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Average daily nitrate and nitrite intake in the Belgian population older than 15 years

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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

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	<p>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.</p>
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4 1 **Average daily nitrate and nitrite intake in the Belgian**
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6 2 **population older than 15 years**
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50 Running title: Nitrate and nitrite intake in Belgium
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53 15 **KEYWORDS**
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56 16 Nitrate, nitrite, food consumption survey, exposure assessment, ADI, Belgium
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19 ABSTRACT

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

38 INTRODUCTION

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

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

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

56 It is worthwhile to assess the exposure of the Belgian population to nitrate and nitrite and its
57 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.

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3 62 The objective of the present study was to re-estimate the nitrate and nitrite intake via the diet
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5 63 in Belgium by use of actual concentrations and individual food consumption data. To
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8 64 complement the existing data on occurrence of nitrate in unprepared vegetables and fruits,
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10 65 obtained from Dejonckheere et al (Dejonckheere et al., 1994), analyses were performed in
11
12 66 processed vegetables and fruits (canned, jarred or deep frozen, as available on the market).
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15 67 For some products, for example spinach, including the processing in intake assessment is
16
17 68 especially important because of the higher consumption of frozen compared to fresh spinach
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19 69 in Belgium. To our knowledge the effects of processing methods on nitrate concentrations in
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21 70 vegetables available on the Belgium market have not been evaluated before. In addition, food
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23 71 groups (cheese, meat products) were analyzed in which nitrate or nitrite is added as a food
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25 72 additive.
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74 **METHODS**

75 **Study design**

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35 76 For the analysis of nitrate in foods, the focus was on fruits and vegetables, potato (products),
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38 77 cheese and processed meat. Food items for nitrate or nitrite analyses were chosen on the basis
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40 78 of data on nitrate concentrations present in foods available in Belgium: from literature, from
41
42 79 the Belgian Federal Agency for the Safety of the Food Chain or available product
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44 80 information. Already existing data on nitrate concentrations in fresh vegetables and fruits
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46 81 from Belgium were used from Dejonckheere et al (Dejonckheere et al., 1994), as well as more
47
48 82 recent data of fresh vegetables from non-targeted official control programmes. In case both
49
50 83 consulted sources contained data for a certain vegetable or fruit, the most recent data were
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52 84 used. In Table 1 it can be found for each vegetable or fruit which source for concentration
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54 85 data has been used.
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3 86 There seemed to be no important evolution in time in concentrations of nitrate in vegetables.
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5 87 Additional analyses were performed that could add to these already existing data. The focus
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8 88 was on the nitrate content of differently processed vegetables and fruits and nitrate used as
9
10 89 additive in cheese and processed meats. The average nitrate content of bottled water, as
11
12 90 communicated by the industry to the Federal Public Service of Health (2.6 mg/L; average of
13
14
15 91 positive concentrations), was used for mineral and source waters. For other drinks based on
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17 92 water (coffee, tea, soup), the nitrate concentration was assumed to be 21 mg/l, which is the
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19 93 average nitrate concentration as communicated by official distributors of tap water in Brussels
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21 94 and Wallonia. For the analysis of nitrite, focus was on the group of processed meats because
22
23 95 of the high allowed levels (residual concentration of 50-175 mg/kg) compared to its presence
24
25 96 in other food items (European Parliament and Council Directive No 95/2/EC of 20 February
26
27 97 1995).
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31 98 Afterwards, the nitrate and nitrite concentrations of the different foods were linked to
32
33 99 corresponding foods recorded in the individual food consumption survey after applying
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35 100 correction factors for peeling, washing, and home cooking as done by Dejonckheere
36
37 101 (Dejonckheere et al., 1994). The effect of processing was taken into account for vegetables
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39 102 using the corrected concentration value whenever the processing or conservation mode of the
40
41 103 food was registered in the food consumption survey. The utilized factors can be found in
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44 104 Table 1.
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3 109 **Nitrate and nitrite concentration of selected foods**
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7 110 **Food sampling**
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10 111 Efforts were made to ensure representative concentration data. Samples of cheese and meat
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12 112 products, however, were targeted towards products for which nitrate or nitrite use was known
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14 113 from the label. Sampling was performed from January till March 2006 in the 5 most
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16 114 frequented supermarket chains (6 for the frozen products).
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20 115 Different seasons were not taken into account because it is impossible to determine the time
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22 116 of harvest. Moreover nitrate or nitrite addition to meat or cheese is not a seasonal practice. A
23
24 117 total of 49 vegetable and fruit, 15 cheese and 43 processed meat pooled samples were
25
26 118 analyzed for nitrate and 15 cheese and 43 processed meat pooled samples for nitrite. A pooled
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28 119 sample contained up to 16 different samples, each from a different trade mark and/or a
29
30 120 different kind of food within the food group. After sampling and before treatment of samples
31
32 121 they were stored in the fridge or in the freezer (-18°C or less) depending on the preservation
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34 122 mode at sampling. If the analyses were not conducted on the same day, the sample was stored
35
36 123 deep frozen (-18°C or less) for a maximum time of 1 month. The stability of nitrate during
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38 124 this type of storage has been demonstrated elsewhere (Chung et al. 2004). In case of fresh
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40 125 products, the edible part was analyzed (eg. celeriac was peeled).
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47 126 **Nitrate and nitrite quantification method**
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51 127 Individual samples from the same kind of foods (type and preservation mode) but from
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53 128 different supermarkets and manufacturers were mixed in equivalent proportions to obtain a
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55 129 pooled sample. 200g of each sample was ground with a Robocoup cutter model 3 000 within
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57 130 a minimum period of time to avoid conversion of nitrate into nitrite (bacterial reduction) or
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3 131 nitrite into nitrate (air oxidation). All samples of the same types were mixed together in a
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6 132 pooled sample and two 200g portions were preserved until analysis.

