

# **Estimate of intake of sulfites in the Belgian adult population**

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#### **Food Additives and Contaminants**



## Estimate of intake of sulphites in the Belgian adult population



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**Abstract** 

 

> 39 An exposure assessment was performed to estimate the usual daily intake of sulfites in the 40 Belgian adult population. Food consumption data were retrieved from the national food 41 consumption survey. In a first step, individual food consumption data were multiplied with 42 the maximum permitted use levels for sulfites, expressed as sulphur dioxide, per food group 43 (Tier 2). In a second step, on the basis of a literature review of the occurrence of sulfites in 44 different foods, the results of the Tier 2 exposure assessment and available occurrence data 45 from the control programme of the competent authority, a refined list of foods was drafted for 46 the quantification of sulphite. Quantification of sulphite was performed by a high 47 performance ion chromatography method with eluent conductivity detector in beers and 48 potato products. Individual food consumption data were then multiplied with the actual 49 average concentrations of sulfite per food group, or the maximum permitted levels in case 50 actual levels were not available (partial Tier 3). Usual intakes were calculated using the 51 Nusser method. The mean intake of sulfites was 0.34 mg/kg bw/day (Tier 2), corresponding 52 with 49 % of the acceptable daily intake (ADI) and 0.19 mg/kg bw/day, corresponding with 53 27 % of the ADI (partial Tier 3). The food group contributing most to the intake of sulfites 54 was the group of wines. The results showed that the intake of sulfites is likely to be below the 55 ADI in Belgium. However, there are indications that high consumers of wine have an intake 56 around the ADI.

## **Introduction**

 There are 3 ways of being exposed to  $SO<sub>2</sub>$ : by inhalation, by skin contact or by ingestion from 80 the food chain (NPI, 2005). Sulfite  $(H_2SO_3)$  is formed by sulphur dioxide (SO<sub>2</sub>) gas which is 81 very soluble in water. The presence of sulphur dioxide in the environment is due to 82 combustion processes of organic compounds, such as natural combustion from forest fires or 83 combustion in energy or transformation plants. Sulfite is also present in the exhaust fumes 84 from cars and trucks. Depending on the pH of the environment, different forms of sulfite can 85 exist or coexist.

ricks. Depending on the pH of the environment, different form<br> **Example 20** concerns because of its toxicity for the lungs and its ability to<br> **For Peer Review Only and Example 20** cally in asthmatic persons (Valley et al. 87 Sulfite has raised concerns because of its toxicity for the lungs and its ability to cause allergic 88 reactions, especially in asthmatic persons (Valley et al., 2009). It was estimated that an 89 exposure of 10 to 50 ppm during 5 to 15 min may have a measurable effect on humans. In the 90 UK, 2 ppm (8 hours time weighted average TWA) or 5 ppm (10 min TWA) were set as 91 maximum occupational exposure values (European Cooperation on School Education, 2004). 92 In Belgium, the maximum allowed concentration of sulfite in the air is 350  $\mu$ g/m<sup>3</sup> during 93 maximum 1 hr or 125 µg/m<sup>3</sup> during maximum 24 hr (European Community 1999a). In a 94 review of the Health Council of the Netherlands sulfite was found to be responsible of chronic 95 urticaria, skin allergy and bronchial asthma in some humans. The NOAEL in rats was set at 96 72 mg SO<sub>2</sub>/kg bw/day (Health Council of the Netherlands, 2005).

98 The Acceptable Daily Intake (ADI) is the amount of  $SO<sub>2</sub>$  in food that can be ingested orally 99 over a lifetime without an appreciable health risk. The ADI is expressed per kg body weight 100 (abbreviated "bw") per day. In the case of sulfites (expressed as  $SO_2$ ), the JECFA (Joint 101 FAO/WHO Expert Committee on Food Additives) has set the group ADI value to 0.7 mg/kg 102 bw/day (JECFA, 1999).

104 Sulfite can be intentionally added to food for its antioxidant or antimicrobial properties. 105 Sulfite inhibits the enzymatic activity of polyphenol oxidase, which is responsible for 106 browning in fruits, vegetables and seafood products. It also prevents oxidation of food due to 107 contact with the air and the browning effect occurring from the Maillard-type reactions. The 108 aim of adding sulfite to wine is to prevent microbial growth and oxidation of volatile odorous 109 compounds which may develop during the fermentation (Ough, 1986). 

111 Sulfite is allowed as a preservative (E220-E228) in a list of different foods (excluding all 112 others) under the condition of respecting the maximum use levels, as specified by the 113 European Directive 95/2/EC as amended (European Community 1995) and for wine in annex 114 V of the Council regulation (EC) No. 1493/1999 (European Community 1999b).

115 The concentration in different foods is expressed as sulphur dioxide  $(SO_2)$  in mg/kg or mg/l, 116 depending on the nature of the food and relates to the total quantity from all sources. The 117 legislation mentions that an  $SO_2$  content of not more than 10 mg/kg or 10 mg/l is not 118 considered to be present. Directive 2000/13/EC on labelling of pre-packaged food requires 119 labelling of products containing sulfite, if the  $SO_2$  concentration is higher than 10 mg/kg or 10 120 mg/l, for the benefit of people with a food intolerance for sulfite (European Community 121 2000).

