



HAL
open science

Dietary Intake of Artificial Sweeteners by the Belgian Population

Kevin Huvaere, Stefanie Marie Vandevijvere, Moez Hasni, Christine Vinkx,
Joris van Loco

► **To cite this version:**

Kevin Huvaere, Stefanie Marie Vandevijvere, Moez Hasni, Christine Vinkx, Joris van Loco. Dietary Intake of Artificial Sweeteners by the Belgian Population. *Food Additives and Contaminants*, 2011, 29 (1), pp.54-65. 10.1080/19440049.2011.627572 . hal-00753038

HAL Id: hal-00753038

<https://hal.science/hal-00753038>

Submitted on 17 Nov 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Dietary Intake of Artificial Sweeteners by the Belgian Population

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2011-249.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	18-Sep-2011
Complete List of Authors:	Huvaere, Kevin; Scientific Institute of Public Health, Food, Medicines and Consumer Safety Vandevijvere, Stefanie; Scientific Institute of Public Health, Epidemiology Hasni, Moez; Scientific Institute of Public Health, Food, Medicines and Consumer Safety Vinkx, Christine; Federal Service of Public Health, Food Chain and the Environment Van Loco, Joris; Scientific Institute of Public Health, Food, Medicines and Consumer Safety
Methods/Techniques:	Exposure assessment, LC/MS, Total diet studies
Additives/Contaminants:	Sweeteners
Food Types:	Beer, Beverages, Confectionary
Abstract:	In this study it was investigated whether the Belgian population older than 15 years was at risk of exceeding ADI levels of acesulfame-K, saccharin, cyclamate, aspartame, and sucralose through assessment of usual dietary intake of artificial sweeteners and specific consumption of table-top sweeteners. The conservative Tier 2 approach, for which an extensive label survey was performed, showed that mean usual intake was significantly lower than the respective ADIs for all sweeteners. Even consumers with high intakes were not exposed to excessive levels, as relative intakes at the 95th percentile (p95) were 31% for acesulfame-K, 13% for aspartame, 30% for cyclamate, 17% for saccharin, and 16% for sucralose of the respective ADIs. Assessment of intake using the Tier 3 approach was preceded by optimization and validation of an analytical method based on liquid chromatography with mass spectrometric detection. Concentrations of sweeteners in various food matrices and table-top sweeteners were thus determined and mean positive concentration values were included in the Tier 3 approach, leading to relative intakes for p95 of 17% for acesulfame-K, 5% for aspartame, 25% for cyclamate, 11% for saccharin, and 7% for

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

	<p>sucralose of the corresponding ADIs. The contribution of table-top sweeteners to the total usual intake (< 1% of ADI) was demonstrated to be negligible. Comparison of observed intake of the total population with intake of diabetics (acesulfame-K: 3.55 vs. 3.75, aspartame: 6.77 vs. 6.53, cyclamate: 1.97 vs. 2.06, saccharine: 1.14 vs. 0.97, and sucralose: 3.08 vs. 3.03, expressed as mg kg⁻¹ bodyweight day⁻¹ for p95) showed that the latter group was not exposed to higher levels. It was thus concluded that the Belgian population was not at risk of exceeding the established ADIs for sweeteners.</p>

SCHOLARONE™
Manuscripts

For Peer Review Only

1
2
3 **1 Dietary Intake of artificial sweeteners by the Belgian population**
4
5
6
7

8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

2

3 Kevin Huvaere,¹ Stefanie Vandevijvere,² Moez Hasni,¹ Christine Vinkx,³ and Joris Van Loco^{1*}

4

5 ¹*Department of Food, Medicines and Consumer Safety, Scientific Institute of Public Health,*
6 *Rue J. Wytsmanstraat 14, B-1050 Brussels, Belgium*

7 ²*Department of Public Health and Surveillance, Scientific Institute of Public Health, Rue J.*
8 *Wytsmanstraat 14, B-1050 Brussels, Belgium*

9 ³*Belgian Federal Service of Public Health, Food Safety and Environment, Place Victor Horta*
10 *40 box 10, B-1060 Brussels, Belgium*

11

12 Tel: +32 2 642 5353

13 Email: Joris.VanLoco@wiv-isp.be

14

15 **ABSTRACT**

16 This study investigated whether the Belgian population older than 15 years was at risk of
17 exceeding ADI levels for acesulfame-K, saccharin, cyclamate, aspartame, and sucralose
18 through assessment of usual dietary intake of artificial sweeteners and specific consumption
19 of table-top sweeteners. The conservative Tier 2 approach, for which an extensive label
20 survey was performed, showed that mean usual intake was significantly lower than the
21 respective ADIs for all sweeteners. Even consumers with high intakes were not exposed to
22 excessive levels, as relative intakes at the 95th percentile (p95) were 31% for acesulfame-K,
23 13% for aspartame, 30% for cyclamate, 17% for saccharin, and 16% for sucralose of the
24 respective ADIs. Assessment of intake using the Tier 3 approach was preceded by
25 optimization and validation of an analytical method based on liquid chromatography with
26 mass spectrometric detection. Concentrations of sweeteners in various food matrices and
27 table-top sweeteners were thus determined and mean positive concentration values were
28 included in the Tier 3 approach, leading to relative intakes for p95 of 17% for acesulfame-K,
29 5% for aspartame, 25% for cyclamate, 11% for saccharin, and 7% for sucralose of the
30 corresponding ADIs. The contribution of table-top sweeteners to the total usual intake (< 1%
31 of ADI) was demonstrated to be negligible. Comparison of observed intake of the total
32 population with intake of diabetics (acesulfame-K: 3.55 vs. 3.75, aspartame: 6.77 vs. 6.53,
33 cyclamate: 1.97 vs. 2.06, saccharine: 1.14 vs. 0.97, and sucralose: 3.08 vs. 3.03, expressed
34 as mg kg⁻¹ bodyweight day⁻¹ for p95) showed that the latter group was not exposed to higher
35 levels. It was thus concluded that the Belgian population was not at risk of exceeding the
36 established ADIs for sweeteners.

37 INTRODUCTION

38 The desire for sweet taste, commonly referred to as *the sweet tooth*, is suggested to be an
39 evolutionary legacy of early humans, who survived by their continuous quest for sugary and
40 therefore energy-dense foods (Shils et al. 2005). However, current understanding of
41 medicine has led to the association between lifetime consumption of sugar and a series of
42 adverse health effects, ranging from tooth decay to the increased risk of developing life-
43 threatening conditions such as obesity and diabetes (Bray et al. 2004; Gross et al. 2004;
44 Ludwig et al. 2001; Touger-Decker et al. 2003). Replacing sugar by intense, artificial
45 sweeteners (also referred to as nonnutritive sweeteners), which possess such sweetening
46 power that relatively small quantities are sufficient to invoke a sweetness equivalent to sugar,
47 is a suggested remedy to reduce sugar intake (Mattes et al. 2009). However, use of such
48 additives in food products is submitted to strict European legislation and maximum permitted
49 levels of sweeteners used in particular foods are stipulated in Directive 94/35/EC (European
50 Parliament and Council 1994). Compounds which are nowadays abundant in sugar-free and
51 sugar-reduced foods as well as in table-top sweeteners include acesulfame-K (E950),
52 aspartame (E951), cyclamate (E952), saccharin (E954), and sucralose (E955). As
53 permissions for applying sweeteners such as neohesperidin dihydrochalcone (E959),
54 neotame (E961), and thaumatin (E957) are more recent, their use is assumed to be less
55 prevalent, while use of steviol glycosides is currently not approved. Beside stating permitted
56 compounds and corresponding maximum levels, the Directive also specifies appropriate food
57 labeling and settles warnings to be listed on foods containing sweeteners. Furthermore,
58 Regulation 1333/2008 of the European Parliament and Council, which includes general
59 guidelines for use of food additives, demands for monitoring use and consumption in different
60 Member States in view of potential legal action if intake levels frequently exceed the advised
61 *acceptable daily intake* (ADI) (European Parliament and Council 2008).

