DRAFT SCIENTIFIC OPINION

Guidance on the data required for the risk assessment of flavourings[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This Opinion follows a request from the European Commission for the data required for the risk assessment of flavourings.

The approach presented takes into account the experience from the previous evaluation programme of flavouring substances.

KEY WORDS

Flavourings, guidelines, risk assessment, dietary exposure.

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SUMMARY

Following a request from the Commission, the Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids was asked to provide scientific advice regarding the data required for the evaluation of flavourings.

Part A of this Opinion provides a proposal concerning the data required for the risk assessment of flavouring substances, i.e. chemically defined substances with flavouring properties.

Part B of this Opinion provides a proposal concerning the data required for the risk assessment of flavouring preparations obtained from material of vegetal, animal or microbiological origin, other than food, by appropriate physical, enzymatic or microbiological processes, the material being taken as such or prepared by one or more of the traditional food preparation processes listed in Annex II of the Regulation.

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BACKGROUND AS PROVIDED BY THE COMMISSION

On 16 December 2008 the following Regulations of the European Parliament and of the Council were adopted:

Regulation (EC) No 1332/2008 on food enzymes[4],

Regulation (EC) No 1333/2008 on food additives[5],

Regulation (EC) No 1334/2008 on flavourings and certain food ingredients with flavouring properties[6] and

Regulation (EC) No 1331/2008 on a common authorisation procedure for food additives, food enzymes and food flavourings[7].

The Regulations entered into force on 20 January 2009.

Regulation (EC) No 1334/2008 on flavourings and certain food ingredients with flavouring properties applies to flavourings which are used or intended to be used in or on foods, food ingredients with flavourings properties, food containing flavourings and/or food ingredients with flavouring properties and source materials and/or source materials for food ingredients with flavouring properties.

The Regulation requires an evaluation by the European Food Safety Authority (EFSA) and an approval by the Commission for the following types of flavourings: 1) flavouring substances, 2) flavouring preparations obtained from material other than food, 3) thermal process flavourings where ingredients for the production of thermal process flavourings are from source material other than food or the production conditions set in Annex V of the Regulation are not met, 4) flavour precursors obtained from source material other than food, 5) other flavourings and 6) source materials other than food.

In order to ensure consistency amongst the new Regulations on food additives, food enzymes and food flavourings, the procedural aspects of approval of substances (such as handling of applications within well defined deadlines, evaluation of substances by EFSA and decision making by the Commission,) are provided in Regulation (EC) No 1331/2008 on the common authorisation procedure on food additives, food enzymes and food flavourings. This Regulation also provides that an implementing measure (Article 9) shall be adopted by the Commission, within 24 months from the adoption of the Regulation on flavourings, which shall concern in particular the content, drafting and presentation of the application for the evaluation and authorisation of flavourings. With a view to the adoption of this implementing measure the Commission consulted EFSA, which, within six months of the date of entry into force of the Regulation on flavourings, i.e. by 20 July 2009, shall present a proposal concerning the data required for risk assessment of flavourings.

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

The European Food Safety Authority (EFSA) is asked to provide the Commission with a proposal concerning the data required for the risk assessment of flavourings with a view to including it in the implementing measure which will lays down amongst other aspects, the content, drafting and presentation of the application for the evaluation and authorisation of flavourings.

[4] OJ L 354, 31.12.2008, p.7
[5] OJ L 354, 31.12.2008, p.16
[6] OJ L 354, 31.12.2008, p.34
[7] OJ L 354, 31.12.2008, p.1

INTRODUCTION

According to the Regulation (EC) No 1334/2008 (European Commission, 2008) which entered into force on 20 January 2009, hereafter referred to as "the Regulation," The European Food Safety Authority (EFSA) is asked to provide the Commission with a proposal concerning the data required for the risk assessment of flavourings with a view to including it in the implementing measure which will lay down amongst other aspects, the content, drafting and presentation of the application for the evaluation and authorisation of flavourings.

The Regulation shall apply to:

(a) flavourings which are used or intended to be used in or on foods, without prejudice to more specific provisions laid down in Regulation (EC) No 2065/2003;

(b) food ingredients with flavouring properties;

(c) food containing flavourings and/or food ingredients with flavouring properties;

(d) source materials for flavourings and/or source materials for food ingredients with flavouring properties.

PART A: FLAVOURING SUBSTANCES

I. GENERAL PRINCIPLES OF THE SAFETY ASSESSMENT OF FLAVOURING SUBSTANCES INTENDED TO BE USED IN OR ON FOODS

1. Background

Regulation (EC) No 2232/96 of the European Parliament and the Council (European Commission, 1996) laid down a Procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all others in the EU. In application of that Regulation, a Register of flavouring substances used in or on foods in the Member States was adopted by Commission Decision 1999/217/EC (European Commission, 1999), as last amended by Commission Decision 2009/163/EC (European Commission, 2009). Each flavouring substance was attributed a FLAVIS-number (FL-number) and all substances were divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common. These chemical groups are covered in Flavouring Group Evaluations (FGEs).

The substances listed in the Register have been evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (European Commission, 2000), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999). After the completion of the evaluation programme, but at the latest by 31 December 2010, the Community list of flavouring substances for use in or on foods in the EU shall be adopted (Article 5 (1) of Regulation (EC) No 2232/96) (European Commission, 1996, 2008).

In the mandate for the current Opinion, the Commission suggests that EFSA takes into account the experience from this programme.

2. General approach

Flavouring substances in the Community Register have been evaluated in accordance with Commission Regulation (EC) No 1565/2000. This Regulation lays down a general approach for the evaluation.

The Panel considered that the elaboration of a proposal concerning the data required for the risk assessment of newly submitted flavouring substances should build upon the experience gained in the course of the evaluation of flavouring substances included in the Community list.

A general principle of this Opinion is that newly submitted flavouring substances that can be assigned to one of the existing Flavouring Group Evaluations (FGEs) on the basis of structural and metabolic similarities should be evaluated according to the scientific principles and to the group-based approach underlying the former evaluation programme.

In addition, the proposal provides a Procedure for the evaluation of flavouring substances which cannot be assigned to one of the existing FGEs. This should allow an individual evaluation of the newly submitted flavouring substance.

The overall strategy for the risk assessment of flavouring substances is outlined in Figure 1.

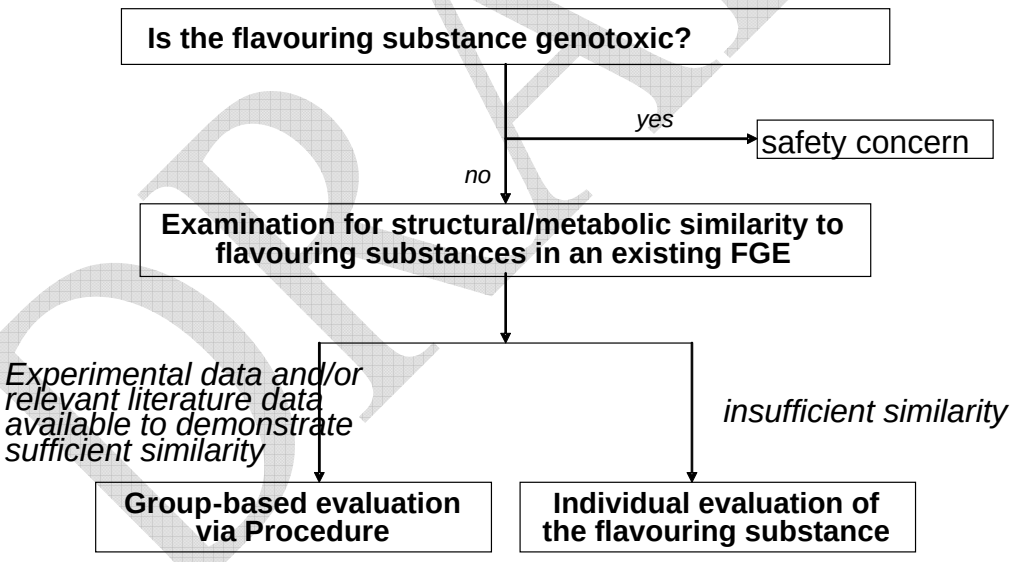


Figure 1: Overall strategy for the risk assessment of flavouring substances.