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10 134 All reagents solutions were prepared from analytical grade reagents with vacuum filtered
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12 135 water showing less residual resistivity than $18.2\text{M}\Omega\text{ cm}^{-1}$ (from a Millipore MilliQ Reagent
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14 136 Grade System coupled after a Millipore Elix 100 deionised water production system). 10g of
15
16 137 the pooled homogenized sample was mixed at about 15 000rpm in an Ultra-Turrax T25 (IKA,
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18 138 Germany) with 100ml of a 6.55mmol l^{-1} Borax (Fluka, Switzerland) buffer heated at 60°C just
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20 139 before extraction. The Ultraturax was rinsed 2 times with a total of 40ml extraction solution
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22 140 which is then added to the sample extract. 2ml of a 15% potassium hexacyanoferrate (II)
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24 141 (Merck, Germany) and 2ml of 30% zinc sulfate (Merck, Germany) are added to the extract
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26 142 which is shaken after each addition. After cooling, the extract is brought up to 200.0ml with
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28 143 MilliQ water. The extract was filtered through a $0.22\mu\text{m}$ PVDF syringe filter after
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30 144 centrifugation at 7 000rpm.
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38 146 The final extract was injected into an High Performance Liquid Chromatography (HPLC)
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40 147 system formed of a 232XL injector (Gilson, USA), a 9010 HPLC pump (Varian inc.,
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42 148 Australia), a column oven (Alltech, USA), a UV detector model 785A (Applied Biosystem –
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44 149 PerkinElmer Inc., USA) operated at 220nm and a signal acquisition system formed of a
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46 150 Star800 interface and Galaxy version 1.7 (Varian Inc, Australia). Further calibration and
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48 151 quality assurance calculations were done in Microsoft Excel version 10 SP3. The analytes
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50 152 were injected isocratically at 1.0ml min^{-1} on a Lichrospher100 RP18 250mm length, 4.6mm
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52 153 internal diameter and $5\mu\text{m}$ particles thickness maintained at 35°C . The eluent was constituted
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54 154 by a mix of tetrabutylammonium sulfate 50mmol l^{-1} (Fluka, Switzerland), purified water and
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56 155 methanol Lichrosolve grade (Merck, Germany) in 10/68/22 proportion.
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3 156 The signal (peak high, in this case) for a particular compound in an unknown sample was
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5 157 compared with the one of the same compound in a standard solution of known concentration.
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8 158 To prove the linearity of the detection system, a minimum of 7 different standard
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10 159 concentrations were used in the range 0.1 to 200mg l⁻¹ of NO₃⁻ or NO₂⁻. The calibration
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12 160 solutions were prepared from purified water with resistivity lower than 18.2MΩ cm⁻¹ and
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15 161 NaNO₃ (Riedel-deHaën, Switzerland) and NaNO₂ (Riedel-deHaën, Switzerland) reagent
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17 162 grade or 1000mg l⁻¹ solutions. Those solutions were prepared on each series of analysis. The
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19 163 relationship between signal and concentration was linear with a first order polynomial
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21 164 equation between 0.1 and 50mg l⁻¹ and also between 10 and 200mg l⁻¹. A second order
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23 165 polynomial regression can also be used over the all range. The purity, accuracy and stability
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25 166 (after 18 days) of the standards were checked with a 1/20 dilution of ready certified standard
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27 167 solutions for ion chromatography 1 000mg l⁻¹ (Fluka, Switzerland) in NO₃⁻ or NO₂⁻ ions. The
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29 168 recovered amounts were 97.7% nitrate and 101.8% nitrite. Each series of analysis was
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31 169 composed of a calibration curve, solvent blank, sample blank, control sample, samples and
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33 170 control standard.
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40 172 The method precision was validated by injecting 9 replicates of the same sample over 3 day's
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42 173 of work. This was done at 3 different concentrations chosen in regards of the expected
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44 174 concentration in the type of food targeted. There were 3 different matrices representing meat
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46 175 products, vegetables and cheese (chopped pork meat, tomatoes and Emmental). For nitrate,
47
48 176 the precision was greater in meat and vegetable products (less than 5% variation for the
49
50 177 highest concentrations tested) than in cheese where very high variation was found at
51
52 178 concentration near the quantification limit (nearly 90%). In the case of nitrite, the precision
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54 179 was lower than for nitrate between 25 and 50mg l⁻¹ (maximum variation result of 36% but
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56 180 once again, for concentrations near the quantification limit, the variation observed (in
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58 181 vegetable) was higher (66%).
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3 182 The quantification limit was 5.0mg kg⁻¹ for nitrate and 6.7mg kg⁻¹ for nitrite. A selectivity test
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5 183 of the method has been carried out and demonstrated that the separation of nitrate and nitrite
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7 184 was achieved from phosphate, chloride, ascorbic acid and citric acid that can also be present
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10 185 in the sample in plus minus high quantities.
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16 187 **Food consumption data**