 

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ducts containing sulfite, if the SO<sub>2</sub> concentration is higher than<br>
penefit of people with a foo 123 Sulfite can be present in foods as free sulfite or react with a variety of organic compounds in 124 foods to form reversibly or irreversibly bound products. In our digestive tract, the free and 125 reversibly bound forms can be liberated (Wedizicha, 1992). García-Alonso et al., (2001) 126 reported that the amount of added sulfite in fresh sausage was reduced by 26 % when S— 127 sulphonate bounds (irreversibly bound) were formed and by 23 % when  $C$ — $O$ — $SO_2$  bounds 128 (reversibly bound) were formed. Those percentages of loss may vary sharply from 10 to 49 % 129 depending on the conditions when adding the sulfite to the meat (time of mixing, presence of 130 oxygen, degree of mincing and fat composition of the meat).

 

132 Cooking was shown to reduce the level of sulfite in beef and chicken burgers (Pena-Egido et 133 al; 2005; Garcia-Alonso et al., 2001) the distribution of the 3 different forms of sulfite (free, 134 reversibly bound and S-sulphonate) remained approximately the same after as before the 135 cooking process. The losses reported for meat burgers were 31 % for the S-sulphonates, 29 % 136 for the free sulfite and 28% for the total sulfite. This study also showed that the frying process 137 reduced the amount of total sulfite on average by 41 %  $(\pm 13\%)$ . A study in shrimps 138 (Armentia-Alvarez et al., 1994) showed that during cooking the loss of free and reversibly 139 bound sulfite varied between 45 and 55 %. The level of total sulfite was about 5 times higher 140 in the non edible parts compared to the edible parts. The authors concluded that the boiling 141 step did not change the distribution of free and total sulfite in shrimps, but a reduction of 142 about 40 % was observed for the total sulfite in the edible parts. From the boiled water it was 

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143 shown that the loss of  $SO_2$  in shrimps during the boiling process was not totally recovered in 144 the water due to oxidation of sulfite and reaction of sulfite with food components.

146 Another study on shrimps (Armentia-Alvarez et al., 1997) showed that during bad freezing 147 storage  $(>-18\degree C)$ , the free sulfite combined with the disulfide bounds of proteins denatured 148 by the freezing process. The longer the storage time, the higher the amount of combined 149 sulfite produced. Reduction of sulfite concentration after a storage period of 1 month was 150 reported to be between 25 and 50 % for fish products, potatoes and dried fruits (Leclercq et 151 al., 2000)

152 Finne et al., (1986) studied the residual sulfite content in shrimps after different days of 153 conservation in ice. They demonstrated that, starting with a concentration of about 80 ppm, 154 the residual content was reduced to less than 10 ppm after 6 days of storage on ice.

between 25 and 50 % for fish products, potatoes and dried frui<br>1986) studied the residual sulfite content in shrimps after dii<br>1986) studied the residual sulfite content in shrimps after dii<br>1986) studied the residual sulf 156 Directive 95/2/EC (European Community, 1995) requires that Member States of the EU 157 monitor the intake and use of food additives in order to allow evaluation of intake trends. 158 Measures can be taken to amend the legislation if intake concerns are identified in one or 159 more Member States. The Report from the European Commission on Dietary Food Additive 160 Intake in the European Union (European Commission, 2001) describes a harmonized, tiered 161 method to estimate additive intakes. In the first step (Tier 1), theoretical food-consumption 162 data (Hansen, 1979) are combined with the maximum permitted usage levels for the additive. 163 This conservative estimate was performed at European level. For the additives exceeding the 164 ADI in Tier 1, in a further step (Tier 2), national food consumption data are combined with 165 maximum permitted usage levels. Such intake estimate will still overestimate the real intake, 166 but can exclude additives, which do not pose a major risk from the most difficult estimate in 167 Tier 3: a combination of actual national food consumption data with actual levels of the 168 additive. The report from the Scientific Committee on Food (SCF) about Dietary Food 169 Additive Intake in the European Union (1998) showed that, based on Tier 1, there were 10 170 additives in the European Union for which a more refined intake estimate was needed. 171 Among those additives also sulfite was mentioned (Scientific Committee on Food). A Tier 2 172 exposure assessment in 7 countries (Belgium not included) showed that the range of estimated 173 intakes was 20-266% of the ADI for adults (European Commission).

174 The objective of this study was to perform a refined exposure assessment for sulfite intake in 175 the Belgian adult population, using the data from the national food consumption survey of 176 2004. Another objective was to identify the food groups contributing most to the total intake 177 of sulfites in Belgium.

## **Materials and Methods**

#### **Study design**

sment, maximum levels of sulphur dioxide in different foods we<br>ion data of these specific foods, derived from the Belgian foo<br>For some foods in the EU legislation, it is unlikely that they an<br>tities or they were too diffic 182 A semi-probabilistic approach was used to perform the exposure assessment. For the Tier 2 183 exposure assessment, maximum levels of sulphur dioxide in different foods were linked to the 184 food consumption data of these specific foods, derived from the Belgian food consumption 185 survey (2004). For some foods in the EU legislation, it is unlikely that they are consumed in 186 significant quantities or they were too difficult to link with the data from the food 187 consumption survey: burger meat with a vegetable or cereal content of minimum 4 %; 188 breakfast sausages; *Longaniza fresca* and *Butifarra fresca*; dried salted *Gadidae* fish; *sago*; 189 pearl barley; dried ginger; pulp of horseradish; pulp of onions, shallots and garlic; yellow 190 paprika in brine; flavourings on the basis of lemon fruit juice; concentrated grape juice for the 191 making of house wine; *mostrarda di frutti*; gelatinized fruit extracts or fluid pectin; glass 192 preserves of cut lemon; white syrup and molasses; orange-, grapefruit-, apple- and pineapple 193 juice for unpackaged sell in catering enterprises; concentrates on the basis of fruit juice with 194 at least 2.5 % pearl barley; *capilhé groselha*; unfermented grape juice for religious use; wine 195 without alcohol; fermented vinegar; marinated nuts; vacuum packaged sugar maize and 196 distilled alcoholic beverages with whole pears. Consequently these products were not taken 197 into account in the intake assessment. This could lead to an underestimation of the intake, but 198 this was supposed to be limited. As stated in the introduction, cooking and freezing can 199 decrease the sulfite concentration of foods. This was not taken into account either due to the 200 lack of reliable processing factors. Consequently this could lead to an overestimation of the 201 intake. 