62
63 According to the requirements for data collection, as stipulated in article 22 of Regulation
64 1333/2008 (European Parliament and Council 2008), the Belgian Federal Service of Public

1
2
3 65 Health, Food Safety and Environment conceived a research project to investigate the
4
5 66 prevalence of sweeteners on the Belgian market and to evaluate the consumption of these
6
7 67 products by the Belgian population. A second objective was to compare intake levels of
8
9 68 diabetics with those of the general population. In response to these questions, a food label
10
11 69 survey was initiated to map the occurrence of sweeteners in foods and in table-top
12
13 70 sweeteners on the Belgian market, while chemical analysis of a representative number of
14
15 71 foods was considered essential to gain insight in the concentrations being used. Resulting
16
17 72 concentration levels were used for subsequent intake estimations, which were based on a
18
19 73 tiered approach in view of harmonization with intake studies of other Member States
20
21 74 (European Commission 2004). Both Tier 2 and Tier 3 approaches were used to assess a
22
23 75 realistic intake of selected sweeteners and to calculate contribution of table-top sweeteners
24
25 76 to the total dietary intake.
26
27
28

77

78 **MATERIALS AND METHODS**

79 **Food label survey**

80 The label survey was approached from the point of view that food additive labeling is
81 mandatory, hence artificially-sweetened products were readily selected from label screening
82 of > 2000 food items in 7 major supermarkets with good market share in Belgium. Screening
83 of table-top sweeteners, a well-defined group of sweetener-based products, was most
84 apparent, but the set of sweetened foods was considerably larger and exceedingly divers.
85 Therefore, Directive 94/35/EC, which includes an overview of foods legally allowed to contain
86 sweeteners (European Parliament and Council 1994), was used as starting point from which
87 food labels were explored. Relevant foods were inserted in an in-house database and
88 organized in groups according to available national food consumption data (Vandevijvere et
89 al. 2009), thus generating following food groups: nonalcoholic beverages, beers and
90 alcoholic beverages, desserts and similar products, sweets including breath-freshening
91 micro-sweets, chewing gums, canned and bottled foods, marmalades, sauces, and cereals.
92 Information from these products, including brand name, producer name, physical state,

1
2
3 93 ingredients, sweetener content, labeled warnings, supermarket name, day of visit, and batch
4
5 94 number were registered in the database. Duplicates, i.e. identical products found at different
6
7 95 locations or supplied in varying packaging (a particular drink, for example, supplied in cans,
8
9 96 PET-bottles or glass bottles) were counted only once. Screening was performed from
10
11 97 December 2009 till February 2010.
12

13 98

14 99 **Chemicals**

15
16
17 100 Acesulfame-K and sodium saccharinate were obtained from Fluka (Buchs, Switzerland),
18
19 101 while sodium cyclamate, sucralose, and sulfadimidine came from Sigma (St. Louis, MO,
20
21 102 USA). Aspartame was purchased from Supelco (Bellefonte, PA, USA), while ammonium
22
23 103 acetate was a product from Acros (Geel, Belgium). All chemicals were of analytical grade.
24
25 104 Methanol (HPLC-grade) was obtained from Biosolve (Valkenswaard, The Netherlands), while
26
27 105 purified, de-ionized water ($R=18\text{ M}\Omega\text{ cm}$, total organic content $< 4\text{ ppb}$) was produced by a
28
29 106 MilliQ unit (Millipore, Billerica, MA, USA).
30

31 107

32 108 **Analytical methodology**

33 109 *Sample Preparation*

34
35
36
37 110 The complexity and heterogeneity of certain foods such as confectionery, biscuits, and dairy
38
39 111 products required thorough sample preparation, which, for the sake of throughput, was
40
41 112 preferentially universally applicable. Initially, solid phase extraction (SPE), which is classic in
42
43 113 analyses of complex samples, was applied for foods, but low affinity of saccharin and
44
45 114 acesulfame salts for the C_{18} -packing material resulted in poor reproducibility. In this respect,
46
47 115 it was recently reported that retention of these compounds on SPE cartridges is highly
48
49 116 dependent on the type and manufacturer of the packing material (Zygler et al. 2010).
50
51 117 Alternative sample preparations were thus investigated and reported methods for beverages
52
53 118 or a specific food group (Koyama et al. 2005; Wasik et al. 2007; Yang et al. 2009) were
54
55 119 further optimized for use with the proposed set of complex and diverse matrices. It is worth
56
57 120 noting that sweeteners in foods and particularly in table-top sweeteners were relatively highly
58
59
60

1
2
3 121 concentrated, hence samples needed dilution prior to analysis. Thus, following preparation
4
5 122 procedures for different matrices based on homogenization and dilution were elaborated.

6
7 123 **Table-top sweeteners.** Pellets or cubes were initially milled in a coffee grinder, while,
8
9 124 obviously, formulations in aqueous solution required no additional homogenization.
10
11 125 Approximately 50 mg, or 0.5 mL for liquid formulations, of the homogenized sample was
12
13 126 taken, followed by addition of 500 μL of a 1000-ppm sulfadimidine (internal standard, IS)
14
15 127 solution in methanol. The resulting mixture was diluted to 25 mL with water/methanol (1:1,
16
17 128 v/v) and subsequently sonicated for 10 min. Then, 1 mL of the solution was centrifuged for 5
18
19 129 min at 4°C and at 5000 rpm (Avanti J25 centrifuge with J-10 rotor, Beckman, Brea, CA,
20
21 130 USA). The supernatant was isolated, 100 μL was taken and diluted with water (1:400, v/v).
22
23 131 After subsequent filtering (0.45 μm), the sample was ready for injection.

24
25 132 **Foods and beverages.** Similar as for table-top sweeteners, solid foods were initially milled
26
27 133 in a grinder. Then, to approximately 0.5 g of the respective sample (or to 1 mL for
28
29 134 beverages), 250 μL of a 100-ppm sulfadimidine solution in methanol was added. The
30
31 135 resulting mixture was diluted to 25 mL with water/methanol (1:1, v/v) and exposed to
32
33 136 ultrasones for 10 min. The sample was then left for cold precipitation (ice bath, 2h), after
34
35 137 which 1 mL of the resulting supernatant was centrifuged for 10 min at 13,000 rpm (Biofuge
36
37 138 Pico, Heraeus, Buckinghamshire, UK). Finally, 200 μL of the thus isolated supernatant was
38
39 139 diluted with water (1:20, v/v) and after filtration, the mixture was ready for injection.

40
41
42 140

43 141 *Chromatography*

44
45 142 Samples for chromatography were placed immediately after preparation in a thermostated
46
47 143 sample rack (at 5°C) of a Surveyor autosampler with built-in LC pump (Thermo Electron
48
49 144 Corp., San Jose, CA, USA). Sample injection (20 μL) occurred in *full loop* mode and was
50
51 145 preceded by washing and flushing of the injection needle with methanol (2 \times 400 μL).
52
53 146 Sweeteners were separated using an analytical Waters Symmetry C₁₈-column (150 mm \times 2.1
54
55 147 mm, 5 μm) (Waters Corporation, Milford, MA, USA), equipped with corresponding guard
56
57 148 column and installed in the column oven (set at 50°C) of the Surveyor station. Gradient
58
59
60

1
2
3 149 elution was optimized using a solvent system consisting of water (solvent A) and methanol
4
5 150 (solvent B), each with 20 mM ammonium acetate added. Eventually, following gradient
6
7 151 profile with satisfying separation was applied: 0 to 2 min at 5% B, raise to 49% B in 12 min,
8
9 152 raise to 100% B in 1 min, stay for 2 min, return to 5% B in 1 min and stay for 10 min.
10
11 153 Retention times of the sweeteners eluting under these conditions are shown in Table 1.
12

