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Estimate of intake of benzoic acid for the Belgian adult population

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Abstract

An exposure assessment was performed to estimate average daily benzoic acid intake for Belgian adults. Food consumption data were retrieved from the national food consumption survey. In a first step, individual food consumption data were multiplied with the maximum permitted use levels for benzoic acid per food group (Tier 2). In a second step, a label survey to identify the foods where benzoic acid is effectively used as an additive and a literature review of the possible occurrence of benzoic acid as a natural substance, were performed. With this information a refined list of foods was drafted for the quantification of benzoic acid, which was performed by an HPLC method, optimized and validated for this purpose. Individual food consumption data were then multiplied with the actual average concentrations of benzoic acid per food group (Tier 3). Usual intakes were calculated using the Nusser method. The mean benzoic acid intake was 1.58 mg/kg bw/day (Tier 2) and 1.25 mg/kg bw/day (Tier 3). In Tier 2, men exceeded the acceptable daily intake (ADI) of 5 mg/kg bw/day at the 99th percentile. The greatest contributors to the benzoic acid intake were soft drinks. Benzoic acid as a natural substance represents only a small percentage of the total intake. The results show that actual benzoic acid intake is very likely to be below the ADI. However, there is a need to collect national food consumption data for children as they might be more vulnerable for an excessive intake.

39 Introduction

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41 Benzoic acid (E210) or its sodium (E211), potassium (E212) or calcium (E213) salt, is intentionally
42 added to certain foods as a food preservative or may naturally be present in specific foods. Sodium
43 benzoate is about 200 times more soluble than benzoic acid and is used preferably as one of the main
44 antimicrobial preservatives in foods and beverages. Benzoic acid is used as an antiseptic, antimycotic
45 and antipyretic agent (Suhr and Nielsen 2004).

46
47 The temporary acceptable daily intake (ADI) was set at 5 mg/kg body weight by the European
48 Scientific Committee on Food in 1994. The Joint WHO/FAO Expert Committee on Food Additives
49 (JECFA) set an ADI of 5 mg/kg body weight in 1996, which was a confirmation of the evaluation in
50 1994. Use of benzoic acid is authorised in the European Union in several food groups on the condition
51 of respecting the maximum use levels (Directive 95/2/EC), which are all expressed as the free acid.
52 The European Commission (Directive 95/2/EC) asks the Member States to monitor the consumption
53 and use of food additives, in order to allow evaluation of the consumption trends as well as differences
54 in consumption patterns among various Member States.

55
56 The Report from the European Commission on Dietary Food Additive Intake in the European Union
57 (European Commission 2001) describes a harmonized, tiered method to estimate additive intakes. In
58 Tier 1, the theoretical food consumption data are combined with the maximum permitted usage levels
59 for the additive. This conservative estimate was done at the EU level and is not a task for the Member
60 States, as no national data are used. Only for a limited list of additives, national estimates are
61 necessary to refine the estimate for those additives for which in Tier 1 an exceeding of the ADI was
62 observed. In the next step (Tier 2), actual national food consumption data are used and combined with
63 maximum permitted usage levels. Such intake estimate will still overestimate the real intake, but can
64 exclude additives which do not pose a major risk from the most difficult estimate in Tier 3: a
65 combination of actual national food consumption data with actual usage levels of the additive.

66
67 A study on the intake of benzoic acid was chosen because of the following elements: (1) high intakes
68 were estimated in other countries; (2) benzoic acid is much less reactive than sulphites or nitrites
69 resulting in residual levels more close to usage levels; (3) feasibility to analyse; (4) benzoic acid is
70 important in soft drinks for which consumption data are high and increasing; (5) there is a long list of
71 food groups in which the additive is authorised and extensions of authorisations are from time to time
72 requested.

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74 In a former study in Belgium, a Tier 2 exposure assessment of benzoic acid was carried out in three
75 different population groups: Flemish preschool children (n=697; age 2-6; 3 days estimated dietary

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3 76 record), adolescents from a major city in Flanders, Ghent (n=341; age 14-18; 7-day estimated dietary
4 77 record) and women of reproductive age from Flanders (n=641; age 18-40; 2-day estimated dietary
5 78 record) (Bilau et al. 2008). The median estimated daily intake was respectively 2.0 mg/kg bw, 1.7
6 79 mg/kg bw and 1.92 mg/kg bw. In all age groups the greatest contributor to total benzoic acid intake
7 80 was the group of non-alcoholic flavoured drinks. At respectively the 92nd percentile (children), the 97th
8 81 percentile (adolescents) and the 94th percentile (women), the ADI was slightly exceeded. This study
9 82 showed the need to perform a more refined estimate, using actual levels.
10 83

11 84 Besides being widely used as a food additive, benzoic acid occurs naturally in different fruits
12 85 (Hegnaur 1992). Appreciable amounts have been detected in most berries (around 0.05%) (Budavari et
13 86 al. 1996), in particular in cranberries where they are present in a range from 300 to 1300 mg/kg
14 87 (Hegnaur 1966). Benzoic acid has also been detected in many animal products, including dairy
15 88 products, as the result of the lactic acid fermentation (Sauh et al. 1990). It is also produced during the
16 89 ripening of cheese by the propionic acid fermentation following the lactic acid fermentation (Thierry
17 90 and Maillard 2002). Naturally occurring benzoic acid has also been found in honey (Stegg and Montag
18 91 1987), mushrooms (Beltran-Garcia 1997) and wine (Matejicek et al. 2003). Further, benzoic acid can
19 92 be used as a plasticizer in the manufacturing of plastic packing materials for yoghurt, butter or
20 93 margarine (Vermeiren et al. 2002). The latter source of contamination with benzoic acid will not be
21 94 further discussed in this paper.
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34 95 The objective of this study was to perform a refined estimate of the benzoic acid intake in the
35 96 Belgian adult population, using the data from the national food consumption survey of 2004.
36 97 Another objective was to identify the food groups contributing most to the total benzoic acid
37 98 intake in Belgium. The national food consumption data and maximum usage levels of benzoic
38 99 acid were first combined to perform a Tier 2 exposure assessment. Analytical results were
39 100 obtained for chosen food groups, and then, as far as possible, actual average levels of benzoic
40 101 acid were combined with consumption data to perform a Tier 3 exposure assessment. Besides
41 102 taking into account additive use, the refined intake estimate also took into account the natural
42 103 presence of benzoic acid in certain foods to obtain the best estimate of real intake. In Tier2, as
43 104 well as in Tier 3, intake of benzoic acid was compared with the ADI,
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54 105 Materials and methods

55 106

56 107 Study design

57 108 For the Tier 2 exposure assessment, maximum usage levels of benzoic acid in different foods were as
58 109 far as possible linked to the food consumption data of these specific foods, derived from the Belgian
59 110 food consumption survey 2004). For some foods in the EU legislation, it is unlikely that they exist on

