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

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

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ABSTRACT

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

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

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

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

INTRODUCTION

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

A major pitfall of dietary exposure assessment to chemicals is the limited availability of the two types of information that are needed, food consumption data and chemical concentration in foods (EFSA 2005). The ideal situation of performing a detailed dietary exposure assessment by collecting information at the individual level for every hazardous substance is neither practical nor cost-effective (Lawrie and Rees 1996), especially when the objective is to verify that a safety limit is not exceeded. As a consequence, dietary exposure should be evaluated through a stepwise approach (WHO 1997).

The stepwise approach follows the premise of an assessment using the least refined method (screening) towards the most refined one, if necessary. The refinement of data is judged necessary when the dietary exposure assessed with a conservative method using highly aggregated data (i.e. the chemical is assumed to be present in specific food groups supposedly ingested by the whole population and there is no information about distribution of the consumption) exceeds the safety limits of the chemical. Once safety limits are surpassed, this indicates there is a possibility of safety concern and further investigation is needed by using less aggregated data (e.g. food consumption collected at the individual level). Then, the next step is performed using more detailed information on food consumption and/or concentration data in order to determine the right hand extreme of the distribution of dietary exposure. On the other hand, when the dietary exposure assessed using screening methods is under the safety limits, further refinement of the assessment is not needed (Gibney and Lambe 1996, Lawrie and Rees 1996, WHO 1997). In this way, wasting of resources by

*Joint Food and Agriculture Organization/World Health Organization

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

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

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

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

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

109 In this paper, we report the results of an explorative study aimed at comparing methods used
110 to estimate the dietary exposure to flavourings in the context of a stepwise approach.

MATERIAL AND METHODS

Flavourings under investigation

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

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

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

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

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

Food consumption data used for the refined assessment of dietary exposure

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

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University Ethical Committee and informed consent was obtained from all study participants.

EPIC-Soft software

EPIC-Soft is a software program that has been developed in the EPIC study (European Prospective Investigation into Cancer and Nutrition) to ensure the highest possible level of standardization of 24-hour dietary recalls. The structure and standardization procedure of EPIC-Soft are described in detail elsewhere (Slimani et al. 1999, Slimani et al. 2000). An important feature of EPIC-Soft is the use of two complementary food description systems: *explicit* and *implicit*. In the explicit description, facets and descriptors are used during the process of food identification, which is based on the Languag coding system initially used to describe technological and toxicological food characteristics (FDA/CFSAN 1993). Facets are used to describe foods in more detail and this is done by means of standardized questions asked to the interviewee each time a food is reported. One of the facets available for a number of food categories is 'flavour or added component'. The descriptors, which are the country-specific terms associated with each facet, are used as pre-defined potential answers built in the database of the software (e.g. strawberry flavour or strawberry added pieces). In addition, the descriptor 'unknown' may be used when the interviewees are not able to provide the expected level of detail (e.g. unknown flavour for a yogurt that has been consumed). In the implicit description, the name of a food provides sufficient information to identify the food and no further detail is collected using the facet/descriptor system. For instance, the food name "liquorice drops" implies the presence of liquorice so that there is no need to use the facet "flavour" to indicate such presence.

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

n.s., forest fruit. No further adaptations were made in the descriptors of glycyrrhizinic acid (i.e. liquorice), caffeine (i.e. coffee) and coumarin (i.e. cinnamon) flavourings.

Dietary exposure assessment

With the use of the stepwise approach, dietary exposure to the four flavourings was assessed in three different steps (Table 1).

Step 1 – Use of screening methods: Maximized Survey-Derived Daily Intake (MSDI) and Single-Portion Exposure Technique (SPET) as used by JECFA and modified Theoretical Added Maximum Daily Intake (mTAMDI) used by EFSA.

MSDI is also known as the 'per capita method' or 'per capita x 10' approach. Assumptions of the method are: that 60% of total production of flavourings is reported by the industry; that 10% of the total population are consumers of the flavouring; and that there is no variation in the intake of the particular flavouring among consumers. Accordingly, the following formula is used:

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

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

As for the safety evaluations performed by JECFA, EU population in this study was assumed to be 32×10^6 . The annual production volumes of the flavourings considered were those used by JECFA (WHO 2001) and SCF (SCF 2003): $19,500 \text{ kg y}^{-1}$ for raspberry ketone and $1,956 \text{ kg y}^{-1}$ for glycyrrhizinic acid, respectively. Poundage data for coumarin is not available since it can not be used as an added flavouring substance. In the absence of EU production volumes for caffeine, per capita dietary exposure in the USA was used as a proxy for per capita dietary exposure in the EU.