18 188 The food consumption data were obtained from the Belgian Food Consumption Survey (FCS)
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20 189 2004 (De Vriese et al. 2005). The target population covered all Belgian inhabitants of 15
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22 190 years or older with no upper limit of age. The sample was stratified by province and included
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24 191 3245 participants randomly selected from the National Register.
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29 193 Information on dietary intake was collected by a repeated non-consecutive 24h recall in
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31 194 combination with a food frequency questionnaire. The respondents reported the quantity of all
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33 195 foods and beverages consumed during the preceding day. Quantification of foods and recipes
34
35 196 was performed using photographs, standard units or ml/g. In order to get information on the
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37 197 within-person variation, two non-consecutive 24h recalls per respondent were collected. The
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39 198 24h recall was carried out using the standardized EPIC-SOFT program (Slimani and Valsta
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41 199 2002).
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49 201 **Statistical analyses**

51 202 Only respondents with two complete 24-h recall interviews were included in the analyses (n=
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53 203 3 083; 1 546 men and 1 537 women). The individual intake of nitrate and nitrite from a
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55 204 certain food product was estimated by:

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

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3 206 where y_i is the intake of nitrate/nitrite by individual i from a particular food (in mg per kg
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6 207 bodyweight and per interview day), x_i is the consumption of a certain food by individual i
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8 208 (kg), c_i is the concentration of nitrate/nitrite in that food (mg per kg; when nitrate
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10 209 concentrations were below the limit of detection (LOD), 0 mg kg⁻¹ concentration was
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12 210 applied)(Kroes et al. 2002) and bw_i is the self-reported body weight of individual i (kg). To
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14 211 estimate the total intake of nitrate/nitrite per group of foods and/or per day, individual daily
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16 212 intakes of the contaminant from different foods were summed. The concentration in a food is
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18 213 either a concentration in a pooled sample, or an average concentration from different
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20 214 individual and/or pooled samples.
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27 216 The *usual* nitrate intake distribution was estimated with the Nusser method (Nusser et al.
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29 217 1996) using the C-side program (Iowa State University 1996). Several statistical methods are
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31 218 available to estimate *usual* intake distributions with the correct mean, variance and skewness.
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33 219 These statistical procedures adjust for within-person or day-to-day variability. The Nusser
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35 220 method eliminates the within-individual variance and additionally transforms the data to
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37 221 obtain approximately normally distributed data. The method is suitable to estimate usual
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39 222 intake distributions in a population both for normally and non-normally distributed foods and
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41 223 nutrients.
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3 225 **RESULTS**
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7 226 **Nitrate concentrations of (conserved) foods**
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10 227 The nitrate concentrations in fruits and vegetables used in the exposure assessment are given
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12 228 in table 1, for cheese and meat products in table 2.
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16 229 **Vegetables and fruits**
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20 230 The highest nitrate concentrations were found for lettuce, some other leafy vegetables and
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22 231 celery, with large differences depending on the method of processing. The highest nitrate
23
24 232 concentrations for all vegetables studied (except for peas and carrots) were found in fresh
25
26 233 vegetables followed by frozen vegetables and canned vegetables or vegetables in glass
27
28 234 respectively. Nitrate concentration in peas in glass or can was 3.8 times higher than in fresh
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30 235 peas while nitrate was not detected in frozen peas. Nitrate concentration in fresh carrots was
31
32 236 higher than in carrots in glass or can and frozen carrots. Nitrate concentrations in frozen
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34 237 vegetables were on average 43% (19%-62%) and in canned or glass packed vegetables 69%
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36 238 (54%-91%) lower than in fresh vegetables.
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41 239 In the previous intake calculations of nitrate performed by Dejonckheere et al (Dejonckheere
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43 240 et al., 1994), only vegetables were considered, for which data were available for the 3
44
45 241 different conservation methods (fresh, frozen and canned or glass).
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50 243 In order to estimate the variation in concentrations obtained in pooled samples, the analysis
51
52 244 was done within samples of the same processing method for some vegetables, namely endive,
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54 245 beans with pods and celery. Nitrate (NO_3^-) concentrations in frozen endive (n=2) were
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56 246 1264 ± 119 mg/kg (CV of 9.4%), in frozen endive with added cream (n=9) 758 ± 95 mg/kg (CV
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58 247 of 13%) and in white celery, in can or glass (n=5) 757 ± 173 mg/kg (CV of 23%).
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3 248 The largest variations were observed for frozen beans (n=12) with a nitrate concentration of
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5 249 429±250 mg/kg (CV of 59%) and beans in glass jar (n=10) 264±79 mg/kg (CV of 30%). The
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7
8 250 average concentrations measured in these additional analyses, however, were similar to the
9
10 251 pooled sample analyses as reported in table 1, except for white celery.
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13 252

