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
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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

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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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Dietary Intake of artificial sweeteners by the Belgian population

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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.

INTRODUCTION

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

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

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

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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,

ingredients, sweetener content, labeled warnings, supermarket name, day of visit, and batch number were registered in the database. Duplicates, i.e. identical products found at different locations or supplied in varying packaging (a particular drink, for example, supplied in cans, PET-bottles or glass bottles) were counted only once. Screening was performed from December 2009 till February 2010.

Chemicals

Acesulfame-K and sodium saccharinate were obtained from Fluka (Buchs, Switzerland), while sodium cyclamate, sucralose, and sulfadimidine came from Sigma (St. Louis, MO, USA). Aspartame was purchased from Supelco (Bellefonte, PA, USA), while ammonium acetate was a product from Acros (Geel, Belgium). All chemicals were of analytical grade. Methanol (HPLC-grade) was obtained from Biosolve (Valkenswaard, The Netherlands), while purified, de-ionized water ($R=18\text{ M}\Omega\text{ cm}$, total organic content $< 4\text{ ppb}$) was produced by a MilliQ unit (Millipore, Billerica, MA, USA).

Analytical methodology

Sample Preparation

The complexity and heterogeneity of certain foods such as confectionery, biscuits, and dairy products required thorough sample preparation, which, for the sake of throughput, was preferentially universally applicable. Initially, solid phase extraction (SPE), which is classic in analyses of complex samples, was applied for foods, but low affinity of saccharin and acesulfame salts for the C_{18} -packing material resulted in poor reproducibility. In this respect, it was recently reported that retention of these compounds on SPE cartridges is highly dependent on the type and manufacturer of the packing material (Zygler et al. 2010). Alternative sample preparations were thus investigated and reported methods for beverages or a specific food group (Koyama et al. 2005; Wasik et al. 2007; Yang et al. 2009) were further optimized for use with the proposed set of complex and diverse matrices. It is worth noting that sweeteners in foods and particularly in table-top sweeteners were relatively highly

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concentrated, hence samples needed dilution prior to analysis. Thus, following preparation procedures for different matrices based on homogenization and dilution were elaborated.

Table-top sweeteners. Pellets or cubes were initially milled in a coffee grinder, while, obviously, formulations in aqueous solution required no additional homogenization. Approximately 50 mg, or 0.5 mL for liquid formulations, of the homogenized sample was taken, followed by addition of 500 μ L of a 1000-ppm sulfadimidine (internal standard, IS) solution in methanol. The resulting mixture was diluted to 25 mL with water/methanol (1:1, v/v) and subsequently sonicated for 10 min. Then, 1 mL of the solution was centrifuged for 5 min at 4°C and at 5000 rpm (Avanti J25 centrifuge with J-10 rotor, Beckman, Brea, CA, USA). The supernatant was isolated, 100 μ L was taken and diluted with water (1:400, v/v). After subsequent filtering (0.45 μ m), the sample was ready for injection.

Foods and beverages. Similar as for table-top sweeteners, solid foods were initially milled in a grinder. Then, to approximately 0.5 g of the respective sample (or to 1 mL for beverages), 250 μ L of a 100-ppm sulfadimidine solution in methanol was added. The resulting mixture was diluted to 25 mL with water/methanol (1:1, v/v) and exposed to ultrasones for 10 min. The sample was then left for cold precipitation (ice bath, 2h), after which 1 mL of the resulting supernatant was centrifuged for 10 min at 13,000 rpm (Biofuge Pico, Heraeus, Buckinghamshire, UK). Finally, 200 μ L of the thus isolated supernatant was diluted with water (1:20, v/v) and after filtration, the mixture was ready for injection.