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3 111 the Belgian market (for example grape juice for sacramental use or liquid tea concentrates). Other
4 112 products were not found in the food consumption survey (e.g. food supplements). For the authorised
5 113 *quantum satis* use as surface treatment of dried meat products, a Tier 2 approach was not possible. The
6 114 exclusion of some food groups could lead to an underestimation of the intake, but this was supposed to
7 115 be very limited.
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13 117 For the Tier 3 exposure assessment, first a food label survey was performed and then samples of
14 118 different foods, in which benzoic acid is actually added or present as a natural substance, were taken
15 119 from different supermarkets. In these samples concentrations of benzoic acid were determined with an
16 120 optimized and validated analytical method. Benzoic acid concentrations in different foods were then
17 121 linked to the consumption quantity of these foods, calculated from the data of the food consumption
18 122 survey. For foods for which no analyses were performed (e.g. shrimps, crustaceans and molluscs,
19 123 which were designated as a less important source of benzoic acid due to its less frequent intake), the
20 124 maximum permitted level of benzoic acid was used.
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28 126 **Food consumption data**

29 127 Consumption data from the national food consumption survey 2004 were used to perform the exposure
30 128 assessments. Aims, design and methods of this survey are described elsewhere (De Vriese et al. 2005).
31 129 The target population comprised all Belgian inhabitants of 15 years or older. The sample included
32 130 3245 participants randomly selected from the National Register, using a multi-stage stratified
33 131 procedure.
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39 133 Information on dietary intake was collected by two non-consecutive 24-hour recalls in combination
40 134 with a food frequency questionnaire. During the 24-hour recall interviews the respondent reported the
41 135 quantity of all foods and beverages consumed during the preceding day. The 24-hour recall was
42 136 carried out using EPIC-SOFT software (Slimani and Valsta 2002). This program allows obtaining very
43 137 detailed information about the foods consumed and the recipes used in a standardized way.
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49 139 3083 participants completed two 24-hour recalls of which 1537 women and 1546 men. Participants
50 140 were categorized into four age groups: 15-18 years (n=760), 19-59 years (n=830), 60-74 years (n=789)
51 141 and 75 years or older (n=704).
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55 143 **Benzoic acid concentration of selected foods**

56 144 Before the development of the sampling plan for the collection of the samples to be analysed, a food
57 145 label survey was performed to identify the food products where benzoic acid is actually used as an
58 146 additive. Five supermarket chains with good market share in Belgium were visited. Based on the
59 147 regulation (Directive 95/2/EC), several food groups have been surveyed, 1245 items in total. Products

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3 148 were selected according to the indications on the labels concerning addition of benzoic acid. Although
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5 149 this was not an absolutely exhaustive survey of all existing categories of food, it was attempted to
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7 150 cover the most important national and marketplace's brands. The final goal of the survey was to get an
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9 151 overview of the general manufacturing practices regarding benzoic acid application as an additive in
10 152 Belgium.

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13 154 Further, a literature review of the possible occurrence of benzoic acid as a natural substance was
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15 155 performed. After this first selection of foods, a refined list of foods was drafted for the quantification
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17 156 of benzoic acid. The food consumption survey 2004 was used to evaluate the various channels used
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19 157 for food provisions (supermarkets), the frequencies of consumption of different foods and the
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21 158 preparations of foods. The Tier 2 exposure assessment provided an indicative list of the most
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23 159 important foods contributing to benzoic acid intake in the Belgian population. The comparison of the
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25 160 Tier 2 acquired list with the refined list from the label survey and knowledge of foods in which
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27 161 benzoic acid may be present as a natural substance, permitted the development of the sampling plan
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29 162 for the collection of the food samples to be analysed.

30 163
31 164 A great effort was put into ensuring representative concentration data of benzoic acid in foods on the
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33 165 Belgian market. The sampling plan included two categories: food items with benzoic acid used as an
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35 166 additive and those where benzoic acid is likely to be present as natural substance. In total for both
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37 167 categories 58 samples (28 and 30, respectively to the category) were collected and analysed. The
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39 168 results of the analyses were grouped to ensure a clear data presentation. The grouping was performed
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41 169 according to the EU directive 95/2/EC as follows: desserts, confectionary, prepared salads, non
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43 170 emulsified sauces, and non-alcoholic drinks. Additionally, two food groups were included, namely
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45 171 dairy products (cheese, yoghurt, and dairy flavoured with fruits) and products based on fruits (berries,
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47 172 grapes, apples, pears and tropical fruits). Collection of samples was performed from August to
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49 173 September 2007 in 5 major supermarket chains with a good market share. These 5 food distributors
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51 174 are visited by approximately 75% of the Belgian population in search for their food provisioning
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53 175 (CRIOC - OIVO 2007). Prepared salads were additionally taken from sandwich delivery snack bars. If
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55 176 the analyses were not conducted on the same day, the sample was stored deep frozen for a maximum
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57 177 time of one month.

58 178
59 179 Extraction from and determination of benzoic acid in different matrices was done by a modified
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180 method described in ISO/FDIS 22855:2007(E) (International Organisation for Standardisation 2007)
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182 where benzoic acid was quantified by a high performance liquid chromatography method optimized
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184 and validated for this purpose. This proposed method is based on a formerly used method (Küppers
and Jans 1988).

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3 185 Sample preparation and extraction of benzoic acid was adjusted to the state (liquid or solid) of the
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5 186 sample. In this manner liquid samples were homogenised prior to opening of the package while
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7 187 carbonated drinks were degassed in an ultrasonic bath for 3-5 min. A sample (10ml) was transferred
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9 188 into a 50ml conical tube where 10ml of methanol-water (80:20, v:v) solution of 3-chlorobenzoic acid
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11 189 (75mg/l) was added. The solution pH was adjusted with a droplet of 36.5% HCl. In the following step
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13 190 the solution was vortex mixed and centrifuged for 5 minutes at 7000rpm. From this 1ml was used for
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15 191 further purification.

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18 193 Solid samples (200g) were homogenised with an Ultra-Turrax mixer. Out of that 10g±0.1g was
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20 194 weighed and transferred into a 50ml conical tube. 10 ml of methanol-water (80:20, v:v) solution of 3-
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22 195 chlorobenzoic acid (75mg/l) was added. After adding a droplet of 36.5% HCl, the tube was closed and
23
24 196 vortex mixed (non fatty solid partially hydrophilic samples, e.g. jams and gums) or mixed with the
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26 197 Ultra-Turrax (fatty solid hydrophobic samples, e.g. cheese, fish, and crustaceans) until dissolution into
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28 198 the aqueous phase was obtained. If necessary, the sample was placed in a warm (45-60°C) water bath
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30 199 for 30 min with frequent agitation. In the following step the solution was vortex mixed, centrifuged
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32 200 for 5 min at 7000 rpm and cooled to room temperature. From this 1ml was used for further
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34 201 purification.