16 253 **Nitrate and nitrite additive use in cheese and meat products**

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19
20 254 Some cheeses were analyzed and the concentrations found in pooled samples are shown in
21
22 255 table 2. Some cheeses were not pooled because they are special foods (“**appellations**”) of
23
24 256 **controlled origin**. The average concentration of 9 pooled Gouda half- and old cheeses was
25
26
27 257 about the same as the average concentration of 11 young ones. This was an unexpected result
28
29 258 because half and old Gouda are more dry than the young one, so the concentration was
30
31 259 expected to be higher. More investigations should be done on individual analysis basis.
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35 260 Meat products were analyzed for nitrate after pooling of individual samples. The results are
36
37 261 given in table 2. Due to the huge number of meat products available on the market, it was
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39 262 decided in some cases to make more than one pooled sample in order to represent major types
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41 263 of processed meat products. Not more than 15 individual samples were pooled per sample.
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47 265 The standard deviation of the mean nitrate concentration found in samples of the same meat
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49 266 product (samples within a pooled sample) varied from 11% of the mean concentration in the
50
51 267 case of creamy pie to up to 81% of the mean concentration in the case of raw meat sausages.
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53 268 Those results suggested that depending on the manufacturer the concentration can vary
54
55 269 substantially. The obtained results of nitrite concentrations in meat products are also given in
56
57 270 table 2. The measured concentrations were between 5 and 34 mg kg⁻¹.
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3 271 This result was unexpected because of the relatively high allowed residual concentrations (50-
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5 272 175 mg/kg). Even for bacon, the concentration was very low (5 mg kg⁻¹).
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9 **273 Nitrate intake**

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12 274 The average age of the population was 47±19 yr: 46±18 yr for men and 48±19 yr for women.

13 275 The average self-reported weight of the population was (71±14) kg; (78±13) kg for men and
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17 276 (65±12) kg for women (De Vriese et al. 2006)
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22 278 The estimated average usual daily intake of selected foods (g/day) is given in table 3. The
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24 279 combination of individual consumption data and average concentration data for the different
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27 280 food items regrouped in categories is given in table 4. The total estimated average daily intake
28
29 281 of nitrate from these foods was 1.38 mg/kg bw/day (or 96 mg/day) when beverages (non-
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31 282 alcoholic and soup) were included and 0.885 mg/kg bw/day (or 61 mg/day) on average when
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33
34 283 only potatoes, vegetables and fruits were included. When all foods were considered, half of
35
36 284 the mean usual nitrate intake was from vegetables and 20% from non-alcoholic beverages.

37
38 285 Potatoes and soups (soups were supposed to consist of 30% vegetable mix and 70% tap water,
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41 286 resulting in a calculated nitrate concentration for soups of 105 mg/kg) contributed each for
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43 287 around 10% to the daily nitrate intake. Of the vegetables, lettuce was the main contributor to
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46 288 total nitrate intake, both because of its high nitrate concentration and high consumption in the
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48 289 Belgian population. Carrots had a relatively lower nitrate concentration but a high
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51 290 consumption; on the other hand spinach has a high nitrate content but was consumed only by
52
53 291 a small part of the population. The category 'other (mixtures) of vegetables' consisted of
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55 292 vegetables, which were not specified by the respondent, during the 24-h recalls, or mixtures
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58 293 of vegetables.
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3 295 Fruits contributed only little to nitrate intake. Of the drinks almost half of the nitrate is from
4
5 296 the tap water used to brew coffee and tea. The estimated daily food additive intake of nitrate,
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8 297 from meat products and cheese, was 1.16 mg (or 0.013 mg/kg bw/day), which was only 0.9%
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10 298 of the total average daily intake. The ADI was not exceeded even in the high percentiles of
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12 299 intake, although it should be noted that average and not maximum concentrations of nitrate
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15 300 were used in the different foods. At total average intake, the intake represented 38% of ADI
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17 301 and at the 97.5 percentile of intake it corresponded to 76% of ADI.
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21 22 23 303 **Nitrite intake from processed meat**

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27 304 Table 5 shows the estimated usual food additive intake of nitrite from meat products, which
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29 305 was on average 0.24mg/day or 0.003mg/kg bw/day, representing 6% of the ADI. Although
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31 306 boiled ham had a nitrite concentration near to the LOD, because of the high consumption of
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34 307 this meat product, it was the main source of intake (24%).
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37 38 39 309 **DISCUSSION**