13
14
154

155 *Detection*

156 Sweetener elution was monitored by ion trap mass spectrometry with electrospray ionization
17
18 156 Sweetener elution was monitored by ion trap mass spectrometry with electrospray ionization
19
20 157 source (LCQ Deca XP mass spectrometer, Thermo Electron Corp.). Ionization parameters
21
22 158 were optimized for the selected sweeteners to obtain reproducible fragmentation of the
23
24 159 respective compounds. The settings for the electrospray source were capillary voltage -34 V
25
26 160 and tube lens offset -10 V for acesulfame-K, saccharin, and cyclamate and -4 V and 20 V for
27
28 161 sucralose, aspartame, and the internal standard. Except for sucralose, ion isolation width
29
30 162 was m/z 1, activation Q was 0.25, and activation time was 30 ms (4, 0.2, and 35,
31
32 163 respectively, for sucralose). For all compounds, absolute spray voltage was 5 kV and
33
34 164 capillary temperature was 375 °C. Indeed, the high concentration of water with first-eluting
35
36 165 compounds required high capillary temperature to overcome surface tension of sprayed
37
38 166 micro-droplets. For the same reason, significant sheath and sweep nitrogen gas flows were
39
40 167 applied (set at 45 and 20 arbitrary units, respectively). Collision energy needed for
41
42 168 fragmenting saccharin and cyclamate was too high to yield stable ions, hence only fictitious
43
44 169 transitions for these compounds were monitored. Unlike other sweeteners, aspartame was
45
46 170 detected in positive ionization mode. Under similar conditions, the molecular ion of
47
48 171 sulfadimidine, the internal standard (m/z 279 in positive mode) was fragmented to give ions
49
50 172 m/z 186.1 and m/z 203.9.
51

52
53
173

54 174 *Calibration*

55
56 175 Quantification of the different compounds was based on external calibration curves
57
58 176 constructed by injecting a series of standard concentrations. Since no matrix effect was
59
60

1
2
3 177 observed for beverages, dairy products, and confectionary, calibration in solution was used.
4
5 178 Sodium saccharinate and sodium cyclamate were used for calibration and analytical results
6
7 179 for these compounds therefore refer to concentrations of the salts rather than the
8
9 180 corresponding free acids. The calibration is controlled by internal standard (sulfadimidine at
10
11 181 50 ppb).
12

182

183 *Validation*

184 The performance of the sweetener quantification method was evaluated by determining
185 selectivity, linear range, recovery, precision, detection and limit of quantification. Risk of
186 interference was practically non-existent for compounds with unique transitions, but for
187 cyclamate and saccharin, for which detection solely relied on retention time and molecular
188 mass, the degree of selectivity might be lower. Nevertheless, blanks from beverages,
189 yoghurt, and sweets lacked signals, while, after spiking these matrices, sweeteners were
190 readily detected at their expected retention time which demonstrated acceptable selectivity.
191

191

192 After injection of blank samples to measure noise levels at relevant retention times, the limit
193 of detection (LOD) was determined by injecting standard solutions to reach a signal-to-noise
194 ratio (S/N) of 3 (Table 2). The limit of quantification (LOQ) was then determined as the
195 concentration at which S/N gives 10. The linearity of detector response was obtained over a
196 large concentration range for the five compounds (Ace-K: 20-4000 ng mL⁻¹, Asp: 20-10000
197 ng mL⁻¹, Cyc: 20-4000 ng mL⁻¹, Sacc: 20-4000 ng mL⁻¹, Sucr: 20-4000 ng mL⁻¹), which is
198 useful due to the high variation in sweetener concentration in sampled foods and table-top
199 sweeteners. Resulting calibration curves followed first order regression with correlation
200 coefficients (R²) of 0.998 for acesulfame-K, 0.994 for saccharin, 0.994 for cyclamate, 0.995
201 for sucralose, and 0.992 for aspartame.
202

202

203 Method precision and accuracy were assessed for a complex matrix (sweets), which did not
204 contain any of the analytes under investigation. To a homogenized matrix was added a mix

1
2
3 205 of the respective sweeteners to reach three different final concentrations, namely 0.5 g kg^{-1}
4 206 (*low*), 1 g kg^{-1} (*medium*), and 1.5 g kg^{-1} (*high*), an experiment that was performed in triplicate
5
6 207 and repeated on three non-consecutive days. The resulting 27 ($3 \times 3 \times 3$) samples were
7
8 208 analyzed according to the reported procedure and validation results were described in Table
9
10 209 2. Herein, recovery was determined by the ratio of the average medium concentration and its
11
12 210 corresponding theoretical value (1 g kg^{-1}).
13
14
15
16

17 212 ***Intake assessment***

18
19 213 A uniform methodology, designed by members of the SCOOP task force in view of realizing
20
21 214 harmonized additive intake assessments, was previously reported and consisted of a 3-
22
23 215 phase approach (European Commission 2004). Tier 1, which is based on theoretical food
24
25 216 consumption combined with maximum permitted sweetener levels according to Directive
26
27 217 94/35/EC (European Parliament and Council 1994), is a conservative intake estimate carried
28
29 218 out at European level and is not a task for Member States. Tier 2 and Tier 3 refer to
30
31 219 assessment at the level of individual Member States and are based on a combination of
32
33 220 national food consumption data with maximum permitted levels (Tier 2) and with actual use
34
35 221 levels (Tier 3), respectively.
36
37
38

39 223 Consumption data from the Belgian Food Consumption Survey (dating from 2004) were used
40
41 224 to perform the intake assessment. Aims, design and methods of this survey can be found
42
43 225 elsewhere (Vandevijvere et al. 2009). The target population comprised all Belgian inhabitants
44
45 226 of 15 years or older. The sample included 3245 participants randomly selected from the
46
47 227 National Register using a multi-stage stratified procedure. Information on dietary intake was
48
49 228 collected by two non-consecutive 24-h recalls in which the respondent reported the quantity
50
51 229 of all foods and beverages consumed during the preceding day. Both 24-h recalls, which
52
53 230 were carried out using EPIC-SOFT software (Slimani et al. 2002), were completed by 3083
54
55 231 participants (hence 6166 recalls including 428 from diabetics).
56
57
58
59
60

$$y_i = \frac{c_i \times x_i}{bw_i} \quad (\text{Eq. 1})$$

234

235 The individual intake of each sweetener from a certain food product was calculated by
236 Equation 1. Herein, y_i is the intake of the particular sweetener by individual i from a particular
237 food on an interview day ($\text{mg kg}^{-1} \text{ bodyweight day}^{-1}$); c_i is the concentration of the sweetener
238 in the food (mg kg^{-1}); x_i is the consumption of a certain food by individual i (kg); and bw_i is the
239 self-reported bodyweight of individual i (kg). To estimate the total intake of each sweetener
240 per food group per day, individual daily intakes of the sweeteners from different foods were
241 added up. In the Tier 2 approach, c_i represents the maximum permitted concentration in each
242 food, while in the Tier 3 approach, c_i represents the actual mean concentration of the
243 sweetener observed in a particular food. Minor food groups that were not sampled were
244 assigned maximum concentrations in Tier 3 (as in Tier 2), while others were excluded due to
245 the lack of consumption of some foods listed in Directive 94/35/EC or to the fact that no
246 sweeteners were found on the labels of food belonging to these products (such as sauces,
247 bakery products for special nutrition, foods intended to be consumed in energy-restricted
248 diets, dietetic foods for special medical purposes, liquid nutritional supplements and solid
249 food supplements, spirits with an alcohol content of less than 15%, tablets with reduced
250 sugar, essoblaten, and Feinkost salat). The usual intake distribution for sweeteners was
251 estimated with the Nusser-method (Nusser et al. 1996) using the C-side software (Iowa State
252 University 1996). Several statistical methods are available to estimate usual intake
253 distributions with the correct mean, variance and skewness. These statistical procedures
254 adjust for within-person or day-to-day variability. The usual intake distribution was weighted
255 for the age and sex distribution of the Belgian population and adjusted for day of the week
256 and season. Usual intake was reported absolutely (as $\text{mg kg}^{-1} \text{ bodyweight day}^{-1}$) or relatively
257 against the ADI of the respective sweeteners.