 

203 In order to perform a more refined estimate of the sulfite intake (partial Tier 3 exposure 204 assessment), on the basis of literature, available concentration values for products analyzed by 205 The Belgian Federal Agency for Safety of the Food Chain (FASFC) and the results from the 206 Tier 2 exposure assessment, it was decided to analyze concentrations of sulphur dioxide in 207 beers and potato products. In these samples concentrations were determined using a high 208 performance ion chromatography method with eluent conductivity detector, optimized and 209 validated for this purpose. In the partial Tier 3 exposure assessment, analyzed actual levels of 

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210 sulphur dioxide in different foods were linked to the food consumption data of these specific 211 foods, derived from the Belgian food consumption survey (2004). For foods for which no 212 analyzed levels were available, maximum concentrations were used, as in the Tier 2 exposure 213 assessment. No illegal uses of sulfites in food groups in which no use of sulfites is authorised, 214 were taken into account in this study.

malyses were grouped to ensure a clear data presentation. The<br>proding to the EU directive 95/2/EC (European Community, 199<br>ugars and glucose-based products; condiments and dessert sauce<br>s; shellfish; cereal-based products 216 Although the regulation specifies maximum levels for specific groups, in this paper, the 217 results of the analyses were grouped to ensure a clear data presentation. The grouping was 218 performed according to the EU directive 95/2/EC (European Community, 1995) as follows: 219 beers; wines; sugars and glucose-based products; condiments and dessert sauces; gelatine and 220 meat substitutes; shellfish; cereal-based products (starches and biscuits); processed potatoes, 221 processed fruits (including dried fruits, candied fruits, jams, jellies and marmalades, lemon 222 and lime juice, fruit fillings and fruits in vinegar, brine, oil) and processed vegetables 223 (including dried tomatoes, mushrooms, vegetables in vinegar, brine and oil and white 224 vegetables).

**Food consumption data** 

228 Consumption data from the national food consumption survey 2004 were used to perform the 229 exposure assessment. Aims, design and methods of this survey are described elsewhere (De Vriese et al. 2005). The target population comprised all Belgian inhabitants of 15 years or 231 older. The sample included 3245 participants randomly selected from the National Register, 232 using a multi-stage stratified procedure.

234 Information on dietary intake was collected by two non-consecutive 24-hour recalls in 235 combination with a food frequency questionnaire. During the 24-hour recall interviews the 236 respondent reported the quantity of all foods and beverages consumed during the preceding 237 day. The 24-hour recall was carried out using EPIC-SOFT software (Slimani & Valsta, 2002). 238 This program allows obtaining very detailed information about the foods consumed and the 239 recipes used in a standardized way.

241 3083 participants, of which 1537 women and 1546 men, completed two 24-hour recalls. 242 Participants were categorized into four age groups: 15-18 years (n=760), 19-59 years (n=830), 243 60-74 years (n=789) and 75 years or older (n=704).

## **Sulphur dioxide concentration of selected foods**

246 The selection of the matrix types to be analyzed was based on the results of the Tier 2 247 calculation, literature and on the available sulfite concentrations obtained from the FASFC in 248 different kinds of foods (Table 1). Both beers and pre-cut fresh potatoes or French fries and 249 frozen prepared potato (precooked or not) products were sampled.

*x* (Belgian Monitor) specifies 4 categories of beer (S and I to III<br>e.e. Beers are characterized according to the fermentation type,<br>e wort density and the alcohol concentration. Those chara<br>he present study, for example 251 The Belgian law (Belgian Monitor) specifies 4 categories of beer (S and I to III) depending on 252 the Plato degree. Beers are characterized according to the fermentation type, the bitterness, 253 the colour, the wort density and the alcohol concentration. Those characteristics were 254 important in the present study, for example in case of ale beers undergoing a second 255 fermentation in the bottle where sulfite can be released by the fermentation process. The 256 presence of fruits can also be a source of sulfite. Therefore, in this study, beers were 257 categorized as follows: beer table blond, beer table brown, beer table triple blond, beer table 258 non specified colour, beer pilsner, beer pilsner light, beer without alcohol, beer special blond, 259 beer special amber, beer special brown, beer special with fruits, beer special unknown colour, 260 beer gueuze, beer gueuze with fruits, beer white, beer white with fruits and beer unknown 261 type and colour.

263 From the Belgian food consumption survey 2004 the frequencies and amounts of beer 264 consumption for all beers were retrieved. Each brand was assigned to a group of beer, as 265 defined earlier; and some simple calculations were made to determine the percentage of 266 consumption for each beer category and each brand within each category. The number of 267 samples was taken proportional to the brand distribution in the respective categories.