Chromatography

Samples for chromatography were placed immediately after preparation in a thermostated sample rack (at 5°C) of a Surveyor autosampler with built-in LC pump (Thermo Electron Corp., San Jose, CA, USA). Sample injection (20 μ L) occurred in *full loop* mode and was preceded by washing and flushing of the injection needle with methanol (2 $\times$ 400 μ L). Sweeteners were separated using an analytical Waters Symmetry C₁₈-column (150 mm $\times$ 2.1 mm, 5 μ m) (Waters Corporation, Milford, MA, USA), equipped with corresponding guard column and installed in the column oven (set at 50°C) of the Surveyor station. Gradient

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

Detection

Sweetener elution was monitored by ion trap mass spectrometry with electrospray ionization source (LCQ Deca XP mass spectrometer, Thermo Electron Corp.). Ionization parameters were optimized for the selected sweeteners to obtain reproducible fragmentation of the respective compounds. The settings for the electrospray source were capillary voltage -34 V and tube lens offset -10 V for acesulfame-K, saccharin, and cyclamate and -4 V and 20 V for sucralose, aspartame, and the internal standard. Except for sucralose, ion isolation width was m/z 1, activation Q was 0.25, and activation time was 30 ms (4, 0.2, and 35, respectively, for sucralose). For all compounds, absolute spray voltage was 5 kV and capillary temperature was 375 °C. Indeed, the high concentration of water with first-eluting compounds required high capillary temperature to overcome surface tension of sprayed micro-droplets. For the same reason, significant sheath and sweep nitrogen gas flows were applied (set at 45 and 20 arbitrary units, respectively). Collision energy needed for fragmenting saccharin and cyclamate was too high to yield stable ions, hence only fictitious transitions for these compounds were monitored. Unlike other sweeteners, aspartame was detected in positive ionization mode. Under similar conditions, the molecular ion of sulfadimidine, the internal standard (m/z 279 in positive mode) was fragmented to give ions m/z 186.1 and m/z 203.9.

Calibration

Quantification of the different compounds was based on external calibration curves constructed by injecting a series of standard concentrations. Since no matrix effect was

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observed for beverages, dairy products, and confectionary, calibration in solution was used. Sodium saccharinate and sodium cyclamate were used for calibration and analytical results for these compounds therefore refer to concentrations of the salts rather than the corresponding free acids. The calibration is controlled by internal standard (sulfadimidine at 50 ppb).

Validation

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

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

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

of the respective sweeteners to reach three different final concentrations, namely 0.5 g kg⁻¹ (*low*), 1 g kg⁻¹ (*medium*), and 1.5 g kg⁻¹ (*high*), an experiment that was performed in triplicate and repeated on three non-consecutive days. The resulting 27 (3×3×3) samples were analyzed according to the reported procedure and validation results were described in Table 2. Herein, recovery was determined by the ratio of the average medium concentration and its corresponding theoretical value (1 g kg⁻¹).

Intake assessment

A uniform methodology, designed by members of the SCOOP task force in view of realizing harmonized additive intake assessments, was previously reported and consisted of a 3-phase approach (European Commission 2004). Tier 1, which is based on theoretical food consumption combined with maximum permitted sweetener levels according to Directive 94/35/EC (European Parliament and Council 1994), is a conservative intake estimate carried out at European level and is not a task for Member States. Tier 2 and Tier 3 refer to assessment at the level of individual Member States and are based on a combination of national food consumption data with maximum permitted levels (Tier 2) and with actual use levels (Tier 3), respectively.

Consumption data from the Belgian Food Consumption Survey (dating from 2004) were used to perform the intake assessment. Aims, design and methods of this survey can be found elsewhere (Vandevijvere et al. 2009). The target population comprised all Belgian inhabitants of 15 years or older. The sample included 3245 participants randomly selected from the National Register using a multi-stage stratified procedure. Information on dietary intake was collected by two non-consecutive 24-h recalls in which the respondent reported the quantity of all foods and beverages consumed during the preceding day. Both 24-h recalls, which were carried out using EPIC-SOFT software (Slimani et al. 2002), were completed by 3083 participants (hence 6166 recalls including 428 from diabetics).

