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Joris van Loco

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Dietary Intake of Artificial Sweeteners by the Belgian Population

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| 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 |

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| | <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> |
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3 **1 Dietary Intake of artificial sweeteners by the Belgian population**
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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).

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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

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3 65 Health, Food Safety and Environment conceived a research project to investigate the
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5 66 prevalence of sweeteners on the Belgian market and to evaluate the consumption of these
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7 67 products by the Belgian population. A second objective was to compare intake levels of
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9 68 diabetics with those of the general population. In response to these questions, a food label
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11 69 survey was initiated to map the occurrence of sweeteners in foods and in table-top
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13 70 sweeteners on the Belgian market, while chemical analysis of a representative number of
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15 71 foods was considered essential to gain insight in the concentrations being used. Resulting
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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
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25 76 to the total dietary intake.
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27 77

28 29 78 **MATERIALS AND METHODS**

30 31 79 **Food label survey**

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33 80 The label survey was approached from the point of view that food additive labeling is
34
35 81 mandatory, hence artificially-sweetened products were readily selected from label screening
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37 82 of > 2000 food items in 7 major supermarkets with good market share in Belgium. Screening
38
39 83 of table-top sweeteners, a well-defined group of sweetener-based products, was most
40
41 84 apparent, but the set of sweetened foods was considerably larger and exceedingly divers.
42
43 85 Therefore, Directive 94/35/EC, which includes an overview of foods legally allowed to contain
44
45 86 sweeteners (European Parliament and Council 1994), was used as starting point from which
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47 87 food labels were explored. Relevant foods were inserted in an in-house database and
48
49 88 organized in groups according to available national food consumption data (Vandevijvere et
50
51 89 al. 2009), thus generating following food groups: nonalcoholic beverages, beers and
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53 90 alcoholic beverages, desserts and similar products, sweets including breath-freshening
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55 91 micro-sweets, chewing gums, canned and bottled foods, marmalades, sauces, and cereals.
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57 92 Information from these products, including brand name, producer name, physical state,
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3 93 ingredients, sweetener content, labeled warnings, supermarket name, day of visit, and batch
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5 94 number were registered in the database. Duplicates, i.e. identical products found at different
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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
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11 97 December 2009 till February 2010.
12

13 98

14 99 **Chemicals**

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17 100 Acesulfame-K and sodium saccharinate were obtained from Fluka (Buchs, Switzerland),
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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*

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37 110 The complexity and heterogeneity of certain foods such as confectionery, biscuits, and dairy
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39 111 products required thorough sample preparation, which, for the sake of throughput, was
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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
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53 118 or a specific food group (Koyama et al. 2005; Wasik et al. 2007; Yang et al. 2009) were
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55 119 further optimized for use with the proposed set of complex and diverse matrices. It is worth
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57 120 noting that sweeteners in foods and particularly in table-top sweeteners were relatively highly
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3 121 concentrated, hence samples needed dilution prior to analysis. Thus, following preparation
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5 122 procedures for different matrices based on homogenization and dilution were elaborated.

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7 123 **Table-top sweeteners.** Pellets or cubes were initially milled in a coffee grinder, while,
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9 124 obviously, formulations in aqueous solution required no additional homogenization.
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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)
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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
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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
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35 137 which 1 mL of the resulting supernatant was centrifuged for 10 min at 13,000 rpm (Biofuge
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37 138 Pico, Heraeus, Buckinghamshire, UK). Finally, 200 μL of the thus isolated supernatant was
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39 139 diluted with water (1:20, v/v) and after filtration, the mixture was ready for injection.

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43 141 *Chromatography*

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45 142 Samples for chromatography were placed immediately after preparation in a thermostated
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47 143 sample rack (at 5°C) of a Surveyor autosampler with built-in LC pump (Thermo Electron
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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
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55 147 mm, 5 μm) (Waters Corporation, Milford, MA, USA), equipped with corresponding guard
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57 148 column and installed in the column oven (set at 50°C) of the Surveyor station. Gradient
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3 149 elution was optimized using a solvent system consisting of water (solvent A) and methanol
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5 150 (solvent B), each with 20 mM ammonium acetate added. Eventually, following gradient
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7 151 profile with satisfying separation was applied: 0 to 2 min at 5% B, raise to 49% B in 12 min,
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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