268 Samples were only taken for the brands with a percentage of consumption higher than 0.5  $\%$ , 269 except for 1 brand of gueuze with fruits and 2 other brands of gueuze because they are 270 representative in their specific categories and they were observed in different stores.

272 Collection of samples was performed in 2007 in 5 major supermarket chains with a good 273 market share, which are visited by approximately 77.5 % of the Belgian population in search 274 for their food provisioning (CRIOC - OIVO). In total for both categories 67 samples (21 for 275 potato products and 46 for beers) were collected and analysed.

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277 The only existing ISO method including sulfite analysis is the method ISO 10304-3:1997. The 278 HPIC/conductivity detector with eluent conductivity suppressor was used in this study to 279 perform the analyses. Ion chromatography is an appropriate technique for the separation of 280 sulfite from other anions like phosphate and sulphate, and it has also been recognised for the 281 quantification of sulfite (Armentia-Alvarez A. et al., 1997; Kim 1989; Kim et al. 1987; Kim et al. 2000; Paino-Campa et al. 1991; Pena-Egido et al., 2005; Pizzoferrato et al. 1990) Pulsed 283 amperometric detection necessitates very specific material and is not so easy to use because of 284 possible fouling of the electrode by matrix compounds (McFeeters and Barish, 2003). The 285 most used detection method in ion chromatography is eluent suppressed conductivity. With 286 the carbonate/bicarbonate eluent, the produced species in the suppressor is  $H_2CO_3$  (which 287 decomposes in  $CO<sub>2</sub>$  and  $H<sub>2</sub>O$ ).

#### *Material*

g of the electrode by matrix compounds (McFeeters and Bariction method in ion chromatography is eluent suppressed conoicarbonate eluent, the produced species in the suppressor is CO<sub>2</sub> and H<sub>2</sub>O).<br> **For Peer Review Only an** 290 For sample preparation, a Robocoup mixer 3000, a T25 Ultra-Turrax (IKA) and a centrifuge 291 Beckman Avanti J-25 operated at 7500rpm were used. All extracts where filtered through 292 0.2µm PVDF syringe filters. The analytical system was composed of a Waters 717+ auto 293 injector, an HP 1050 pump, an Alltech Suppressor model 640 and an Alltech conductivity 294 detector model 650. The acquisition of the signal and data treatment (calibration curve and 295 extract concentration) were made by a computer equipped with an analogue/digital converter 296 and ChromQuest software version 4.1. The separation column was composed of a Dionex 297 RFIC analytical column type IonPac AS14A-5 $\mu$ m 3x150mm (particle size of 5 $\mu$ m) and 298 precolumn RFIC IonPac AG14A-5µm 3x30mm.

## *Reagents and solutions*

301 All reagents were at least from analytical grade. Sodium carbonate and sodium bicarbonate 302 where purchased from VWR International. D-Mannitol, disodium salt of ethylenediamine 303 tetra acetic acid (EDTA) and formaldehyde were purchased from Sigma-Aldrich and 304 anhydrous sulfite was purchased from Fluka.

305 The aqueous solutions where prepared with a MilliQ Reagent Grade System's water with 306 conductivity lower then 18.2 M $\Omega$ cm<sup>-1</sup> (from Millipore coupled after a Millipore Elix 100 307 deionised water production system). Stock solutions NaHCO<sub>3</sub> 0.3 M, Na<sub>2</sub>CO<sub>3</sub> 0.2M and D-308 Mannitol 0.5 M were prepared. Those solutions were kept in the fridge. The eluent used for 309 the anion separation was the buffer  $NaHCO<sub>3</sub>/Na<sub>2</sub>CO<sub>3</sub> 3mM/2mM$ . The sulfite extraction 

310 buffer was a mixture of NaHCO<sub>3</sub>/Na<sub>2</sub>CO<sub>3</sub>/D-Mannitol 9mM/16mM/10mM. This resulted in a 311 final extraction pH between 8 and 10, as proposed in literature for free sulfite and reversible 312 bound sulfite extraction (Armentia-Alvarez A. et al. 1993; Wedzicha, 1992). The 1000 mg/l 313 sulphur dioxide standard stock solution was prepared by weighting 0.1968g anhydrous sulfite 314 and making a 100.0 ml solution with extraction buffer.

## *Sample collection, storage and pre-treatment*

317 After collection of the samples, they were identified by a unique identification number and 318 registered in a Microsoft Excel sheet. Following characteristics were added: brand name, 319 product description, lot number, temperature of conservation in the store and any relevant 320 information for the project (presence or absence of sulfite mentioned on the label or usage of 321 ingredients containing sulfite). The liquid samples where stored in a fridge and the solid 322 samples where stored in the freezer (-18°C or less) until pre-treatment or analysis.

324 The pre-treatment of the solid samples consisted of grinding at least 200 g collected sample 325 with a kitchen robot (Robot Coupe model R 301 Ultra) in order to get piece cuts less than 3~5 326 mm. Afterwards the prepared samples were stored deep frozen. All analyses where conducted 327 within 3 weeks after collection of the samples. 