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36 202
37 203 All prepared samples were subjected to purification through SPE C18ec (Chromabond, 500mg, 3ml)
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39 204 which was conditioned by 1 cartridge volume methanol followed by 1 cartridge volume HCl 0.1M.
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41 205 Next, 1ml of the sample was applied (at the flow rate 0.4ml/min) and the cartridge was successively
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43 206 washed twice with 2ml of 0.1M HCl. and additionally washed with 1ml hexane where before and after
44
45 207 this step the cartridge was left to dry for 3 or 1 min (respectively). The extract was eluted with 3ml
46
47 208 methanol and concentrated under nitrogen at 40°C to approximately 1ml.

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49 209
50 210 Finally the extracts were filtered through PTFE 0.45µm and injected into HPLC. HPLC analysis was
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52 211 conducted using an Agilent 1100 Series (Waldbronn, Germany) equipped with a quaternary pump, an
53
54 212 auto sampler and DAD detector operated by ChemStation software (version B.01.03) A LiChrospher
55
56 213 RP Select B (5µm; 250x4.6mm) HPLC column with Lichrospher RP Select B pre-column was used
57
58 214 throughout the study.

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60 215
61 216 The separation was obtained by gradient elution with three mobile phases. Mobile phase A
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63 217 (ammonium acetate aqueous solution, pH 3.9, 0.05M) was kept constant at 10% while mobile phase B
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65 218 (acetonitrile) and C (MiliQ water) were kept constant first 10 min: 25% B and 65% C; then the
66
67 219 gradient changed 10-13min 60%B and 30%C and kept constant for 7 min; 20-21min: 25% B and

220 65% C and the system was equilibrated for 4 min. The flow rate was 1ml/min at 35°C. The detection
221 was carried out by DAD detector at 235 and 255nm.

222
223 Identification of benzoic acid was done by comparing the retention time and peak shape to that of
224 reference standard whereas quantification was performed according to the calibration curve
225 ($y=1.48x+0.03$; $R^2=0.99$) plotted as the respond ratio of benzoic acid and 3-chlorobenzoic acid versus
226 concentration of benzoic acid in the range 0-250mg/l for nine calibration points (0; 0.2; 0.5; 1; 5; 10;
227 25; 50; 100; 250mg/l).

228 The method was validated on 3 different matrices: sugared liquids, emulsified solids and dairy
229 products (mayonnaise and plain yoghurt respectively). The limit of detection (LOD) and limit of
230 quantification (LOQ) for benzoic acid were 2.5 and 5 mg/l respectively. The intra-day and inter-day
231 precision of the method showed a coefficient of variation (%CV) between 0.25 and 16% for spiked
232 samples (taking $\leq 10\%$ as acceptable limit) and the recovery of the method was found to be between 87
233 to 113%.. The precision of the method was dependent on the matrix and the concentration. For both
234 non-alcoholic flavoured drinks (soft drinks) and dairy products (yoghurt) the lowest level (10 mg/l and
235 5 mg/kg respectively) resulted in higher %CV's (12-16 %) whereas CV's for determination of benzoic
236 acid in fatty samples (mayonnaise) were low at all three levels.

237 **Statistical analysis**

238 Only respondents with two completed 24-hour recall interviews were included in the analyses (n=
239 3083; 1546 men and 1537 women).

240
241 The individual intake of benzoic acid from a certain food product was estimated by the following
242 equation:

$$243 \quad y_i (\text{mg / kgbw / day}) = \frac{c_i \times x_i}{bw_i} ,$$

244 where y_i is the intake of benzoic acid by individual i from a particular food (in mg per kg bodyweight
245 per interview day), c_i is the concentration of benzoic acid in that food (mg per kg), x_i is the
246 consumption of a certain food by individual i (kg) and bw_i is the self-reported body weight of
247 individual i (kg). To estimate the total intake of benzoic acid per food group or per day, individual
248 daily intakes of benzoic acid from different foods were added up.

249
250 As mentioned before, two approaches were used for the calculation of benzoic acid intake. In the Tier
251 2 approach, c_i represents the maximum allowed concentration in each food. The maximum permitted
252 levels of benzoic acid used in different foods are listed in the European Directive 95/2/EC (Table 1) (

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3 253 Directive 95/2/EC). In the Tier 3 approach, c_i represents the actual average concentration of benzoic
4 254 acid that is observed in a particular food.

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8 256 The usual intake distribution for benzoic acid was estimated with the Nusser method (Nusser et al.
9
10 257 1996) using the C-side software (Iowa State University 1996). Several statistical methods are available
11 258 to estimate usual intake distributions with the correct mean, variance and skewness. These statistical
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13 259 procedures adjust for within-person or day-to-day variability. Of all different statistical procedures, the
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15 260 Nusser method (Nusser et al., 1996) is highly recommended because it eliminates the intra-individual
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17 261 variance and additionally transforms the data to obtain approximately normally distributed data. The
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19 262 method is suitable to estimate usual intake distributions in a population both for normally and non-
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21 263 normally distributed foods and nutrients. The usual intake distribution was weighted and adjusted for
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23 264 the age and sex distribution of the Belgian population and adjusted for day of the week and season.
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25 265

26 266 Results

27 267
28 268 The label survey revealed that in biscuits, cakes and confectionary no benzoic acid is used on the
29 269 Belgian market, although it is allowed. In the category of emulsified sauces the difference between
30 270 regular and light sauces was remarkable. Of 55 light emulsified sauces, 19 contained benzoic acid
31 271 (35%) whereas only 10% of the regular products had the additive indicated on the label. From the
32 272 results it appeared that benzoic acid may more often be used in light sauces. Similarly, in relation to
33 273 the fat percentage of the product, it was observed that benzoic acid was more often used in products
34 274 containing less than 60% fat.
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40 276 The concentration of benzoic acid in samples suspected to contain benzoic acid as a natural substance,
41 277 was under official limits. Red and white wine, fruit juices and syrups, in particular those from berries,
42 278 did not contain benzoic acid above the LOQ. Among dairy products, the highest amount of benzoic
43 279 acid was detected in cottage cheese. The average amounts in yoghurts were around 25 mg/kg which
44 280 was comparable to the amounts detected in different yoghurts collected from stores in Belgium in
45 281 1982 (Guyot et al. 1998; Sieber et al. 1995; Van Renterghem 1982). On the other hand, the amount of
46 282 the additive in all analysed soft drinks (coke and lemonade) was between 100 mg/l and the maximum
47 283 permitted level of 150 mg/l. As a result, the use of actual levels instead of maximum levels did not
48 284 reduce the intake estimate dramatically (Table 1). Intake estimates of consumption of food groups can
49 285 be found in Table 2.
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58 287 The Tier 2 approach, which is the most conservative approach, revealed a mean usual daily benzoic
59 288 acid intake of 1.58 mg/kg bw/day (Table 3; Figure 1). At the 97.5th percentile, the usual intake of
60 289 benzoic acid was 3.97 mg/kg bw/day which was still below the ADI. The most important contributor

290 to the intake of benzoic acid was the group of non-alcoholic flavoured beverages (31.07%). Other
291 important contributors at the upper end of the intake distribution were non-emulsified sauces
292 (16.16%), vegetables in vinegar, brine or oil (13.24%), prepared salads (8.08%), semi-preserved fish
293 products (6.90%) and shrimps, molluscs and crustaceans (6.84%).

