



HAL
open science

Dietary exposure to flavouring substances: from screening methods to detailed assessments using food consumption data collected with EPIC-Soft software

Sandra Patricia Crispim, Anouk Geelen, Cinzia Le Donne, Jeanne H. M. de Vries, Stefania Sette, Antonio Raffo, Els Siebelink, Marga C Ocke, Pieter Van'T Veer, Catherine Leclercq, et al.

► To cite this version:

Sandra Patricia Crispim, Anouk Geelen, Cinzia Le Donne, Jeanne H. M. de Vries, Stefania Sette, et al.. Dietary exposure to flavouring substances: from screening methods to detailed assessments using food consumption data collected with EPIC-Soft software. Food Additives and Contaminants, 2010, 27 (04), pp.433-446. 10.1080/19440040903420614 . hal-00576994

HAL Id: hal-00576994

<https://hal.science/hal-00576994>

Submitted on 16 Mar 2011

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

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



Dietary exposure to flavouring substances: from screening methods to detailed assessments using food consumption data collected with EPIC-Soft software

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2009-223.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	12-Oct-2009
Complete List of Authors:	Crispim, Sandra; Wageningen University, Division of Human Nutrition; National Research Institute for Food and Nutrition Geelen, Anouk; Wageningen University, Division of Human Nutrition Le Donne, Cinzia; National Research Institute for Food and Nutrition de Vries, Jeanne; Wageningen University, Division of Human Nutrition Sette, Stefania; National Research Institute for Food and Nutrition Raffo, Antonio; National Research Institute for Food and Nutrition Siebelink, Els; Wageningen University, Division of Human Nutrition Ocke, Marga; National Institute for Public Health and the Environment van't Veer, Pieter; Wageningen University, Division of Human Nutrition Leclercq, Catherine; National Research Institute for Food and Nutrition on behalf of EFCOVAL consortium, -
Methods/Techniques:	Exposure assessment
Additives/Contaminants:	Flavourings
Food Types:	Ingredients

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

SCHOLARONE™
Manuscripts

For Peer Review Only

1
2
3 1 **DIETARY EXPOSURE TO FLAVOURING SUBSTANCES: FROM SCREENING METHODS**
4
5 2 **TO DETAILED ASSESSMENTS USING FOOD CONSUMPTION DATA COLLECTED WITH**
6
7 3 **EPIC-SOFT SOFTWARE**
8
9 4

10
11 5 Sandra P. Crispim¹, Anouk Geelen¹, Cinzia Le Donne², Jeanne H. M. de Vries¹, Stefania
12 6 Sette², Antonio Raffo², Els Siebelink¹, Marga Ocke³, Pieter van't Veer¹, Catherine Leclercq²
13
14
15 7 on behalf of the EFCOVAL consortium
16
17 8

18
19 9 ¹ Division of Human Nutrition, Wageningen University (WU),
20
21 10 Bomenweg 2, Wageningen 6703 HD, The Netherlands.

22
23 11 ² National Research Institute for Food and Nutrition (INRAN)
24
25 12 Via Ardeatina, 546, 00178, Rome, Italy

26
27 13 ³ National Institute for Public Health and the Environment (RIVM)
28
29 14 PO Box 1, Bilthoven, 3720 BA, The Netherlands.

30
31 15
32
33 16 Corresponding author:

34
35 17 Dr. Catherine Leclercq

36
37 18 National Research Institute for Food and Nutrition (INRAN)
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

19

20 ABSTRACT

21 AIM: To compare different methods of assessing dietary exposure to flavourings in the
22 context of a stepwise approach.

23 METHODS: The dietary exposure to four flavourings was determined: raspberry ketone,
24 glycyrrhizinic acid, coumarin, and caffeine. When dietary exposure exceeded the safety limits,
25 the need for more detailed assessment using less aggregated data was judged necessary.
26 First, screening methods (Maximized Survey-Derived Daily Intake - MSDI, Single-Portion
27 Exposure Technique - SPET and modified Theoretical Added Maximum Daily Intake -
28 mTAMDI) were applied. Next, individual food consumption data were used for creating
29 models with different levels of detail to identify the foods: a model based on food groups and
30 models based on food items. These were collected from 121 Dutch adults using a
31 standardized 2x24h-dietary recall (EPIC-Soft) in the European Food Consumption Validation
32 (EFCOVAL) study. Three food item models were developed: without improvements of the
33 flavouring descriptor built in the software; with improvements; with use of non-specified
34 flavour descriptors.

35 RESULTS: Based on results of at least one of the three screening methods, refined
36 assessment was necessary for raspberry ketone, glycyrrhizinic acid and caffeine. When
37 applying the food group model, the need for refinement was indicated for the four flavourings.
38 When applying the food item models, only glycyrrhizinic acid and caffeine presented dietary
39 exposure above the safety limits. In the raspberry ketone case, dietary exposure increased
40 when improvements in food description were considered. The use of non-specified flavour
41 descriptors hardly changed the results.

42 CONCLUSION: The collection of detailed food consumption data at the individual level is
43 useful in the dietary exposure assessment of these flavourings.

44

45

46

1
2
3 47 **INTRODUCTION**
4
5

6 48 More than 2700 flavouring substances (hereafter called 'flavourings') are currently registered
7
8 49 and can be added to foods and beverages in the European Union (European Commission
9
10 50 1999, European Commission 2002, European Commission 2004, European Commission
11 51 2005). Accordingly, the Joint FAO/WHO* Expert Committee on Food Additives (JECFA) and
12 52 the European Food Safety Authority (EFSA) have been working towards the safety evaluation
13 53 of flavourings in order to provide a positive list of these substances (EFSA 2009, WHO 2009).
14 54 Within the safety evaluation procedure of any chemical substance, one crucial step is the
15 55 dietary exposure assessment.

16
17
18
19
20
21
22 56 A major pitfall of dietary exposure assessment to chemicals is the limited availability of the
23 57 two types of information that are needed, food consumption data and chemical concentration
24 58 in foods (EFSA 2005). The ideal situation of performing a detailed dietary exposure
25 59 assessment by collecting information at the individual level for every hazardous substance is
26 60 neither practical nor cost-effective (Lawrie and Rees 1996), especially when the objective is to
27 61 verify that a safety limit is not exceeded. As a consequence, dietary exposure should be
28 62 evaluated through a stepwise approach (WHO 1997).

29
30
31
32
33
34
35
36 63 The stepwise approach follows the premise of an assessment using the least refined method
37 64 (screening) towards the most refined one, if necessary. The refinement of data is judged
38 65 necessary when the dietary exposure assessed with a conservative method using highly
39 66 aggregated data (i.e. the chemical is assumed to be present in specific food groups
40 67 supposedly ingested by the whole population and there is no information about distribution of
41 68 the consumption) exceeds the safety limits of the chemical. Once safety limits are surpassed,
42 69 this indicates there is a possibility of safety concern and further investigation is needed by
43 70 using less aggregated data (e.g. food consumption collected at the individual level). Then, the
44 71 next step is performed using more detailed information on food consumption and/or
45 72 concentration data in order to determine the right hand extreme of the distribution of dietary
46 73 exposure. On the other hand, when the dietary exposure assessed using screening methods
47 74 is under the safety limits, further refinement of the assessment is not needed (Gibney and
48 75 Lambe 1996, Lawrie and Rees 1996, WHO 1997). In this way, wasting of resources by

49
50
51
52
53
54
55
56
57
58
59
60

* Joint Food and Agriculture Organization/World Health Organization

1
2
3 76 collecting a large amount of unneeded data is avoided. The most important characteristic of
4
5 77 screening methods is that conservative assumptions regarding food consumption and
6
7 78 concentration levels in food should be used in order to provide a good level of protection for
8
9 79 the whole population by intentionally overestimating chronic dietary exposure (FAO/WHO
10
11 80 2005).

12
13
14 81 The assessment is said to be refined when dietary exposure evaluations go beyond
15
16 82 conservative assumptions of screening methods. In a refined assessment, the purpose of the
17
18 83 evaluation often changes to provide an estimate of dietary exposure based on observed food
19
20 84 consumption patterns and/or measured chemical concentration data rather than assumed
21
22 85 values (EFSA 2006, FAO/WHO 2005). The refinement of dietary exposure to chemicals
23
24 86 should be designed in such a way that non-average individuals are considered in the
25
26 87 assessment, and in particular those who consume relatively large quantities of foods
27
28 88 containing higher concentrations of substances that may potentially lead to a health risk
29
30 89 (FAO/WHO 2005).