The Procedure applied for the group-based evaluation is shown and explained in chapter V (see also Figure 2), the individual evaluation of a flavouring substance is outlined in chapter VI (see also Figure 3).

II. INFORMATION TO BE SUPPLIED WITH AN APPLICATION FOR THE AUTHORISATION OF FLAVOURING SUBSTANCES

1. Description of the production process

The process employed to produce the flavouring substance (e.g. chemical synthesis, enzyme-catalysis, fermentation or isolation from a natural source) should be described. The information should specifically focus on the potential of the applied process to result in by-products, impurities or contaminants.

2. Specification

The following information have to be provided for the flavouring substance:

- Chemical name (IUPAC name, synonyms)

- CAS-, E-, EINECS-, CoE- and FEMA numbers (if assigned)

- Chemical and structural formula, molecular weight

- Physical form/odour

- Solubility data

- Identity test (infra red-, nuclear magnetic resonance- and/or mass spectrum)

- Purity/Minimum assay value

- Impurities

- Physical parameters related to purity: boiling point (for liquids), melting point (for solids), refractive index (for liquids), specific gravity (for liquids)

- Information on the configuration of the flavouring substance: It is recognised that geometrical and optical isomers of substances may have different properties. Their organoleptic properties may be different and they may have different chemical properties resulting in differences in their absorption, distribution, metabolism, elimination and toxicity. Thus, information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers, or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number, etc.)

- Stability and decomposition products, if relevant

- Interaction with food components, if relevant

- Any other relevant information.

3. Data on use levels and occurrence levels

3.1 Levels in food (as added flavouring substances and from other sources)

In order to assess total dietary exposure to the flavouring substance from all sources, for each of the categories reported in Table 1, the applicant needs to provide:

- Normal and maximum use levels as added flavouring substance (first two columns of Table 1).
  For each specific food/beverage category, "normal use level", expressed in mg/kg of product, is intended as the median of anticipated concentration values of added flavouring substance for products belonging to the category. On the other hand, "maximum use level", expressed in mg/kg of product, is intended as the concentration of added flavouring substance that, according to the applicant, will not be exceeded in any product belonging to the category.

- Normal and maximum occurrence levels of the substance from other sources: as natural constituent, as substance developed during the processing of foods (such as Maillard products or products resulting from hydrolysis or oxidation reactions) or as carry over originating from their use in animal feed (medium two columns of Table 1).
  In order to estimate normal values in each category, only foods and beverages in which the substance is present in significant amounts will be considered (i.e. for the category "Fresh fruit" 04.1.1., the normal concentration is the median concentration observed in all kinds of fruit where the flavouring substance is known to occur).

- Normal and maximum combined occurrence levels of the substance, taking into account all sources; as added flavourings and from other sources (last two columns of Table 1).

Most categories reported in Table 1 correspond to the sub-categories of the Codex GSFA (General Standard for Food Additives available at http://www.codexalimentarius.net/gsfaonline/CXS_192e.pdf) that were used by the JECFA in the "Single Portion Exposure Technique" (SPET) technique (FAO/WHO, 2008). No use levels are requested for the category infant formulae and follow-on formulae since, based on the Commission Directive 2006/141/EC of 22 December 2006, flavouring substances are not expected to be added to these products. For the category 13.2 (complementary foods for infants and young children), further refined categories have been added so that a specific assessment of dietary exposure can be performed for infants and small children. If these data should not be provided by the applicant it will be assumed that the flavouring substance is not intended to be used in products specifically designed for infants and/or small children and that the safety evaluation of the substance is referred only to its use in regular food.

In case of foods marketed as powder or as concentrates, occurrence levels must be reported for the reconstituted product, considering the instructions reported on the product label or one of the standard dilution factors established by the JECFA (FAO/WHO 2008).

Occurrence levels as added flavouring substance (first two columns of Table 1) must be provided at the same level of detail as in the International Organization of the Flavour Industry (IOFI) provision of use levels to the Commission for all substances present in the Register and belonging to class III (IOFI - DG SANCO, 2008). However, refined use levels cannot be obtained through an Industry survey performed in the EU since newly submitted flavouring substances are not yet in use in this area. Therefore, refined use levels will need to be provided from Industry surveys performed in other areas of the world. For newly submitted flavouring substances that would not have been used in other areas of the world and for which no similar substance could be identified, "anticipated" use levels will be

provided. The conclusion of the safety evaluation may then be revised in the future to take into account updated uses and use levels. The safety evaluation of the substance will be considered provisional until post marketing confirmation of the levels of use.

Occurrence levels from other sources (as natural constituent and/or as substance developed during the normal processing of foods) will be retrieved by the applicant from all available databases. In particular, all quantitative data present in databases listed in Appendix 2 need to be considered.

A flavouring substance can be concomitantly present in a product as an added flavouring and from other sources (thus a substance may be present in a fruit-based beverage both as added flavouring substance and as a natural constituent of the fruit ingredient). The last two columns of Table 1 ("Combined occurrence level from all sources") can be filled by the applicant by adding up the columns "Occurrence level as added flavouring substance" and "Occurrence level from other sources". Alternatively, these columns can be filled based on an expert judgment of the likelihood of the concomitant presence of the substance from the two sources. This will be done both for normal use levels and for maximum use levels.

3.2 Non-food sources of exposure

The applicant needs to indicate the non-food uses of the flavouring substance. Available information on annual volumes of production in the EU for non-food uses (e.g. in cosmetics and detergents), the content of the substance in these products and its absorption rates via skin and/or inhalation should be provided by the applicant to identify non-food sources of exposure.

The Panel is aware that for many flavouring substances, quantitative data on their occurrence in non-food sources may be limited. Any available information on potential non-food sources, i.e. the exposure via dermal or inhalational routes should be provided.

4. Assessment of dietary exposure

In the evaluation of flavouring substances to be included in the Community list, the dietary exposure considered by EFSA within the Procedure to assess their safety has been a *per capita* estimate, the "Maximised Survey-Derived Daily Intake" (MSDI), based on the annual volume of production reported by the applicant. In addition, the "modified Theoretical Added Maximum Daily Intake" (mTAMDI) was calculated, based on the normal added use levels of the substances as reported by the applicant in the 18 food categories of Annex III of Commission Regulation EC (No) 1565/2000. The mTAMDI value was not considered in the Procedure but was only used as a tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004). Both the MSDI and the mTAMDI approach take into consideration the dietary exposure of a 60 kg adult.

For flavouring substances that are also naturally present in foods or beverages, qualitative and quantitative information related to natural occurrence was reported by the applicant but was not considered for the assessment of dietary exposure.

The limitations of the MSDI approach have been frequently reiterated by the Scientific Committee for Food, the former Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) of the EFSA, and the FAO/WHO Joint Expert Committee on Food Additives (JECFA). In particular, the use of the MSDI value calculated on the basis of anticipated volumes of production (for flavouring substances that were not yet on the market at the time of the safety evaluation) was questioned by the JECFA (FAO/WHO, 2008).