294
295 The Tier 3 approach is more accurate than the Tier 2 since it takes into account the actual average
296 concentrations of benzoic acid in different foods and it also considers the presence of benzoic acid as a
297 natural substance. Nevertheless the results of Tier 3 were comparable to those of Tier 2 (Table 4,
298 Figure 2), where no exceeding of the ADI was observed. Non-alcoholic flavoured drinks (34.28%)
299 like coke and lemonade were again the most important contributors to the intake of benzoic acid.
300 Benzoic acid present as a natural substance in food only contributed to a very small percentage (2.7%)
301 to the total benzoic acid intake (Table 4).

302
303 It was observed that the benzoic acid intake in men was higher than in women (Figure 1; Figure 2).
304 Mean usual benzoic acid intake in men (Tier 3) was 1.41 mg/kg bw/day. For women mean usual
305 benzoic acid intake (Tier 3) was 1.22 mg/kg bw/day. Men consumed more non-alcoholic flavoured
306 beverages than women (288 ± 307 g/day versus 177 ± 198 g/day for women), which were the main
307 contributors to the benzoic acid intake. Men only exceeded the acceptable daily intake at the 99th
308 percentile for the Tier 2 estimation at an intake of 5.18 mg/kg bw/day (while women only reached
309 4.22 mg/kg bw/day at the 99th percentile). However, men did not exceed the ADI at the 99th percentile
310 for the Tier 3 estimation where the intake was 4.64 mg/kg bw/day (while 4.10 mg/kg bw/day for
311 women).

312 Discussion

313
314
315 The food label survey showed that in some products, like confectionary, although allowed, no benzoic
316 acid is used in Belgium. Remarkable was that benzoic acid is used more frequently in light sauces than
317 in regular sauces. A possible explanation could be the higher water content of these products which in
318 turn require higher levels of benzoic acid to exert a sufficient preservative effect. The analyses of the
319 food samples for quantification of benzoic acid showed that no products with higher levels of benzoic
320 acid than allowed were present in selected food stores in Belgium.

321
322 In the most conservative method (Tier 2), both mean usual benzoic acid intake and the usual benzoic
323 acid intake at the higher percentiles were found to be lower than the ADI. When a Tier 3 exposure
324 assessment was performed, the mean usual intake of benzoic acid and the usual intake of benzoic acid
325 at the higher percentiles were also lower than the ADI, which means there is no direct concern of an
326 excessive benzoic acid intake in the Belgian adult population. Benzoic acid intake in this population

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3 327 may be regarded as safe and does not need further consideration if dietary patterns do not change
4 328 tremendously. However, there are some minor concerns which may be raised.

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8 330 The most important contributor to the benzoic acid intake in the Belgian adult population is the food
9 331 group of non-alcoholic flavoured drinks. People might be loyal to one brand and hence not consume
10 332 an average concentration. Using maximum levels in the calculation might better protect consumers. As
11 333 explained above, some food groups in which use of benzoic acid is authorised were not taken into
12 334 account. Drugs and cosmetics were not taken into account either.

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17 336 In the Belgian population 15 years and older, there is no direct threat to consumers regarding benzoic
18 337 acid intake. However, there may be special groups which are at risk for exceeding the ADI. First, men
19 338 seem to have a higher intake of benzoic acid than women, due to a higher intake of soft drinks. Men
20 339 exceed the ADI at the 99th percentile in a Tier 2 exposure assessment. Second, soft drinks are usually
21 340 related to children's preferences and children are particularly vulnerable to food safety issues. On the
22 341 other hand sodium benzoate will be phased out as additive in soft drinks, resulting in lower intake of
23 342 benzoic acid in the near future (Nyman et al. 2008). Moreover it is remarkable that no benzoic acid is
24 343 used in Belgium in cakes, biscuits and confectionary, food groups which are frequently consumed by
25 344 children.

26 345
27 346 Unfortunately, the lack of national data about children's consumption habits posed a limitation to the
28 347 study. The EU Scientific committee on Food has recommended that special attention should be given
29 348 to additive intake in children, since the intake is expressed on a body weight basis and may be
30 349 markedly higher than that of adults.

31 350
32 351 Benzoic acid has also been linked to hyperactivity in children when mixed with other artificial food
33 352 colours (McCann et al., 2007). In Flemish preschool children, the calculated (Tier 2) intake estimate
34 353 was only slightly higher than in adolescents or adult women (Bilau et al., 2008).

35 354
36 355 The intake as well as the pattern of benzoic acid in the Belgian population was comparable with that in
37 356 some other countries but somewhat higher. The average intake of benzoic acid in Australia and New
38 357 Zealand was between 10 and 15% of the ADI for the consumers older than 2 years whereas for the 95th
39 358 percentile the intake was between 50% and 65% of the ADI. The most exposed population group were
40 359 children between 2 and 12 years. In this age group, benzoic acid exposure was estimated to be about
41 360 1.2-1.4 times the ADI (with higher intake for boys than for girls). The major intake for the average
42 361 population came from soft drinks (55%), regular cordial (21%) and orange juice (18%) (Food
43 362 Standards Australia New Zealand 2005).