31
32 90 To consider the distribution of dietary exposure, it is important to collect food consumption
33
34 91 information from individuals rather than base the assessment on average population data.
35
36 92 Methods available to collect individual dietary data include food records, food frequency
37
38 93 questionnaires and 24-hour dietary recalls (Kroes et al. 2002). Monitoring surveys aim to
39
40 94 provide such type of information for nationally representative populations. However, dietary
41
42 95 assessment methods are not standardized across countries (Verger et al. 2002) and the level
43
44 96 of detail available in the data may differ considerably.

45
46 97 Furthermore, challenges may be encountered during refined dietary exposure assessment
47
48 98 using information at the individual level. One of these challenges is the presence of
49
50 99 uncertainties in the process of identifying and describing the consumption of foods. The non-
51
52 100 identification of potential consumers of interest may occur due to the lack of ability of dietary
53
54 101 methods, such as 24-hour dietary recalls, on capturing sufficient information for the
55
56 102 assessment of chemicals in the diet (EFSA 2006). Additionally, the ability of interviewees on
57
58 103 providing such information can be limited, resulting in misreporting or non-reporting of foods.
59
60

1
2
3 104 The 'European Food Consumption Validation' (EFCOVAL) project aims at validating a method
4
5 105 for future monitoring surveys on the dietary intake in European countries. For this purpose, a
6
7 106 duplicate 24-hour recall using EPIC-Soft software has been chosen. A secondary objective is
8
9 107 to adapt EPIC-Soft in such a way that food safety issues can be investigated. To explore this,
10
11 108 the flavouring substances category has been chosen.

12
13 109 In this paper, we report the results of an explorative study aimed at comparing methods used
14
15 110 to estimate the dietary exposure to flavourings in the context of a stepwise approach.
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

111 MATERIAL AND METHODS

112 Flavourings under investigation

113 Four flavourings were selected for the exercise of assessing dietary exposure to flavourings in
114 the diet: raspberry ketone, glycyrrhizinic acid (excluding ammonium glycyrrhizinate), coumarin
115 and caffeine. These flavourings represent different origins (naturally contained in food and/or
116 added flavouring) and different production volumes when used as added flavouring.

117 Raspberry Ketone (4-(4-Hydroxyphenyl)butan-2-one; Chemical Abstracts Service (CAS)
118 number 5471-51-2) is the primary aroma compound of raspberry and is also found naturally in
119 other berry fruits such as cranberry, blackberry, and loganberry (Borejsza-Wysocki 1994,
120 Gallois 1982). It is also used in flavour formulations of mixed berries and strawberries added
121 to processed foods such as yoghurt and beverages (Burdock 2005, Gerasimov 2001). The
122 safety limit for raspberry ketone is assumed to be $0.03 \text{ mg kg}^{-1} \text{ body weight (bw) day}^{-1}$,
123 considering the Threshold of Toxicological Concern (TTC) of $1800 \text{ } \mu\text{g person}^{-1} \text{ day}^{-1}$ for
124 flavourings classified in structural class I (Cramer et al. 1978) and assuming a 60 kg adult.
125 Structural class I suggests the lowest of three classes of toxicity of flavourings in their safety
126 evaluation procedure by JECFA and was assigned to raspberry ketone in 2001 (WHO 2001).

127 Glycyrrhizinic acid (CAS number 1405-86-3) is found in foods and beverages as a natural
128 constituent or as an added flavouring. Glycyrrhizinic acid is present in extracts of roots and
129 rhizomes of the Liquorice plant, *Glycyrrhiza glabra*. Liquorice confectionery and herbal teas
130 are the main sources of dietary exposure to this substance (Fenwick et al. 1990, Stormer et
131 al. 1993). Although an acceptable daily intake (ADI) is not determined, safety evaluations of
132 glycyrrhizinic acid performed by JECFA and the Scientific Committee on Food (SCF) have
133 suggested that a dietary exposure to 100 mg day^{-1} would be unlikely to cause adverse effects
134 in the majority of adults (SCF 1991, 2003, WHO 2006). A safety factor of 10 has been used
135 by Stormer et al. (1993) to establish a safety limit with the 100 mg day^{-1} figure. This safety
136 factor is used to account for inter-individual variability in susceptibility when toxicological
137 information is available for humans. Based on this reference, a safety limit of $0.16 \text{ mg kg}^{-1} \text{ bw}$
138 day^{-1} , considering a 60 kg bw was used in the present paper for the sole scope of this study.

1
2
3 139 Coumarin (1,2-benzopyrone; CAS number 91-64-5) is a naturally occurring flavouring present
4
5 140 in plants and spices. The main source of coumarin in the diet is cinnamon (Rychlik 2008)
6
7 141 although coumarin content can greatly differ between different types of cinnamon. Cassia
8
9 142 cinnamon can contain up to 3000 mg kg⁻¹ of coumarin whereas the most refined type of
10
11 143 cinnamon, the Ceylon cinnamon, contains only about 8 mg kg⁻¹ (BfR 2006). Other sources of
12
13 144 coumarin include bilberry, celery, and green tea (Felter et al. 2006). According to both EU and
14
15 145 USA legislation, coumarin can not be added as such to foodstuffs, whereas it may be present
16
17 146 in a foodstuff following the addition of cinnamon. For this reason, maximum permitted levels
18
19 147 of coumarin in foodstuffs have been set (European Commission 2008). Furthermore, EFSA
20
21 148 suggests a Tolerable Daily Intake (TDI) of 0.1 mg kg⁻¹ bw (EFSA 2004a, EFSA 2008a).

22
23
24 149 Caffeine (1,3,7-trimethylxanthine; CAS number 58-08-2) may be naturally present in foods or
25
26 150 added to them. Beverages and foods containing caffeine include coffee, tea, guarana, cola
27
28 151 nuts, cocoa, chocolate, energy drinks, and some plants (e.g. mate) (Gilbert 1984). In addition,
29
30 152 caffeine may be added to a variety of both prescription and over-the-counter drugs, which
31
32 153 were not part of the present assessment. An officially established TDI or ADI for caffeine does
33
34 154 not exist. A review published by Nawrot and colleagues (2003), concluded that for the healthy
35
36 155 adult population, moderate daily caffeine intake at a dose level up to 400 mg day⁻¹ was not
37
38 156 associated with adverse effects. Thus, for the sole scope of this study, the safety limit to
39
40 157 caffeine was estimated to be 6.7 mg kg⁻¹ bw when using an individual bw of 60 kg.

41 42 43 158 **Food consumption data used for the refined assessment of dietary exposure**

44
45
46 159 Food consumption data used in the refined dietary exposure assessment was collected in the
47
48 160 Dutch sample of the EFCOVAL validation study. Between May and July 2007, trained
49
50 161 dieticians carried out interviews using a standardized 24-hour dietary recall method (EPIC-
51
52 162 Soft software) on two non-consecutive days. The two 24-hour dietary recalls were collected
53
54 163 with at least one month in-between, taking into account weekday variations. The sample
55
56 164 consisted of a total of 121 healthy Dutch adults (62 women and 59 men), aged between 45
57
58 165 and 65 years old and with all educational levels being represented. However, the participants
59
60 166 in the EFCOVAL validation study could not be considered a representative sample of the
167
167 general population in these strata. The study protocol was approved by the Wageningen

1
2
3 168 University Ethical Committee and informed consent was obtained from all study participants.
4
5

6 169 **EPIC-Soft software**
7
8

9
10 170 EPIC-Soft is a software program that has been developed in the EPIC study (European
11
12 171 Prospective Investigation into Cancer and Nutrition) to ensure the highest possible level of
13
14 172 standardization of 24-hour dietary recalls. The structure and standardization procedure of
15
16 173 EPIC-Soft are described in detail elsewhere (Slimani et al. 1999, Slimani et al. 2000). An
17
18 174 important feature of EPIC-Soft is the use of two complementary food description systems:
19
20 175 *explicit* and *implicit*. In the explicit description, facets and descriptors are used during the
21
22 176 process of food identification, which is based on the Languag coding system initially used to
23
24 177 describe technological and toxicological food characteristics (FDA/CFSSAN 1993). Facets are
25
26 178 used to describe foods in more detail and this is done by means of standardized questions
27
28 179 asked to the interviewee each time a food is reported. One of the facets available for a
29
30 180 number of food categories is 'flavour or added component'. The descriptors, which are the
31
32 181 country-specific terms associated with each facet, are used as pre-defined potential answers
33
34 182 built in the database of the software (e.g. strawberry flavour or strawberry added pieces). In
35
36 183 addition, the descriptor 'unknown' may be used when the interviewees are not able to provide
37
38 184 the expected level of detail (e.g. unknown flavour for a yogurt that has been consumed). In
39
40 185 the implicit description, the name of a food provides sufficient information to identify the food
41
42 186 and no further detail is collected using the facet/descriptor system. For instance, the food
43
44 187 name "liquorice drops" implies the presence of liquorice so that there is no need to use the
45
46 188 facet "flavour" to indicate such presence.
47