40 41 42 43 310 **Nitrate concentrations of foods**

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47 311 Concentrations of nitrate were the highest for fresh rucola (rocket salad), lamb's lettuce,
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49 312 lettuce, radish, celery, spinach and endive (Table 1). Nitrate concentrations of most vegetables
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51 313 analyzed during this study fall within the wide range of values reported before by
52
53 314 Dejonckheere et al, 1996) However, other vegetables contained higher nitrate concentrations
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55 315 (e.g. turnip) and for others no concentrations have been reported so far (rhubarb, courgette)
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57 316 (Belitz H-D et al. 1999; Chung et al. 2003; Penttilä PL 1995; Petersen and Stoltze 1999;
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59 317 Tamme et al. 2006).
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3 318 Concentrations of nitrate in vegetables vary, in addition, according to the processing method
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5 319 with the highest concentrations in fresh, then frozen and the lowest in vegetables preserved in
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7 320 can or glass. This was confirmed in this study, except for peas and carrots. The nitrate content
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9 321 in processed carrots (frozen, canned or jarred) was lower than in fresh carrots, but the level in
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11 322 frozen carrots was not higher than the level in canned or jarred carrots. A likely explanation is
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13 323 that nitrate is lost during blanching of frozen vegetables and into the water in the jar, as far as
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15 324 the nitrate content in the vegetable is higher than in water. For vegetables with low nitrate
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17 325 content such as peas and carrots, a transfer of nitrate to the vegetables might be possible from
18
19 326 the added water used for preparation (if the nitrate concentration in water is higher than that in
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21 327 the vegetables). Variation of concentrations even within the same vegetable and processing
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23 328 method is high; within the group of processed vegetables studied, the highest variation of
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25 329 58% was noticed for frozen beans with pods. This spread may be explained by different
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27 330 varieties, growing and processing conditions. Food additive nitrate concentrations of cheese
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29 331 and meat products are low compared to the possible contamination observed in vegetables
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31 332 and tap water.
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40 333 **Nitrate intake**

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43 334 In the current intake assessment, using individual consumption data and average
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45 335 concentration data, the nitrate intake was estimated to be 96 mg per day or 1.38 mg/kg bw/day
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47 336 (38% of the ADI). The intake assessment took into account potatoes, vegetables and fruits,
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49 337 processed meat, cheese, water and drinks based on water. Half of the intake was from
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51 338 vegetables, of which 20% from lettuce. When comparing these figures with previous ones,
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53 339 different aspects have to be taken into account, like the type of food consumption data and the
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55 340 food groups included in the analyses.
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3 342 Comparing our results with the results of the previous Belgian intake estimate (Dejonckheere
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5 343 et al., 1994), a higher intake of 2.11 mg/kg bw/day was recorded in the previous Belgian
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7 344 study. The data used in the latter study were derived from household budget surveys and food
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10 345 balance sheets for Belgium. These types of food consumption data are collected at group level
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12 346 with no information on the actual amounts consumed by individuals, including waste,
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15 347 resulting thus in a higher estimate of the daily intake. In addition, dietary patterns might have
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17 348 changed during the past years. In the previous study for instance, the intake of vegetables and
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19 349 potatoes was 202 and 250g per person per day respectively (Dejonckheere et al., 1994), while
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21
22 350 the consumed quantities currently measured were only 132 and 98 g per person per day,
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24
25 351 respectively.

26
27 352 The recommended intake of vegetables (Vlaams Instituut voor Gezondheidspromotie 2007) is
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29 353 much higher than the amounts actually consumed. In a scenario where vegetable intake would
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31 354 comply with the recommended amount of 350g/day instead of 132g/day, the mean intake of
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34 355 nitrate would be 2.49mg/kg bw/day (68% of ADI) instead of 1.38mg/kg bw/day.

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36 356 As the ADI of nitrate will not be exceeded by complying with the recommendation for
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38 357 vegetable consumption, the beneficial effects of increasing vegetable intake outweigh the
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41 358 possible adverse effects. In the current food consumption survey only 1% of the respondents
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43 359 reached the recommendations for vegetables (Vandevijvere et al. 2008). In addition, certain
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45 360 nutritional habits, such as changing especially in the winter season from green house head
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48 361 lettuce to iceberg lettuce with lower nitrate concentration could reduce intake. Among
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51 362 beneficial effects might be the effects of nitrate and nitrite themselves. Recent prospective
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53 363 epidemiologic studies have shown that green leafy vegetables are among the foods most
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55 364 protective against coronary heart disease and ischemic stroke risk (Joshiyura et al. 1999). The
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58 365 authors hypothesize that: the content of inorganic nitrate in certain vegetables and fruit can
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60 366 provide a physiologic substrate for reduction to nitrite, nitric oxide, and other metabolic