The SPET method provides a dietary exposure assessment based on normal use levels and identifies the single food category containing the flavouring agent of interest that is likely to contribute to the highest dietary exposure from one 'standard portion'. The standard portion is taken to represent the mean food consumption amount within one eating event for consumers

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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:

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

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

amounts in two types of cinnamon (cassia cinnamon: 0.3%; Ceylon cinnamon: 0.008% (BfR 2006)).

Step 2 – Use of food consumption data aggregated in food groups.

At this step, food consumption data at the individual level (Dutch EFCOVAL sample) were grouped in food categories based on the EPIC-Soft grouping system (Slimani, et al. 1999). It was assumed that all foods within a given 'flavourable food category' contained the flavouring of interest (see Appendix 1). For instance, raspberry ketone may be added to some foods in the dairy food group (e.g. yogurts). Thus, in the assessment of step 2, all foods belonging to the yogurt category, a subgroup category of dairy products, were assumed to contain raspberry ketone, even though some foods are known not to contain it.

Concentration levels used in step 2 were called 'refined concentrations' (see Appendix 2). First choice for the concentration data was normal use levels reported by industry. An exception was made for caffeine contents in non-alcoholic beverages since reported industry levels (0.13 mg/kg) were clearly underestimated as compared to the analytical determinations gathered in the literature (see Appendix 2). For glycyrrhizinic acid, upper use levels were used. Analytical determinations from literature were also used in the cases where the flavouring was known to occur in its natural form or when levels of added flavourings were not reported by industry. For instance, glycyrrhizinic acid is known to be added to soy sauce, but use levels in sauces have not been reported. List of references used to collect the flavouring concentration data in foods can be provided upon request.

Step 3 - Use of food consumption data at the level of foods items.

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

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study, while step 3a gave information that would have been available before the extension. In the last of the three models (3c), foods were identified in the same way as for step 3a (for glycyrrhizinic acid, caffeine and coumarin) and 3b (for raspberry ketone), but the descriptor ‘unknown flavour’ was assumed to include the flavouring of interest.

Concentration levels used in step 3 were the same as used in step 2: ‘refined concentration’.

Data analysis

To estimate dietary exposure to the flavourings, food consumption was multiplied by the concentration of the chemical in the food and then divided by the body weight to be expressed in $\text{mg kg}^{-1} \text{bw day}^{-1}$. In step 1, a body weight of 60 kilos was assumed whereas for steps 2 and 3, individually measured body weights were used. Food consumption data in steps 2 and 3 were based, for each individual, on the average of the two 24-hour dietary recalls. In these two steps, potential dietary exposure to the flavourings was estimated for each subject. Besides the mean and the median intake of the total group, the 95th percentile of the population distribution was used to characterize highly exposed subjects. As stated by EFSA (2008b), the 95th percentile can be assessed with approximately 130 subjects when using a binominal distribution (Conover 1971). Furthermore, the average contribution of the different food groups to the overall dietary exposure in steps 2 and 3 was estimated in percentages. Data processing and descriptive statistical analyses were performed using SAS software (version 9.1; SAS Institute, Cary NC, USA).

RESULTS

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

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

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

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mean dietary exposure to raspberry ketone and glycyrrhizinic acid was slightly higher as compared to step 3a and 3b. In the case of coumarin and caffeine, dietary exposure was the same in all of these steps.

By comparing tables 2 and 3 it appears that in some cases the screening techniques lead to a dietary exposure lower than that of the refined exposure assessment. It was the case for raspberry ketone where SPET was 0.03 mg/kg versus 0.04 and 0.05 mg/kg at the 95th percentile at step 3b and 3c, respectively.

In the investigation of food groups contributing to the exposure in each step of the assessment (Figure 1), it can be seen that for raspberry ketone the main sources of the flavourings were the same in almost all steps: 'dairy products' and 'non-alcoholic beverages'. Yet, while 'non-alcoholic beverages' and 'cakes' were the food groups most contributing to the dietary exposure of raspberry flavouring in step 3a, dietary exposure to raspberry contained in 'dairy products' became an important source with the use of facets and descriptors in step 3b. In the case of glycyrrhizinic acid, 'sugar and confectionery' and 'non-alcoholic beverages' (most herbal teas) were the bigger contributors of the substance in all steps, with a probable overestimation of the contribution from 'sugar and confectionery' in step 2 (group level) as compared to the other steps. The same pattern of overestimation at food group level is seen in the assessment of coumarin and caffeine (figures not shown). Main contributors to dietary exposure were 'cakes', 'biscuits' and 'tea' for coumarin and 'non-alcoholic beverages' for caffeine at all steps.