48 189 A pre-existing list of facets and descriptors was available in the Dutch software's database
49
50 190 since EPIC-Soft has been used in the Dutch National Food Consumption Survey (Ocké et al.
51
52 191 2005). However, this list was not aimed at the assessment of dietary exposure to flavourings.
53
54 192 Therefore, within the EFCOVAL study adjustments were made in the list of descriptors and
55
56 193 facets for the identification of foods containing raspberry flavouring. The facet 'flavour' was
57
58 194 assigned to new food groups where raspberry may be present, and fourteen new descriptors
59
60 195 were included: raspberry, blackberry, blueberry, cranberry, strawberry, cloudberry,
196 loganberry, thimbleberry, bilberry, blackberry, mulberry, berries non-specified (n.s.), red fruits

1
2
3 197 n.s., forest fruit. No further adaptations were made in the descriptors of glycyrrhizinic acid (i.e.
4
5 198 liquorice), caffeine (i.e. coffee) and coumarin (i.e. cinnamon) flavourings.
6
7

8
9 **199 Dietary exposure assessment**

10
11 200 With the use of the stepwise approach, dietary exposure to the four flavourings was assessed
12
13 201 in three different steps (Table 1).
14

15
16
17 202 Step 1 – Use of screening methods: Maximized Survey-Derived Daily Intake (MSDI) and
18
19 203 Single-Portion Exposure Technique (SPET) as used by JECFA and modified Theoretical
20
21 204 Added Maximum Daily Intake (mTAMDI) used by EFSA.
22

23
24 205 *MSDI* is also known as the 'per capita method' or 'per capita x 10' approach. Assumptions of
25
26 206 the method are: that 60% of total production of flavourings is reported by the industry; that
27
28 207 10% of the total population are consumers of the flavouring; and that there is no variation in
29
30 208 the intake of the particular flavouring among consumers. Accordingly, the following formula is
31
32 209 used:
33

34
35 210
$$\text{Intake} = \frac{\text{annual production of the flavouring, kg} \times 10^9 \mu\text{g kg}^{-1}}{\text{population of consumers} \times 365 \text{ days}}$$

36 211
37 212

38 213 Final figures were converted into $\text{mg kg}^{-1} \text{ bw day}^{-1}$
39

40 214 As for the safety evaluations performed by JECFA, EU population in this study was assumed
41
42 215 to be 32×10^6 . The annual production volumes of the flavourings considered were those used
43
44 216 by JECFA (WHO 2001) and SCF (SCF 2003): 19.500 kg y^{-1} for raspberry ketone and 1.956
45
46 217 kg y^{-1} for glycyrrhizinic acid, respectively. Poundage data for coumarin is not available since it
47
48 218 can not be used as an added flavouring substance. In the absence of EU production volumes
49
50 219 for caffeine, per capita dietary exposure in the USA was used as a proxy for per capita dietary
51
52 220 exposure in the EU.
53

54
55 221 The *SPET* method provides a dietary exposure assessment based on normal use levels and
56
57 222 identifies the single food category containing the flavouring agent of interest that is likely to
58
59 223 contribute to the highest dietary exposure from one 'standard portion'. The standard portion is
60
224 taken to represent the mean food consumption amount within one eating event for consumers

of that food category, assuming daily consumption of one portion over a long period. These standard portions can be found in the 67th and 69th report of JECFA (WHO 2007, WHO 2009). Thus, the general formula to derive the SPET figure is:

$$\text{Intake (mg kg}^{-1}\text{)} = \text{Maximum (standard portion size (mg) x normal use level of the flavouring (mg/kg))}$$

The industry normal use levels used in this study were the ones reported in the Fenaroli's handbook (Burdock 2005), except for glycyrrhizinic acid, for which only upper use levels are reported by industry (EFFA 2003). For coumarin, the maximum permitted level in foods containing cinnamon (European Commission 2008) was used as replacement of the absent upper use level.

mTAMDI is calculated on the basis of standard portions and normal use levels for flavourable beverages and foods in general, i.e. foods and beverages that may contain the flavouring substance, and for five particular foods groups (exceptions a to e). For instance, exception_a used in this calculation refers to candies and confectioneries (EFSA 2004b). The use levels considered for SPET calculations were also applied to calculate *mTAMDI*. The general formula used to estimate the *mTAMDI* (EFSA 2004b) is:

$$\begin{aligned} \text{Intake (mg kg}^{-1}\text{)} = & \\ & (\text{normal use levels in beverages} \times 324) + (\text{normal use levels in foods} \times 133) + (\text{normal use} \\ & \text{levels in exception}_a \times 27) + (\text{normal use levels in exception}_b \times 20) + (\text{normal use levels in} \\ & \text{exception}_c \times 20) + (\text{normal use levels in exception}_d \times 20) + (\text{normal use levels in exception}_e \times \\ & 2) \\ & \text{normal use levels in mg kg}^{-1} \end{aligned}$$

248 *Screening assessment of Coumarin*

Because literature has shown that observed levels of coumarin in food products containing cinnamon can be in fact higher than the maximum permitted level (BfR 2006), further screening calculations were made to assess the dietary exposure to coumarin by considering the observed coumarin content in cinnamon products. Therefore, extra calculations of SPET and *mTAMDI* were done with use levels of cinnamon as reported by the Flavour and Extract Manufacturers' Association - FEMA (Burdock 2005) and assuming a constant of coumarin

1
2
3 255 amounts in two types of cinnamon (cassia cinnamon: 0.3%; Ceylon cinnamon: 0.008% (BfR
4
5 256 2006)).
6
7

8
9 257 Step 2 – Use of food consumption data aggregated in food groups.
10

11 258 At this step, food consumption data at the individual level (Dutch EFCOVAL sample) were
12
13 259 grouped in food categories based on the EPIC-Soft grouping system (Slimani, et al. 1999). It
14
15 260 was assumed that all foods within a given ‘flavourable food category’ contained the flavouring
16
17 261 of interest (see Appendix 1). For instance, raspberry ketone may be added to some foods in
18
19 262 the dairy food group (e.g. yogurts). Thus, in the assessment of step 2, all foods belonging to
20
21 263 the yogurt category, a subgroup category of dairy products, were assumed to contain
22
23 264 raspberry ketone, even though some foods are known not to contain it.
24
25

26 265 Concentration levels used in step 2 were called ‘refined concentrations’ (see Appendix 2).
27
28 266 First choice for the concentration data was normal use levels reported by industry. An
29
30 267 exception was made for caffeine contents in non-alcoholic beverages since reported industry
31
32 268 levels (0.13 mg/kg) were clearly underestimated as compared to the analytical determinations
33
34 269 gathered in the literature (see Appendix 2). For glycyrrhizinic acid, upper use levels were
35
36 270 used. Analytical determinations from literature were also used in the cases where the
37
38 271 flavouring was known to occur in its natural form or when levels of added flavourings were not
39
40 272 reported by industry. For instance, glycyrrhizinic acid is known to be added to soy sauce, but
41
42 273 use levels in sauces have not been reported. List of references used to collect the flavouring
43
44 274 concentration data in foods can be provided upon request.
45
46

47 275 Step 3 - Use of food consumption data at the level of foods items.
48
49

50
51 276 Within this step, three models were created based on the consumption of food items from the
52
53 277 Dutch EFCOVAL sample. The first two models (3a and 3b in Table 1) considered the
54
55 278 consumption of foods that, according to the name of the product or to the use of facets and
56
57 279 descriptors available in EPIC-Soft, do contain the flavouring. The difference between the two
58
59 280 steps was that step 3b included information from flavourings after the descriptors of the facet
60
281 flavour had been extended (for the assessment of raspberry ketone only) in the EFCOVAL

1
2
3 282 study, while step 3a gave information that would have been available before the extension. In
4
5 283 the last of the three models (3c), foods were identified in the same way as for step 3a (for
6
7 284 glycyrrhizinic acid, caffeine and coumarin) and 3b (for raspberry ketone), but the descriptor
8
9 285 'unknown flavour' was assumed to include the flavouring of interest.