258

259 **RESULTS & DISCUSSION**

1
2
3 260 **Label survey**

4
5 261 For the presentation of the results of the label survey, it was decided to treat table-top
6
7 262 sweeteners and foods separately due to differences in both legislation and practical use of
8
9 263 the two groups. Indeed, according to regulation 1333/2008, table-top sweeteners are defined
10
11 264 as *preparations of permitted sweeteners, which may contain other food additives and/or food*
12
13 265 *ingredients and which are intended for sale to the final consumer as a substitute for sugars*
14
15 266 (European Parliament and Council 2008). The latter implies that, unlike in foods,
16
17 267 concentrations of sweeteners in table-top sweeteners are not determined by legislation and
18
19 268 usage is according to consumer preference. Aspartame was most frequently found (Figures
20
21 269 1A and 1C) and was used as single sweetener or in combination with primarily acesulfame-K
22
23 270 (Figure 1B). Saccharin and cyclamate were another popular combination and, due to their
24
25 271 stability and pronounced solubility, they were exclusively used in aqueous sweetener
26
27 272 solutions. Products based on neohesperidine dihydrochalcone, thaumatin, or neotame were
28
29 273 not found.

30
31 274

32
33 275 Unlike table-top sweeteners, which basically contained bulk materials with high
34
35 276 concentrations of sweeteners, ingredient lists of the heterogeneous set of sweetened foods
36
37 277 were considerably more complicated. A total of 270 labels, divided over 10 different food
38
39 278 groups according to consumption data, were examined. The most important group was the
40
41 279 set of beverages, including beer and non-alcoholic drinks, which, together, represented more
42
43 280 than half of the total supply of sweetened foods (Figure 2A). Acesulfame-K and aspartame
44
45 281 were used most frequently (Figure 2B), while also a relatively large number of beverages
46
47 282 was sweetened with sucralose compared to the 5 items found during a similar study in 2004
48
49 283 (Belgian Scientific Institute of Public Health 2005). This sweetener was also found in a
50
51 284 significant number of other foods (67 in total), which nicely complied with recent findings on
52
53 285 high sucralose presence (up to 365 ng L⁻¹) in Belgian river waters (Loos et al. 2009). Similar
54
55 286 as observed for table-top sweeteners, results showed that compounds were often combined
56
57 287 in an attempt to mask or eliminate unwanted aftertastes (Figure 2C). Only 1 food item
58
59
60

1
2
3 288 (beverage) was found to contain neohesperidin dihydrochalcone, hence the decision to leave
4
5 289 this and other recently approved sweeteners out of the study was justified.
6
7 290

8 9 291 **Analysis method**

10 292 Despite the fact that various methods have been described, development and optimization of
11
12 293 an analytical methodology was pursued to meet the specific objectives set for this work. A
13
14 294 method based on simple dilution followed by filtration, which was previously found effective
15
16 295 for drinks and table-top sweeteners (Zygler et al. 2009), was optimized for more complicated
17
18 296 matrices by introduction of a cooling phase followed by ultracentrifugation of the resulting
19
20 297 supernatant. As for the chromatography, use of triethylamine in the mobile phase (as in
21
22 298 methods by Huang et al. 2006; Wasik et al. 2007; Yang et al. 2009) was avoided due to
23
24 299 persistent memory effects with mass spectrometric detection (Rutters et al. 2000). Instead,
25
26 300 ammonium acetate was preferred as additive (Scheurer et al. 2009). Details of
27
28 301 chromatography and mass spectrometric detection of sweeteners are given in Table
29
30
31
32

33 303 **Concentrations of sweeteners in table-top sweeteners and foods**

34
35 304 Samples of table-top sweeteners were prepared and analyzed according to the procedure
36
37 305 described in the experimental section. All table-top sweeteners found during the food label
38
39 306 survey ($n=30$) were sampled and qualitative analysis results complied with data extracted
40
41 307 from the label. Liquid table-top sweeteners were exclusively composed of cyclamate and
42
43 308 saccharin and doses amounting to $\sim 80 \text{ g L}^{-1}$ for the sum of the two sweeteners were
44
45 309 measured. Next to cyclamate and saccharin, solid table-top sweeteners contained high
46
47 310 levels of aspartame so that a total sweetener content of up to 40% of the total mass was
48
49 311 reached. In this respect, pellets were significantly more concentrated than powders, which
50
51 312 are normally consumed in larger portions. Average concentrations of sweeteners in positive
52
53 313 samples (Table 3) confirmed the notion that cyclamate, having the weakest sweetening
54
55 314 power, is used in the highest concentrations.
56
57
58
59
60

1
2
3 316 For analyses of foods, 89 samples, which were a representative distribution of sweetened
4
5 317 products found on the market, were selected for analysis (Table 3). In one case, aspartame
6
7 318 was labeled but was not detected as such, probably due to degradation during storage
8
9 319 (despite that analysis was carried out prior to expiry date) (Hutchinson et al. 1999). Only one
10
11 320 sample (beer) was found in which the listed sweetener (acesulfame-K) differed from the one
12
13 321 detected (saccharin), a result of inconsistent labeling as confirmed by the involved brewery.
14
15 322 Reconstituting sweetener composition of sampled beer and beverages based on analytical
16
17 323 data, showed that sweeteners in beer were relatively low concentrated compared to non-
18
19 324 alcoholic drinks. Beers mostly contained saccharin or acesulfame-K, which were favored
20
21 325 over cyclamate (not allowed in beer) and aspartame (unstable in acidic media). Acesulfame-
22
23 326 K was also abundantly used in nonalcoholic drinks, despite its unpleasant bitter aftertaste
24
25 327 (Kuhn et al. 2004). For this reason, it often appears in combination with aspartame and
26
27 328 cyclamate, the latter, due to its low sweetening power, is higher concentrated. The former
28
29 329 combination (i.e., aspartame and acesulfame-K) dominates other food groups as well and
30
31 330 particularly chewing gum was characterized by high quantities of these compounds. Indeed,
32
33 331 Directive 94/35/EC (European Parliament and Council 1994) permits acesulfame-K levels up
34
35 332 to 2000 mg kg⁻¹ while aspartame can be concentrated as high as 5500 mg kg⁻¹. In this
36
37 333 respect, one sample was found to contain a significant excess of acesulfame-K, while in
38
39 334 canned fruit (3 of 4 samples) acesulfame-K levels slightly exceeding the maximum permitted
40
41 335 level were found. Permitted concentrations of these and other sweeteners, as stated in the
42
43 336 sweetener directive, were not exceeded in other foods.
44
45
46
47

337

338 **Intake of artificial sweeteners in Belgium**

339 From the food label survey, it became clear that recently approved sweeteners
340 neohesperidine dihydrochalcone, thaumatin, and neotame were not found on the local
341 market, hence only intake of aspartame, cyclamate, acesulfame-K, saccharin, and sucralose
342 was relevant. The Tier 2 was based on maximum permitted levels but despite the
343 conservative nature of this approach, usual intake of sweeteners for the 95th percentile was