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3 367 products (NO_x) that produce vasodilatation, decrease blood pressure, and support
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5 368 cardiovascular function (McKnight et al. 1999). These studies call into question the rationale
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7 369 for recommendations to limit nitrate and nitrite intake from plant foods. EFSA's
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9 370 Contaminants Panel (CONTAM) has assessed the risks and benefits to consumers from
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11 371 nitrates in vegetables and concluded that the beneficial effects of eating vegetables and fruit
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13 372 outweigh potential risk to human health from exposure to nitrate through vegetables.
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20 374 In studies with similar individual dietary assessments as in the current study (24h recall, food
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22 375 records), intake is largely influenced by whether water and water based drinks are taken into
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24 376 account. Taking into account only potatoes, vegetables and fruits and some drinks, results are
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26 377 similar to a Finnish study that reported an intake of 55mg/day (or 0.92mg/kg bw/day)
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28 378 (Penttilä et al. 1990) . Penttilä et al (Penttilä et al., 1990) probably underestimated the intake
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30 379 of nitrate by drinks. Calculating back the intake of coffee and soft drinks is on average
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32 380 50ml/day. In the present study the intake of coffee was 270ml/day. Another important
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34 381 difference is that the nitrate content of water was estimated to be 10mg/kg whereas in the
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36 382 current study an average content of 21mg/L was used. Individual food consumption data
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38 383 (dietary history) estimated a total intake of 77mg/day from foods for Finnish adults (Dich et
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40 384 al. 1996). Nitrate from drinking water was not taken into account. Of this estimate 90% was
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42 385 derived from vegetables (including potatoes). Our results are very similar; the average
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44 386 estimated nitrate intake is 76 mg when the non alcoholic beverages are not taken into account,
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46 387 60% of this intake is from vegetables.
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55 389 In a recent French study among adults (Menard et al. 2008) the average nitrate intake was 3.7
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57 390 mg per kg bw/day or 40% of the ADI. The major contributors were, vegetables (24% of
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59 391 ADI), potatoes (5% of ADI), and water (5% of ADI). Intake of nitrite was 0.06 mg per kg
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3 392 bw/day (33-67% of the ADI), with major contributions of additive food vectors. Another
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5 393 dietary assessment method was used (7-day food record) than in our study.
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10 395 To estimate the intake from water and water based drinks it was assumed that the
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12 396 concentration of nitrate in water was on average 21mg/L. This average is based on
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15 397 concentrations communicated for tap water in the Wallonia and Brussels regions. For
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17 398 different food groups (soft drinks, juices reconstituted or not) it was assumed that they
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19 399 consisted for 100% of drinking water, which might be an overestimation. A maximal level of
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22 400 50mg/kg is allowed for drinking water. In case of this upper level, intakes will be higher
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24 401 (average of 1.76mg/kg bw/day instead of 1.38mg/kg bw/day or 48% of ADI). Drinking water
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26 402 in Belgium, however, rarely exceeds this limit although regional differences might exist (van
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29 403 Grinsven et al. 2006).
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34 405 In a combined situation of both an assumed median vegetable intake which equals the
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36 406 recommended vegetable intake and water with maximum nitrate concentrations, the average
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38 407 nitrate intake will be 2.59 mg/kg bw/day (75.7% of ADI) instead of 1.38 mg/kg bw/day. It is
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40
41 408 thus clear that the recommendation for vegetable consumption is safe with regard to nitrate
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43 409 intake. In such unlikely scenario in which all consumers increase their vegetable intake with
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45 410 the same percentage as the median consumer would do to reach the recommended vegetable
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48 411 intake, and in which all tap water (also used to make coffee, tea, to reconstitute fruit juice and
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50 412 to make soft drinks) reaches the maximum nitrate level, almost 25% of the adult population in
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53 413 Belgium would exceed the acceptable daily intake. Whether this is beneficial or harmful must
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55 414 be further assessed.
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3 417 **Nitrite**
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7 418 Intake of the additive nitrite from processed meats was 0.24mg/day. This is only 6% of the
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9 419 ADI. For nitrite the main issue is the use as additive. Further evaluation must be focused on
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11 420 more vulnerable groups such as children, with possibly high consumption of certain products
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13 421 like ham sausages.
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20 423 **Methodological issues**
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23 424 In this study, the intake of nitrate and nitrite in the adult Belgian was estimated as accurately
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25 425 as possible. The nitrate concentration data for potatoes, vegetables and fruits took into
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27 426 account, if available, waste and preparation method. Consumption data are reported as
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29 427 consumed. In many cases, however, information on correction factors is not available,
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31 428 resulting in an overestimation of the nitrate concentration.
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37 430 In only a very limited number of foods analyzed, only some cheeses, nitrate concentrations
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39 431 were below the limit of detection and in these cases a concentration of 0 mg/kg was applied.
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41 432 This could have lead to an underestimation of the intake but the foods concerned were not
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43 433 consumed at a high level and not frequently. Additionally, the mean concentration values
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45 434 were used in the intake assessment; no maximum concentrations.
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49 435 The variation in concentration among the different variety or brands of one particular food
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51 436 could expose some consumers to higher nitrate concentrations in time than the mean
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53 437 concentration value if brands or varieties are not regularly varied and if consumers choose
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55 438 regularly those ones with the higher nitrate concentrations.
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3 440 A common problem in dietary assessment in general (Zhang et al. 1999; Zhang et al. 2000)
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5 441 and also in the present study is the possibility of underreporting of foods. It was estimated,
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7 442 based on energy requirements, that 12% of the respondents under recorded their real food
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9 443 intake. This might affect nitrate intake assessment. On the other hand large efforts were made
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11 444 in this study to include a representative sample of the Belgian population older than 15 years
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13 445 of age by choosing respondents among all categories of age, regions and social classes of
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15 446 Belgium. In addition, this study is reliable because it takes into account the most important
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17 447 foods consumed in Belgium and food concentrations were reported not only in the case of an
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19 448 additive usage but also when it could be assumed to be present on a natural way
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21 449 (“contaminant”) in the food.
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28 450 **CONCLUSION**