10
11
12 286 Concentration levels used in step 3 were the same as used in step 2: 'refined concentration'.
13
14

15 287 **Data analysis**

16
17
18 288 To estimate dietary exposure to the flavourings, food consumption was multiplied by the
19
20 289 concentration of the chemical in the food and then divided by the body weight to be
21
22 290 expressed in $\text{mg kg}^{-1} \text{bw day}^{-1}$. In step 1, a body weight of 60 kilos was assumed whereas for
23
24 291 steps 2 and 3, individually measured body weights were used. Food consumption data in
25
26 292 steps 2 and 3 were based, for each individual, on the average of the two 24-hour dietary
27
28 293 recalls. In these two steps, potential dietary exposure to the flavourings was estimated for
29
30 294 each subject. Besides the mean and the median intake of the total group, the 95th percentile
31
32 295 of the population distribution was used to characterize highly exposed subjects. As stated by
33
34 296 EFSA (2008b), the 95th percentile can be assessed with approximately 130 subjects when
35
36 297 using a binominal distribution (Conover 1971). Furthermore, the average contribution of the
37
38 298 different food groups to the overall dietary exposure in steps 2 and 3 was estimated in
39
40 299 percentages. Data processing and descriptive statistical analyses were performed using SAS
41
42 300 software (version 9.1; SAS Institute, Cary NC, USA).
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

301 **RESULTS**

302 All three screening methods (Step 1) provided exposure estimates at or over the safety limit
303 for raspberry ketone and, therefore, further refinement of the dietary exposure assessment
304 was needed for this flavouring (Table 2). For glycyrrhizinic acid, the MSDI method indicated a
305 dietary exposure at least 16 times lower than the safety limit, whereas the two other methods
306 (SPET and mTAMDI) provided estimates above the safety limit, indicating the need of
307 refinement. Caffeine presented an estimate above the safety limit based on the MSDI
308 method. Additional refinement of coumarin dietary exposure was not necessary based on the
309 screening methods. However, dietary exposure assessment of coumarin using models of step
310 2 and 3 was carried out given that the four selected flavourings were meant to be examples
311 for practical testing of dietary exposure assessment through the use of EPIC-Soft.

312 Descriptive analyses of dietary exposure assessment using highly aggregated consumption
313 data at the food group level are presented in Table 3 (step 2). Average and high (95th
314 percentile) levels of exposure to raspberry ketone, glycyrrhizinic acid and caffeine were above
315 the safety limit, indicating the need for more detailed assessment of these three flavourings.
316 Average dietary exposure to coumarin was below the safety limit, despite of the conservative
317 model on food consumption used in Step 2, but above the safety limit at the 95th percentile.
318 Therefore, additional investigation of dietary exposure to coumarin was necessary within the
319 stepwise approach.

320 Table 3 also presents results of the dietary exposure done at the food item level (step 3).
321 When identifying foods by the name of the product and without using the extended facets and
322 descriptors (step 3a), the mean dietary exposure was under the safety limits, except for
323 caffeine (\cong eight times higher). At the 95th percentile, the dietary exposure to glycyrrhizinic
324 acid and caffeine were three and twenty times higher than the safety limit, respectively. In the
325 case of raspberry ketone, if considering the adjustments made in the database for facets and
326 descriptors (step 3b), dietary exposure was higher than values obtained in step 3a. In the next
327 step (3c), when not only foods that surely contained the flavouring substance were included in
328 the model but also those for which the flavour was not specified (use of descriptor 'unknown'),

1
2
3 329 mean dietary exposure to raspberry ketone and glycyrrhizinic acid was slightly higher as
4
5 330 compared to step 3a and 3b. In the case of coumarin and caffeine, dietary exposure was the
6
7 331 same in all of these steps.
8
9

10 332 By comparing tables 2 and 3 it appears that in some cases the screening techniques lead to a
11
12 333 dietary exposure lower than that of the refined exposure assessment. It was the case for
13
14 334 raspberry ketone where SPET was 0.03 mg/kg versus 0.04 and 0.05 mg/kg at the 95th
15
16 335 percentile at step 3b and 3c, respectively.
17
18

19 336 In the investigation of food groups contributing to the exposure in each step of the
20
21 337 assessment (Figure 1), it can be seen that for raspberry ketone the main sources of the
22
23 338 flavourings were the same in almost all steps: 'dairy products' and 'non-alcoholic beverages'.
24
25 339 Yet, while 'non-alcoholic beverages' and 'cakes' were the food groups most contributing to the
26
27 340 dietary exposure of raspberry flavouring in step 3a, dietary exposure to raspberry contained in
28
29 341 'dairy products' became an important source with the use of facets and descriptors in step 3b.
30
31 342 In the case of glycyrrhizinic acid, 'sugar and confectionery' and 'non-alcoholic beverages'
32
33 343 (most herbal teas) were the bigger contributors of the substance in all steps, with a probable
34
35 344 overestimation of the contribution from 'sugar and confectionery' in step 2 (group level) as
36
37 345 compared to the other steps. The same pattern of overestimation at food group level is seen
38
39 346 in the assessment of coumarin and caffeine (figures not shown). Main contributors to dietary
40
41 347 exposure were 'cakes', 'biscuits' and 'tea' for coumarin and 'non-alcoholic beverages' for
42
43 348 caffeine at all steps.
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 349 **DISCUSSION**
4
5

6 350 The dietary exposure to raspberry ketone, glycyrrhizinic acid, coumarin and caffeine was
7
8 351 estimated in this study using a stepwise approach. It has been shown that the refinement of
9
10 352 food consumption data in the assessment of dietary exposure to flavourings might be
11
12 353 necessary, but dependent of the chosen screening method for the assessment. When using
13
14 354 data from the 24h-dietary recall by means of EPIC-Soft software, the dietary exposure to
15
16 355 raspberry ketone was higher in the model where descriptors have been extended as
17
18 356 compared to the model where no adjustments have been considered.

19
20
21 357 The dietary exposure calculated using the screening methods exceeded the safety limits and
22
23 358 therefore implied the need of more refined assessment for raspberry ketone, glycyrrhizinic
24
25 359 acid and caffeine, but with somewhat different results depending on the method used and on
26
27 360 the flavouring under assessment. In particular, variation in outcomes using different screening
28
29 361 methods was observed; whereas by the MSDI method the exposure to glycyrrhizinic acid was
30
31 362 evaluated to be of no safety concern, the dietary exposure assessed by SPET and mTAMDI
32
33 363 indicated the need of further refinement. On the other hand, dietary exposure to caffeine
34
35 364 assessed by MSDI indicated the need of refined assessment while the other two methods did
36
37 365 not indicate it. One of the reasons for the variation in results from the screening methods is
38
39 366 probably the difference in assumptions between them (e.g. the percentage of consumers in
40
41 367 the dietary exposure and how conservative they are, i.e. whether individuals, who consume
42
43 368 large quantities of flavoured foods, are considered in the dietary exposure assessed by the
44
45 369 different methods). Although it is beyond the scope of this study to investigate the accuracy of
46
47 370 such estimates, this topic deserves further attention. In fact, many of the conservative
48
49 371 assumptions and default values that are currently used in screening assessments were
50
51 372 established some time ago and in some cases they were originally based on subjective or
52
53 373 arbitrary estimates (EFSA 2006). In the case of the MSDI, which until recently was the unique
54
55 374 method used by JECFA to assess dietary exposure within the safety evaluation of flavourings,
56
57 375 the insufficient conservativeness of the method has been discussed in a number of scientific
58
59 376 publications (Arcella and Leclercq 2005, Hall and Ford 1999, Lambe et al. 2002, Leclercq
60
377 2007, Munro and Danielewska-Nikiel 2006). Most recently, JECFA has acknowledged the

1
2
3 378 likely underestimation of the MSDI method in the assessment of some flavourings and
4
5 379 developed a new method (SPET), which takes into account different food patterns of
6
7 380 consumers and the uneven distribution of dietary exposure in consumers of flavourings (WHO
8
9 381 2007). Furthermore, according to EFSA, the appropriateness of the conservative assumptions
10
11 382 and default values that are used in screening assessments of chemicals, including flavourings
12
13 383 may require further investigation. Analysis of uncertainty in the screening assessment may
14
15 384 not be required, provided they include proper conservative assumptions to take account of
16
17 385 uncertainty (EFSA 2006).

18
19
20 386 Once the need of further refinement in the dietary exposure is identified, other limitations
21
22 387 might be encountered in the assessment of exposure to chemicals in the diet. For instance,
23
24 388 the knowledge of chemical concentration data in foods is limited and the ability of dietary
25
26 389 methods to assess dietary exposure to chemicals can be uncertain. In our study, this last
27
28 390 issue has been explored through the different models created to assess the dietary exposure
29
30 391 to flavourings in the Dutch population.