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3 364 An intake survey of different food additives in Finland (Pentilla et al. 1988) identified fruit and berry
4 365 juices, soft drinks and semi-preserved fish as the major sources of intake of benzoic acid which was
5 366 estimated to be 40 mg/day representing 13% of the acceptable daily intake. Those results were
6 367 obtained from the food balance sheets of the Government statistical office and actual concentration in
7 368 1400 foods products was used.
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13 370 At last, there are some important methodological limitations that should be taken into account when
14 371 interpreting the findings of this study. First, it is very difficult to exactly match the food groups or
15 372 items defined in the legislation with the food groups or items defined in the food consumption survey.
16 373 Secondly, some potentially important food groups, for example liquid eggs used in industrial
17 374 processes, to which benzoic acid is commonly added as a preservative, were neglected in this study. It
18 375 was not possible to disaggregate food consumption data up to this level. Further, benzoic acid in
19 376 seasonings and condiments and in grape juice fermented for sacramental juice was neglected because
20 377 of an assumed low consumption and a difficult match with food consumption data.
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28 379 In conclusion, the intake of benzoic acid in the Belgian adult population does not imply an immediate
29 380 threat to consumers. Nevertheless, the intake is close to the accepted daily intake level and this
30 381 maximum level may be exceeded if dietary habits change for example when soft drink consumption
31 382 increases or when authorisations are extended. No data about children are available at this moment
32 383 while children are considered the most vulnerable population group. In the future, a food consumption
33 384 monitoring system should be organised in Belgium to be able to evaluate additive intakes of different
34 385 population groups on a regular basis.
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49 395 Environment.
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Table 1 Maximum permitted level (mg/kg or mg/l) and average concentration level (SD) (mg/kg or mg/l) of benzoic acid in different food groups

FOOD GROUP	Maximum permitted level	Average concentration level	SD	number of samples
<i>use of benzoic acid as an additive</i>				
non-alcoholic flavoured drinks	150	120.5	17.0	10
coca cola	150	128.3	21.7	3
lemonade	150	112.1	10.3	5
ice-tea	150	114.1		1
aromatised water	150	145.9		1
prepared salads	1500	705.0	205.5	10
low-sugar marmalades	500	*		0
confectionary	1500	**		0
chewing gum	1500	**		0
non heat-treated dairy-based desserts	300	**		0
emulsified sauces (>60% fat)	500	**		0
emulsified sauces (<60% fat)	1000	819.0	185.2	5
light sauces	1000	844.6	7.0	2
light mayonnaise	1000	498.6	99.2	2
dressings	1000	768.6		1
non-emulsified sauces	1000	594.3	228.6	3
salted dried fish	200	*		0
semi-preserved fish products	2000	*		0
shrimps, crustaceans and molluscs	1000 [#]	*		0
vegetables in vinegar/oil/brine	2000	*		0
olives and olive-based preparations	500	*		0
liquid soups and broth (not canned)	500	*		0
cooked red beet	2000	*		0
candied fruit	1000	*		0
mustard	1000	*		0
<i>presence of benzoic acid as a natural substance</i>				
wines		2.5 ^{&}		3
fruit juices		3.8 [§]		3
yoghurts total				10
yoghurts with berries		25.7	6.5	7
yoghurts nature		24.1	0.2	3
butter		7.5 [§]		3
syrops		3.8 [§]		6
hard (Gouda) cheeses		13.4	0.5	2
cottage cheese		31.9 [°]		3

SD standard deviation

*No analyses were performed because the Tier 2 showed that the contribution of these products to the total benzoic acid intake was low

** No benzoic acid was found on the labels of these products and consequently no analyses were performed

[#] 6000 mg/kg for grey shrimps

& lower bound method used (3 samples below LOD)

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§ middle bound method used (for fruit juices: 2 samples below LOD and 1 sample below LOQ, for syrups 3 samples below LOD and 3 samples below LOQ)
\$ Upper bound method used (3 samples below LOQ)
° Only one sample above LOQ

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Table 2 The mean (SD; P50; P97.5; P99) usual consumption (g/day or ml/day) and the number of consumers (n) of the food groups in which addition of benzoic acid is permitted or in which benzoic acid is present as a natural substance (Belgian food consumption survey, 2004)

FOOD GROUP	Intake (g/day or ml/day)						n
	mean	SD	P50	P95	P97.5	P99	
<i>use of benzoic acid as an additive</i>							
non-alcoholic flavoured drinks	228	251	164	719	884	1107	1540
coca cola	140	201	23	545	673	846	975
lemonade	51	97	0	242	324	446	603
ice-tea	14	45	0	119	169	223	203
aromatised water	23	82	0	174	277	417	211
prepared salads	6	9	0	25	32	41	373
low-sugar marmalades	0.6	3.1	0.0	0.9	8.9	16.8	121
confectionary	1.2	3.6	0.0	7.7	11.5	17.4	256
chewing gum*	0.03	0.87	0.00	0.00	0.00	0.00	15
non heat-treated dairy-based desserts	16	17	11	49	60	77	166
emulsified sauces (>60% fat)	5.8	4.6	4.8	14.8	17.6	21.4	1139
emulsified sauces (<60% fat)	3.9	3.6	3.1	10.9	13.3	16.8	924
light sauces	1.4	2.8	0.0	7.0	9.4	12.8	273
light mayonnaise	0.6	1.1	0.0	2.8	3.6	4.7	254
dressings	1.9	2.3	1.2	6.4	7.6	9.3	503
non-emulsified sauces	17.2	11.1	15.2	38.4	44.5	52.5	1317
salted dried fish*	0.1	3.1	0.0	0.0	0.0	0.0	4
semi-preserved fish products	3.8	3.1	3.1	9.9	11.8	14.4	411
shrimps, crustaceans and molluscs	5.3	5.6	3.9	16.5	20.0	24.7	404
vegetables in vinegar/oil/brine	7.6	8.1	5.0	23.9	30.2	39.0	579
olives and olive-based preparations	0.7	1.8	0.0	4.4	6.3	8.9	99
liquid soups and broth (not canned)*	3.1	35.0	0.0	0.0	0.0	0.0	54
cooked red beet*	0.3	5.8	0.0	0.0	0.0	0.0	29
candied fruit*	0.001	0.038	0.000	0.000	0.000	0.000	1
mustard	0.3	0.2	0.2	0.7	0.9	1.2	289
<i>Presence of benzoic acid as a natural substance</i>							
wines	67	95	20	260	320	402	885
fruit juices	59	79	29	215	269	344	1072
yoghurts: total	39	49	23	129	162	213	943
yoghurts: with fruit	25	39	0	100	125	168	588
yoghurt: plane	14	30	0	78	101	131	440
butter	7	12	1	33	43	58	1164
syrops	3	17	0	20	41	77	124
hard (gouda) cheeses	14	11	12	36	42	51	1568
cottage cheeses	11	26	0	67	92	124	429

The average usual intakes are weighted and adjusted for the age and sex distribution of the Belgian population 2004 and adjusted for interview day and season

The total number of consumers (n) is 3083

* Average intake of the two interview days in stead of usual intake (Nusser method could not be applied due to low number of observations), not adjusted for the age and sex distribution of the Belgian population, neither for interview day and season

Table 3. The mean estimated usual intake (mg/kg bw/day) (SD, % contribution of food groups to the benzoic acid intake, P50, P95, P97.5, P99, % contribution of food groups to the ADI) of benzoic acid in the Belgian population via intake of different food groups, the Tier 2 approach