1
2
3 344 significantly below established ADI values (Table 4). For diabetics, which were suspected of
4
5 345 consuming higher amounts of sweeteners, observed intake (based on mean intake of 2
6
7 346 interview days) rather than usual intake was calculated because of the smaller subpopulation
8
9 347 ($n = 428$). Thus, for p95 the observed intake was found to be 3.75 ± 1.30 , 6.53 ± 2.32 , $2.06 \pm$
10
11 348 0.80 , 0.97 ± 0.39 , and 3.03 ± 1.07 mg kg⁻¹ bodyweight day⁻¹ for acesulfame-K, aspartame,
12
13 349 cyclamate, saccharin, and sucralose, respectively, which was not considerably different from
14
15 350 observed intake of the entire population (3.55 ± 1.41 , 6.77 ± 2.61 , 1.97 ± 0.86 , 1.14 ± 0.43 ,
16
17 351 and 3.08 ± 1.20 mg kg⁻¹ bodyweight day⁻¹, respectively). Intake of table-top sweeteners was
18
19 352 however not included in these figures because the lack of maximum permitted levels and
20
21 353 their contribution could only be taken into account in Tier 3. This approach uses actual, mean
22
23 354 concentrations (Table 3) (or maximum permitted levels for minor foods) for combination with
24
25 355 food consumption data and is expected to be more accurate. Indeed, resulting lower intakes
26
27 356 (Table 5) emphasize the conservative character of Tier 2. Contributions of table-top
28
29 357 sweeteners were low and of minor significance: 0.06 %, 0.05 %, 0.44 %, and 0.17 % of the
30
31 358 respective ADI for acesulfame-K, aspartame, cyclamate, and saccharin, respectively. No
32
33 359 intake data was obtained for sucralose due to its practical absence in table-top sweeteners.
34
35 360 Similar as in the Tier 2, observed intake was calculated for diabetics and compared with the
36
37 361 entire population. At p95, 1.48 ± 0.60 , 1.36 ± 0.72 , 1.20 ± 0.47 , 0.57 ± 0.23 , and 1.13 ± 0.46
38
39 362 mg kg⁻¹ bodyweight day⁻¹ were consumed of acesulfame-K, aspartame, cyclamate,
40
41 363 saccharin, and sucralose, respectively, by diabetics versus 1.96 ± 0.78 , 2.46 ± 1.01 , $1.18 \pm$
42
43 364 0.52 , 0.60 ± 0.23 , and 1.53 ± 0.59 mg kg⁻¹ bodyweight day⁻¹ by the total population. Intake
44
45 365 through consumption of table-top sweeteners was slightly higher for diabetics (0.004 ± 0.002 ,
46
47 366 0.019 ± 0.008 , 0.027 ± 0.011 , and 0.008 ± 0.003 mg kg⁻¹ bodyweight day⁻¹ of acesulfame-K,
48
49 367 aspartame, cyclamate, and saccharin, respectively) compared to the entire population (0.002
50
51 368 ± 0.001 , 0.008 ± 0.005 , 0.011 ± 0.008 , and 0.003 ± 0.002 mg kg⁻¹ bodyweight day⁻¹,
52
53 369 respectively), but was negligible with respect to established ADIs. Since usual intake
54
55 370 remained significantly lower than reported ADIs, it was safely assumed that the adult Belgian
56
57 371 population was not exposed to excessive intakes.
58
59
60

1
2
3 3724
5 373 *Sweetener intake in other Member States*

6
7 374 Belgian results were compared with contemporary intake assessments from other countries,
8
9 375 including Italy (Arcella et al. 2004; Leclercq et al. 1999), the UK (UK Food Standards Agency
10
11 376 2003; Wilson et al. 1999), France (Bemrah et al. 2008; Garnier-Sagne et al. 2001), Spain
12
13 377 (Serra-Majem et al. 2003), the Netherlands (van Rooij-van den Bos et al. 2004), and
14
15 378 Denmark (Leth et al. 2008). These studies showed intakes significantly below the established
16
17 379 ADI for the respective sweeteners (Table 6). Worst-case scenarios were evaluated as well
18
19 380 (Hendriksen et al. 2011; Husoy et al. 2008; Ilback et al. 2003), but no significant exceeding of
20
21 381 ADI was reported. As for table-top sweeteners, only few data on consumption were found.
22
23 382 Arcella et al. (2004) reported a worst case scenario from Italy, in which only sugar-free
24
25 383 alternatives were used at the highest observed concentration, but, despite the highly
26
27 384 conservative assessment, p95 level of intake was far below the respective ADIs (12%, 29%,
28
29 385 24%, and 38% of ADI for aspartame, acesulfame-K, saccharin, and cyclamate, respectively).
30
31 386 In the same study, a realistic assessment based on a questionnaire gave slightly higher
32
33 387 intake than for the Belgian population because the survey group was a selection of
34
35 388 consumers with high reported table-top sweetener intake (Table 6).

36
37 389

38
39 390 Although adults were not at risk of exceeding ADIs for the respective sweeteners, intake by
40
41 391 children was possibly problematic according to a British study (UK Food Standards Agency
42
43 392 2003). Similar risks for cyclamate were purported in worst case scenarios from The
44
45 393 Netherlands (van Rooij-van den Bos et al. 2004) and from Sweden (Ilback et al. 2003), but,
46
47 394 on the other hand, intake assessments in Denmark (Leth et al. 2008), France (Bemrah et al.
48
49 395 2008), and Portugal (Lino et al. 2008) demonstrated that cyclamate intake in small children
50
51 396 and young teenagers remained well below the ADI. Unfortunately, few data was available for
52
53 397 this subgroup and the latter was certainly true for the Belgian Food Consumption Survey,
54
55 398 which did not include individuals younger than 15 years.

56
57 399
58
59
60

1
2
3 400 Few intake data were found from countries outside of EU, but comparison with European
4
5 401 results is difficult because of differences in both legislation and advised ADI levels. The latter,
6
7 402 which are set by the WHO/FAO Joint Expert Committee on Food Additives, differ for
8
9 403 acesulfame-K ($15 \text{ mg kg}^{-1} \text{ day}^{-1}$ instead of $9 \text{ mg kg}^{-1} \text{ day}^{-1}$) and for cyclamate ($11 \text{ mg kg}^{-1} \text{ day}^{-1}$
10
11 404 ¹ instead of $7 \text{ mg kg}^{-1} \text{ day}^{-1}$). Still, intake studies from Australia and New-Zealand and from
12
13 405 Korea showed that sweetener intake was below the respective ADIs (Chung et al. 2005;
14
15 406 Food Standards Australia New-Zealand 2004).

16
17
18 40719
20 408 **Conclusions**

21
22 409 A thorough food label survey, initiated to screen the presence of sweeteners in foods,
23
24 410 showed that acesulfame-K, saccharin, cyclamate, aspartame, and sucralose were well
25
26 411 distributed in the Belgian market. Intake assessment of these additives by the Belgian
27
28 412 population older than 15 years was carried out and eventually demonstrated that the risk of
29
30 413 the adult Belgian population (including possibly high consumers such as diabetics) for being
31
32 414 excessively exposed to acesulfame-K, saccharin, cyclamate, aspartame, and sucralose was
33
34 415 of no concern. The intake of other permitted intense sweeteners such as neohesperidin
35
36 416 dihydrochalcone (E959), neotame (E961), and thaumatin (E957), as well as the expected
37
38 417 steviol glycosides, was currently not relevant, but expected future developments in
39
40 418 sweetener formulation obviously justify future monitoring. Contrary to the safe consumption
41
42 419 by adults, the possibility of exceeding ADI by children at the higher percentiles has been
43
44 420 suggested in the literature. Accordingly, the Scientific Committee on Food urged to focus on
45
46 421 additive intake by children, which, on a body weight basis, are at higher risk than adults for
47
48 422 being exposed to excessive levels. However, as ADI is based on life-long exposure, a
49
50 423 possible exceeding of the ADI in childhood is very likely compensated by a very low intake in
51
52 424 adulthood (as shown by this work) and will thus not compromise the conclusions on the
53
54 425 safety of the intake of sweeteners in Belgium.