31
32
33 392 In the first model created (step 2), the dietary exposure was characterized by investigating the
34
35 393 consumption of flavourings at the food group level. As noted in Table 3, the dietary exposures
36
37 394 of the four flavourings were high as compared to all other steps of the assessment.
38
39 395 Considering that in this model, foods that do not contain the flavouring may have been
40
41 396 quantified as part of the dietary exposure, we recognize a certain degree of overestimation in
42
43 397 the estimate. This should be, however, an indication of safe dietary exposure, in case the
44
45 398 estimate would be below the safety limit. However, the need for further refinement of the food
46
47 399 consumption data collected at the individual level appeared necessary for the four flavourings
48
49 400 under assessment.

50
51
52 401 With the data on food items collected with the 24-hour dietary recall, it has been noted that
53
54 402 the adjustments made in the software databases for the raspberry ketone case, resulted in a
55
56 403 higher dietary exposure to this flavouring. The number of consumers in step 3b, where the
57
58 404 new raspberry ketone descriptors have been included, was eight times higher as compared to
59
60 405 the step with no modifications in EPIC-Soft (data not shown). This is the result of food
406 406 consumption data collected at lower aggregation level and with more details. Assuming that

1
2
3 407 the 24-hour dietary recall provided an accurate estimate of the intake of flavoured foods, the
4
5 408 high dietary exposure to raspberry ketone in step 3b suggests that such adjustments, which
6
7 409 characterize the consumption of foods in more detail, is useful when assessing dietary
8
9 410 exposure to flavourings. Nonetheless, 24-hour dietary recalls are known to underestimate the
10
11 411 dietary intake of some individuals (Bingham 1987, Willett 1998) and because of the lack of
12
13 412 proper validated biomarkers for these flavourings, the accuracy of the estimate of dietary
14
15 413 exposure to flavourings, as assessed, cannot be ensured without further research.

16
17
18 414 No alterations in the Dutch EPIC-Soft version were implemented for glycyrrhizinic acid,
19
20 415 coumarin and caffeine, and evaluation of such alterations was therefore not possible.
21
22 416 However, for some types of flavourings, such as glycyrrhizinic acid, the use of facets and
23
24 417 descriptors might not be that important for an accurate dietary exposure assessment given
25
26 418 that the food name itself often indicates the presence of the flavouring, which would be
27
28 419 enough for the food identification. Nevertheless, additional exploration is needed for this
29
30 420 conclusion. Moreover, we do not know to what extent the consumption of cinnamon was
31
32 421 correctly identified. First, the use of spices, including cinnamon, during home cooking is not
33
34 422 collected during the 24-hour dietary recall using Epic-Soft software. Second, this spice, in
35
36 423 particular, may not be easily identified by the name of the product and neither by the use of
37
38 424 descriptors since it does not seem to be clear to the population whether a certain food would
39
40 425 contain cinnamon or not. The dieticians of our study reported that for the food group most
41
42 426 expected to contain cinnamon (cereals and biscuits), subjects were not able to provide this
43
44 427 kind of detail and that they, as interviewers, had no experience in collecting information about
45
46 428 flavourings. Third, the authors of this study may have been not correctly identified the
47
48 429 presence of cinnamon in certain culinary products such as soups since the presence of
49
50 430 cinnamon is not always evident. Because of these reasons, the dietary exposure to coumarin
51
52 431 may have been underestimated in this assessment. As a check whether the descriptors of the
53
54 432 four flavourings may have been sufficiently identified, the potential flavoured foods with
55
56 433 descriptor 'non-specified' were assumed to include the flavouring of interest (Step 3c). The
57
58 434 dietary exposure did not considerably change for any of the four flavourings in this step.

59
60 435 The assessment of dietary exposure by the different steps and their food group sources gives

1
2
3 436 an indication that such changes in the database (in facets and descriptors) may be food group
4
5 437 dependent. Most probable, some degree of uncertainty was present in the assessment at
6
7 438 food group level, which tends to overestimate the dietary exposure to flavourings. When the
8
9 439 need of more detailed dietary exposure assessment to a specific flavour is identified, the
10
11 440 more detailed approach could be limited to a number of food groups, for which it is known that
12
13 441 the flavouring can be present and descriptors should be added.

14
15
16 442 It is important to mention that the estimates presented in steps 2 and 3 of the assessment are
17
18 443 not representative of the usual Dutch food consumption. Because of the lack of
19
20 444 representativeness of the sample and the limited number of survey days, chronic dietary
21
22 445 exposure may not have been correctly estimated in this assessment. In fact, the collection of
23
24 446 only two days of 24-h recalls does not allow to assess chronic exposure but short term
25
26 447 exposure. This is probably the reason why the refined exposure assessment performed for
27
28 448 raspberry ketone leads to higher values than that obtained with the SPET technique. An
29
30 449 improved refined assessment could be performed by using additional information on usual
31
32 450 intake of flavoured foods, such as a food propensity questionnaire. Subar and colleagues
33
34 451 (2006) have shown that food propensity questionnaires may offer important covariate
35
36 452 information in supplementing 24-h recalls for estimating usual intake of food groups. This is
37
38 453 possibly true for assessing chemicals in the diet as well. Furthermore, only dietary exposure
39
40 454 has been considered in our assessment and contribution from other sources (e.g. medicines)
41
42 455 may lead to an additional exposure. In fact, the safety limits we have used in this
43
44 456 assessment should refer to the total exposure to the flavourings but with the study we
45
46 457 performed we can only conclude on exposure from the diet. In addition, the small number of
47
48 458 evaluated flavourings limits the possibilities to extrapolate the results of our study to other
49
50 459 types of flavourings. Another limitation is the scarce availability of concentration data on
51
52 460 chemicals. These are relatively seldom published in open literature and therefore difficult to
53
54 461 retrieve(EFSA 2005). In the flavouring case, few analytical data are currently available and
55
56 462 little is know about the influence of storage and processing on the residues of theses
57
58 463 substances in food (EFSA 2006). Consequently, a high variability in the available
59
60 464 concentration data is expected.. This study, however, was a first exploration of the
465 possibilities to assess dietary exposure to food chemicals by using data collected at the

1
2
3 466 individual level with the standardized 24-h recall.
4
5

6 467 In summary, this study showed that the collection of detailed food consumption data at the
7
8 468 individual level is useful and should be further explored for other flavourings. In addition, the
9
10 469 possibility of further adaptations of the databases used in EPIC-Soft software seemed to
11
12 470 provide a higher dietary exposure to raspberry ketone as compared to the non-modified
13
14 471 databases, which may also be true for other flavourings. Yet, the need for alterations may still
15
16 472 differ depending on the nature of the flavouring under assessment. To further study the
17
18 473 usefulness of detailed food consumption data in the dietary exposure assessment of
19
20 474 flavourings and other chemicals, research should include biological markers and analytical
21
22 475 determination in flavoured foods, which would warrant the check of accuracy of such
23
24 476 estimates. Finally, the benefit of assessing usual intake of chemicals in the diet by combining
25
26 477 24-h recalls and food propensity questionnaires is a topic that deserves more exploration.
27

28
29 478
30

31
32 479
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

480 **ACKNOWLEDGMENTS**

481 The Community funding under the Sixth Framework Program for the EFCOVAL project is
482 acknowledged (FOOD-CT-2006-022895). Authors wish to thank Davide Arcella (EFSA) and
483 EFCOVAL partners for their useful advices.

484 This document reflects only the author's views and the Community is not liable for any use
485 that may be made of the information contained therein.