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

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

RESULTS & DISCUSSION

260 ***Label survey***

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

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

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(beverage) was found to contain neohesperidin dihydrochalcone, hence the decision to leave this and other recently approved sweeteners out of the study was justified.

Analysis method

Despite the fact that various methods have been described, development and optimization of an analytical methodology was pursued to meet the specific objectives set for this work. A method based on simple dilution followed by filtration, which was previously found effective for drinks and table-top sweeteners (Zygler et al. 2009), was optimized for more complicated matrices by introduction of a cooling phase followed by ultracentrifugation of the resulting supernatant. As for the chromatography, use of triethylamine in the mobile phase (as in methods by Huang et al. 2006; Wasik et al. 2007; Yang et al. 2009) was avoided due to persistent memory effects with mass spectrometric detection (Rutters et al. 2000). Instead, ammonium acetate was preferred as additive (Scheurer et al. 2009). Details of chromatography and mass spectrometric detection of sweeteners are given in Table

Concentrations of sweeteners in table-top sweeteners and foods

Samples of table-top sweeteners were prepared and analyzed according to the procedure described in the experimental section. All table-top sweeteners found during the food label survey ($n=30$) were sampled and qualitative analysis results complied with data extracted from the label. Liquid table-top sweeteners were exclusively composed of cyclamate and saccharin and doses amounting to $\sim 80 \text{ g L}^{-1}$ for the sum of the two sweeteners were measured. Next to cyclamate and saccharin, solid table-top sweeteners contained high levels of aspartame so that a total sweetener content of up to 40% of the total mass was reached. In this respect, pellets were significantly more concentrated than powders, which are normally consumed in larger portions. Average concentrations of sweeteners in positive samples (Table 3) confirmed the notion that cyclamate, having the weakest sweetening power, is used in the highest concentrations.

For analyses of foods, 89 samples, which were a representative distribution of sweetened products found on the market, were selected for analysis (Table 3). In one case, aspartame was labeled but was not detected as such, probably due to degradation during storage (despite that analysis was carried out prior to expiry date) (Hutchinson et al. 1999). Only one sample (beer) was found in which the listed sweetener (acesulfame-K) differed from the one detected (saccharin), a result of inconsistent labeling as confirmed by the involved brewery. Reconstituting sweetener composition of sampled beer and beverages based on analytical data, showed that sweeteners in beer were relatively low concentrated compared to non-alcoholic drinks. Beers mostly contained saccharin or acesulfame-K, which were favored over cyclamate (not allowed in beer) and aspartame (unstable in acidic media). Acesulfame-K was also abundantly used in nonalcoholic drinks, despite its unpleasant bitter aftertaste (Kuhn et al. 2004). For this reason, it often appears in combination with aspartame and cyclamate, the latter, due to its low sweetening power, is higher concentrated. The former combination (i.e., aspartame and acesulfame-K) dominates other food groups as well and particularly chewing gum was characterized by high quantities of these compounds. Indeed, Directive 94/35/EC (European Parliament and Council 1994) permits acesulfame-K levels up to 2000 mg kg⁻¹ while aspartame can be concentrated as high as 5500 mg kg⁻¹. In this respect, one sample was found to contain a significant excess of acesulfame-K, while in canned fruit (3 of 4 samples) acesulfame-K levels slightly exceeding the maximum permitted level were found. Permitted concentrations of these and other sweeteners, as stated in the sweetener directive, were not exceeded in other foods.