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31 451 Usual exposure to nitrate in Belgian adults was on average 96 mg per day or 1.38 mg/kg
32
33 452 bw/day and represented 38% of the ADI. Half of the intake was derived from vegetables
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35 453 (especially lettuce) and 20% of water and drinks based on water. Daily intake of the food
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37 454 additives nitrate (0.2% of ADI) and nitrite (6% of ADI) from cheese and meat products was
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39 455 low. Scenario analyses showed the possible effect of a higher vegetable intake and higher
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41 456 nitrate content of water on estimated nitrate intake.
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54 459 **ACKNOWLEDGEMENTS**

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59 461 Environment.
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Table 1. Concentrations of nitrate ($[\text{NO}_3^-]$) (mg/kg) in selected vegetables, potatoes and fruit.

Food item	Number of samples	Number of samples per pooled sample	Preparation or Preparation correction	$[\text{NO}_3^-]$ (mg/kg) after correction	Source
VEGETABLES					
Salad Rocket	6		Washed without exterior leaves (-13.9%)	3488	FASFC(2006)
Lettuce, lambs	41		Washed (-9.4%)	2736	FASFC(2006)
Lettuce	305		Washed without exterior leaves (-13.9%)	2351	FASFC (2006)
Lettuce, Iceberg	12		Washed without exterior leaves (-13.9%)	1084	FASFC (2006)
Celery (green, white)	73			2110	FASFC (2006)
Celery white, frozen		1		1624	IPH (2006)
Celery white, can/glass		5		964	IPH (2006)
Spinach	44		Stewed without stems and midribs (-30.6%)	1509	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%)	1414	FASFC (2006)
Endive, frozen		1		1320	IPH (2006)
Endive with cream, frozen		4		684	IPH (2006)
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		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)

Food item	Number of samples	Number of samples per pooled sample	Preparation or Preparation correction	[NO ₃] ⁻ (mg/kg) after correction	Source
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	*
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 carrots)		15		300	IPH (2006)
POTATOES					
Fresh potatoes			Peeled (-33.8%)	102	*
Potato crips		13		223	IPH (2006)
Potato croquettes, frozen		11	Frozen	72	IPH (2006)
French fries, frozen		13	Frozen	40	IPH (2006)
FRUITS					
Melon			Peeled (-41.1%)	221	*
Strawberry				156	*
Banana			Peeled (-61.9%)	153	*
Grape				46	*
Pear				14	*
Orange				13	*
Nectarine				12	*
Apple				11	*
Peach				10	*
Kiwi		7	Peeled	0	IPH (2006)

Food item	Number of samples	Number of samples per pooled sample	Preparation or Preparation correction	[NO ₃ ⁻] (mg/kg) after correction	Source
Pineapple, can/glass		10		7	IPH (2006)

IPH2006 : Sampling and analyses performed by the scientific institute of Public health in the frame of the current study

* Dejonckheere et al, 1996

FASFC 2006: Non-targeted official control data from 2004-2005 received from the Belgian Federal Agency for Safety of the Food Chain, unpublished data, personal communication.

Table 2. Concentration of nitrate (NO_3^- (mg/kg) and nitrite (NO_2^-) (mg/kg)) in meat products and cheese ^a.

Food item	Number of samples in pools	Number of pooled samples	$[\text{NO}_3^-]$ (mg/kg)	$[\text{NO}_2^-]$ (mg/kg)
Meat products				
Sausages, ham	11	1	31.6	34.4
Sausages, boiled	24	3	35.3±5.9	21.6±11.8
Sausage, raw meat	81	8	40.4±32.9	13.2±7.7
Sausages boiled in can	45	6	23.5±6.7	12.0±3.1
Poultry meat	33	4	32.0±11.6	19.5±7.8
Horse meat	6	1	187.0	18.2
Beef	6	1	59.1	17.8
Ham, raw	43	6	149.0±76.4	8.5±2.5
Ham, boiled	22	3	18.0±4.2	6.6±0.8
Ham, boiled Magistral	8	1	14.7	<LOQ**
Hure	13	1	18.4	8.8
Liver paste	24	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	11	1	15.8	<LOQ**
Gouda, matured	9	1	16.1	<LOQ**
Emmentaler	9	1	<LOQ*	<LOQ**
Edam, Leerdam, Maasdam	6	1	7.0	<LOQ**
Gruyères	5	1	<LOQ*	<LOQ**
Parmesan	5	1	<LOQ*	<LOQ**
Melted cheeses	9	1	16.0	<LOQ**
Passendale	1	1	9.3	<LOQ**

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Père Joseph	1	1	28.8	<LOQ**
Vieux-Bruges	1	1	23.8	<LOQ**
Vieux-Bruxelles	1	1	8.5	<LOQ**
Nazareth Classic	1	1	22.8	<LOQ**
Mi-vieux de Bruges	1	1	<LOQ*	<LOQ**
Gouda with mustard seeds	1	1	<LOQ*	<LOQ**

LOQ* = 5.0 mg NO₃⁻/kgLOQ** = 6.67 mg NO₂⁻/kg^aAll analyses performed by the Scientific Institute of Public Health (2006).