486 **EFCOVAL partners**

487	Belgium	Ghent University (DPH)
488	Croatia	Academy of Medical Sciences (AMZH)
489	Czech Republic	National Institute of Public Health (NIPH)
490	Denmark	National Institute, Technical University of Denmark (DTU)
491	France	French Food Safety Authority (AFSSA)
492		International Agency for Research on Cancer (IARC)
493		National Institute for Agricultural Research (INRA)
494	Germany	German Institute of Human Nutrition (DIfE)
495	Italy	National Research Institute for Food and Nutrition (INRAN)
496	Netherlands	Wageningen University (WU)
497		National Institute for Public Health and the Environment (RIVM)
498	Norway	University of Oslo
499	Spain	Basque Foundation for Health Innovation and Rsearch (BIOEF)
500	United Kingdom	Prima informatics limited (Primainfo)

501

502

503

504

505

506 **REFERENCES**

- 507 Arcella D and Leclercq C. 2005. Assessment of dietary intake of flavouring substances within
508 the procedure for their safety evaluation: advantages and limitations of estimates obtained by
509 means of a per capita method. *Food Chem Toxicol.* 43(1):105-116.
- 510 BfR. 16 June 2006. Consumers, who eat a lot of cinnamon, currently have an overly high
511 exposure to coumarin. Federal Institute for Risk Assessment. BfR Health Assessment No.
512 043/2006. Berlin (Germany).
- 513 Bingham S. 1987. The dietary assessment of individuals; methods, accuracy, new techniques
514 and recommendations. *Nutrition Abstracts and Reviews.* 9(1).
- 515 Borejsza-Wysocki W. 1994. Biosynthesis of p-hydroxyphenylbutan-2-one in raspberry fruits
516 and tissue cultures. *Phytochemistry (Oxford).* 35(3):623-628.
- 517 Burdock GA. 2005. Fenaroli's handbook of flavor ingredients. 5th ed. Florida (US): CRC
518 Press.
- 519 Conover WJ. 1971. *Practical Nonparametric Statistics.* New York: Wiley.
- 520 Cramer GA, Ford RA and Hall RL. 1978. Estimation of toxic hazard—a decision tree
521 approach. *Food Cosmet Toxicol.* 16(3):255–276.
- 522 EFFA. 2003. European Flavour and Fragrance Association (EFFA). Submission to the
523 European Commission, February 20th, 2003 [document number unknown].
- 524 EFSA. 2004a. Opinion of the Scientific Panel on food additives, flavourings, processing aids
525 and materials in contact with food (AFC) related to Coumarin. European Food Safety
526 Authority. EFSA-Q-2003-118. *The EFSA Journal.* 104:1-36.
- 527 EFSA. 2004b. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing
528 Aids and Materials in contact with Food (AFC) on a request from the Commission related to
529 Flavouring Group Evaluation 3 (FGE.03): Acetals of branched- and straight-chain aliphatic
530 saturated primary alcohols and branched- and straight-chain saturated aldehydes, and an
531 orthoester of formic acid, from chemical groups 1 and 2. Question number EFSA-Q-2003-146.
532 *The EFSA Journal.* 107:1-59.
- 533 EFSA. 2005. Opinion of the Scientific Committee on a request from EFSA related to Exposure
534 Assessments. European Food Safety Authority. EFSA-Q-2003-107. *The EFSA Journal.*
535 249:1-26.
- 536 EFSA. 2006. Guidance of the Scientific Committee on a request from EFSA related to

- 1
2
3 537 Uncertainties in Dietary Exposure Assessment. European Food Safety Authority. EFSA-Q-
4 538 2004-019. The EFSA Journal. 438:54.
5
6
7 539 EFSA. 2008a. Coumarin in flavourings and other food ingredients with flavouring properties
8 Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials
9 540 in Contact with Food (AFC)(Question No EFSA-Q-2008-677) Adopted on 8 July 2008. The
10 541 EFSA Journal. 793:1-15.
11
12
13
14 543 EFSA. 2008b. Guidance document for the use of the Concise European Food Consumption
15 544 Database in Exposure Assessment, Parma, EFSA/DATEX/2008/01. The EFSA Journal:1-8.
16
17
18 545 EFSA. 2009. Flavouring Group Evaluation 18, Revision 1 (FGE. 18 Rev1):Aliphatic, alicyclic
19 546 and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their
20 547 esters from chemical groups 6 and 8. European Food Safety Authority. EFSA-Q-2003-150B.
21 548 The EFSA Journal. 978:1-85.
22
23
24
25 549 European Commission. 1999. Commission Decision 1999/217/EC of 23 February 1999
26 550 adopting a register of flavourings substances used in or on foodstuffs drawn up in application
27 551 of Regulation (EC) No 2232/96 of European Parliament and of the Council of 28 October
28 552 1996. In: Off J Eur Comm. p. 1-137.
29
30
31
32 553 European Commission. 2002. Commission Decision 2002/113/EC of 23 January 2002
33 554 amending Commission Decision 1999/217/EC as regards the register of flavouring
34 555 substances used in or on foodstuffs. In: Off J Eur Comm. p. 1-160.
35
36
37
38 556 European Commission. 2004. Commission Decision of 7 April 2004 amending Decision
39 557 1999/217/EC as regards the register of flavouring substances. In: Off J Eur Comm. p. 28-36.
40
41
42 558 European Commission. 2005. Commission Decision of 18 May 2005 amending Decision
43 559 1999/217/EC as regards the register of flavouring substances used in or on foodstuffs. In: Off
44 560 J Eur Comm. p. 73-76.
45
46
47
48 561 European Commission. 2008. Regulation (EC) No 1334/2008 of the European Parliament and
49 562 of the council of 16 December 2008 on flavourings and certain food ingredients with
50 563 flavouring properties for use in and on foods and amending Council Regulation (EEC) No
51 564 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. In:
52 565 Off J Eur Comm. p. 34-50.
53
54
55
56 566 Fabech B, Bryhni K, Forshell LP, Georgsson F, Gry J, Hansen BT, Hallström H, Hatakka M,
57 567 Holene E, Kapperud G, Kristinsson J, Maijala R, Nielsen NL, Nordström U, Schultz AC,
58 568 Solheim C and Thorkelsson Æ. Date. A practical approach to the application of the risk
59 569 analysis process - Illustrated with two examples Caffeine and Campylobacter [internet]. cited
60 570 2009 Sep 15. Journal:[place unknown].Available from:

- 1
2
3 571 http://www.norden.org/da/publikationer/publikationer/2002-510/at_download/publicationfile
4
5
6 572 FAO/WHO. 2005. Consultations and workshops : dietary exposure assessment of chemicals
7 573 in food : report of a joint FAO/WHO consultation, 2-6 May 2005. Annapolis, Maryland (USA):
8 574 FAO/WHO.
- 10
11 575 FDA/CFSSAN. 1993. LanguaL User's Manual. version 1993.
- 13
14 576 Felter SP, Vassalo JD, Carlton BD and Daston GP. 2006. A safety assessment of coumarin
15 577 taking into account species-specificity of toxicokinetics. Food Chem Toxicol. 44(4):462-475.
- 17
18 578 Fenwick GR, Lutomski J and Nieman C. 1990. Liquorice, Glycyrrhiza glabra L. - composition,
19 579 uses and analysis. Food Chem. 38(2):119-143.
- 21
22 580 Gallois A. 1982. Rapid determination of p-hydroxyphenyl-1-butanone-3 in raspberries by TLC.
23 581 Sciences des Aliments. 2(1):99-106.
- 25
26 582 Gerasimov AV. 2001. Application of planar chromatography with computer processing of
27 583 chromatograms to the analysis of flavoring materials, using the determination of 4-(n-
28 584 hydroxyphenyl)-2-butanone (raspberry ketone) as an example. Zhurnal Analiticheskoi Khimii.
29 585 56(4):419-424.
- 31
32 586 Gibney MJ and Lambe J. 1996. Estimation of food additive intake: methodology overview.
33 587 Food Addit Contam. 13(4):405-410.
- 35
36 588 Gilbert RM. 1984. Caffeine consumption. In: The Methylxanthine beverages and foods:
37 589 Chemistry, consumption, and health effects Berlin (GE): Spring Verlag. p. 185-213.
- 39
40 590 Hall RL and Ford RA. 1999. Comparison of two methods to assess the intake of flavouring
41 591 substances. Food additives and contaminants. 16(11):481-495.
- 43
44 592 Kroes R, Muller D, Lambe J, Lowik MR, van Klaveren J, Kleiner J, Massey R, Mayer S, Urieta
45 593 I, Verger P and Visconti A. 2002. Assessment of intake from the diet. Food Chem Toxicol.
46 594 40(2-3):327-385.
- 48
49 595 Lambe J, Cadby P and Gibney M. 2002. Comparison of stochastic modelling of the intakes of
50 596 intentionally added flavouring substances with theoretical added maximum daily intakes
51 597 (TAMDI) and maximized survey-derived daily intakes (MSDI). Food Addit Contam. 19(1):2-14.
- 53
54 598 Lawrie CA and Rees NM. 1996. The approach adopted in the UK for the estimation of the
55 599 intake of food additives. Food Addit Contam. 13(4):411-416.
- 57
58 600 Leclercq C. 2007. Issues arising when methods used to assess dietary exposure to flavouring
59 601 substances are compared. Food Chem Toxicol. 45(11):2336-2337.