A complementary method was recently developed by the JECFA (FAO/WHO, 2008) to assess dietary exposure to flavouring substances: the SPET (Single Portion Exposure Technique). The SPET is calculated by combining, in each food category, added use levels for a flavouring substance with standard portion sizes of flavourable foods. For flavouring substances with usages in multiple food categories (most cases), only the food category resulting in the highest potential dietary exposure is considered. This dietary exposure is taken to represent that of a 60 kg adult, regular consumer of a flavoured food, who is loyal to a specific product containing the specific flavouring of interest.

The CEF Panel considered that a new approach to dietary exposure assessment should be developed for newly submitted flavourings in order to cover the limitations of the methods that have been used until now. The following issues should be taken into account:

4.1 Chronic dietary exposure to flavouring substances from the consumption of flavoured foods and beverages in adults and children

Dietary exposure should be assessed in adults and children consuming foods and beverages containing the substance of interest. The highest of these values among adults and children, expressed per kg body weight (bw), should be used as the basis for the safety evaluation of the substance.

Poundage data are not considered to be useful for the quantitative assessment of dietary exposure to newly submitted flavourings. Surveyed poundage data of newly submitted flavourings used in foods and beverages could be provided only for non-EU regions (e.g. in the case of flavouring substances currently used in the United States) and these data may not be relevant for the EU situation. On the other hand, "anticipated annual production volumes" in the EU cannot be used since they bear a very high uncertainty.

The new method used to estimate the dietary exposure for adults and children is an adaptation of the TAMDI method called "Added Portions Exposure Technique" (APET). It is based on the standard portions developed by the JECFA when developing the SPET method and on the assumption that the consumer will daily consume a fixed amount of flavoured solid foods and liquids and that these items will always contain the flavouring substance under evaluation. Dietary exposure is then assessed based on the normal combined occurrence levels provided by the applicant in each of the food sub-categories reported in Table 1, with the exclusion of categories 13.1 and 13.2 (formulae for infants and complementary foods for infants and young children).

Sub-categories are classified in two groups: "Beverages", including all sub-categories of the category 14.0 ("Beverages, excluding dairy products") and "Solid foods", including all others sub-categories. For both, "Beverages" and "Solid foods", only the food category resulting in the highest potential dietary exposure is considered. This latest procedure is the same as that used by the SPET method. For both "Beverages" and "Solid foods" the dietary exposure is taken to represent that of a regular consumer of one flavoured product among the group, who is loyal to a brand containing the specific flavour of interest at the normal combined occurrence level.

The APET is calculated by summing the highest potential dietary exposure within each of the two groups ("Beverages" and "Solid foods"). Such an estimate, based on daily consumption of one single standard portion of beverage and one single standard portion of solid food is likely to provide a conservative assessment of long-term average dietary exposure for consumers of flavoured products. The APET is expressed in mg/kg bw per day. For an adult a body weight of 60 kg is considered and the portions are those established by the JECFA (FAO/WHO, 2008) when developing the SPET technique.

A child of 3 years of age will be considered in order to provide a conservative scenario for all children aged more than 3, since the consumption of foods and beverages per kg bw decreases with age. In the 3-year old child, a 15 kg bw is considered. Moreover, all sub-categories included in the sub-category 14.2 ("Alcoholic beverages, including alcohol-free and low-alcoholic counterparts") and the sub-category 13.4 ("Dietetic formulae for slimming purposes and weight reduction") are excluded *a priori* since it can be assumed that these products are not consumed by children. *Ad hoc* standard portions are used for each of the other sub-categories. These are derived from the adult standard portions. A correction factor was calculated to take into account their lower consumption of foods and beverages, based on their lower energy requirement. On average, a 3-year old child weighing 15 kg requires 6 MJ of energy whereas on average a sedentary adult requires 9.5 MJ (mean of 10.7 MJ in males and 8.3 MJ in females aged 30-59 years) (Commission for the European Communities, 1993). Standard portion sizes for children are therefore obtained by multiplying the adult standard portion sizes by a factor of 0.63. The value of 15 kg bw is then used to assess exposure in mg/kg bw in a 3 year old child.

The APET in the adult could be higher than the APET in the child only in few cases: if the highest dietary exposure from one portion of beverages is found in the sub-categories 14.2 or if the highest dietary exposure from one portion of solid food is found in the sub-category 13.4.

4.2 Dietary exposure to flavouring substances that may be used in foods specifically designed for infants and young children

The age class to which "infants" (0-12 months) and "young children" (12-36 months) refer are defined in the Commission Directive 2006/125/EC on processed cereal-based foods and baby foods for infants and young children (European Commission, 2006). Flavouring substances may be used in products specifically designed for these consumer subgroups. The potential dietary exposure to flavourings per kg bw is likely to be higher than that of adults in these two age classes. Dietary exposure will be assessed taking into consideration only the consumption of foods specifically designed for these two subgroups.

The diets of infants and young children tend to be less varied than those of older children and adults; an *ad hoc* method is therefore needed for estimating the exposure in this age group.

A specific exposure assessment will be performed based on the model diet of a 12-month infant fed milk and a variety of processed baby foods flavoured with the substance of interest.

Due to the high brand loyalty in infants and young children the maximum combined occurrence levels will be considered in this exposure assessment.

Details on how dietary exposure will be assessed in the 12-month infant are reported in Appendix 1.

4.3 Acute dietary exposure

Data on acute toxicity and acute dietary exposure will not be needed on a regular basis. However, in certain cases, acute dietary exposure may need to be assessed. Then, the assessment must be based on maximum concentration of flavouring substances in foods and beverages and on an estimate of the largest quantities (high percentiles) of foods or beverages that can be consumed by a subject within one day.

The General Standard for Food Additives (GSFA) category leading to the highest potential dietary exposure in one day will be identified and this value will be used as an estimate of acute dietary exposure. To this aim the maximum occurrence levels from all sources as reported in Table 1 will be used.

"Large portions" representing the 97.5th percentile of consumption on a single day have been established for Global Environment Monitoring System (GEMS) diet to assess acute dietary exposure to pesticides in adults and children (WHO, 2008) but are referred to agricultural commodities and not directly applicable. Large portions per kg bw were developed in the EFSA Opinion on camphor (EFSA, 2008b) for a limited number of foods and beverages, based on the large single day amounts of commodities (observed 97.5th percentile in one day among eaters only) in the INCA French individual consumption survey (Volatier *et al.*, 2006). The observed levels of consumption correspond, in a 60 kg bw adult to the consumption within one day of 840 g of soft drink, 96 g of candies, 144 g of cheese or 72 g of sauces. These values are about three times as high as the standard portions used in the APET for the corresponding categories (respectively 300 g, 30 g, 40 g and 30 g). Based on this observation, the acute dietary exposure will be assessed considering as large portions the consumption of three standard portions in one day of any of the food or beverage listed in Table 1.

The same will be done for 3-year old children with the use of their specific portions for all relevant foods and beverages categories. The highest value obtained among adults and children will be used in the safety evaluation as an estimate of potential acute dietary exposure.

These estimates of large portions may be refined by EFSA in the future on the basis of the databases of food consumption in EU countries that will be made available to the Data Collection and Exposure (DATEX)unit of EFSA.

No specific assessment of acute dietary exposure is required for infants and young children since the model diet used to assess chronic dietary exposure in this age class already takes into account maximum occurrence levels and a high level of consumption of flavoured foods specifically designed for them. In fact, due to the low day to day variability in consumption patterns in infants and young children, the model used to assess chronic dietary exposure is also adequate to assess acute dietary exposure.

The acute dietary exposure is calculated as follows:

Acute dietary exposure in adults and children (mg/kg bw) =

3 X highest value among (Standard portion for food category i (g) X upper use level in category i (mg/kg of product))/standard body weight.

