GUIDANCE

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Scientific Guidance on the data required for the risk assessment of flavourings to be used in or on foods

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Abstract

Following a request from the European Commission, EFSA developed a new scientific guidance to assist applicants in the preparation of applications for the authorisation of flavourings to be used in or on foods. This guidance applies to applications for a new authorisation as well as for a modification of an existing authorisation of a food flavouring, submitted under Regulation (EC) No 1331/2008. It defines the scientific data required for the evaluation of those food flavourings for which an evaluation and approval is required according to Article 9 of Regulation (EC) No 1334/2008. This applies to *flavouring substances*, *flavouring preparations*, *thermal process flavourings*, *flavour precursors*, *other flavourings* and *source materials*, as defined in Article 3 of Regulation (EC) No 1334/2008. Information to be provided in all applications relates to: (a) the characterisation of the food flavouring, including the description of its identity, manufacturing process, chemical composition, specifications, stability and reaction and fate in foods; (b) the proposed uses and use levels and the assessment of the dietary exposure and (c) the safety data, including information on the genotoxic potential of the food flavouring, toxicological data other than genotoxicity and information on the safety for the environment. For the toxicological studies, a tiered approach is applied, for which the testing requirements, key issues and triggers are described. Applicants should generate the data requested in each section to support the safety assessment of the food flavouring. Based on the submitted data, EFSA will assess the safety of the food flavouring and conclude whether or not it presents risks to human health and to the environment, if applicable, under the proposed conditions of use.

Keywords: Food flavourings, flavouring substances, flavouring preparations, thermal process flavourings, flavour precursors, other flavourings, source materials

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Introduction

Background and Terms of Reference as provided by the requestor

In the European Union, flavourings are subject to Regulation (EC) No 1334/2008[1] on flavourings and certain food ingredients with flavouring properties for use in and on foods. This Regulation lays down among other elements the general requirements for the safe use of flavourings and defines different types of flavourings, amongst which the following categories are identified: flavouring substances, flavouring preparations, thermal process flavourings, flavour precursors, other flavourings, and source materials. It also sets out flavourings for which an evaluation and approval is required.

The flavourings for which an evaluation and approval are required are listed in Article 9 (a) - (f) of the Regulation (EC) No 1334/2008. Although Regulation (EC) No 1334/2008 specifies those flavourings for which an evaluation and an approval prior to being placed on the market is not required according to its Article 8 (a) – (d), under certain circumstances, the European Food Safety Authority (EFSA) can also be asked to evaluate these flavourings.

EFSA was asked in 2009 to provide the Commission with a document concerning the data required for the risk assessment of flavourings laying down amongst other aspects, the content, drafting and presentation of the application for the evaluation and authorisation of flavourings.

EFSA prepared the guidance in response to this request, which is essentially based on the two following main EFSA documents:

- Guidance on the data required for the risk assessment of flavourings to be used in or on foods of the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (EFSA CEF Panel, 2010)

and

- Proposed template to be used in drafting scientific opinions on flavouring substances (explanatory notes for guidance included) (EFSA, 2012).

EFSA is asked to update the above mentioned guidance documents and compile them in a single comprehensive document describing the data required for the risk assessment of new applications on flavourings submitted under Regulation (EC) No 1334/2008 and Regulation (EC) No 1331/2008[2] on the Common Authorisation Procedures for food additives, food enzymes and food flavourings and its implementing Commission Regulation (EC) No 234/2011.[3] The updated guidance is also expected to take into account the latest cross-sectional documents relevant for flavouring evaluations that have been developed by EFSA since the adoption of the current guidance documents on the risk assessment of flavourings.

Regulatory aspects

EFSA should also take into account the legislation on Food for Special Groups, Regulation (EU) 609/2013[4] in particular as regards infants and young children as well as the EFSA Scientific Committee's guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age (EFSA Scientific Committee, 2017a) so that the updated guidance addresses possible use and consumption of flavourings by that population group.

Whenever possible and appropriate the updated EFSA guidance should be consistent with the relevant guidance documents on food additives, as the two areas are closely related, taking also into

[1] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34–50.

[2] Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, p. 1–6.

[3] Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.3.2011, p. 15–24.

[4] Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. OJ L 181, 29.6.2013, p. 35–56.

account their differences in legislative aspects and safety requirements and the fact that both food additives and food flavourings are assessed by the same EFSA panel, the FAF panel.

In preparing this updated guidance, EFSA should take into account Regulation (EC) No 178/2002[5] and Regulation (EC) No 1331/2008, as amended by Regulation (EU) No 2019/1381[6] of the European Parliament and of the Council on the transparency and sustainability of the EU risk assessment in the food chain as well as Commission Regulation 234/2011 as amended by Commission Implementing Regulation (EU) 2020/1823[7]. Consistency should be ensured with other sectors where similar updates will be done.