- 1
2
3 602 Munro IC and Danielewska-Nikiel B. 2006. Comparison of estimated daily intakes of
4 603 flavouring substances with no-observed-effect levels. *Food Chem Toxicol.* 44(6):758-809.
5
6
7 604 Nawrot P, Jordan S, Eastwood J, Rotstein J, Hugenholtz A and Feeley M. 2003. Effects of
8 605 caffeine on human health. *Food Addit Contam.* 20(1):1-30.
9
10
11 606 Ocké M, Hulshof K and van Rossum C. 2005. The Dutch national food consumption survey
12 607 2003. Methodological issues. *Archives of Public Health.* 63:227-241.
13
14
15 608 Rychlik M. 2008. Quantification of Free Coumarin and Its Liberation from Glucosylated
16 609 Precursors by Stable Isotope Dilution Assays Based on Liquid Chromatography-Tandem
17 610 Mass Spectrometric Detection. *Journal of Agricultural and Food Chemistry.* 56(3):796-801.
18
19
20
21 611 SCF. 1991. Scientific Committee for Food. Report (29th series). Commission of the European
22 612 Communities, Food Science and Techniques. SCF/CS/ADD/FLAV/61final. Luxembourg:
23 613 Office of Official Publications of the EC.
24
25
26 614 SCF. 2003. Opinion of the Scientific Committee on Food on glycyrrhizic acid and its
27 615 ammonium salt. SCF/CS/ADD/EDUL/225. In:
28 616 Luxembourg: Office of Official Publications of the EC. p. 41.
29
30
31 617 Slimani N, Deharveng G, Charrondiere RU, van Kappel AL, Ocke MC, Welch A, Lagiou A,
32 618 van Liere M, Agudo A, Pala V, Brandstetter B, Andren C, Stripp C, van Staveren WA and
33 619 Riboli E. 1999. Structure of the standardized computerized 24-h diet recall interview used as
34 620 reference method in the 22 centers participating in the EPIC project. *European Prospective*
35 621 *Investigation into Cancer and Nutrition. Comput Methods Programs Biomed.* 58(3):251-266.
36
37
38 622 Slimani N, Ferrari P, Ocke M, Welch A, Boeing H, Liere M, Pala V, Amiano P, Lagiou A,
39 623 Mattisson I, Stripp C, Engeset D, Charrondiere R, Buzzard M, Staveren W and Riboli E. 2000.
40 624 Standardization of the 24-hour diet recall calibration method used in the european prospective
41 625 investigation into cancer and nutrition (EPIC): general concepts and preliminary results. *Eur J*
42 626 *Clin Nutr.* 54(12):900-917.
43
44
45
46
47
48 627 Stormer FC, Reistad R and Alexander J. 1993. Glycyrrhizic acid in liquorice - evaluation of
49 628 health hazard. *Food Chem Toxicol.* 31(4):303-312.
50
51
52
53 629 Subar AF, Dodd KW, Guenther PM, Kipnis V, Midthune D, McDowell M, Tooze JA, Freedman
54 630 LS and Krebs-Smith SM. 2006. The Food Propensity Questionnaire: Concept, Development,
55 631 and Validation for Use as a Covariate in a Model to Estimate Usual Food Intake. *Journal of*
56 632 *the American Dietetic Association.* 106(10):1556-1563.
57
58
59 633 Verger P, Ireland J, Møller A, Abravicius J, De Henauw S and Naska A. 2002. Improvement
60 634 of comparability of dietary intake assessment using currently available individual food

- 1
2
3 635 consumption surveys. European Journal of Clinical Nutrition. 56(2):S18-S24.
4
5
6 636 WHO. 1997. Food Consumption and Exposure Assessment of Chemicals. WHO/FSF/FOS/97
7 637 5. Geneva (Switzerland): FAO/WHO.
8
9
10 638 WHO. 2001. Safety evaluation of certain food additives and contaminants. Prepared by the
11 639 fifth five meeting of the Joint FAO/WHO Committee on Food Additives (JECFA). WHO
12 640 Technical report series (901). Geneva (Switzerland): WHO.
13
14
15 641 WHO. 2006. Safety evaluation of certain food additives and contaminants. Prepared by the
16 642 sixty-three meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA).
17 643 WHO technical report series (54). Geneva (Switzerland): WHO.
18
19
20
21 644 WHO. 2007. Safety evaluation of certain food additives and contaminants. Prepared by the
22 645 sixty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA).
23 646 WHO technical report series (940) 2006. Rome (Italy): WHO.
24
25
26 647 WHO. 2009. Evaluation of certain food additives. Prepared by the sixty-ninth meeting of the
27 648 Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO technical report series
28 649 (952) 2008. Rome (Italy): WHO.
29
30
31 650 Willett W. 1998. Nutritional epidemiology. New York: Oxford University Press.
32
33 651
34 652
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1 - Stepwise approach used for the assessment of dietary exposure to flavourings in the EFCOVAL study

	Methods	Data Assumptions		Step
		Food Consumption	Concentration in food	
Screening	MSDI [*] or per capita method	Assumption of 10% eaters in the population	Poundage data (industry)	
	Modified TAMDI [†]	Assumption of fixed amount of foods and beverages that could contain the flavour (portion sizes per food categories)	Normal use levels (industry)	1 [†]
	SPET [§]	Assumption of daily consumption of a single food category containing the flavouring agent of interest (highest dietary exposure based on a 'standard portion' size)	Normal use levels (industry)	
Data at individual level using EPIC-Soft	Food group level	Data aggregated in food groups that MAY contain the flavour	'Refined' concentration ^{**}	2
	Food item level	Data disaggregated: food items that DO contain the flavour – without alterations in the list of descriptors in the EPIC-Soft database	'Refined' concentration	3a
		Data disaggregated: food items that DO contain the flavour with alterations in the list of descriptors in the EPIC-Soft database	'Refined' concentration	3b ^{††}
		Data disaggregated: food items that DO contain the flavour plus foods that MAY contain the flavour – Same as 3a/3b plus use of descriptor 'unknown' in the facet flavour	'Refined' concentration	3c

* Maximized Survey-Derived Daily Intake

† Dietary exposure is expressed in $\text{mg kg}^{-1}\text{bw day}^{-1}$, considering an individual weighing 60 kg

‡ Modified Theoretical Added Maximum Daily Intake

§ Single-Portion Exposure Technique

** Concentration values from industry (normal use levels) and analytical determinations found in the literature

†† For raspberry ketone only

Table 2 – Dietary exposure assessment to flavourings (mg kg⁻¹ body weight day⁻¹) using screening methods

Flavouring	Safety limit [*]	Screening method [†]					
		MSDI		SPET		mTAMDI	
		Production volume	Dietary Exposure	Concentration source	Dietary Exposure	Concentration source	Dietary Exposure
Raspberry ketone	0.03	IOFI [‡]	0.05	FEMA [§]	0.03	FEMA	0.06
Glycyrrhizinic acid	0.16	EFFA ^{**}	<0.01	EFFA	3.3	EFFA	3.5
Coumarin	0.10	-	-	EU legislation ^{††}	0.05	EU legislation	0.02
-from <i>Cassia cinnamon</i>		-	-	FEMA	0.03	FEMA	0.04
-from <i>Ceylon cinnamon</i>		-	-	FEMA	<0.01	FEMA	<0.01
Caffeine	6.7	FEMA	7.3 [°]	FEMA	<0.01	FEMA	<0.01

^{*} Raspberry ketone: Threshold of Toxicological Concern in relation to structural class I (Cramer, et al. 1978); Glycyrrhizinic acid: Provisional LOAEL, (SCF 2003); Coumarin: Tolerable Daily Intake (EFSA 2004b); Caffeine: Tolerable daily intake (Fabech, et al. 2002).

[†] MSDI: Maximized Survey-Derived Daily Intake; SPET: Single-Portion Exposure Technique; mTAMDI: modified Theoretical Added Maximum Daily Intake.

[‡] International Organisation of Flavour Industry.

[§] Flavour and Extract Manufacturers' Association (US).

^{**} European Flavour and Fragrance Association.

^{††} Use of maximum permitted levels instead of absent use levels.