<u>FOOD GROUP</u>	<u>mean</u>	<u>SD</u>	<u>%</u>	<u>P50</u>	<u>P95</u>	<u>P97.5</u>	<u>P99</u>	<u>% from ADI</u>
<u>non-alcoholic flavoured drinks</u>	<u>0.50</u>	<u>0.31</u>	<u>31.1</u>	<u>0.35</u>	<u>1.59</u>	<u>1.96</u>	<u>2.47</u>	<u>10.0</u>
<u>coca cola</u>	<u>0.30</u>	<u>0.44</u>	<u>18.6</u>	<u>0.05</u>	<u>1.18</u>	<u>1.46</u>	<u>1.85</u>	<u>6.0</u>
<u>lemonade</u>	<u>0.11</u>	<u>0.22</u>	<u>6.8</u>	<u>0.00</u>	<u>0.54</u>	<u>0.74</u>	<u>1.03</u>	<u>2.2</u>
<u>ice-tea</u>	<u>0.03</u>	<u>0.10</u>	<u>1.9</u>	<u>0.00</u>	<u>0.27</u>	<u>0.38</u>	<u>0.50</u>	<u>0.6</u>
<u>aromatised water</u>	<u>0.05</u>	<u>0.19</u>	<u>3.1</u>	<u>0.00</u>	<u>0.40</u>	<u>0.64</u>	<u>0.97</u>	<u>1.0</u>
<u>prepared salads</u>	<u>0.13</u>	<u>0.20</u>	<u>8.1</u>	<u>0.00</u>	<u>0.54</u>	<u>0.68</u>	<u>0.88</u>	<u>2.6</u>
<u>low-sugar marmalades</u>	<u>0.004</u>	<u>0.023</u>	<u>0.3</u>	<u>0.000</u>	<u>0.007</u>	<u>0.063</u>	<u>0.121</u>	<u>0.1</u>
<u>confectionary</u>	<u>0.07</u>	<u>0.08</u>	<u>4.4</u>	<u>0.00</u>	<u>0.17</u>	<u>0.26</u>	<u>0.39</u>	<u>1.4</u>
<u>chewing gum*</u>	<u>0.00073</u>	<u>0.02321</u>	<u>0.05</u>	<u>0.00000</u>	<u>0.00000</u>	<u>0.00000</u>	<u>0.00000</u>	<u>0.02</u>
<u>non heat-treated dairy-based desserts</u>	<u>0.0690</u>	<u>0.0760</u>	<u>4.3</u>	<u>0.0450</u>	<u>0.2190</u>	<u>0.2750</u>	<u>0.3530</u>	<u>1.4</u>
<u>emulsified sauces (>60% fat)</u>	<u>0.0420</u>	<u>0.0340</u>	<u>2.6</u>	<u>0.0340</u>	<u>0.1080</u>	<u>0.1300</u>	<u>0.1600</u>	<u>0.8</u>
<u>emulsified sauces (<60% fat)</u>	<u>0.0570</u>	<u>0.0530</u>	<u>3.5</u>	<u>0.0440</u>	<u>0.1590</u>	<u>0.1950</u>	<u>0.2440</u>	<u>1.1</u>
<u>light sauces</u>	<u>0.0190</u>	<u>0.0370</u>	<u>1.2</u>	<u>0.0000</u>	<u>0.0950</u>	<u>0.1250</u>	<u>0.1680</u>	<u>0.4</u>
<u>light mayonnaise</u>	<u>0.0089</u>	<u>0.0143</u>	<u>0.6</u>	<u>0.0000</u>	<u>0.0380</u>	<u>0.0485</u>	<u>0.0630</u>	<u>0.2</u>
<u>dressings</u>	<u>0.0280</u>	<u>0.0340</u>	<u>1.7</u>	<u>0.0170</u>	<u>0.0950</u>	<u>0.1140</u>	<u>0.1390</u>	<u>0.6</u>
<u>non-emulsified sauces</u>	<u>0.2600</u>	<u>0.1700</u>	<u>16.2</u>	<u>0.2240</u>	<u>0.5940</u>	<u>0.6970</u>	<u>0.8330</u>	<u>5.2</u>
<u>salted dried fish*</u>	<u>0.00018</u>	<u>0.00710</u>	<u>0.01</u>	<u>0.00000</u>	<u>0.00000</u>	<u>0.00000</u>	<u>0.00000</u>	<u>0.004</u>
<u>semi-preserved fish products</u>	<u>0.111</u>	<u>0.096</u>	<u>6.9</u>	<u>0.086</u>	<u>0.302</u>	<u>0.365</u>	<u>0.449</u>	<u>2.2</u>
<u>shrimps, crustaceans and molluscs</u>	<u>0.110</u>	<u>0.150</u>	<u>6.8</u>	<u>0.066</u>	<u>0.404</u>	<u>0.521</u>	<u>0.689</u>	<u>2.2</u>
<u>vegetables in vinegar/oil/brine</u>	<u>0.213</u>	<u>0.220</u>	<u>13.2</u>	<u>0.147</u>	<u>0.657</u>	<u>0.823</u>	<u>1.050</u>	<u>4.3</u>
<u>olives and olive-based preparations</u>	<u>0.0050</u>	<u>0.0122</u>	<u>0.3</u>	<u>0.0000</u>	<u>0.0304</u>	<u>0.0423</u>	<u>0.0585</u>	<u>0.1</u>
<u>liquid soups and broth (not canned)*</u>	<u>0.023</u>	<u>0.280</u>	<u>1.4</u>	<u>0.000</u>	<u>0.000</u>	<u>0.000</u>	<u>0.000</u>	<u>0.5</u>
<u>cooked red beet*</u>	<u>0.010</u>	<u>0.170</u>	<u>0.6</u>	<u>0.000</u>	<u>0.000</u>	<u>0.000</u>	<u>0.000</u>	<u>0.2</u>
<u>candied fruit*</u>	<u>5.449E-06</u>	<u>0.00043</u>	<u>0.0</u>	<u>0.00000</u>	<u>0.00000</u>	<u>0.00000</u>	<u>0.00000</u>	<u>0.0</u>
<u>mustard</u>	<u>0.0039</u>	<u>0.0037</u>	<u>0.2</u>	<u>0.0029</u>	<u>0.0104</u>	<u>0.0137</u>	<u>0.0191</u>	<u>0.1</u>
<u>TOTAL (% from ADI)</u>	<u>1.58 (31.6)</u>	<u>0.95</u>	-	<u>1.39 (27.8)</u>	<u>3.42 (64.8)</u>	<u>3.97 (79.4)</u>	<u>4.72 (94.4)</u>	-

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3 The average usual intakes are weighted and adjusted for the age and sex distribution of the Belgian population 2004 and adjusted for interview day and season