Scientific and technical developments

When updating the guidance, EFSA should take into account the scientific and technical progress. For example, there have been significant developments in considerations on Threshold of Toxicological Concern related to flavourings. The so-called JECFA procedure for the assessment of flavouring substances has been modified at the 82nd JECFA meeting (JECFA, 2016). New methods for the dietary exposure assessment, as well as for the acceptability of the read across are now available for flavourings. New developments in the assessment of genotoxicity of substances and mixtures should be considered, together with new and/or updated OECD test guidelines.

There have also been developments in the techniques/approaches applied in the manufacturing of food flavourings and improvements in the performances of the analytical methods, which allow an in-depth characterisation of the final product, and its source materials. It also allows defining more accurately specifications for the material of commerce.

In addition, EFSA has gained very substantial experience as regards the safety assessment of flavouring substances and other flavourings both, on so-called existing flavouring substances under the old evaluation program and new flavouring substances.

Concerning dietary exposure assessment, the updated guidance should take into account that a number of substances and products can be, in addition to their use as flavourings, also be used in foods for other purposes. For example, they can be used, as food additives (e.g. sorbates, neohesperidin), food ingredients with physiological effects (e.g. caffeine), and food contact materials (e.g. ethyl acrylate), or may be related to plant protection products or cosmetics.

In the dietary exposure assessment specific consideration should be given to infants and young children, representing a particular vulnerable part of the population. Where relevant, this should reflect not only the consumption of foods intended for infants and young children defined in Regulation (EU) 609/2013, but also foods typically consumed by adults that may be consumed by infants and young children from a certain age.

The updated guidance should also take into consideration the scientific guidance from the EFSA Scientific Committee applicable for the assessment of substances intentionally added to foods intended for use by infants below 16 weeks of age.

Furthermore, EFSA should also take into account that the food categories used for regulatory purposes in flavourings are those mentioned in Part D of Annex II of Regulation 1333/2008[8] on food additives. This may be particularly relevant when carrying out more refined dietary exposure assessments based on actual use levels and detailed food consumption data across different population groups and scenarios.

Besides the safety aspects derived from the general requirements for flavourings, the protection of the environment should also be considered, where appropriate. In particular, experience shows that persistence in the environment may be a relevant issue for some products.

[5] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

[6] Regulation (EU) 2019/1381 of the European Parliament and of the Council of 20 June 2019 on the transparency and sustainability of the EU risk assessment in the food chain and amending Regulations (EC) No 178/2002, (EC) No 1829/2003, (EC) No 1831/2003, (EC) No 2065/2003, (EC) No 1935/2004, (EC) No 1331/2008, (EC) No 1107/2009, (EU) 2015/2283 and Directive 2001/18/EC. OJ L 231, 6.9.2019, p. 1–28.

[7] Commission Implementing Regulation (EU) 2020/1823 of 2 December 2020 amending Regulation (EU) No 234/2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 406, 3.12.2020, p. 43–50.

[8] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.

Smoke flavourings

Although smoke flavourings are a category of flavourings covered by Regulation 1334/2008, there are specific provisions, specific conditions of use and also specific EFSA guidance documents for this category of flavourings. The guidance on flavourings should therefore consider the specific guidance for smoke flavourings to ensure consistency but not to address their safety requirements as these are covered by specific guidance documents developed by EFSA (EFSA, 2021; EFSA FAF Panel, 2021).

Terms of reference as provided by the requestor

In accordance with Article 29 of Regulation (EC) No 178/2002, the Commission requests EFSA to update the Guidance on the data required for the risk assessment of applications on flavourings to be used in or on foods submitted under Regulation (EC) No 1331/2008.

It should take into account the information provided in the background and the experience gained with the assessment of the currently authorised flavourings. Where possible, EFSA should ensure consistency with guidance documents in other sectors.

The Commission requests EFSA to carry out this updating within 18 months from the receipt of this letter.

Interpretation of the Terms of Reference

This document is intended to provide guidance to applicants for the preparation of applications for the authorisations of new food flavourings as well as for modifications of existing authorisations of food flavourings, submitted under Regulation (EC) No 1331/2008. Such modifications may involve changes in the conditions of use, production processes or in the specifications.

All administrative information related to the preparation and submission of an application for a new authorisation or for a modification of an existing authorisation of food flavouring is addressed in a separate EFSA document, i.e. Administrative guidance for the preparation of applications on food improvement agents (food enzymes, food additives and food flavourings) (EFSA, 2021).