[°] In the absence of EU production volumes for caffeine, per capita dietary exposure in the USA (based on USA production volumes) was used as a proxy for per capita dietary exposure in the EU

Table 3 – Estimated dietary exposure to flavourings (expressed as mg kg⁻¹ body weight day⁻¹) in a sample of 121 adults from The Netherlands* (food consumption combined with refined concentration data[†])

Dietary exposure assessment (Steps 2 and 3)													
Flavourings	Safety limits [‡]	Food item level											
		2: Food group level			3a: without improvement of descriptors in the EPIC-Soft			3b: with improvements of descriptors in the EPIC-Soft			3c: all foods from step 3a and 3b plus the foods where 'unknown' descriptor in the facet flavour was reported		
		Mean	Median	P95 th	Mean	Median	P95 th	Mean	Median	P95 th	Mean	Median	P95 th
Raspberry Ketone	0.03	0.08	0.07	0.18	<0.01	0	<0.01	<0.01	<0.01	0.04	0.01	<0.01	0.05
Glycyrrhizinic acid	0.16	0.46	0.34	1.37	0.11	<0.01	0.46	-	-	-	0.13	<0.01	0.52
Coumarin	0.10	0.07	0.06	0.12	<0.01	<0.01	0.02	-	-	-	<0.01	<0.01	0.02
Caffeine	6.7	18.6	17.0	43.5	6.81	5.47	16.8	-	-	-	6.82	5.47	16.8

* Sample population is part of the European Food Consumption Validation (EFCHOVAL) project

[†] Concentration values from normal use levels and analytical determinations

[‡] Raspberry ketone: Threshold of Toxicological Concern in relation to structural class I (Cramer, et al. 1978); Glycyrrhizinic acid: Provisional LOAEL, (SCF 2003); Coumarin: Tolerable Daily Intake (EFSA 2004b); Caffeine: Tolerable daily intake (Fabech, et al. 2002).

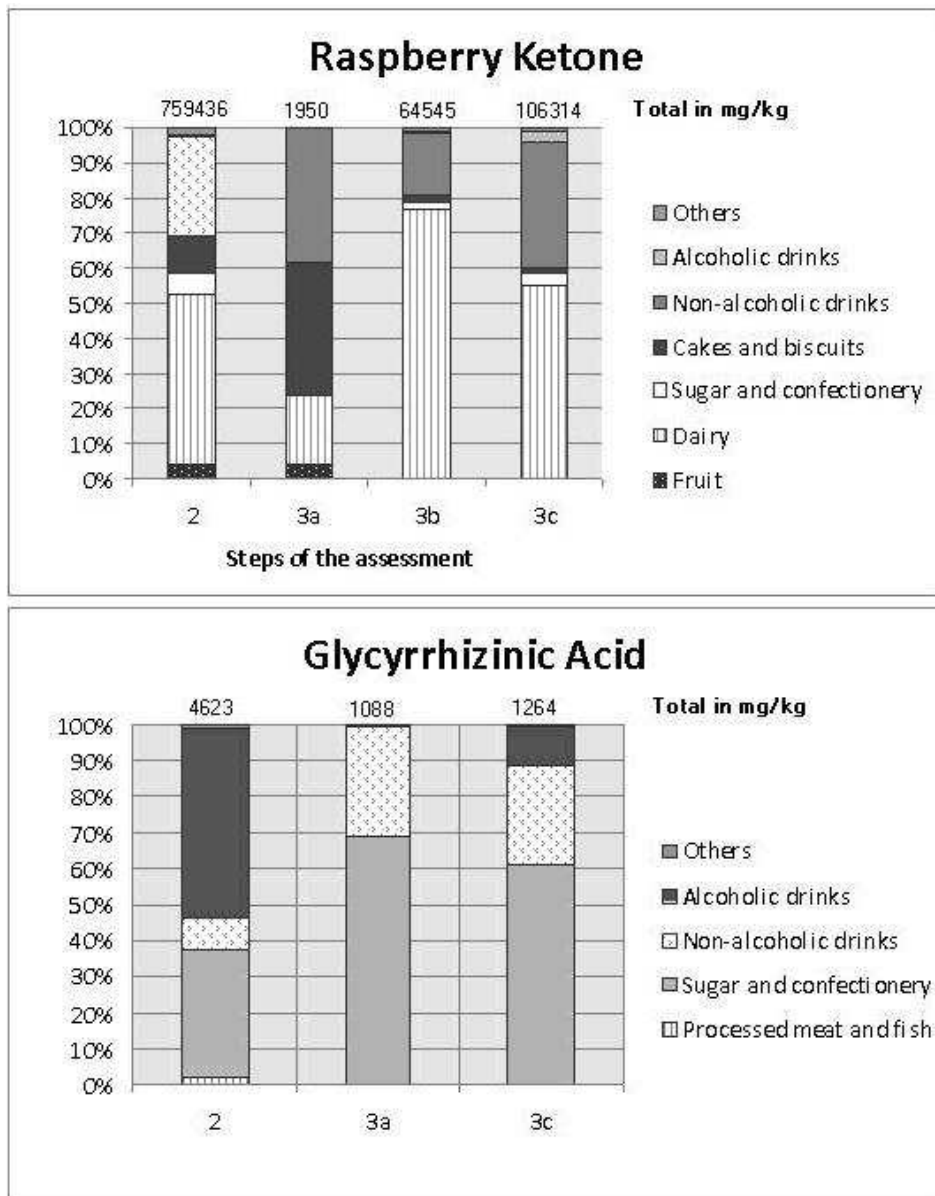


Figure 1 - Dietary exposure to raspberry ketone and glycyrrhizinic acid and their food group sources in each step (2, 3a, 3b and 3c) of the assessment.

2 - Food group level: All foods belonging to a flavourable food group are included in the model.

3a - Food item without modifications in EPIC-Soft

3b - Food item with modifications in EPIC-Soft

3c - Steps 3a and 3b plus foods which were reported as non-specified flavour.

141x179mm (96 x 96 DPI)

Appendix 1 – Flavourable food groups considered in the dietary exposure assessment of step 2

<i>Raspberry Ketone</i>	<i>Glycyrrhizinic Acid</i>	<i>Coumarin</i>	<i>Caffeine</i>
Alcoholic beverages (liqueurs, brandies, gin subgroups)	Alcoholic beverages (spirit, aniseed drinks and liqueur subgroups)	Alcoholic beverages (wine, beer subgroups)	Alcoholic beverages (liqueur subgroup)
Biscuits	Dressing sauces	Biscuits	Breakfast cereals
Breakfast cereals	Fish products	Breads	Cakes and biscuits
Cakes	Liquorice confectionery (non-confectionery chocolate and ice cream subgroups)	Breakfast cereals	Dairy products (milk beverages and cream desserts subgroups)
Dairy products (yogurt, milk beverages, cream desserts, puddings subgroups)	Non-alcoholic beverages (herbal tea subgroup)	Cakes	Dessert sauces
Dressing and dessert sauces	Processed meats	Dairy products (desserts subgroup)	Dessert sauces
Fruits		Dessert sauces	Non alcoholic beverages (carbonated drinks, coffee, tea subgroups)
Non-alcoholic beverages		Fruits	
Sugar and confectionery (jams, non-chocolate, ice cream, sorbet, water ice subgroups)		Non-alcoholic beverages	
		Root vegetables	Sugar and confectionery (syrup, chocolate bar, ice cream subgroups)
		Sugar confectionery	

Appendix 2 – Refined concentrations* used in the dietary exposure assessment of steps 2 (food group level) and 3 (food item level)

<i>Raspberry Ketone</i>		<i>Glycyrrhizinic Acid</i>		<i>Coumarin</i> [†]		<i>Caffeine</i>	
Foods	mg kg ⁻¹	Foods	mg kg ⁻¹	Foods	mg kg ⁻¹	Foods	mg kg ⁻¹
Baked goods	13.1	Alcoholic beverages	135	Baked goods	16.6	Baked goods	0.06
Chocolate (n=2)	9.3 ± 9.1	Liquorice confectionery	1500	Bilberry	0.005	Brewed Coffee	680
Ice cream	2.6	Non-alcoholic beverages	50	Breakfast cereal	7.5	Cocoa powder	340
Jam	0.3	Soy sauce (n=5)	37 ± 19.4	Celery	16.6	Chocolate milk	60
Non-alcoholic beverages	2.8			Cinnamon powder	3000	Chocolate syrup	106
Raspberry (n=39)	1.3 ± 1.2			Dairy products	1.1	Cola drinks	125
Sauce	0.9			Frozen dairy	1.1	Dark chocolate	700
Yogurt	20.2			Jam	2.9	Espresso	2473
				Non-alcoholic beverages	0.06	Energy drinks	240
				Pudding	3.8	Puddings	0.3
						Frozen dairy	0.3
						Liquor	170
						Milk chocolate	220
						Tea	205
						White chocolate	14

* Number of samples used = 1 unless otherwise specified; in that case mean +/- SD are reported. See methods section.

[†] Based on percentage of coumarin in cinnamon products (cassia cinnamon: 0.3% (BfR 2006)) and in the use levels of cinnamon reported by FEMA (Flavour and Extract Manufacturers' Association) at Fenaroli's Handbook of flavour ingredients (Burdock 2005), except for bilberry and celery, which were collected from the literature.