Mushrooms	0.003	0.003	0.002	0.012	
	Other (mixtures of) vegetables	0.144	0.147	0.096	0.552	
Fruits		<u>0.082</u>	0.091	0.051	<u>0.343</u>	
	Banana	0.033	0.051	0.000	0.170	
	Melon (excl water melon)	0.017	0.036	0.000	0.124	
	Strawberry	0.009	0.028	0.000	0.096	
	Apple	0.006	0.006	0.004	0.022	
	Grapes	0.004	0.009	0.000	0.032	
	Citrus fruits	0.004	0.005	0.000	0.019	
	Mixed fruits	0.004	0.016	0.000	0.060	
	Other fruits	0.007	0.015	0.001	0.044	
Cheese		0.003	0.003	0.002	0.009	
Meat product	ts	<u>0.013</u>	<u>0.011</u>	0.010	0.043	
Nonalcoholic	beverages	0.278	0.148	0.249	0.632	
	Mineral and source water	0.020	0.017	0.016	0.061	
	Other nonalcoholic beverages					
	Coffee and tea	0.134	0.107	0.115	0.404	
	Soft drinks	0.064	0.080	0.040	0.276	
	Tap water	0.030	0.064	0.000	0.221	
	Juices	0.019	0.026	0.009	0.091	
Beer		<u>0.001</u>	<u>0.003</u>	<u>0.000</u>	<u>0.011</u>	
Total intake		1.380	0.570	1.290	2.760	

Nitrate expressed as nitrate ion, the acceptable daily intake (ADI) for nitrate is 3,65 mg/kg bw/day

- The habitual dietary intakes are weighted and adjusted for the age and
- sex distribution of the Belgian population 2004 and adjusted for interview day and season.
- Total number of consumption days is 6166
- * Habitual intake could not be estimated by the Nusser method because of too few replicate intakes.

for the total population									
	Nitrite (mg/day)			Nitrite (mg/day/kg bodyweight)					% of ADI
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Total meat products	0.242	0.189			0.003	0.003			6
Ham sausages*			0.037	0.282			0.001	0.004	
Sausages boiled*			0.030	0.277			0.000	0.004	
Sausages raw meat*			0.029	0.146			0.000	0.002	
Sausages boiled in can*			0.019	0.174			0.000	0.003	
Poultry meat*			0.014	0.111			0.000	0.002	
Pate*			0.011	0.076			0.000	0.001	
Horse meat*			0.010	0.089			0.000	0.001	

Table 5. Estimated habitual nitrite intake (mg/day and mg/kg bw/day) from meat products in Belgium for the total population

acceptable daily intake

The average dietary intakes are weighted and adjusted for the age and

sex distribution of the Belgian population 2004 and adjusted for interview day and season.

The acceptable daily intake for nitrite is 0,07 mg/kg bw/day

* The habitual intake could not be estimated by the Nusser method because of too few replicate intakes.