## *Sample preparation*

**Fourage and pre-treatment**<br>
in of the samples, they were identified by a unique identification<br>
Microsoft Excel sheet. Following characteristics were added<br>
toon, lot number, temperature of conservation in the store and<br> 329 For the preparation of the liquid samples 100 µl of D-Mannitol 0.5 M was added to about 100 330 ml of the sample before degassing in an ultrasound bath for 5 minutes; 5.0 ml of sample was 331 then diluted in the extraction buffer by a factor of 5 (final volume = 25.0 ml) and the extract 332 was filtered through a PVDF syringe filter with 0.2 µm pore size before injection on the HPIC 333 system. For the preparation of the solid samples, after a short period of unfreezing, about 5 g 334 of sample (weighted with at least 3 digits after the point) was weighted in a P.E. tube of 50 ml 335 to be centrifuged; 25.0 ml of extraction buffer (measured with a graduate cylinder of 25 ml) 336 was added to the sample; the sample was then homogenated in an Ultra-Turrax for a 337 minimum of time, so that the mixture looked like creamy (10 to 15 seconds suffice). 

338 The tube was closed and set in a centrifuge at 7500 rpm for 10 minutes; the surnatant extract 339 was filtered through a PVDF syringe filter with 0.2 µm pore size before injection on the HPIC 340 system. 

5, 10, 50, 100, and 200 mg  $SO<sub>2</sub>/l$ ). Blank was used as 0 mg  $SO<sub>2</sub>/l$ . The best fit was used in



ize the coefficient of determination (> 98 %) and to minimise<br>s validated for linearity, precision (repeatability, reproducibility<br>on according to the procedure described by Van Loco et al (4). After multiple tests for con

9.3 and of 8.7 when prepared with frozen French fries. NaOH/formol was tested for the

the extract concentration. This type of regression resulted in a determination coefficient

phosphate, sulfite, and sulphate with a total runtime period of 25 minutes (stabilization

373 The range for the calibration was 0.1 to 100 mg/l extract (The injection of the point 200 mg/l 374 was kept in order to be able to quantify higher amounts from a new regression). The range in 375 the extract corresponded to a range in the sample going from 0.5 to 500 mg/kg (or mg/l, 376 depending of the nature of sample) if the dilution factor was taken into account.

378 In order to test the repeatability and reproducibility a sample (table beer) was fortified at 4 379 levels of concentration corresponding to 0, 5, 10 and 15 mg/l of the sample (which 380 corresponds to 0, 1, 2 and 3mg/l in the extract). The test was conducted in triplicate for each 381 level and repeated for each level at 3 different days. The non fortified sample did not result in 382 a detectable response and the obtained result of reproducibility was lower than 12.6 %.

0, 1, 2 and 3mg/l in the extract). The test was conducted in tripted for each level at 3 different days. The non fortified sample c ponse and the obtained result of reproducibility was lower than recovery was, on average, 384 The apparent recovery was, on average, for the 3 levels of concentration,  $73 \pm 6$  at 3 385 concentration levels. A lower recovery may be explained by a possible reaction of  $SO<sub>2</sub>$  to 386 some compounds present in the matrix and/or by oxidation after the dissolution of ambient 387 oxygen into the extracts. Therefore, it was decided to correct the results for the recovery of 388 the control standard included in the series of analysis.

390 In beer sample, the sulfite peak was poorly separated from matrix interferences. The 391 minimum detectable concentration in the extract was about 0.5 mg/l. This corresponds to a 392 quantification limit (LOQ) of 1 mg/l extract which corresponds to 5 mg/l (or mg/kg) in the 393 sample. This is also the lowest tested concentration.

**Exposure assessment** 

396 Only respondents with two completed 24-hour recall interviews were included in the analyses 397 (n= 3083; 1546 men and 1537 women).

399 The individual intake of sulphur dioxide from a certain food product was estimated using the 400 following equation:

401 
$$
y_i (mg / kgbw / day ) = \frac{c \times x_i}{bw_i}
$$

402 where  $y_i$  is the intake of sulphur dioxide by individual *i* from a particular food (in mg per kg 403 bodyweight per interview day), *c* is the concentration of sulphur dioxide in that food (mg per 404 kg),  $x_i$  is the consumption of a certain food by individual *i* (kg) and  $bw_i$  is the self-reported 405 body weight of individual *i* (kg). To estimate the total intake of sulphur dioxide per food

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406 group or per day, individual daily intakes of sulphur dioxide from different foods were added 407 up.

408 As mentioned before, two approaches were used for the calculation of sulphur dioxide intake. 409 In the Tier 2 approach, *ci* represents the maximum allowed concentration in each food. The 410 maximum permitted levels of sulphur dioxide used in different foods are listed in the 411 European Directive 95/2/EC (European Community, 1995). In the Tier 3 approach, *c<sup>i</sup>* 412 represents the actual average concentration of sulphur dioxide that is observed in a particular 413 food.

ke distribution for sulphur dioxide was estimated with the 1996) using the C-side software (lowa State University, ods are available to estimate usual intake distributions with the kewness. These statistical procedures ad 415 The usual intake distribution for sulphur dioxide was estimated with the Nusser method 416 (Nusser et al. 1996) using the C-side software (Iowa State University, 1996). Several 417 statistical methods are available to estimate usual intake distributions with the correct mean, 418 variance and skewness. These statistical procedures adjust for within-person or day-to-day 419 variability. Of all different statistical procedures, the Nusser method (Nusser et al., 1996) is 420 highly recommended because it eliminates the intra-individual variance and additionally 421 transforms the data to obtain approximately normally distributed data. The method is suitable 422 to estimate usual intake distributions in a population both for normally and non-normally 423 distributed foods and nutrients. The usual intake distribution was weighted and adjusted for 424 the age and sex distribution of the Belgian population and adjusted for day of the week and 425 season.