4.4 Cumulative dietary exposure

Cumulative dietary exposure to flavouring substances structurally related to the substance under study is assessed within the Procedure for their safety assessment in order to ensure that the concomitant dietary exposure to all flavouring substances belonging to the same group does not exceed the capacity of the organism to metabolise them. To this aim an assessment of cumulative dietary exposure within one day is needed. Until now it was assessed by adding up MSDIs (the so called "combined intake"). A technique based on occurrence levels in foods is needed for newly submitted flavourings. In order to assess potential cumulative dietary exposure within one day the applicant shall provide occurrence levels not only for the newly submitted substance but also for structurally related substances which have already been evaluated in an FGE.

Potential cumulative dietary exposure within one day to flavouring substances structurally related to the newly submitted substance will be assessed.

The applicant shall identify all flavouring substances structurally related to the newly submitted substance and shall retrieve the most recent EU poundage data for these substances. Substances will be

ordered according to their poundage data. Those substances with the highest poundage data and responsible altogether for 30 % of the total poundage of the chemical group will be identified ("high poundage substances"). The applicant shall retrieve maximum occurrence levels for these substances used as added flavouring substances and use them to calculate the APET in adults and children, as described above, and to assess potential dietary exposure in infants (as described in Appendix 1).

The APET of the "high poundage substances" and of the newly submitted substance will be added up and used as an estimate of potential cumulative dietary exposure within one day, expressed in mg/kg bw per day, in adults and children, respectively.

For infants and young children, the potential cumulative dietary exposure within one day will be calculated by adding up the dietary exposure to the "high poundage substances" (assessed with the techniques described in Appendix 1) to that of the newly submitted substance and expressed in mg/kg bw per day.

The EFSA Scientific Committee on Exposure Assessments gave an Opinion (EFSA, 2005) that all sources of exposure should be taken into account, including non-food sources of exposure.

Any information on potential non-food sources, i.e. the exposure via dermal or inhalational routes should therefore be considered on the basis of data described in Section 3.2. The Panel is aware that at present for most flavouring substances quantitative data on their occurrence in non-food sources are rather limited. Nevertheless, the applicant should provide all available information on the exposure to the flavouring substance from consumer products allowing the estimation of the overall exposure and an evaluation of potential health risks arising from the addition of the flavouring substance to food.

III. ASSESSMENT OF THE GENOTOXIC POTENTIAL OF THE FLAVOURING SUBSTANCE

For any newly submitted flavouring substance its genotoxic potential has to be assessed in the first step. This assessment should start with *in vitro* tests, covering all three genetic endpoints, i.e. gene mutations, structural and numerical chromosomal aberrations. The following three *in vitro* tests would normally be required:

- a test for induction of gene mutations in bacteria (Ames test; OECD guideline 471)
- a test for induction of gene mutations in mammalian cells (preferably the mouse lymphoma *tk* assay with colony sizing; OECD guideline 476)
- an *in vitro* chromosomal aberration test (OECD guideline 473) or an *in vitro* micronucleus assay (Draft OECD guideline 487).

There may be circumstances under which it may be justified to deviate from the above-mentioned core set. In such cases a scientific justification should be provided and additional types of considerations or mechanistic studies may be needed.

One or more positive *in vitro* tests normally require follow-up by *in vivo* testing, unless it can be adequately demonstrated that the positive *in vitro* findings are not relevant for the *in vivo* situation. This is in line with the general strategy elaborated in the updated WHO/IPCS Harmonised Scheme on mutagenicity testing (Eastmond *et al.*, 2009).

The choice of the appropriate *in vivo* test is critical, due to different sensitivities, different endpoints and other variables. It requires expert judgement based on all available information, to be applied case-by-case. For this reason, a flexible approach is preferable to a fixed decision tree.

Guidance for the follow-up of positive results from *in vitro* assays could be taken from a guidance document issued recently by the European Chemicals Agency (ECHA, 2008) which recommends that any of the following tests may be conducted:

- a rodent bone marrow or mouse peripheral blood micronucleus test (OECD 474) or a rodent bone marrow clastogenicity study (OECD 475)

- a Comet (single cell gel electrophoresis) assay

- a test for gene mutations in a transgenic rodent model, *e.g.* using *lacI*, *lacZ* or *cII* as reporter gene

- a rat liver Unscheduled DNA synthesis (UDS) test.

According to this ECHA guidance (ECHA 2008), "the nature of the original *in vitro* response(s) (i.e. gene mutation, structural or numerical chromosome aberration) should be considered when selecting the *in vivo* study. For example, if the test substance showed evidence of *in vitro* clastogenicity, then it would be most appropriate to follow this up with either a micronucleus test or chromosomal aberration test or a Comet assay. However, if a positive result were obtained in the *in vitro* micronucleus test, the rodent micronucleus test would be appropriate to best address clastogenic and aneugenic potential. The rat liver UDS test may be appropriate for substances that appear preferentially to induce gene mutations, although the Comet and transgenic tests are also suitable (Kirkland D and Speit, 2008). These latter test systems offer greater flexibility, most notably the possibility of selecting a range of tissues for study on the basis of what is known of the toxicokinetics and toxicodynamics of the substance. It should be realised that the UDS and Comet tests are indicator assays detecting putative DNA lesions. In contrast, the transgenic test measures permanent mutations."

A combination of the *in vivo* micronucleus assay and the Comet assay in a single study as suggested by Pfuhler et al. (2007) would also be acceptable.

Other studies (e.g. DNA adduct studies) could also be relevant in order to clarify the mechanism of genotoxicity.

It should also be taken into account that the sensitivity (ability to detect carcinogens as positive) and specificity (ability to give negative results with non-carcinogens) of such assays have recently been analysed by Kirkland and Speit (2008).

Studies should be conducted using internationally agreed protocols. Test methods described by OECD or in European Commission Directives are recommended. The most up-to-date edition of any test guideline should be followed. Studies should be carried out according to the principles of Good Laboratory Practice (GLP) described in Council Directives 87/18/EEC and 88/320/EEC and accompanied by a statement of GLP compliance. Use of any methods differing from internationally agreed protocols should be justified. An OECD guideline is not yet available for the Comet assay. However, recommendations for an appropriate performance of the assay using OECD guidelines for other *in vivo* tests have been published and a standard protocol and acceptance criteria for this assay have been developed through the International Workshop on Genotoxicity Working Parties and international Comet assay workshops (Tice *et al.*, 2000; Hartman *et al.*, 2003; Burlinson *et al.*, 2007). Additional information could be taken from a website on the Comet assay (http://cometassay.com).

IV. EXAMINATION FOR STRUCTURAL/METABOLIC SIMILARITY TO FLAVOURING SUBSTANCES IN AN EXISTING FGE

The applicant should provide a proposal for the assignment of the newly submitted flavouring substance to an existing Flavouring Group Evaluation (FGE). This proposal has to be substantiated by appropriate experimental data or relevant evidence from the literature in order to demonstrate the structural/metabolic similarity to the substances in this FGE. The Panel will decide on these proposals on a case-by-case basis.

V. GROUP-BASED EVALUATION VIA THE PROCEDURE

If sufficient structural/metabolic similarity of the flavouring substance to flavouring substances in an existing FGE has been demonstrated, a group-based evaluation using the Procedure can be performed. The Procedure, referred to as the approach for a safety evaluation of chemically defined flavouring substances in Commission Regulation (EC) No 1565/2000 (EC, 2000), is shown in Figure 2 and explained in the following text.