DISCUSSION

The dietary exposure to raspberry ketone, glycyrrhizinic acid, coumarin and caffeine was estimated in this study using a stepwise approach. It has been shown that the refinement of food consumption data in the assessment of dietary exposure to flavourings might be necessary, but dependent of the chosen screening method for the assessment. When using data from the 24h-dietary recall by means of EPIC-Soft software, the dietary exposure to raspberry ketone was higher in the model where descriptors have been extended as compared to the model where no adjustments have been considered.

The dietary exposure calculated using the screening methods exceeded the safety limits and therefore implied the need of more refined assessment for raspberry ketone, glycyrrhizinic acid and caffeine, but with somewhat different results depending on the method used and on the flavouring under assessment. In particular, variation in outcomes using different screening methods was observed; whereas by the MSDI method the exposure to glycyrrhizinic acid was evaluated to be of no safety concern, the dietary exposure assessed by SPET and mTAMDI indicated the need of further refinement. On the other hand, dietary exposure to caffeine assessed by MSDI indicated the need of refined assessment while the other two methods did not indicate it. One of the reasons for the variation in results from the screening methods is probably the difference in assumptions between them (e.g. the percentage of consumers in the dietary exposure and how conservative they are, i.e. whether individuals, who consume large quantities of flavoured foods, are considered in the dietary exposure assessed by the different methods). Although it is beyond the scope of this study to investigate the accuracy of such estimates, this topic deserves further attention. In fact, many of the conservative assumptions and default values that are currently used in screening assessments were established some time ago and in some cases they were originally based on subjective or arbitrary estimates (EFSA 2006). In the case of the MSDI, which until recently was the unique method used by JECFA to assess dietary exposure within the safety evaluation of flavourings, the insufficient conservativeness of the method has been discussed in a number of scientific publications (Arcella and Leclercq 2005, Hall and Ford 1999, Lambe et al. 2002, Leclercq 2007, Munro and Danielewska-Nikiel 2006). Most recently, JECFA has acknowledged the

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likely underestimation of the MSDI method in the assessment of some flavourings and developed a new method (SPET), which takes into account different food patterns of consumers and the uneven distribution of dietary exposure in consumers of flavourings (WHO 2007). Furthermore, according to EFSA, the appropriateness of the conservative assumptions and default values that are used in screening assessments of chemicals, including flavourings may require further investigation. Analysis of uncertainty in the screening assessment may not be required, provided they include proper conservative assumptions to take account of uncertainty (EFSA 2006).

Once the need of further refinement in the dietary exposure is identified, other limitations might be encountered in the assessment of exposure to chemicals in the diet. For instance, the knowledge of chemical concentration data in foods is limited and the ability of dietary methods to assess dietary exposure to chemicals can be uncertain. In our study, this last issue has been explored through the different models created to assess the dietary exposure to flavourings in the Dutch population.

In the first model created (step 2), the dietary exposure was characterized by investigating the consumption of flavourings at the food group level. As noted in Table 3, the dietary exposures of the four flavourings were high as compared to all other steps of the assessment. Considering that in this model, foods that do not contain the flavouring may have been quantified as part of the dietary exposure, we recognize a certain degree of overestimation in the estimate. This should be, however, an indication of safe dietary exposure, in case the estimate would be below the safety limit. However, the need for further refinement of the food consumption data collected at the individual level appeared necessary for the four flavourings under assessment.

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

the 24-hour dietary recall provided an accurate estimate of the intake of flavoured foods, the high dietary exposure to raspberry ketone in step 3b suggests that such adjustments, which characterize the consumption of foods in more detail, is useful when assessing dietary exposure to flavourings. Nonetheless, 24-hour dietary recalls are known to underestimate the dietary intake of some individuals (Bingham 1987, Willett 1998) and because of the lack of proper validated biomarkers for these flavourings, the accuracy of the estimate of dietary exposure to flavourings, as assessed, cannot be ensured without further research.