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15 155 *Detection*

16
17 156 Sweetener elution was monitored by ion trap mass spectrometry with electrospray ionization
18
19 157 source (LCQ Deca XP mass spectrometer, Thermo Electron Corp.). Ionization parameters
20
21 158 were optimized for the selected sweeteners to obtain reproducible fragmentation of the
22
23 159 respective compounds. The settings for the electrospray source were capillary voltage -34 V
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25 160 and tube lens offset -10 V for acesulfame-K, saccharin, and cyclamate and -4 V and 20 V for
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27 161 sucralose, aspartame, and the internal standard. Except for sucralose, ion isolation width
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29 162 was m/z 1, activation Q was 0.25, and activation time was 30 ms (4, 0.2, and 35,
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31 163 respectively, for sucralose). For all compounds, absolute spray voltage was 5 kV and
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33 164 capillary temperature was 375 °C. Indeed, the high concentration of water with first-eluting
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35 165 compounds required high capillary temperature to overcome surface tension of sprayed
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37 166 micro-droplets. For the same reason, significant sheath and sweep nitrogen gas flows were
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39 167 applied (set at 45 and 20 arbitrary units, respectively). Collision energy needed for
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41 168 fragmenting saccharin and cyclamate was too high to yield stable ions, hence only fictitious
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43 169 transitions for these compounds were monitored. Unlike other sweeteners, aspartame was
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45 170 detected in positive ionization mode. Under similar conditions, the molecular ion of
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47 171 sulfadimidine, the internal standard (m/z 279 in positive mode) was fragmented to give ions
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49 172 m/z 186.1 and m/z 203.9.
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53 174 *Calibration*

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55 175 Quantification of the different compounds was based on external calibration curves
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57 176 constructed by injecting a series of standard concentrations. Since no matrix effect was
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3 177 observed for beverages, dairy products, and confectionary, calibration in solution was used.
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5 178 Sodium saccharinate and sodium cyclamate were used for calibration and analytical results
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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
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11 181 50 ppb).
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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

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

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

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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
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6 207 and repeated on three non-consecutive days. The resulting 27 ($3 \times 3 \times 3$) samples were
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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
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12 210 corresponding theoretical value (1 g kg^{-1}).
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17 212 ***Intake assessment***

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19 213 A uniform methodology, designed by members of the SCOOP task force in view of realizing
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21 214 harmonized additive intake assessments, was previously reported and consisted of a 3-
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23 215 phase approach (European Commission 2004). Tier 1, which is based on theoretical food
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25 216 consumption combined with maximum permitted sweetener levels according to Directive
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27 217 94/35/EC (European Parliament and Council 1994), is a conservative intake estimate carried
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29 218 out at European level and is not a task for Member States. Tier 2 and Tier 3 refer to
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31 219 assessment at the level of individual Member States and are based on a combination of
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33 220 national food consumption data with maximum permitted levels (Tier 2) and with actual use
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35 221 levels (Tier 3), respectively.
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38
39 223 Consumption data from the Belgian Food Consumption Survey (dating from 2004) were used
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41 224 to perform the intake assessment. Aims, design and methods of this survey can be found
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43 225 elsewhere (Vandevijvere et al. 2009). The target population comprised all Belgian inhabitants
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45 226 of 15 years or older. The sample included 3245 participants randomly selected from the
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47 227 National Register using a multi-stage stratified procedure. Information on dietary intake was
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49 228 collected by two non-consecutive 24-h recalls in which the respondent reported the quantity
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51 229 of all foods and beverages consumed during the preceding day. Both 24-h recalls, which
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53 230 were carried out using EPIC-SOFT software (Slimani et al. 2002), were completed by 3083
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55 231 participants (hence 6166 recalls including 428 from diabetics).
56
57 232

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

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 (mg kg^{-1} 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 mg kg^{-1} bodyweight day^{-1}) or relatively
257 against the ADI of the respective sweeteners.