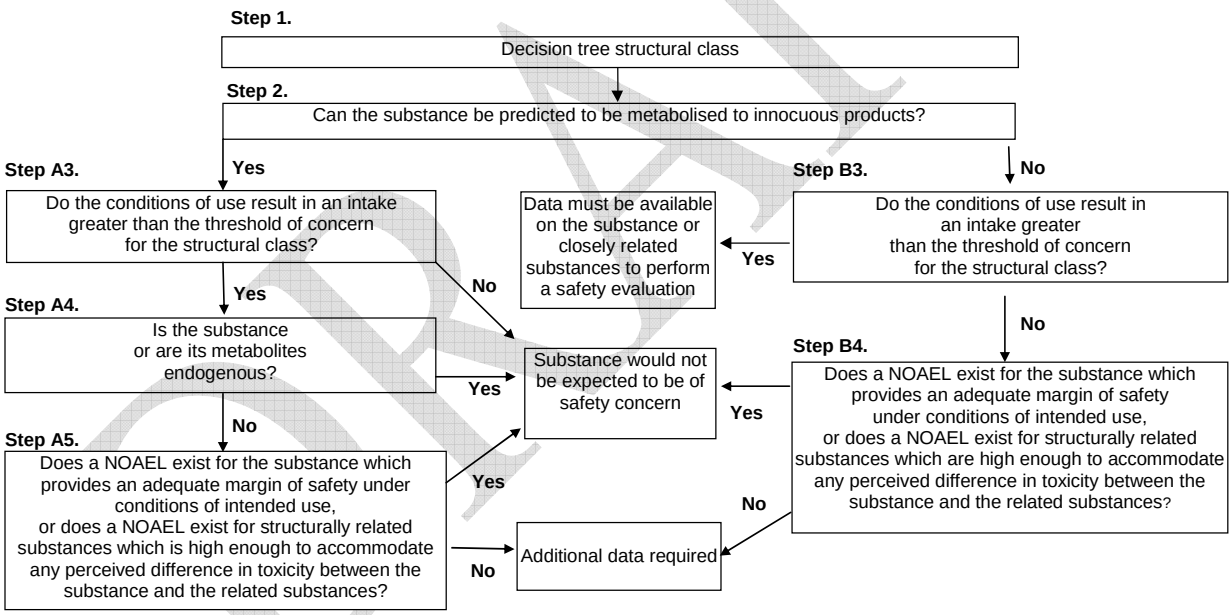


Figure 2: Procedure for the safety evaluation of chemically defined flavouring substances.

It is based on the Opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999), which is derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995; JECFA, 1996; JECFA, 1997; JECFA, 1999).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure-activity relationships, metabolism and, when needed, toxicity. One of the key elements in the

Procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) that are not considered to present a safety concern have been specified. Class I contains flavouring substances that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavouring substances that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavouring substances that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 1800, 540 or 90 microgram/person/day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996).

In addition to the data provided for the flavouring substance to be evaluated (candidate substance), toxicological background information available for compounds structurally related to the candidate substance is considered (supporting substances).

The Panel is of the Opinion that the principles of the above described Procedure should be applied to the evaluation of newly submitted flavouring substances if the substance can be assigned to one of the existing FGEs on the basis of structural and metabolic similarities.

Based on the experience gained from the evaluation of flavouring substances to be included into the Community list, particular attention should be paid to the following issues when applying the Procedure:

Step 2 of the Procedure

At Step 2 of the Procedure the question "Can the substance be predicted to be metabolised to innocuous products?" has to be answered.

"Innocuous products" are defined as metabolites that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring substance. The application of this definition requires that quantitative aspects related to the anticipated chronic exposure should be taken into account at this step of the Procedure (see Section 4.1). The assessment of the metabolites has to be substantiated by appropriate experimental data or relevant evidence from the literature.

Step A4 of the Procedure

At step A4 of the Procedure the question "Is the substance or are its metabolites endogenous?" has to be answered.

"Endogenous" substances are intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included.

The dietary exposure to flavouring substances that are, or are metabolised to, endogenous substances should be sufficiently low not to be expected to give rise to perturbations outside the physiological range.

Required toxicological data (Steps A5 and B4)

At step A5 and B4 of the Procedure the question "Does a No Observed Adverse Effect Level (NOAEL) exist for the substance which provides an adequate margin of safety under conditions of intended use, or does a NOAEL exist for structurally related substances which are high enough to accommodate any perceived difference in toxicity between the substance and the related substances?" has to be answered.

Regarding the first part of this question, generally, the minimum toxicological data required to establish a NOAEL to be used at these steps of the Procedure should be based on a repeated-dose oral (usually dietary) study in rodents of at least 90 days duration on the candidate substance or on an appropriate structurally and metabolically related substance in accordance with the most recent OECD guidelines.

The second part of the question envisages the situation where there is a NOAEL and a dietary exposure estimate, and the margin of safety under the conditions of intended use, resulting from these two parameters, is inadequate. Under these circumstances the default position would be that there is a safety concern.

If the outcome at step B3 of the Procedure is "Data must be available on the substance or closely related substances to perform a safety evaluation", more information, e.g. further toxicity data going beyond a 90-day study in rodents (see above) is generally required. Such a study may not be necessary if adequate biotransformation studies according to OECD guidelines are available. If, on the basis of these studies, the substance can be demonstrated to be metabolized to innocuous products, the substance could be evaluated via the A-side of the Procedure.

If multiple structurally/metabolically related flavouring substances refer to a NOAEL from the same chemical at step A5 or B4, these structurally/metabolically related flavouring substances should be identified and the applicant shall retrieve for all of them the most recent EU poundage data. The "high poundage ones" will be selected (those responsible altogether for 30 % of the cumulative poundage of these substances) and the applicant shall retrieve their normal use levels as added flavourings so as to calculate their APET. The APET of the high poundage substances will be added up for comparison with the NOAEL.

Intake data (Steps A3/B3 and A5/B4)