**Results and discussion** 

429 As an adult inhales about 15  $m<sup>3</sup>$  of air per day, this means that in the worst case the amount of 430 SO<sub>2</sub> intake from the environment is 215  $\mu$ g/m<sup>3</sup> x 15 m<sup>3</sup> = 3225  $\mu$ g/ day = 3.2 mg/day. 215 431  $\mu$ g/m<sup>3</sup> is the highest concentration of SO<sub>2</sub> measured in Belgium in 2005. Since the average 432 body weight of a Belgian adult is around 70 kg, the final  $SO_2$  intake from the environment is 433 estimated to be 0.045 mg/kg bw/day. From those results it was decided to neglect the 434 environmental contribution to  $SO<sub>2</sub>$  intake in Belgium.

436 The usual sulfite intake of the Belgian adult population estimated by the Tier 2 approach was 437 0.34 mg/kg bw/day (49 % of the ADI) at the mean level of consumption (Table 2). At the 438 97.5<sup>th</sup> percentile of the population, the sulfite intake was estimated to be 1.1 mg  $SO_2/kg$ 439 bw/day (157 % of the ADI). It was observed that the ADI was exceeded beyond the  $90<sup>th</sup>$  

440 percentile of the population. At this percentile the contribution of wine consumption to the 441 total intake was estimated to be 71 %. Including only wine consumers (55.1%), the ADI was 442 reached at the  $57.5^{\text{th}}$  percentile of population (data not shown).

444 Wine contributed for more than 50 % to the total intake of sulfite. Sugar and glucose-based 445 products, beers, processed fruits and processed potatoes contributed each between 7-13 % to 446 the total intake, while shellfish and processed vegetables contributed each about 4 % to the 447 total intake. Condiments and cereal products contributed to less than 1 % to the total average 448 sulfite intake (Table 2).

and ments and cereal products contributed to less than 1 % to the cable 2).<br> **For Solution** in beer samples (n=46) were all under the detection line of sulfite in beer samples (n=46) were all under the detection line unlu 450 Concentrations of sulfite in beer samples (n=46) were all under the detection limit (LOD) of 5 451 mg/l; these results were different from the concentrations found in other countries. For 452 example, in France (Mareschi et al. 1992) and Italy (Leclercq et al., 2000) the average sulfite 453 concentrations found in beer were respectively 7.5 and 15 mg  $SO<sub>2</sub>/l$ . In these studies the 454 Monier-Williams method was used to perform the analyses. It is known that this method has 455 possible interferences with volatile compounds. The method used in the present study does 456 not have this disadvantage. With the limit of quantification of 5 mg  $SO<sub>2</sub>/l$  it was not possible 457 to determine any residual  $SO<sub>2</sub>$  levels in the beers analyzed. Provided this and the results 458 reported by other countries obtained by more sensitive electrochemical methods of analysis 459 (Dvorak et al. 2006) it was decided not to perform an intake estimation of sulfite from beer 460 using a null concentration of sulfite in beers. It is known that sulfite could be produced during 461 the natural fermentation process in the bottle. Therefore half of the LOQ concentration was 462 used (medium bound approach) instead of the null concentration to perform the intake 463 estimation.

465 For potato products 6 samples out of 21 analyzed samples resulted in  $SO_2$  levels above the 466 quantification limit (LOQ) of 5 mg/l and only one sample with no sulfite declared on the label 467 resulted in a concentration higher than the LOQ. This positive sample was a fresh pre-cut 468 product in which the concentration was 9.9 mg  $SO_2/kg$ . For the samples with a concentration 469 above LOQ, an average concentration value of 11.6 mg  $SO<sub>2</sub>/kg$  was found and the results 470 were spread between 9.0 and 13.5 mg  $SO<sub>2</sub>/kg$ . In total there were 83 samples checked for 471 sulfite on the label, while only 21 samples were analyzed. If all samples checked on the label 472 for sulfite would be considered (n=83) instead of only the analyzed ones, and if it is assumed 473 there are no frauds on sulfite usage, only 6 of 83 products were found to have a sulfite 

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474 concentration above LOQ. For the evaluation of the sulfite intake from potato products it was 475 decided to use the average concentration of the samples having a concentration above LOQ. 476 This might result in an overestimation of the intake since only 7 % of selected and analyzed 477 potato products had a  $SO_2$  concentration above the LOQ. However, some consumers might by 478 loyal to these particular kinds of brands. In addition this actual level used (11.6 mg/kg) is still 479 far below the maximum permitted levels (50-100 mg/kg  $SO<sub>2</sub>$ ).