55
56
57 426
58
59
60

1
2
3 427 **Acknowledgments**
4

5 428 This work was funded by the Directorate-General Animal, Plant, and Food (DG 4) of the

6
7 429 Federal Service of Public Health, Safety of the Food Chain, and Environment.
8

9
10 430
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review Only

431 **References**

432 Arcella D, Le Donne C, Piccinelli R, Leclercq C. 2004. Dietary estimated intake of intense
433 sweeteners by Italian teenagers. Present levels and projections derived from the INRAN-RM-
434 2001 food survey. *Food and Chemical Toxicology* 42:677-685.

435 Belgian Scientific Institute of Public Health. 2005. Estimation de la dose journalière
436 d'édulcorants artificiels consommée par la population belge par l'intermédiaire de la
437 consommation des boissons rafraîchissantes - Estimation à partir des quantités réelles
438 d'édulcorants mesurées dans les boissons rafraîchissantes du marché belge (Projet additif
439 2004):1-123.

440 Bemrah N, Leblanc JC, Volatier JL. 2008. Assessment of dietary exposure in the french
441 population to 13 selected food colours, preservatives, antioxidants, stabilizers, emulsifiers
442 and sweeteners. *Food Additives and Contaminants: Part B Surveillance* 1:2-14.

443 Bray GA, Nielsen SJ, Popkin BM. 2004. Consumption of high-fructose corn syrup in
444 beverages may play a role in the epidemic of obesity. *The American Journal of Clinical
445 Nutrition* 79:537-543.

446 Chung MS, Suh HJ, Yoo W, Choi SH, Cho YJ, Cho YH, Kim CJ. 2005. Daily intake
447 assessment of saccharin, stevioside, D-sorbitol and aspartame from various processed foods
448 in Korea. *Food Additives and Contaminants* 22:1087-1097.

449 European Commission. 2004. Report from the Commission on Dietary Food Additive Intake
450 in the European Union. COM(2001) 542 final:1-27.

451 European Parliament and Council. 1994. Directive 94/35/EC of 30 June 1994 on sweeteners
452 for use in foodstuffs. *Official Journal of the European Union* L237:3-12.

453 European Parliament and Council. 2008. Regulation (EC) No 1333/2008 of 16 December
454 2008 on food additives. *Official Journal of the European Union* L354:16-33.

455 Ferrer I, Thurman EM. 2010. Analysis of sucralose and other sweeteners in water and
456 beverage samples by liquid chromatography/time-of-flight mass spectrometry. *Journal of
457 Chromatography A* 1217:4127-4134.

458 Food Standards Australia New-Zealand. 2004. Consumption of intense sweeteners in
459 Australia and New-Zealand: Benchmark Survey 2003. Evaluation Report Series N° 8.

- 1
2
3 460 Garnier-Sagne I, Leblanc JC, Verger P. 2001. Calculation of the intake of three intense
4 461 sweeteners in young insulin-dependent diabetics. *Food and Chemical Toxicology* 39:745-
5 462 749.
6
7
8 463 Gross LS, Li L, Ford ES, Liu S. 2004. Increased consumption of refined carbohydrates and
9 464 the epidemic of type 2 diabetes in the United States: an ecologic assessment. *The American*
10 465 *Journal of Clinical Nutrition* 79:774-779.
11
12
13 466 Hendriksen MA, Tijhuis MJ, Fransen HP, Verhagen H, Hoekstra J. 2011. Impact of
14 467 substituting added sugar in carbonated soft drinks by intense sweeteners in young adults in
15 468 the Netherlands: Example of a benefit-risk approach. *European Journal of Nutrition* 50:41-51.
16
17
18 469 Huang ZQ, Ma JY, Chen B, Zhang Y, Yao SZ. 2006. Determination of cyclamate in foods by
19 470 high performance liquid chromatography-electrospray ionization mass spectrometry.
20 471 *Analytica Chimica Acta* 555:233-237.
21
22
23 472 Husoy T, Mangschou B, Fotland TO, Kolset SO, Jakobsen HN, Tommerberg I, Bergsten C,
24 473 Alexander J, Andersen LF. 2008. Reducing added sugar intake in Norway by replacing sugar
25 474 sweetened beverages with beverages containing intense sweeteners - A risk benefit
26 475 assessment. *Food and Chemical Toxicology* 46:3099-3105.
27
28
29 476 Hutchinson SA, Ho GS, Ho CT. 1999. Stability and degradation of the high-intensity
30 477 sweeteners: Aspartame, alitame, and sucralose. *Food Reviews International* 15:249-261.
31
32
33 478 Ilback NG, Alzin M, Jahrl S, Enghardt-Barbieri H, Busk L. 2003. Estimated intake of the
34 479 artificial sweeteners acesulfame-K, aspartame, cyclamate and saccharin in a group of
35 480 Swedish diabetics. *Food Additives and Contaminants* 20:99-114.
36
37
38 481 Iowa State University. 1996. A user's guide to C-SIDE. Software for Intake Distribution
39 482 Estimation. Department of Statistics and Center for Agricultural and Rural Development,
40 483 Iowa State University, IA.
41
42
43 484 Koyama M, Yoshida K, Uchibori N, Wada I, Akiyama K, Sasaki T. 2005. Analysis of nine
44 485 kinds of sweeteners in foods by LC/MS. *Journal of the Food Hygienic Society of Japan*
45 486 46:72-78.
46
47
48 487 Kuhn C, Bufe B, Winnig M, Hofmann T, Frank O, Behrens M, Lewtschenko T, Slack JP,
49 488 Ward CD, Meyerhof W. 2004. Bitter taste receptors for saccharin and acesulfame K. *Journal*
50 489 *of Neuroscience* 24:10260-10265.
51
52
53
54
55
56
57
58
59
60

- 1
2
3 490 Leclercq C, Berardi D, Sorbillo MR, Lambe J. 1999. Intake of saccharin, aspartame,
4 491 acesulfame K and cyclamate in Italian teenagers: present levels and projections. Food
5 492 Additives and Contaminants 16:99-109.
6
7
8 493 Leth T, Jensen U, Fagt S, Andersen R. 2008. Estimated intake of intense sweeteners from
9 494 non-alcoholic beverages in Denmark, 2005. Food Additives and Contaminants 25:662-668.
10
11
12 495 Lino CM, Costa IM, Pena A, Ferreira R, Cardoso SM. 2008. Estimated intake of the
13 496 sweeteners, acesulfame-K and aspartame, from soft drinks, soft drinks based on mineral
14 497 waters and nectars for a group of Portuguese teenage students. Food Additives and
15 498 Contaminants Part A-Chemistry Analysis Control Exposure & Risk Assessment 25:1291-
16 499 1296.
17
18
19
20
21 500 Loos R, Gawlik BM, Boettcher K, Locoro G, Contini S, Bidoglio G. 2009. Sucralose screening
22 501 in European surface waters using a solid-phase extraction-liquid chromatography-triple
23 502 quadrupole mass spectrometry method. Journal of Chromatography A 1216:1126-1131.
24
25
26
27 503 Ludwig DS, Peterson KE, Gortmaker SL. 2001. Relation between consumption of sugar-
28 504 sweetened drinks and childhood obesity: a prospective, observational analysis. The Lancet
29 505 357:505-508.
30
31
32 506 Mattes RD, Popkin BM. 2009. Nonnutritive sweetener consumption in humans: effects on
33 507 appetite and food intake and their putative mechanisms. American Journal of Clinical
34 508 Nutrition 89:1-14.
35
36
37
38 509 Nusser SM, Carriquiry AL, Dodd KW, Fuller WA. 1996. A semiparametric transformation
39 510 approach to estimating usual daily intake distributions. Journal of the American Statistical
40 511 Association 91:1440-1449.
41
42
43 512 Renwick AG. 2006. The intake of intense sweeteners - an update review. Food Additives and
44 513 Contaminants A 23:327-338.
45
46
47 514 Rutters H, Mohring T, Rullkotter J, Griep-Raming J, Metzger JO. 2000. The persistent
48 515 memory effect of triethylamine in the analysis of phospholipids by liquid
49 516 chromatography/mass spectrometry. Rapid Communications in Mass Spectrometry 14:122-
50 517 123.
51
52
53
54 518 Scheurer M, Brauch HJ, Lange FT. 2009. Analysis and occurrence of seven artificial
55 519 sweeteners in German waste water and surface water and in soil aquifer treatment (SAT).
56 520 Analytical and Bioanalytical Chemistry 394:1585-1594.
57
58
59
60