4 The total number of consumers (n) is 3083

5 *Average in stead of usual intake (due to low number of observations), not adjusted for the age and sex distribution of the Belgian population, neither for interview
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Table 4. The mean estimated usual benzoic acid intake (mg/kg bw/day) (SD, P50, % contribution of food groups to benzoic acid intake, P95, P97.5, P99, % contribution of food groups to ADI) as an additive (97.3%) and as a natural substance (2.7%) in the Belgian population via intake of different food groups, the Tier 3 approach

FOOD GROUP	mean	SD	%	P50	P95	P97.5	P99	% from ADI
<i>Benzoic acid as additive</i>								
non-alcoholic flavoured drinks	0.40	0.53	34.3	0.28	1.28	1.58	1.99	8.0
coca cola	0.240	0.350	20.6	0.040	0.950	1.180	1.490	4.8
lemonade	0.090	0.180	7.7	0.000	0.433	0.591	0.828	1.8
ice-tea	0.025	0.081	2.1	0.000	0.213	0.303	0.404	0.5
aromatised water	0.043	0.150	3.7	0.000	0.320	0.510	0.780	0.9
prepared salads	0.059	0.095	5.1	0.000	0.252	0.320	0.414	1.2
low-sugar marmalades	0.004	0.023	0.3	0.000	0.007	0.063	0.121	0.1
emulsified sauces (>60% fat)*	0.006	0.015	0.5	0.000	0.038	0.054	0.075	0.1
emulsified sauces (<60% fat)	0.044	0.042	3.8	0.034	0.125	0.154	0.194	0.9
-light sauces	0.0150	0.0300	1.3	0.0000	0.0780	0.1030	0.1370	0.3
-light mayonnaise	0.0044	0.0071	0.4	0.0000	0.0190	0.0242	0.0314	0.1
-dressings	0.0230	0.0270	2.0	0.0140	0.0770	0.0930	0.1140	0.5
non-emulsified sauces	0.1540	0.1000	13.2	0.1330	0.3530	0.4140	0.4950	3.1
salted dried fish**	0.00018	0.00710	0.02	0.00000	0.00000	0.00000	0.00000	0.0
semi-preserved fish products	0.111	0.096	9.5	0.086	0.302	0.365	0.449	2.2
Shrimps, crustaceans, molluscs	0.110	0.150	9.4	0.066	0.404	0.521	0.689	2.2
vegetables in vinegar/oil/brine	0.213	0.220	18.3	0.147	0.657	0.823	1.050	4.3
olives and olive-based preparations	0.0050	0.0122	0.4	0.0000	0.0304	0.0423	0.0585	0.1
soups and broth (not canned)**	0.023	0.280	2.0	0.000	0.000	0.000	0.000	0.5
cooked red beet**	0.0103	0.1700	0.9	0.0000	0.0000	0.0000	0.0000	0.2
candied fruit**	0.00001	0.00043	0.0	0.00000	0.00000	0.00000	0.00000	0.0
mustard	0.0039	0.0037	0.3	0.0029	0.0104	0.0137	0.0191	0.1

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Benzoic acid as natural substance

wines	0.0023	0.0033	0.2	0.0007	0.0091	0.0113	0.0141	0.05
fruit juices	0.0033	0.0045	0.3	0.0015	0.0121	0.0155	0.0201	0.07
yoghurts total	0.0146	0.0191	1.3	0.0085	0.0490	0.0620	0.0833	0.3
-yoghurts with fruit	0.0095	0.0161	0.8	0.0000	0.0380	0.0487	0.0679	0.2
-yoghurts plane	0.0051	0.0108	0.4	0.0000	0.0283	0.0367	0.0483	0.1
butter	0.00081	0.00140	0.1	0.00012	0.00359	0.00481	0.00656	0.02
syrups	0.000048	0.000190	0.0	0.000000	0.000360	0.000610	0.000960	0.0
hard (gouda) cheeses	0.0028	0.0021	0.2	0.0023	0.0068	0.0080	0.0096	0.06
cottage cheese	0.0049	0.0119	0.4	0.0000	0.0306	0.0415	0.0559	0.01
TOTAL (% from ADI)	1.25 (25.)	0.83	-	1.05 (21)	2.87 (57)	3.4 (68)	4.11(82)	-

The average usual intakes are weighted and adjusted for the age and sex distribution of the Belgian population 2004 and adjusted for interview day and season
 The total number of consumers (n) is 3083
 ** Average in stead of usual intake (The Nusser method could not be applied due to low number of observations), not adjusted for the age and sex distribution of the Belgian population, neither for interview day and season;
 *without mayonnaise because no benzoic acid was found on the labels
 Confectionary, chewing gum and non heat-treated dairy-based desserts were set at 0 mg/kg because no benzoic acid was found on the labels

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wines	0.0023	0.0033	0.20	0.0000	0.0007	0.0037	0
fruit juices	0.0033	0.0045	0.28	0.0000	0.0015	0.0051	0
yoghurts total	0.0146	0.0191	1.25	0.0000	0.0085	0.0232	0
-yoghurts with fruit	0.0095	0.0161	0.81	0.0000	0.0000	0.0153	0
-yoghurts plane	0.0051	0.0108	0.44	0.0000	0.0000	0.0051	0
butter	0.00081	0.00140	0.07	0.00000	0.00012	0.00111	0
syrups	0.000048	0.000190	0.00	0.000000	0.000000	0.000000	0
hard (gouda) cheeses	0.0028	0.0021	0.24	0.0012	0.0023	0.0039	0
cottage cheese	0.0049	0.0119	0.42	0.0000	0.0000	0.0000	0
% as natural contaminant= 2.7%							
TOTAL	1.25 (0.25)	0.83		0.65 (0.13)	1.05 (0.21)	1.63 (0.33)	0