481 From the Belgian Federal Agency for the Safety of the Food Chain (FASFC), sulfite analysis 482 results for different kinds of foods sampled between 2003 and 2006 were obtained (Table 1). 483 These results show that average actual levels found are often much lower than maximum 484 levels.

an Federal Agency for the Safety of the Food Chain (FASFC),<br>rent kinds of foods sampled between 2003 and 2006 were obta<br>how that average actual levels found are often much lower to<br>bahow that average actual levels found ar 485 After considering the data from different years, it was decided to take the average results from 486 the last year only or from different previous years. For each food group, the average 487 concentration of the samples having a concentration above LOQ (5 mg/l) was used, in order 488 to account for consumers being loyal to certain brands of products. There was a drastic 489 decrease (about factor 10) in  $SO_2$  concentration in dried tomatoes from 2004 to 2005 due to 490 the fact that in 2004 there were found levels above the maximum while no levels above the 491 maximum were found in 2005; therefore only the 2 last year's results were taken into account 492 (2005 and 2006). The available number of data for each kind of dried fruits, except dried 493 apricots, is low; therefore it was decided to use the average concentration of sulfite in dried 494 fruits (excluding dried apricots) (data from 2006) for dried fruits other than apricots. The 495 concentration assigned to dried apricots was the average of the 2005 and 2006 measurements 496 in dried apricots. For wines, the average concentration found by the FASFC in white and red 497 wines for the years 2004 and 2006 was assigned.

499 Measurements made in France (Mareschi et al., 1992) during the period 1989-1992, showed 500 that the highest average  $SO_2$  concentrations were found in salted fish fillets and dried fruits 501 (more than 625 mg  $SO_2/kg$ ), followed by mustard (225 mg  $SO_2/kg$ ) and peeled potatoes (100 502 mg  $SO_2/kg$ ). Wines resulted in an average concentration of 75 mg  $SO_2/l$  (no differences where 503 made between red and white wines). Other measurements made during the same period in the 504 United States (Daniels et al. 1992) showed higher concentrations in dried fruits, mashed 505 potatoes and wines. More recent (2000-2005) studies made in Italy (Leclercq et al., 2000) and 

506 Australia (Food Standards Australia New Zealand, 2005), showed that the sulfite level content 507 in food tends to diminish in some kinds of foods like cider, beer, died fruits and wines.

509 The results of the partial Tier 3 based on the analyzed products and on the maximum levels 510 for the other food groups, are given in Table 3. The mean intake of sulfites in the Belgian 511 adult population was estimated to be 0.19 mg/kg bw/day, corresponding to 27 % of the ADI. 512 At the 97.5<sup>th</sup> percentile of the population the usual intake was 0.61 mg SO<sub>2</sub>/kg bw/day (87 %) 513 of the ADI). It was found that for the average consumer wine contributed up to 14 % of the 514 ADI). The sugar group still had a relative significant contribution to the intake, but actual 515 concentrations were not available. It is known, however, that food producers try to avoid the 516 need for allergen labelling of sulfite, by reducing the levels below 10 mg/kg.

was found that for the average consumer wine contributed up<br>ar group still had a relative significant contribution to the int<br>were not available. It is known, however, that food producers<br>that habelling of sulfite, by redu 518 Some groups of the population could be more exposed than others to sulfite intake, in 519 particular wine consumers. The probability of approaching the ADI by wine consumers will 520 depend on the particular choice of wine (sulfite concentration) and the consumption level. 521 This is hard to estimate. An exceeding of the ADI can not be excluded for a limited 522 percentage of the population of wine consumers, who might consume wine with a higher 523 average concentration than the average concentration used here in the tier 3 calculations, as 524 can be shown from the tier 2 estimates (where the consumption of wine alone can reach the 525 ADI).

527 The fact that some food groups were not taken into account, could have led to a certain 528 underestimation of the intake. However, the reduction that takes place during further 529 preparation of foods was not taken in to account but is a factor of overestimation of the intake 530 of sulfites. Further, the fact that no illegal uses of sulfites in certain food groups were taken 531 into account, could also have led to an underestimation. In 2005 1002 tests with malachite 532 green were performed in chopped meat by the FASCA of which 54 were positive. 43 of these 533 were confirmed in the laboratory. The average sulfite content in the samples exceeding the 534 LOQ was on average 474.1 +/-487.1 mg/kg and 1782.6 mg/kg at the 97.5 percentile.

 

536 In 1999, JECFA reviewed the intake estimation of sulfite from different countries (JECFA, 537 1999): Aus-NZ, China, Finland, France, India, Japan, Spain, United Kingdom and United 538 States. The estimated range of intake of sulfite (expressed as sulphur dioxide) was between 10 539 and 1400 % of ADI. In should be noted that data modelling differed between countries. The

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540 different used models are the budget method, the poundage data method, the FBS/HES/sales 541 data method, model diets method and individual dietary records method. A summary of the 542 mean intake results *per capita* for those countries is given in Table 4. All of these results are 543 based on a Tier 2 like approach. Comparatively to those results, in the present study lower 544 intakes were found, except when compared to Finland, India and USA.

difite intake was estimated to be 1.34 mg SO<sub>2</sub>/kg bw/day (191<br>at the 95th percentile of the population to be 3.13 mg SO<sub>2</sub>/kg bw<br>f these estimates exceeded the ADI of 0.7 mg/kg bw/day and wa<br>alculated data. The estimatio 546 A more recent study in France (1998) performed a Tier 2 intake estimation (Verger et al. 1998). 547 The average sulfite intake was estimated to be 1.34 mg  $SO_2/kg$  bw/day (191 % of the ADI) 548 and the intake at the 95th percentile of the population to be 3.13 mg  $SO_2/kg$  bw/day (447 % of 549 the ADI). All of these estimates exceeded the ADI of 0.7 mg/kg bw/day and were comparable 550 to the earlier calculated data. The estimation of the intake of sulfite in France (Verger et al. 551 1998) on basis of actual concentrations for the global population was 20 mg SO<sub>2</sub>/day *per capita* in 1992 (48 % of the ADI). The authors concluded that the major contributors to the 553 intake of sulfite were alcoholic beverages (wine, cider and beer). They performed an intake 554 calculation for alcoholic beverage consumers only and for non alcoholic beverage consumers. 555 They found an intake of sulfite respectively of 31.5 mg  $SO_2/day$  (75.0 % of the ADI) and 1.96 556 mg SO<sub>2</sub>/day (4.67 % of the ADI).