No alterations in the Dutch EPIC-Soft version were implemented for glycyrrhizinic acid, coumarin and caffeine, and evaluation of such alterations was therefore not possible. However, for some types of flavourings, such as glycyrrhizinic acid, the use of facets and descriptors might not be that important for an accurate dietary exposure assessment given that the food name itself often indicates the presence of the flavouring, which would be enough for the food identification. Nevertheless, additional exploration is needed for this conclusion. Moreover, we do not know to what extent the consumption of cinnamon was correctly identified. First, the use of spices, including cinnamon, during home cooking is not collected during the 24-hour dietary recall using Epic-Soft software. Second, this spice, in particular, may not be easily identified by the name of the product and neither by the use of descriptors since it does not seem to be clear to the population whether a certain food would contain cinnamon or not. The dieticians of our study reported that for the food group most expected to contain cinnamon (cereals and biscuits), subjects were not able to provide this kind of detail and that they, as interviewers, had no experience in collecting information about flavourings. Third, the authors of this study may have been not correctly identified the presence of cinnamon in certain culinary products such as soups since the presence of cinnamon is not always evident. Because of these reasons, the dietary exposure to coumarin may have been underestimated in this assessment. As a check whether the descriptors of the four flavourings may have been sufficiently identified, the potential flavoured foods with descriptor 'non-specified' were assumed to include the flavouring of interest (Step 3c). The dietary exposure did not considerably change for any of the four flavourings in this step.

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

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

442 It is important to mention that the estimates presented in steps 2 and 3 of the assessment are
443 not representative of the usual Dutch food consumption. Because of the lack of
444 representativeness of the sample and the limited number of survey days, chronic dietary
445 exposure may not have been correctly estimated in this assessment. In fact, the collection of
446 only two days of 24-h recalls does not allow to assess chronic exposure but short term
447 exposure. This is probably the reason why the refined exposure assessment performed for
448 raspberry ketone leads to higher values than that obtained with the SPET technique. An
449 improved refined assessment could be performed by using additional information on usual
450 intake of flavoured foods, such as a food propensity questionnaire. Subar and colleagues
451 (2006) have shown that food propensity questionnaires may offer important covariate
452 information in supplementing 24-h recalls for estimating usual intake of food groups. This is
453 possibly true for assessing chemicals in the diet as well. Furthermore, only dietary exposure
454 has been considered in our assessment and contribution from other sources (e.g. medicines)
455 may lead to an additional exposure. In fact, the safety limits we have used in this
456 assessment should refer to the total exposure to the flavourings but with the study we
457 performed we can only conclude on exposure from the diet. In addition, the small number of
458 evaluated flavourings limits the possibilities to extrapolate the results of our study to other
459 types of flavourings. Another limitation is the scarce availability of concentration data on
460 chemicals. These are relatively seldom published in open literature and therefore difficult to
461 retrieve(EFSA 2005). In the flavouring case, few analytical data are currently available and
462 little is know about the influence of storage and processing on the residues of theses
463 substances in food (EFSA 2006). Consequently, a high variability in the available
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

individual level with the standardized 24-h recall.

In summary, this study showed that the collection of detailed food consumption data at the individual level is useful and should be further explored for other flavourings. In addition, the possibility of further adaptations of the databases used in EPIC-Soft software seemed to provide a higher dietary exposure to raspberry ketone as compared to the non-modified databases, which may also be true for other flavourings. Yet, the need for alterations may still differ depending on the nature of the flavouring under assessment. To further study the usefulness of detailed food consumption data in the dietary exposure assessment of flavourings and other chemicals, research should include biological markers and analytical determination in flavoured foods, which would warrant the check of accuracy of such estimates. Finally, the benefit of assessing usual intake of chemicals in the diet by combining 24-h recalls and food propensity questionnaires is a topic that deserves more exploration.

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- Spain Basque Foundation for Health Innovation and Rsearch (BIOEF)
- United Kingdom Prima informatics limited (Primainfo)

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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)	1†
	Modified TAMDI‡	Assumption of fixed amount of foods and beverages that could contain the flavour (portion sizes per food categories)	Normal use levels (industry)	
	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 mg kg⁻¹bw 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</i> cinnamon		-	-	FEMA	0.03	FEMA	0.04
-from <i>Ceylon</i> cinnamon		-	-	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 (Fabeck, 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

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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 (EFCOVAL) 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)

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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 confectionary	

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.