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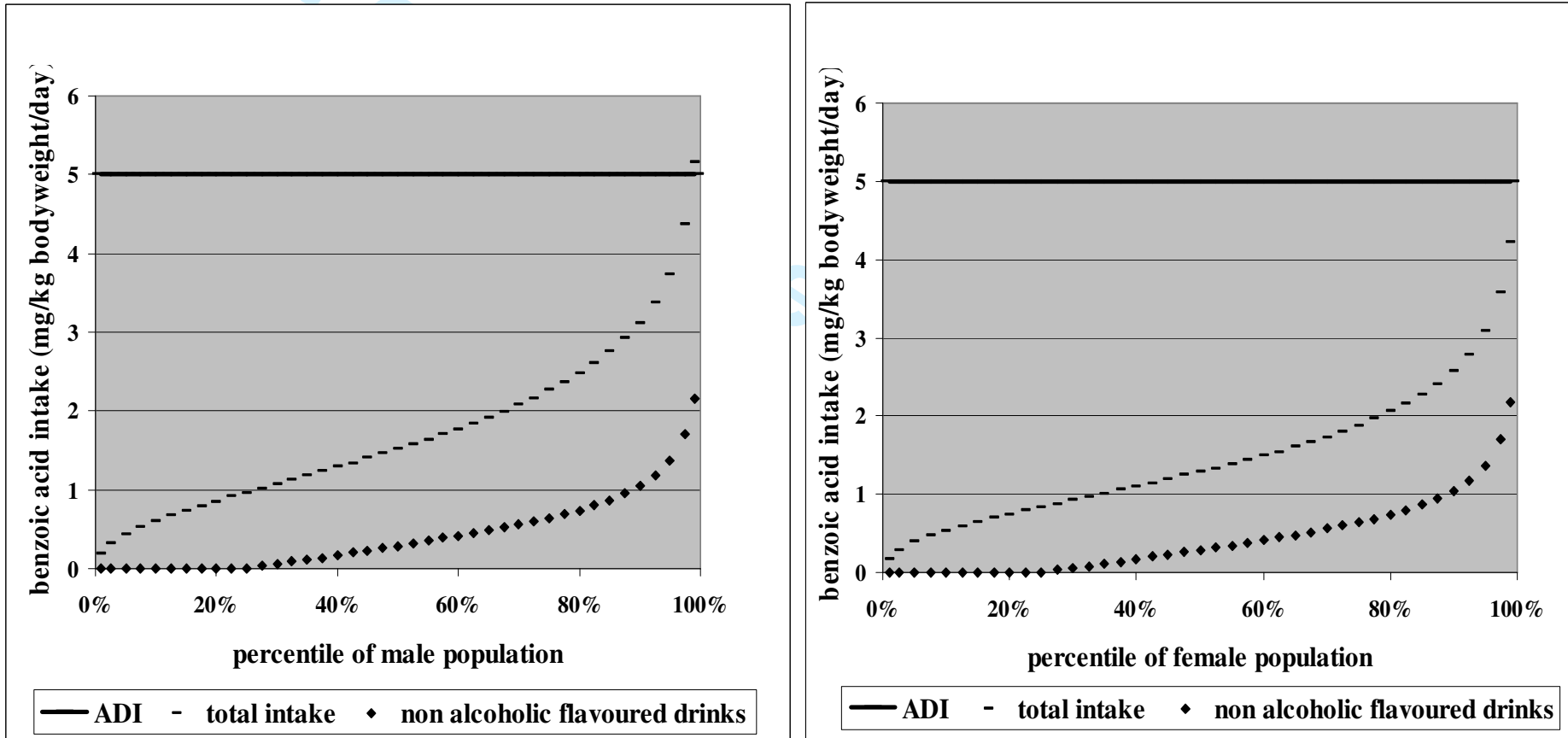


Figure 1. Cumulative distribution functions of the estimated benzoic acid intake (corrected for age) by the Belgian population (mg/kg bw/day), for men and women separately, Tier 2 approach

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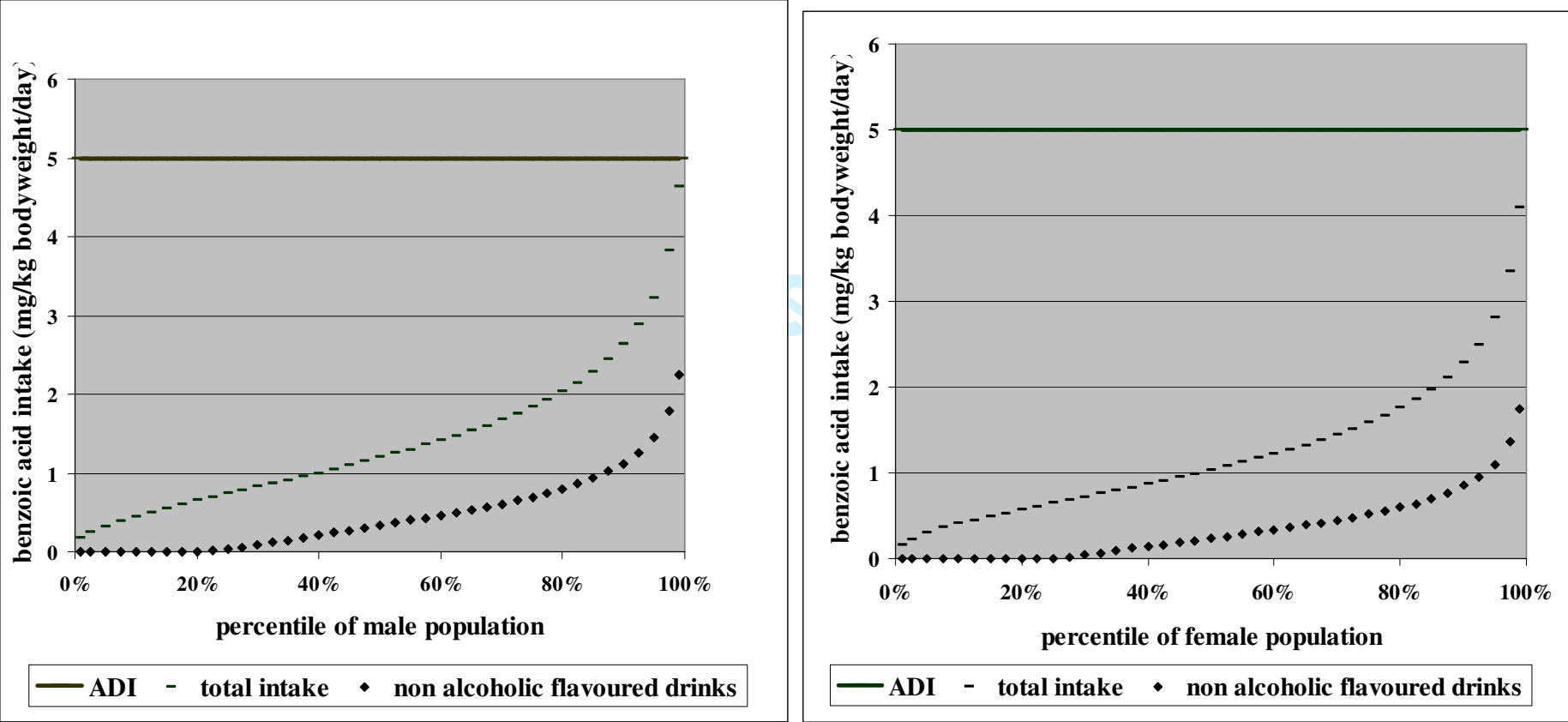


Figure 2. Cumulative distribution functions of the estimated benzoic acid intake (corrected for age) by the Belgian population (mg/kg bw/day) for men and women separately, Tier 3 approach

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