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

	Habitual consumption (g/day)				Consumption days
	Mean	SD	P50	P97,5	%
Potatoes	<u>97.9</u>	<u>46.4</u>	<u>90.0</u>	<u>211.0</u>	65
Soup	<u>93.3</u>	<u>89.0</u>	<u>77.0</u>	<u>296.0</u>	35
Vegetables	<u>131.8</u>	<u>49.7</u>	<u>126.0</u>	<u>246.0</u>	81
Tomato	33.4	21.2	30.0	84.0	28
Carrot	12.8	11.1	9.8	42.6	19
Chicory	9.8	13.8	1.6	46.2	8
Lettuce (incl iceberg lettuce)	8.3	6.9	6.5	26.6	21
Broccoli and cauliflower	8.2	7.6	6.6	27.4	6
Onion	6.6	4.5	5.7	17.9	25
Beans with pods	5.9	4.9	4.6	18.6	7
Head cabbage	4.2	2.9	3.6	11.7	4
Mushrooms	4.2	3.9	3.1	14.9	8
Cucumber	3.5	5.8	0.0	19.1	4
Spinach*	3.1	20.3	0.0	52.0	3
Sweet pepper	2.8	2.8	2.1	10.2	7
Leek	2.3	2.9	1.4	10.3	4
Courgette	1.8	2.1	1.2	7.5	2
Other (mixtures of) vegetables	24.6	17.9	20.2	71.3	30
Fruits	<u>118.2</u>	<u>84.3</u>	<u>105.0</u>	<u>251.0</u>	57
Apple	39.0	39.8	30.0	135.0	27
Citrus fruit	18.8	28.8	3.0	98.0	17
Banana	14.5	22.1	0.0	73.0	9
Grapes	6.0	13.7	0.0	46.4	4
Melon (excl water melon)	4.9	10.4	0.0	35.7	2
Strawberry	3.7	11.5	0.0	40.1	3
Mixed fruits	2.5	9.7	0.0	36.0	1
Other fruits	30.2	38.0	18.0	131.0	21
Cheese	<u>30.1</u>	<u>18.0</u>	<u>27.0</u>	<u>74.0</u>	57
Meat products	<u>32.2</u>	<u>18.9</u>	<u>29.0</u>	<u>78.0</u>	53
Nonalcoholic beverages	<u>1420.6</u>	<u>588.8</u>	<u>1336.0</u>	<u>2823.0</u>	100
Mineral and source water	541.1	448.2	451.0	1636.0	64
Other nonalcoholic beverages					
Coffee and tea	445.8	351.0	385.0	1331.0	75
Soft drinks	212.3	260.9	134.0	911.0	37
Tap water	97.1	206.9	0.0	716.0	19
Juices	62.5	83.2	32.0	286.0	25
Beer	<u>122.1</u>	<u>228.6</u>	<u>0.0</u>	<u>778.0</u>	17

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.

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

		Nitrate (mg/kg bodyweight/day) % of ADI				
		Mean	SD	P50	P97,5	
Potatoes		<u>0.132</u>	<u>0.065</u>	<u>0.121</u>	<u>0.290</u>	4
Soup		<u>0.156</u>	<u>0.254</u>	<u>0.112</u>	<u>0.487</u>	4
Vegetables		<u>0.675</u>	<u>0.382</u>	<u>0.600</u>	<u>1.630</u>	19
	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>	<u>0.091</u>	<u>0.051</u>	<u>0.343</u>	2
	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		<u>0.003</u>	<u>0.003</u>	<u>0.002</u>	<u>0.009</u>	
Meat products		<u>0.013</u>	<u>0.011</u>	<u>0.010</u>	<u>0.043</u>	
Nonalcoholic beverages		<u>0.278</u>	<u>0.148</u>	<u>0.249</u>	<u>0.632</u>	8
	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	38

ADI acceptable daily intake

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

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3 The habitual dietary intakes are weighted and adjusted for the age and
4 sex distribution of the Belgian population 2004 and adjusted for interview day and season.

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6 Total number of consumption days is 6166

7 * Habitual intake could not be estimated by the Nusser method because of too few replicate intakes.
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Table 5. Estimated habitual nitrite intake (mg/day and mg/kg bw/day) from meat products in Belgium for the total population

	Nitrite (mg/day)		Nitrite (mg/day/kg bodyweight)				% of ADI
	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

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.