Intake of artificial sweeteners in Belgium

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

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significantly below established ADI values (Table 4). For diabetics, which were suspected of consuming higher amounts of sweeteners, observed intake (based on mean intake of 2 interview days) rather than usual intake was calculated because of the smaller subpopulation ($n = 428$). Thus, for p95 the observed intake was found to be 3.75 ± 1.30 , 6.53 ± 2.32 , 2.06 ± 0.80 , 0.97 ± 0.39 , and 3.03 ± 1.07 mg kg⁻¹ bodyweight day⁻¹ for acesulfame-K, aspartame, cyclamate, saccharin, and sucralose, respectively, which was not considerably different from observed intake of the entire population (3.55 ± 1.41 , 6.77 ± 2.61 , 1.97 ± 0.86 , 1.14 ± 0.43 , and 3.08 ± 1.20 mg kg⁻¹ bodyweight day⁻¹, respectively). Intake of table-top sweeteners was however not included in these figures because the lack of maximum permitted levels and their contribution could only be taken into account in Tier 3. This approach uses actual, mean concentrations (Table 3) (or maximum permitted levels for minor foods) for combination with food consumption data and is expected to be more accurate. Indeed, resulting lower intakes (Table 5) emphasize the conservative character of Tier 2. Contributions of table-top sweeteners were low and of minor significance: 0.06 %, 0.05 %, 0.44 %, and 0.17 % of the respective ADI for acesulfame-K, aspartame, cyclamate, and saccharin, respectively. No intake data was obtained for sucralose due to its practical absence in table-top sweeteners. Similar as in the Tier 2, observed intake was calculated for diabetics and compared with the 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 mg kg⁻¹ bodyweight day⁻¹ were consumed of acesulfame-K, aspartame, cyclamate, saccharin, and sucralose, respectively, by diabetics versus 1.96 ± 0.78 , 2.46 ± 1.01 , 1.18 ± 0.52 , 0.60 ± 0.23 , and 1.53 ± 0.59 mg kg⁻¹ bodyweight day⁻¹ by the total population. Intake through consumption of table-top sweeteners was slightly higher for diabetics (0.004 ± 0.002 , 0.019 ± 0.008 , 0.027 ± 0.011 , and 0.008 ± 0.003 mg kg⁻¹ bodyweight day⁻¹ of acesulfame-K, aspartame, cyclamate, and saccharin, respectively) compared to the entire population (0.002 ± 0.001 , 0.008 ± 0.005 , 0.011 ± 0.008 , and 0.003 ± 0.002 mg kg⁻¹ bodyweight day⁻¹, respectively), but was negligible with respect to established ADIs. Since usual intake remained significantly lower than reported ADIs, it was safely assumed that the adult Belgian population was not exposed to excessive intakes.

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373 *Sweetener intake in other Member States*

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

389

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

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

Conclusions

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

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3 427 **Acknowledgments**
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6 428 This work was funded by the Directorate-General Animal, Plant, and Food (DG 4) of the
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8 429 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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Tables

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).

Table 2. Method performance parameters

Compound	Iod ($\mu\text{g L}^{-1}$)	Iod ($\mu\text{g L}^{-1}$)	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

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

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Table 3. Mean sweetener concentrations of positive samples

Food group (n)	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.

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

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

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

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>p</i> ₉₅ 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>p</i> ₉₅ 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>p</i> _{97.5} 2.9% (Cyc)	(Bemrah et al. 2008)
	Diabetics (2-20 y)	9% (Ace), 5% (Asp), 6% (Sac)	<i>p</i> _{97.5} 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>p</i> ₉₉ 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>p</i> ₉₅ 45% (Asp), 114% (Cyc), 46% (Sac)	(Ilback et al. 2003)
Norway	Men (16-79 y)	2.5% (Asp), 3 % (Ace)	<i>p</i> ₉₅ 11% (Asp), 12% (Ace)	(Husoy et al. 2008)
	Women (16-79 y)	3% (Asp), 4% (Ace)	<i>p</i> ₉₅ 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>p</i> ₉₅ 0.7% (Ace), 1% (Asp), 6% (Cyc), 0.4% (Sac)	(van Rooij-van den Bos et al. 2004)
	Men (19-30 y)	-	<i>p</i> ₉₅ 29% (Ace), 6% (Asp), 37% (Cyc), 4% (Sac)	(Hendriksen et al. 2011)
	Women (19-30 y)	-	<i>p</i> ₉₅ 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.