258

259 **RESULTS & DISCUSSION**

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3 260 **Label survey**
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5 261 For the presentation of the results of the label survey, it was decided to treat table-top
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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.
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32
33 275 Unlike table-top sweeteners, which basically contained bulk materials with high
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35 276 concentrations of sweeteners, ingredient lists of the heterogeneous set of sweetened foods
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37 277 were considerably more complicated. A total of 270 labels, divided over 10 different food
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39 278 groups according to consumption data, were examined. The most important group was the
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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
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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
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3 288 (beverage) was found to contain neohesperidin dihydrochalcone, hence the decision to leave
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5 289 this and other recently approved sweeteners out of the study was justified.
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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
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14 294 method based on simple dilution followed by filtration, which was previously found effective
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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
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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
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39 306 survey ($n=30$) were sampled and qualitative analysis results complied with data extracted
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41 307 from the label. Liquid table-top sweeteners were exclusively composed of cyclamate and
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43 308 saccharin and doses amounting to $\sim 80 \text{ g L}^{-1}$ for the sum of the two sweeteners were
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45 309 measured. Next to cyclamate and saccharin, solid table-top sweeteners contained high
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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
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51 312 are normally consumed in larger portions. Average concentrations of sweeteners in positive
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53 313 samples (Table 3) confirmed the notion that cyclamate, having the weakest sweetening
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55 314 power, is used in the highest concentrations.
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3 316 For analyses of foods, 89 samples, which were a representative distribution of sweetened
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5 317 products found on the market, were selected for analysis (Table 3). In one case, aspartame
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7 318 was labeled but was not detected as such, probably due to degradation during storage
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9 319 (despite that analysis was carried out prior to expiry date) (Hutchinson et al. 1999). Only one
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11 320 sample (beer) was found in which the listed sweetener (acesulfame-K) differed from the one
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13 321 detected (saccharin), a result of inconsistent labeling as confirmed by the involved brewery.
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15 322 Reconstituting sweetener composition of sampled beer and beverages based on analytical
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17 323 data, showed that sweeteners in beer were relatively low concentrated compared to non-
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19 324 alcoholic drinks. Beers mostly contained saccharin or acesulfame-K, which were favored
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21 325 over cyclamate (not allowed in beer) and aspartame (unstable in acidic media). Acesulfame-
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23 326 K was also abundantly used in nonalcoholic drinks, despite its unpleasant bitter aftertaste
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25 327 (Kuhn et al. 2004). For this reason, it often appears in combination with aspartame and
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27 328 cyclamate, the latter, due to its low sweetening power, is higher concentrated. The former
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29 329 combination (i.e., aspartame and acesulfame-K) dominates other food groups as well and
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31 330 particularly chewing gum was characterized by high quantities of these compounds. Indeed,
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33 331 Directive 94/35/EC (European Parliament and Council 1994) permits acesulfame-K levels up
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35 332 to 2000 mg kg⁻¹ while aspartame can be concentrated as high as 5500 mg kg⁻¹. In this
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37 333 respect, one sample was found to contain a significant excess of acesulfame-K, while in
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39 334 canned fruit (3 of 4 samples) acesulfame-K levels slightly exceeding the maximum permitted
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41 335 level were found. Permitted concentrations of these and other sweeteners, as stated in the
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43 336 sweetener directive, were not exceeded in other foods.
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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

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3 344 significantly below established ADI values (Table 4). For diabetics, which were suspected of
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5 345 consuming higher amounts of sweeteners, observed intake (based on mean intake of 2
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7 346 interview days) rather than usual intake was calculated because of the smaller subpopulation
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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,
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13 349 cyclamate, saccharin, and sucralose, respectively, which was not considerably different from
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15 350 observed intake of the entire population (3.55 ± 1.41 , 6.77 ± 2.61 , 1.97 ± 0.86 , 1.14 ± 0.43 ,
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17 351 and 3.08 ± 1.20 mg kg⁻¹ bodyweight day⁻¹, respectively). Intake of table-top sweeteners was
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19 352 however not included in these figures because the lack of maximum permitted levels and
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21 353 their contribution could only be taken into account in Tier 3. This approach uses actual, mean
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23 354 concentrations (Table 3) (or maximum permitted levels for minor foods) for combination with
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25 355 food consumption data and is expected to be more accurate. Indeed, resulting lower intakes
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27 356 (Table 5) emphasize the conservative character of Tier 2. Contributions of table-top
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29 357 sweeteners were low and of minor significance: 0.06 %, 0.05 %, 0.44 %, and 0.17 % of the
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31 358 respective ADI for acesulfame-K, aspartame, cyclamate, and saccharin, respectively. No
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33 359 intake data was obtained for sucralose due to its practical absence in table-top sweeteners.
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35 360 Similar as in the Tier 2, observed intake was calculated for diabetics and compared with the
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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
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39 362 mg kg⁻¹ bodyweight day⁻¹ were consumed of acesulfame-K, aspartame, cyclamate,
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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
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45 365 through consumption of table-top sweeteners was slightly higher for diabetics (0.004 ± 0.002 ,
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47 366 0.019 ± 0.008 , 0.027 ± 0.011 , and 0.008 ± 0.003 mg kg⁻¹ bodyweight day⁻¹ of acesulfame-K,
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49 367 aspartame, cyclamate, and saccharin, respectively) compared to the entire population (0.002
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51 368 ± 0.001 , 0.008 ± 0.005 , 0.011 ± 0.008 , and 0.003 ± 0.002 mg kg⁻¹ bodyweight day⁻¹,
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53 369 respectively), but was negligible with respect to established ADIs. Since usual intake
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55 370 remained significantly lower than reported ADIs, it was safely assumed that the adult Belgian
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57 371 population was not exposed to excessive intakes.
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5 373 *Sweetener intake in other Member States*

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

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

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

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20 408 **Conclusions**

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

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3 427 **Acknowledgments**
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5 428 This work was funded by the Directorate-General Animal, Plant, and Food (DG 4) of the

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7 429 Federal Service of Public Health, Safety of the Food Chain, and Environment.
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556 **Captions to figures**

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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).
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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).

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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.
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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.