- 1
2
3 521 Serra-Majem L, Bassas L, Garcia-Glosas R, Ribas L, Ingles C, Casals I, Saavedra P,
4 522 Renwick AG. 2003. Cyclamate intake and cyclohexylamine excretion are not related to male
5 523 fertility in humans. *Food Additives and Contaminants* 20:1097-1104.
6
7
8 524
9
10
11 525 Shils ME, Shike M, Ross AC, Caballero B, Cousins RJ. 2005. Modern nutrition in health and
12 526 disease. 10th ed. Philadelphia, PA: Lippincott Williams & Wilkins. Slimani N, Valsta L. 2002.
13 527 Perspectives of using the EPIC-SOFT programme in the context of pan-European nutritional
14 528 monitoring surveys: Methodological and practical implications. *European Journal of Clinical*
15 529 *Nutrition* 56: S63-S74.
16
17
18
19 530 Touger-Decker R, van Loveren C. 2003. Sugars and dental caries. *The American Journal of*
20 531 *Clinical Nutrition* 78:881S-892S.
21
22
23 532 UK Food Standards Agency. 2003. Diary survey of the intake of intense sweeteners by
24 533 young children from soft drinks. 36/03
25
26
27 534 van Rooij-van den Bos, L, Konings, EJM, Heida, P, van Hamersveld, ICM, van der Wielen, J,
28 535 Kooijman, M. 2004. Onderzoek naar de kunstmatige zoetstoffen Saccharine, Aspartaam,
29 536 Acesulfaam-K en Cyclamaat in levensmiddelen. Gehaltebepaling en inname door de
30 537 Nederlandse populatie. SD 03 K120
31
32
33
34 538 Vandevijvere S, De Vriese S, Huybrechts I, Moreau M, Temme E, De Henauw S, De Backer
35 539 G, Kornitzer M, Leveque A, Van Oyen H. 2009. The gap between food-based dietary
36 540 guidelines and usual food consumption in Belgium, 2004. *Public Health Nutrition* 12:423-431.
37
38
39 541 Wasik A, McCourt J, Buchgraber M. 2007. Simultaneous determination of nine intense
40 542 sweeteners in foodstuffs by high performance liquid chromatography and evaporative light
41 543 scattering detection - Development and single-laboratory validation. *Journal of*
42 544 *Chromatography A* 1157:187-196.
43
44
45
46 545 Wilson LA, Wilkinson K, Crews HM, Davies AM, Dick CS, Dumsday VL. 1999. Urinary
47 546 monitoring of saccharin and acesulfame-K as biomarkers of exposure to these additives.
48 547 *Food Additives and Contaminants* 16:227-238.
49
50
51
52 548 Yang DJ, Chen B. 2009. Simultaneous Determination of Nonnutritive Sweeteners in Foods
53 549 by HPLC/ESI-MS. *Journal of Agricultural and Food Chemistry* 57:3022-3027.
54
55
56
57
58
59
60

1
2
3 550 Zyglar A, Wasik A, Namiesnik J. 2009. Analytical methodologies for determination of artificial
4 551 sweeteners in foodstuffs. *Trac-Trends in Analytical Chemistry* 28:1082-1102.

5
6
7 552 Zyglar A, Wasik A, Namiesnik J. 2010. Retention behaviour of some high-intensity
8 553 sweeteners on different SPE sorbents. *Talanta* 82:1742-1748.

9
10 554

11
12
13 555

14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review Only

556 **Captions to figures**

557

558

559 **Figure 1.** Total distribution of the various sweeteners (*panel A*, graph labels refer to
560 **absolute counting; relative counting %**), individual sweeteners or sweetener combinations
561 applied in table-top sweeteners (*panel B*), and distribution per physical form (*panel C*) as
562 determined from the label survey (Ace-K: Acesulfame-K; Asp: Aspartame; Cyc: Cyclamate;
563 Sacc: Saccharin; Sucr: Sucralose).
564

565 **Figure 2.** Overview of foods with sweeteners added (*panel A*), total distribution of different
566 sweeteners in these foods (*panel B*) (for both diagrams, graph labels refer to **absolute**
567 **counting; relative counting %**), and individual sweeteners or sweetener combinations
568 applied in foods (*panel C*) as determined from the label survey (Ace-K: Acesulfame-K; Asp:
569 Aspartame; Cyc: Cyclamate; N.I.: Not indicated, i.e. presence of sweeteners mentioned
570 without further details; Neo: Neohesperidin dihydrochalcone; Sacc: Saccharin; Sucr:
571 Sucralose).
572

573

574

574

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Unable to Convert Image

The dimensions of this image (in pixels) are too large to be converted. For this image to convert, the total number of pixels (height x width) must be less than 40,000,000 (40 megapixels).

Peer Review Only

Unable to Convert Image

The dimensions of this image (in pixels) are too large to be converted. For this image to convert, the total number of pixels (height x width) must be less than 40,000,000 (40 megapixels).

Peer Review Only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 **Tables**2 **Table 1.** Details of chromatography and mass spectrometric detection of sweeteners

Compound	RT ^a (min)	Mode ^b	MI ^c (m/z)	CE ^d (%)	Fragments (m/z)	Loss of
Acesulfame-K	2.6	-	162.0	30	82.0	sulfonate
Saccharin	4.8	-	182.0	30	182.0	-
Cyclamate	8.1	-	178.1	30	178.1	-
Sucralose	11.1	-	395.2	24	359.0	chlorine
			397.1		361.0	chlorine
Aspartame	12.6	+	295.1	30	277	water
					262.9	methanol
					235.0	methyl ester

^aRT: Retention time. ^bIonization mode. ^cMolecular ion. ^dCE: Normalized collision energy (expressed relative to the maximum value of 80 eV).

For Peer Review Only

9 **Table 2.** Method performance parameters

Compound	Iod ($\mu\text{g L}^{-1}$)	Iog ($\mu\text{g L}^{-1}$)	Recovery (%)	RSD WD ^a (%)			RSD BD ^e (%)
				L ^b	M ^c	H ^d	
Acesulfame-K	2.5	10	102	4	3	3	4
Saccharin	5	20	99	5	1	3	4
Cyclamate	5	20	97	11	1	3	5
Sucralose	20	50	98	8	11	4	2
Aspartame	5	10	98	4	5	2	12

10
11 ^aRSD WD: Within day relative standard deviation. ^bL: low concentration (0.5 g kg⁻¹).
12 ^cM: medium (1 g kg⁻¹). ^dH: high (1.5 g kg⁻¹). ^eRSD BD: Between day relative standard
13 deviation.
14
15