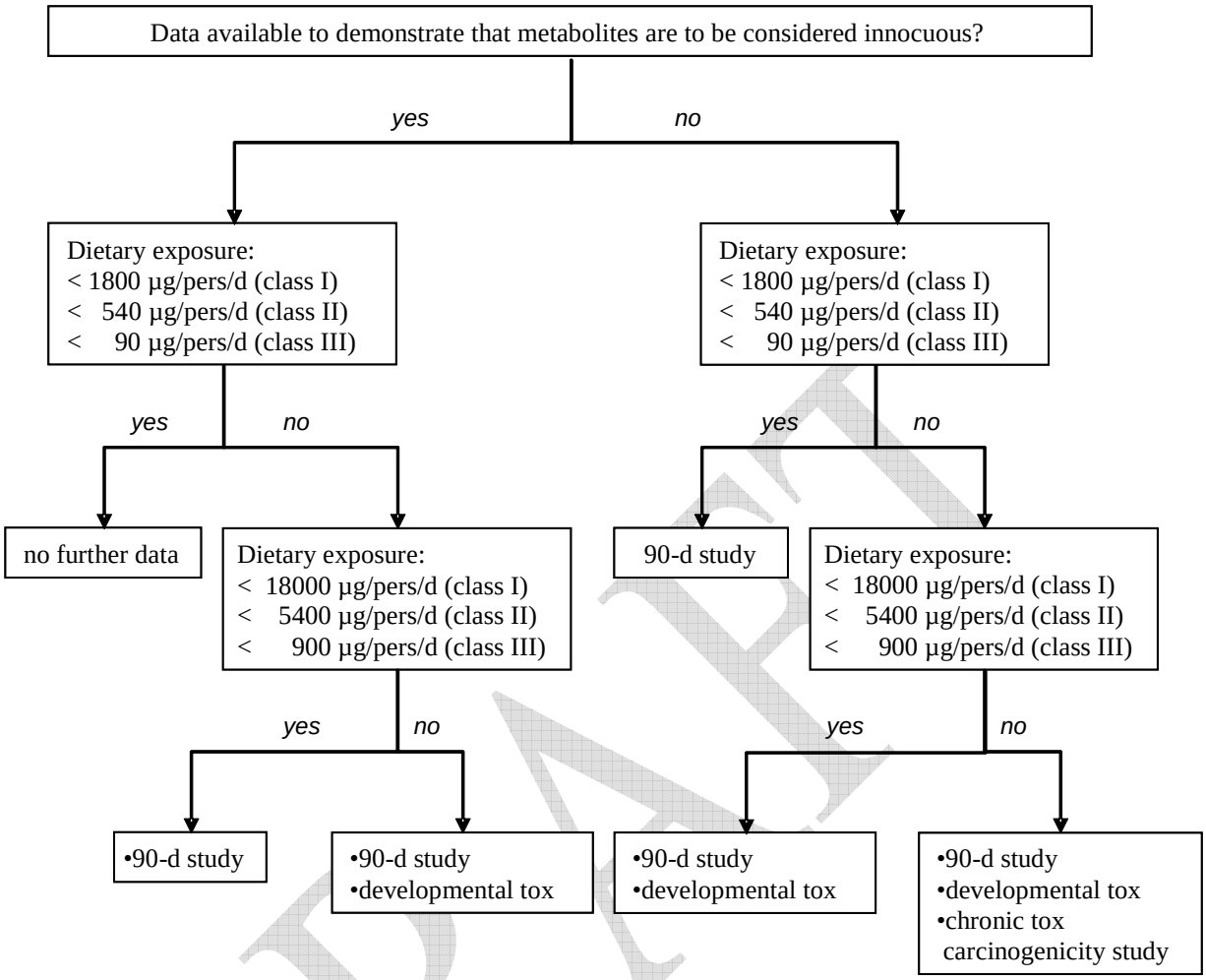
When applying the decision tree to the safety evaluation of a chemically defined flavouring substance used as a food improvement agent, the assessment of the "intake" as referred to at steps A3/B3 and of the "intended use" as referred to at steps A5/B4 should be based on the exposure resulting from the proposed addition of the flavouring substance to foods (See Chapter II). The conclusion drawn in this first part of the safety evaluation has to clearly reflect the underlying approach by stating, for example: "*The proposed use is not expected to be of safety concern at the estimated level of dietary exposure arising from its addition as a flavouring substance to foods*".

VI. INDIVIDUAL EVALUATION OF THE FLAVOURING SUBSTANCE

If a newly submitted flavouring substance cannot be assigned to one of the existing FGEs on the basis of structural and metabolic similarities, an individual evaluation has to be performed, given no safety concern with respect to genotoxicity (See Figure 1). A scheme outlining the principles of this evaluation is shown in Figure 3.

The type of toxicological data required depends on (i) whether there are experimental data available for the substance to demonstrate that the metabolites can be considered innocuous and (ii) whether the chronic dietary exposure, based on added use levels, is below or above the threshold of concern of the structural class to which the flavouring substance belongs.

Experimental data on the flavouring substance as such or on closely structurally related substances can be used as a basis to provide evidence that the metabolites of the flavouring substance are to be considered as innocuous.



596

597 **Figure 3: Individual evaluation of the flavouring substance.**

598 The experimental data for the various tests as indicated in Figure 3 should be provided for the parent
599 flavouring substance. Such data would implicitly cover the toxicity of the putative metabolites. When
600 studies from the past are available, such studies can be taken into consideration, but their acceptability
601 will depend upon their quality and the quality of the respective study report. New studies must be
602 performed according to current OECD or EU guidelines and must be in compliance with GLP.

603 As can be seen from Figure 3, the requirements for further toxicity data depend on the level of
604 exposure in comparison with the respective Cramer class threshold. For exposures below the
605 respective class threshold no additional toxicity data (innocuous metabolites) or a 90-day toxicity
606 study (metabolites not innocuous) should be available. For exposures up to 10-fold above the class
607 threshold a 90-day study or a 90-day study + a developmental toxicity study would suffice, depending
608 on whether metabolites are considered innocuous or not.

609 For higher exposures (i.e. more than 10-fold the respective class threshold) a more extensive data
610 package will be required. For substances which will be converted to noxious metabolites the data
611 requirements include also chronic toxicity and carcinogenicity data. Some background for the factor of
612 10 which determines the extent of the data requirements can be found in a paper by Cheeseman et al

(1999) in which a 3-tiered approach for thresholds of regulation is presented. The tier thresholds differ by a factor of 10.

Detailed considerations underlying the toxicological requirements outlined in this Chapter as well as in Chapter V have been elaborated in the Annex to the guidance on submissions for food additive evaluations by the Scientific Committee on Food (SCF, 2001).

Other studies may also be helpful or necessary for certain flavouring substances. Decisions on whether other studies are needed should be taken on a case-by-case basis. Examples of other areas of investigation which might be appropriate include, but are not limited to: immunotoxicity, allergenicity, intolerance reactions, neurotoxicity, human volunteer studies and predictive mechanistic studies.

There are also other toxicity studies that are not required for evaluation of the safety of flavouring substances, but which may have been conducted for other purposes, such as worker safety (e.g. acute toxicity, irritation and sensitisation studies). If such studies are available, they should be submitted as they may provide useful background information.

VII. CONSIDERATION OF THE NATURAL OCCURRENCE OF A FLAVOURING SUBSTANCE AND THE TOTAL EXPOSURE FROM FOOD AND NON-FOOD SOURCES

Total dietary exposure to flavouring substances should be assessed based on the overall concentrations of flavouring substances in foods and beverages derived from all possible sources (either naturally present, added as flavouring substance or present as residue from other uses) and the value obtained should be considered in the safety evaluation. Moreover, other non-food sources of exposure to flavouring substances will have to be considered.

As an important part of the overall safety assessment, the estimated level of exposure arising from the proposed addition of the flavouring substance to food should therefore be put into the context of any other sources of exposure. On the basis of the data described in Sections I. 3.1 and I. 3.2, the total exposure to the substance should be estimated. The Panel is aware that at present for most flavouring substances quantitative data on their natural occurrence in foods and on their occurrence in non-food products are rather limited. In its evaluation the Panel will take into account the amount of information made available and the level of uncertainty in the data. If the estimates of total exposure are high or if the estimates have a high level of uncertainty, the Panel may request further information on total exposure or may ask for more toxicological data.

PART B: FLAVOURINGS OTHER THAN FLAVOURING SUBSTANCES

In addition to flavouring substances, Article 9 of Regulation (EC) No 1334/2008 of the European Parliament specifies the following categories of flavourings for which an evaluation is required:

Flavouring preparations obtained from material of vegetal, animal or microbiological origin, other than food, by appropriate physical, enzymatic or microbiological processes, the material being taken as such or prepared by one or more of the traditional food preparation processes listed in Annex II of the Regulation;

Thermal process flavourings obtained by heating ingredients where ingredients for the production of thermal process flavourings are from source material other than food or the production conditions and/or the maximum levels for certain undesirable substances set out in Annex V of the Regulation are not met;

Flavour precursors obtained from source material other than food. Flavour precursor is a product not necessarily having flavouring properties itself, intentionally added to food for the sole purpose of producing flavour by breaking down or reacting with other components during food processing;

Other flavourings which are added or intended to be added to food in order to impart odour and/or taste and which do not fall under other definitions of flavourings;

Source materials other than food. These are materials of vegetable, animal, microbiological or mineral origin from which flavourings or food ingredients with flavouring properties may be produced.

As a first approach, for flavouring other than flavouring substances the following data should be provided:

Full description of the production process, with emphasis on the parameters that might influence the composition of the flavouring;

Identification and quantification of all substances present in the flavouring;

Specifications of the flavouring.