558 In Italy during 2000, exposure to sulfite was estimated for adults and children (Leclercq et al., 559 2000). A first Tier 2 calculation resulted in an exposure of 0.82 mg  $SO_2/kg$  bw/day or 117 % 560 of the ADI, of which 32 mg was due to wine consumption (65 % of total intake), 8 mg from 561 dried fruits (16 %) and 2 mg from fish (4 %).

563 Since the ADI is exceeded by the Tier 2 in Italy, calculation with actual data was performed 564 by the same authors. Leclercq *et al*. (2000) made 2 different modelled diets rich in sulfited 565 foods in order to calculate estimation of the intake on bases of actual concentrations in food. 566 The models differed for children and adults, where it was assumed that the body weight is 30 567 kg for a child and 60 kg for an adult. The ADI was not exceeded using the example diets 568 proposed for calculation, although nearly (more then 95 % of the ADI) for adults in the worst 569 case scenario. Real food consumption data would have increased the amount of information 570 and improved the intake estimates.

572 A study conducted in Slovakia (Sinko & Janekova, 2006) demonstrated that the intake of 573 sulfite in a group of children aged between 7 and 10 years old (lowest body weight 21 kg; 574 average weight 26 kg) was between 27 and 93 % of the ADI for a child of 21 kg. The study 575 was based on the actual food consumption during 4 months and maximum allowed sulfite 576 content in food.

(30 % of the ADI). The same calculation for the 95th pe<br>wed an intake estimate of 0.9 mg SO<sub>2</sub>/kg bw/day (lower b<br>presponded to 130 % of the ADI. It was shown that in Tier *i*<br>for high consumers was 1400 % of the ADI (JEC 578 A recent survey in Australia (Food Standards Australia New Zealand, 2005) showed an 579 average intake of sulfite in the population of 0.2 mg  $SO_2/kg$  bw/day (lower bound estimation; 580 concentrations not detected set to zero). With the upper bound estimate, the intake obtained 581 was the same (30 % of the ADI). The same calculation for the 95th percentile of the 582 population showed an intake estimate of 0.9 mg  $SO_2/kg$  bw/day (lower bound or upper 583 bound), that corresponded to 130 % of the ADI. It was shown that in Tier 2 approach the 584 intake estimated for high consumers was 1400 % of the ADI (JECFA) while with actual data, 585 the intake for the high consumers became 130 %, which is more than 10-fold difference (yet 586 still above the ADI).

588 In this study, actual typical levels of sulfites were much lower than the maximum levels set in 589 the legislation for several food groups. This resulted in average intake estimates going from 590 0.34 mg/kg bw/day in Tier 2 to 0.19 mg/kg bw/day in the partial Tier 3. Wine showed a 591 decrease in the sulfite intake estimate from 24 % (Tier 2) to 14 % (partial Tier 3) of the ADI 592 for the mean consumer and from 116% (Tier 2) to 69% (partial Tier 3) for the 97.5 percentile. 593 However, the choice of wine of a consumer might be different than average.

#### **Conclusion**

596 In conclusion, it was shown that the intake of sulfites in the Belgian adult population is likely 597 to be below the ADI with the possible exception for some high consumers of wines with high 598 sulfite levels. As a consequence, the intake of sulfites by wine consumers remains a point of 599 attention. Further it was shown that for several food groups actual typical levels of sulfites are 600 much lower than the maximum levels set in the legislation.

 

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Table 1. Average sulfite concentrations \* in different foods (data from the control program of the Federal Agency for Safety of the Food Chain, Belgium) 



\* Including only samples exceeding the LOQ of 5 mg/L to calculate the average

## **Table 2**

## Usual intake of sulfites (mg/kg bw/day); results from the Tier 2 exposure assessment (national food consumption survey, 2004)



ADI acceptable daily intake

SD standard deviation

bw body weight

The usual dietary intakes were 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; total number of consumers is 3083

The acceptable daily intake (ADI) for sulphite is 0.7 mg/kg bw/day

\* including dried fruits (including dried apricots), candied fruits, jams, jellies and marmalades, lemon and lime juice, fruit fillings, fruits in vinegar, brine, oil.

\*\* including dried tomatoes, mushrooms, vegetables in vinegar, brine and oil, and white vegetables

**Table 3** Usual intake of sulfites (mg/kg bw/day); results from the partial Tier 3 exposure assessment (national food consumption survey, 2004)



ADI acceptable daily intake

SD standard deviation

bw body weight

The usual dietary intakes were 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; total number of consumers is 3083

The acceptable daily intake (ADI) for sulphite is 0.7 mg/kg bw/day

\* including dried fruits (including dried apricots), candied fruits, jams, jellies and marmalades, lemon and lime juice, fruit fillings, fruits in vinegar, brine, oil.

\*\* including dried tomatoes, mushrooms, vegetables in vinegar, brine and oil, and white vegetables

° Maximum concentrations used instead of analyzed concentrations

<sup>oo</sup> Combination of maximum concentrations and analyzed concentrations used depending on the availability of analyzed concentrations for the food items in the considered food groups