For Peer Review Only

16 **Table 3.** Mean sweetener concentrations of positive samples

Food group (<i>n</i>)	Sweeteners ^a					unit
	Ace-K	Sacc	Cyc	Sucr	Asp	
Table-top sweetener (30)						
Cube (1)	0.8	- ^b	-	-	5	g kg ⁻¹
Pellet (10)	58	103	356	-	243	g kg ⁻¹
Powder (14)	5	4	66	-	22	g kg ⁻¹
Liquid (5)	-	6	58	-	-	g L ⁻¹
Nonalc. drinks - total (46)	89	31	153	53	48	mg L ⁻¹
Energy (2)	20	-	61	81	15	mg L ⁻¹
Aromatized water (5)	35	-	-	28	47	mg L ⁻¹
Carbonated (19)	88	27	173	54	45	mg L ⁻¹
Based on fruit juice (14)	107	33	152	30	59	mg L ⁻¹
Based on milk (4)	201	41	158	166	85	mg L ⁻¹
Sports (2)	55	-	-	-	-	mg L ⁻¹
Beers - total (16)	28	13	-	-	32	mg L ⁻¹
Reduced alcohol (2)	36	-	-	-	-	mg L ⁻¹
Table beer (4)	14	10	-	-	-	mg L ⁻¹
Lambic beer (10)	27	13	-	-	32	mg L ⁻¹
Desserts (2)	-	-	-	235	-	mg kg ⁻¹
Chewing gum (6)	1747	-	-	-	2151	mg kg ⁻¹
Sweets (12)	1087	-	-	802	756	mg kg ⁻¹
Marmalade (3)	-	134	512	-	-	mg kg ⁻¹
Canned fruit (4)	501	168	623	-	294	mg kg ⁻¹

^a Ace-K: Acesulfame-K; Sacc: Saccharin; Cyc: Cyclamate; Sucr: Sucralose; Asp: Aspartame. ^b No positive samples detected.

Table 4. Usual sweetener intake (mg kg^{-1} bodyweight day^{-1}) by the adult Belgian population, as assessed by Tier 2.

Sweetener (ADI)	Average ($\text{mg kg}^{-1} \text{day}^{-1}$)	SD ^a ($\text{mg kg}^{-1} \text{day}^{-1}$)	Percentile	Intake ($\text{mg kg}^{-1} \text{day}^{-1}$)	
				AD ^b	%ADI
Acesulfame-K (9 $\text{mg kg}^{-1} \text{day}^{-1}$)	1.01	0.63	p50	0.87	10
			p95	2.22	25
			p97.5	2.61	29
			p99	3.13	35
Aspartame (40 $\text{mg kg}^{-1} \text{day}^{-1}$)	1.95	1.13	p50	1.74	4
			p95	4.11	10
			p97.5	4.76	12
			p99	5.63	14
Cyclamate (7 $\text{mg kg}^{-1} \text{day}^{-1}$)	0.44	0.40	p50	0.33	5
			p95	1.21	17
			p97.5	1.52	22
			p99	1.95	28
Saccharin (5 $\text{mg kg}^{-1} \text{day}^{-1}$)	0.34	0.18	p50	0.31	6
			p95	0.68	14
			p97.5	0.77	15
			p99	0.90	18
Sucralose (15 $\text{mg kg}^{-1} \text{day}^{-1}$)	0.84	0.54	p50	0.73	5
			p95	1.87	12
			p97.5	2.21	15
			p99	2.66	18

^a SD: Standard deviation. ^b AD: All days.

46 **Table 5.** Usual sweetener intake (mg kg^{-1} bodyweight day^{-1})
 47 by the adult Belgian population, as assessed by Tier 3.
 48

Sweetener (ADI)	Average ($\text{mg kg}^{-1} \text{day}^{-1}$)	SD ^a ($\text{mg kg}^{-1} \text{day}^{-1}$)	Percentile	Intake ($\text{mg kg}^{-1} \text{day}^{-1}$)	
				AD ^c	%ADI
Acesulfame-K (9 $\text{mg kg}^{-1} \text{day}^{-1}$)	0.53	0.35	p50	0.46	5
			p95	1.21	13
			p97.5	1.42	16
			p99	1.71	19
Aspartame (40 $\text{mg kg}^{-1} \text{day}^{-1}$)	0.60	0.42	p50	0.51	1
			p95	1.40	4
			p97.5	1.68	4
			p99	2.07	5
Cyclamate (7 $\text{mg kg}^{-1} \text{day}^{-1}$)	0.23	0.34	p50	0.10	1
			p95	0.89	13
			p97.5	1.19	17
			p99	1.61	23
Saccharin (5 $\text{mg kg}^{-1} \text{day}^{-1}$)	0.15	0.11	p50	0.12	2
			p95	0.37	7
			p97.5	0.43	9
			p99	0.52	10
Sucralose (15 $\text{mg kg}^{-1} \text{day}^{-1}$)	0.42	0.22	p50	0.39	3
			p95	0.82	5
			p97.5	0.93	6
			p99	1.07	7

49 ^a SD: Standard deviation. ^b AD: All days.

50

51

Table 6. Overview of sweetener intake studies, based on a review by Renwick (Renwick 2006) but with inclusion of recent values reported in literature. Relative intakes are based on ADI levels as set by the Scientific Committee on Food (i.e., 9, 40, 7, 5, and 15 mg kg⁻¹ for acesulfame-K, aspartame, cyclamate, saccharin, and sucralose, respectively).

Country	Group	Average intake	High consumer intake	Ref.	
Italy	Teenager (13-19 y)	0.1% (Ace), 0.1% (Asp), 3% (Cyc), 4% (Sac)	<i>max</i>	2% (Ace), 1% (Asp), 9% (Cyc), 11% (Sac)	(Leclercq et al. 1999)
	Teenager (14-17 y)	0.5% (Ace), 0.2% (Asp), 1% (Cyc), 0% (Sac) ^a	<i>p95</i>	3% (Ace), 0.7% (Asp), 8% (Cyc), 0% (Sac) ^a	(Arcella et al. 2004)
		0.5% (Ace), 0.4% (Asp), 0.7% (Cyc), 0.6% (Sac) ^b	<i>p95</i>	3% (Ace), 2% (Asp), 4% (Cyc), 5% (Sac) ^b	
UK	Population (3-74 y)	9% (Ace), 14% (Sac)	-	-	(Wilson et al. 1999)
France	Adults (≥ 15 y)	0.3% (Cyc)	<i>p97.5</i>	2.9% (Cyc)	(Bemrah et al. 2008)
	Diabetics (2-20 y)	9% (Ace), 5% (Asp), 6% (Sac)	<i>p97.5</i>	27% (Ace), 20% (Asp), 26% (Sac)	(Garnier-Sagne et al. 2001)
Denmark	Population (1-80 y)	15% (Ace), 4% (Asp), 37% (Cyc), 8% (Sac)	<i>p99</i>	26% (Ace), 5% (Asp), 51% (Cyc), 1% (Sac)	(Leth et al. 2008)
Sweden	Adult diabetics (16-90 y)	< ADI (Ace, Asp, Cyc, and Sac)	<i>p95</i>	45% (Asp), 114% (Cyc), 46% (Sac)	(Ilback et al. 2003)
Norway	Men (16-79 y)	2.5% (Asp), 3% (Ace)	<i>p95</i>	11% (Asp), 12% (Ace)	(Husoy et al. 2008)
	Women (16-79 y)	3% (Asp), 4% (Ace)	<i>p95</i>	14% (Asp), 18% (Ace)	
Spain	Men (30-50 y)	6% (Cyc)	-	-	(Serra-Majem et al. 2003)
The Netherlands	Population (1-97 y)	<0.5% (Ace), <0.3% (Asp), 1% (Cyc), 0.4% (Sac)	<i>p95</i>	0.7% (Ace), 1% (Asp), 6% (Cyc), 0.4% (Sac)	(van Rooij-van den Bos et al. 2004)
	Men (19-30 y)	-	<i>p95</i>	29% (Ace), 6% (Asp), 37% (Cyc), 4% (Sac)	(Hendriksen et al. 2011)
	Women (19-30 y)	-	<i>p95</i>	27% (Ace), 5% (Asp), 29% (Cyc), 3% (Sac)	

^a Calculated based on consumption of soft drinks. ^b Calculated based on consumption of table-top sweeteners by a group of females with high reported table-top sweetener intake.