A risk assessment has to be performed on the basis of toxicological and exposure data.

NOTE: The Panel expects that this Part B would be fine tuned at the end of the public consultation.

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APPENDICES

APPENDIX 1

Dietary exposure in a 12 month young child

The method that needs to be used to estimate the dietary exposure is a model diet which uses standard portions sizes specific for young children. The USDA document that was used by the JECFA as a basis for the development of standard portion sizes in the adult also provides standard portions sizes for infants and toddlers up to 4 years(http://edocket.access.gpo.gov/cfr_2001/aprqtr/21cfr101.12.htm). The portions expressed as "ready to serve" were considered and an additional category was considered: growing up infant formula (one baby bottle corresponding to 200 g).

Dry instant cereals (with or without milk), including pasta: 110 g

Biscuits and cookies: 20 g

Fruit purée: 110 g

Fruit juice, herbal tea: 120 g

Meat based or fish based meal: 170 g

Dairy based dessert: 110 g

Vegetables, potatoes, broth, soups, pulses: 170 g

Milk for young children, ready to serve: 200 g.

Dietary exposure will be assessed by adding up the exposure from one standard portion of each of these foods and beverage categories, at the maximum combined occurrence level, as reported by the Applicant in Table 1. The value obtained will represent the dietary exposure in a 12 month infant consuming every day products containing the flavouring substance at its maximum use level. A standard bw of 10 kg will be used to assess dietary exposure in mg/kg bw per day.

APPENDIX 2.

Databases to be used by petitioners to assess normal and upper levels of concentration of flavourings naturally occurring in the different categories of foods and beverages (the list is not exhaustive):

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826 **Table 1 - Normal and maximum occurrence levels for refined sub categories8 of foods and beverages**

Group CODEX code	Food categories §	Standard portions for adults* (g)	Occurrence level as added flavouring substance		Occurrence level from other sources: as natural constituent and/or developed during the processing and/or as carry over resulting from their use in animal feed		Combined occurrence level from all sources (as added flavouring or from other sources) #	
			Normal	Maximum	Normal	Maximum	Normal	Maximum
01.1	Milk and dairy-based drinks	200						
01.2	Fermented and renneted milk products (plain), excluding food category 01.1.2 (dairy-based drinks)	200						
01.3	Condensed milk and analogues	70						
01.4	Cream (plain) and the like	15						
01.5	Milk powder and cream powder and powder analogues (plain)	30						
01.6	Cheese and analogues	40						
01.7	Dairy-based desserts (e.g., pudding, fruit or flavoured yogurt)	125						
01.8	Whey and whey products, excluding whey cheeses	200						
02.1	Fats and oils essentially free from water	15						
02.2	Fat emulsions mainly of type water-in-oil	15						

8

Group CODEX code	Food categories §	Standard portions for adults* (g)	Occurrence level as added flavouring substance		Occurrence level from other sources: as natural constituent and/or developed during the processing and/or as carry over resulting from their use in animal feed		Combined occurrence level from all sources (as added flavouring or from other sources) #	
			Normal	Maximum	Normal	Maximum	Normal	Maximum
02.3	Fat emulsions mainly of type water-in-oil, including mixed and/or flavoured products based on fat emulsions	15						
02.4	Fat-based desserts excluding dairy-based dessert products of category 1.7	50						
03.0	Edible ices, including sherbet and sorbet	50						
04.1.1	Fresh fruit	140						
04.1.2	Processed fruit	125						
04.1.2.5	Jams, jellies, marmalades	30						
04.2.2	Processed vegetables and nuts and seeds	200						
04.2.2.5	Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed purees and spreads (e.g. peanut butter)	30						
05.1	Cocoa products and chocolate products, including imitations and chocolate substitutes	40						
05.2	Confectionery, including hard and soft candy and nougats, etc., other than 05.1, 05.3 and 05.4	30						

Group CODEX code	Food categories §	Standard portions for adults* (g)	Occurrence level as added flavouring substance		Occurrence level from other sources: as natural constituent and/or developed during the processing and/or as carry over resulting from their use in animal feed		Combined occurrence level from all sources (as added flavouring or from other sources) #	
			Normal	Maximum	Normal	Maximum	Normal	Maximum
05.3	Chewing gum	3						
05.4	Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces	35						
06.1	Whole, broken or flaked grain, including rice	200						
06.2	Flours and starches (including soya bean powder)	30						
06.3	Breakfast cereals, including rolled oats	30						
06.4	Pastas and noodles and like products (e.g. rice paper, rice vermicelli, soya bean pasta and noodles)	200						
06.5	Cereal and starch-based desserts (e.g. rice pudding, tapioca pudding)	200						
06.6	Batters (e.g. for breading or batters for fish or poultry)	30						
06.7	Pre-cooked or processed rice products, including rice cakes (oriental type only)	200						
06.8	Soya bean products (excluding soya bean products of food category 12.9 and fermented soya bean products of food category 12.10)	100						
07.1	Bread and ordinary bakery wares	50						

Group CODEX code	Food categories §	Standard portions for adults* (g)	Occurrence level as added flavouring substance		Occurrence level from other sources: as natural constituent and/or developed during the processing and/or as carry over resulting from their use in animal feed		Combined occurrence level from all sources (as added flavouring or from other sources) #	
			Normal	Maximum	Normal	Maximum	Normal	Maximum
07.2	Fine bakery wares (sweet, salty, savoury) and mixed	80						
08.1	Fresh meat, poultry and game	200						
08.2	Processed meat, poultry and game products in whole pieces or cuts	100						
08.3	Processed comminuted meat, poultry and game products	100						
08.4	Edible casings (e.g. sausage casings)	1						
09.1.1	Fresh fish	200						
09.1.2	Fresh molluscs, crustaceans and echinoderms	200						
09.2	Processed fish and fish products, including molluscs, crustaceans and echinoderms	100						
09.3	Semi-preserved fish and fish products, including molluscs, crustaceans and echinoderms	100						
09.4	Fully preserved, including canned or fermented, fish and fish products, including molluscs, crustaceans and echinoderms	100						
10.1	Fresh eggs	100						
10.2	Egg products	100						

Group CODEX code	Food categories §	Standard portions for adults* (g)	Occurrence level as added flavouring substance		Occurrence level from other sources: as natural constituent and/or developed during the processing and/or as carry over resulting from their use in animal feed		Combined occurrence level from all sources (as added flavouring or from other sources) #	
			Normal	Maximum	Normal	Maximum	Normal	Maximum
10.3	Preserved eggs, including alkaline. salted and canned eggs	100						
10.4	Egg-based desserts (e.g. custard)	125						
11.1	Refined and raw sugar	10						
11.2	Brown sugar excluding products of food category 11.1	10						
11.3	Sugar solutions and syrups, and (partially) inverted sugars, including molasses and treacle, excluding products of food category 11.1.3	30						
11.4	Other sugars and syrups (e.g. xylose, maple syrup, sugar toppings)	30						
11.5	Honey	15						
11.6	Table-top sweeteners, including those containing high-intensity sweeteners	1						
12.1	Salt and salt substitutes	1						
12.2	Herbs, spices, seasonings and condiments (e.g. seasoning for instant noodles)	1						
12.3	Vinegars	15						

Group CODEX code	Food categories §	Standard portions for adults* (g)	Occurrence level as added flavouring substance		Occurrence level from other sources: as natural constituent and/or developed during the processing and/or as carry over resulting from their use in animal feed		Combined occurrence level from all sources (as added flavouring or from other sources) #	
			Normal	Maximum	Normal	Maximum	Normal	Maximum
12.4	Mustards	15						
12.5	Soups and broths	200						
12.6	Sauces and like products	30						
12.7.1	Salads 120 g (e.g. macaroni salad, potato salad) excluding cocoa- and nut-based spreads of food categories	120						
12.7.2	Sandwich spreads (20 g), excluding cocoa- and nut-based spreads of food categories	20						
12.8	Yeast and like products	1						
12.9	Protein productsa	15						
12.1	Fermented soya bean products	40						
13.2. a	Complementary foods for infants and young children: Dry instant cereals (with or without milk), including pasta							
13.2. b	Complementary foods for infants and young children: Meat based or fish based dinner							
13.2. c	Complementary foods for infants							

Group CODEX code	Food categories §	Standard portions for adults* (g)	Occurrence level as added flavouring substance		Occurrence level from other sources: as natural constituent and/or developed during the processing and/or as carry over resulting from their use in animal feed		Combined occurrence level from all sources (as added flavouring or from other sources) #	
			Normal	Maximum	Normal	Maximum	Normal	Maximum
	and young children: Dairy based dessert							
13.2. d	Complementary foods for infants and young children: Vegetables, potatoes, broth, soups, pulses							
13.2. e	Complementary foods for infants and young children: Biscuits and cookies							
13.2. f	Complementary foods for infants and young children: Fruit purée							
13.2. g	Complementary foods for infants and young children: Fruit juice							
13.2. h	Milk for young children							
13.3	Dietetic foods intended for special medical purposes (excluding food products of category 13.1)	200						
13.4	Dietetic formulae for slimming purposes and weight reduction	200						
13.5	Dietetic foods (e.g. supplementary foods for dietary use), excluding products of food categories 13.1–13.4 and 13.6	200						
13.6	Food supplements	5						
14.1	Non-alcoholic (“soft”) beverages	300						
14.2.1	Beer and malt beverages	300						

Group CODEX code	Food categories §	Standard portions for adults* (g)	Occurrence level as added flavouring substance		Occurrence level from other sources: as natural constituent and/or developed during the processing and/or as carry over resulting from their use in animal feed		Combined occurrence level from all sources (as added flavouring or from other sources) #	
			Normal	Maximum	Normal	Maximum	Normal	Maximum
14.2.2	Grape wines	150						
14.2.3	Mead	150						
14.2.4	Spirituous beverages	30						
15.1	Snacks, potato-, cereal-, flour- or starch-based (from roots and tubers, pulses and legumes)	30						
15.2	Processed nuts, including coated nuts and nut mixtures (with e.g. dried fruit)	30						
15.3	Snacks – fish based	30						
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) – foods that could not be placed in categories 01–15	300						

§ Most of the categories reported are the sub-categories of Codex GSFA (General Standard for Food Additives, available at http://www.codexalimentarius.net/gsfaonline/CXS_192e.pdf) used by JECFA in the SPET technique (FAO/WHO, 2008). In the case of category 13.2 (complementary foods for infants and young children), further refined categories have been created so that a specific assessment of dietary exposure can be performed in infants and small children.

* In case of foods marketed as powder or as concentrates, occurrence levels must be reported for the reconstituted product, considering the instructions reported on the product label or one of the standard dilution factors established by JECFA (FAO/WHO 2008):

- 1/25 for powder used to prepare water-based drinks such as coffee, containing no additional ingredients,
- 1/10 for powder used to prepare water-based drinks containing additional ingredients such as sugars (ice tea, squashes, etc.),
- 1/7 for powder used to prepare milk, soups and puddings,
- 1/3 for condensed milk.

$ In order to estimate normal values in each category, only foods and beverages in which the substance is present in significant amount will be considered (e.g. for the category “Fresh fruit” 04.1.1., the normal concentration will be the median concentration observed in all kinds of fruit where the flavouring substance is known to occur).

The normal and maximum combined occurrence levels of the substance will be assessed by the applicant either by adding up occurrence levels from added use to that from other sources or by expert judgment based on the likelihood of their concomitant presence. This will be done both for normal use levels and for maximum use levels.

842 **Table 2 - Normal and maximum occurrence levels in the main9 GSFA food categories**

Group CODEX code	Main GSFA food categories §	Standard portions for adults * (g)	Occurrence level as added flavouring substance		Occurrence level from other sources: as natural constituent and/or developed during the processing and/or as residue from animal feed		Combined occurrence level from all sources (as added flavouring or from other sources) #	
			Normal	Maximum	Normal$	Maximum	Normal	Maximum
01.0	Dairy products and analogues, excluding products of category 02.0	200						
02.0	Fats and oils and fat emulsions	50						
03.0	Edible ices, including sherbet and sorbet	50						
04.0	Fruits and vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes and aloe vera), seaweeds, and nuts and seeds	200						
05.0	Confectionery	40						
06.0	Cereals and cereal products derived from cereal grains, roots and tubers, and pulses and legumes, excluding bakery wares of food category 07.0	200						
07.0	Bakery wares	80						
08.0	Meat and meat products, including poultry and game	200						
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	200						
10.0	Eggs and egg products	125						
11.0	Sweeteners, including honey	30						

[9] The standard portions reported in the present table are, for each main GSFA category, the largest standard portion size among its sub-categories. They will be used to estimate dietary exposure assessment if the applicant can not provide occurrence data at the level of sub-categories as listed in table 1.

12.0	Salts, spices, soups, sauces, salads, protein products (including soya bean protein products) and fermented soya bean products	200						
13.0	Foodstuffs intended for particular nutritional uses	200						
14.0	Beverages, excluding dairy products	300						
15.0	Ready-to-eat savouries	30						
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) – foods that could not be placed in categories 01–15	300						

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844 § The categories reported are the main categories of Codex GSFA (General Standard for Food Additives, available at http://www.codexalimentarius.net/gsfaonline/CXS_192e.pdf).
845 * In case of foods marketed as powder or as concentrates, occurrence levels must be reported for the reconstituted product, considering the instructions reported on the product label or one of
846 the standard dilution factors established by JECFA (FAO/WHO 2008).
847 $ In order to estimate normal values in each category, only foods and beverages in which the substance is present in significant amount will be considered (e.g. for the category "Fruit and
848 vegetables" 04.0., the normal concentration will be the median concentration observed in all kinds of fruit and vegetables where the flavouring substance is known to occur).
849 # The normal and maximum combined occurrence levels of the substance will be assessed by the applicant either by adding up occurrence levels from added use to that from other sources or by
850 expert judgment based on the likelihood of their concomitant presence. This will be done both for normal use levels and for maximum use levels.

851 **ABBREVIATIONS**

852

AFC	Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
APET	Added Portions Exposure Technique
CAP	Common Authorisation Procedure
CAS	Chemical Abstract Service
CEF	Panel on Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CoE	Council of Europe
DATEX	Data Collection and Exposure unit, EFSA
DG SANCO	Directorate General for Health and Consumers
DNA	Deoxyribonucleic acid
EC	European Commission
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EINECS	European INventory of Existing Commercial chemical Substances
EP	European Parliament
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS	Flavour Information System database
GEMS	Global Environment Monitoring System
GLP	Good Laboratory Practice
GSFA	General Standard for Food Additives
IOFI	The International Organization of the Flavor Industry
IUPAC	International Union of Pure and Applied Chemistry

JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MoS	Margin of safety
MSDI	Maximised Survey-Derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NOAEL	No-observed-adverse-effect level
OECD	Organisation for Economic Co-operation and Development
SCF	Scientific Committee on Food
SPET	Single Portion Exposure Technique
TGD	Technical Guidance Document on Risk Assessment of Chemical Substances and Biocides
UDS	Unscheduled DNA synthesis
USDA	United Stated Department of Agriculture
WHO	World Health Organisation

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