This guidance defines the data required for the evaluation of those food flavourings for which an evaluation and approval is required according to Article 9 of by Regulation (EC) No 1334/2008. This applies to (for more details, please refer to the section 'Definitions'):

- *flavouring substances*;
- *flavouring preparations* referred to in Article 3(2)(d)(ii) of Regulation (EC) No 1334/2008, i.e. obtained from material of vegetable, animal or microbiological origin, other than food;
- *thermal process flavourings* obtained by heating ingredients which fall partially or totally within Article 3(2)(e)(ii) of Regulation (EC) No 1334/2008, i.e. obtained from source material other than food, and/or for which the conditions for the production of thermal process flavourings and/or the maximum levels for certain undesirable substances set out in Annex V of the same Regulation are not met;
- *flavour precursors* referred to in Article 3(2)(g)(ii) of Regulation (EC) No 1334/2008, i.e. obtained from source material other than food;
- *other flavourings*;
- *source materials* other than food referred to in Article 3(2)(j)(ii) of Regulation (EC) No 1334/2008.

According to Article 8 of Regulation (EC) No 1334/2008, in case the Commission, a Member State or the Authority expresses doubts concerning the safety of a food flavouring for which an evaluation and approval are not required by default, a risk assessment of such food flavouring or food ingredient with flavouring properties shall be carried out by the Authority. This applies to (for more details, please refer to the section 'Definitions'):

- *flavouring preparations* referred to in Article 3(2) (d) (1) of Regulation (EC) No 1334/2008, i.e. obtained from food;
- *thermal process flavourings* referred to in Article 3(2)(e)(i) of Regulation (EC) No 1334/2008, i.e. obtained from food and which comply with the conditions for the production of thermal process flavourings and maximum levels for certain substances in thermal process flavourings set out in Annex V of the same Regulation;

- *flavour precursors* referred to in Article 3(2)(g)(i) of Regulation (EC) No 1334/2008, i.e. obtained from food;
- *food ingredients with flavouring properties*.

The data requirements for the evaluation of the above-mentioned food flavourings will follow the same principles as detailed in Sections 1–4 of this guidance document, which will apply mutatis mutandis.

As mentioned under the background and terms of reference as provided by the European Commission, smoke flavourings are excluded from the scope of this guidance, since specific EFSA guidance documents apply in that case, i.e. (EFSA, 2021a; EFSA FAF Panel, 2021).

Finally, it is reminded that the safety assessment of potential industrial emissions of food flavourings is not within the remit of EFSA and thus beyond the scope of the present guidance. The same applies for the evaluation of workers' safety.

Scope of the guidance and general principles

This guidance provides information on the type and quality of the data that are required by EFSA to assess whether a new food flavouring submitted for authorisation or a proposed modification of an already authorised flavouring is safe under the proposed conditions of use. Adherence to this guidance will help EFSA to carry out its evaluation and to deliver its scientific opinions in an effective and consistent way.

The main objective of applications for new food flavourings, as well as for the modification of existing authorisations, is to demonstrate that in the light of the current knowledge, they do not present risks to human health or to the environment, under the conditions of use, in line with Articles 1 and 4 of Regulation (EC) No 1334/2008.

This guidance has four main sections which reflect the structure that should be followed by applicants when preparing the scientific content of a technical dossier to support an application for the authorisation of a new food flavouring and/or for the modification of an existing authorisation.

- Section 1 contains the information specific to the characterisation of the food flavouring, including, depending on the type of flavouring, data on its identity, production process, compositional data, stability, reaction and fate in foods and specifications.
- Section 2 contains the information on existing evaluations from other regulatory bodies, if applicable.
- Section 3 contains the information on proposed uses and use levels and the exposure assessment.
- Section 4 contains the information on the safety of the food flavouring, including data on its genotoxic potential and other toxicological information, and information on the safety for the environment.

This document should be read in conjunction with the following Regulations, which are listed in chronological order:

- Regulation (EC) 178/2002, as amended by Regulation (EU) 2019/1381 of the European Parliament and of the Council of 20 June 2019 on the transparency and sustainability of the EU risk assessment in the food chain;
- Regulation (EC) 1,334/2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods.

In addition, the following guidance documents should be considered:

- Administrative guidance for the preparation of applications on food improvement agents (food enzymes, food additives and food flavourings) (EFSA, 2021).
- All relevant cross-sectional EFSA guidance documents cited throughout this guidance documents.

Applicants are advised to follow the most up-to-date scientific knowledge, the current scientific/methodological approaches and the latest versions of EFSA guidance documents and of any other relevant guidance document, including OECD test guidelines.

If applicable, the methods used to identify relevant scientific data or published literature, including the scope and the criteria for literature searches, should be described in line with the principles of the systematic review methodology (EFSA, 2010). In particular, the search methodology (search strategy,

search terms and databases searched) and the relevance and reliability assessment for any retrieved paper should be fully documented.

The data requirements described in this document will become applicable from the date of publication of the guidance in the EFSA Journal.

Definitions

As per Article 3 of Regulation (EC) No 1334/2008, the following definitions apply:

a) *'flavourings'* shall mean products: (i) not intended to be consumed as such, which are added to food in order to impart or modify odour and/or taste; (ii) made or consisting of the following categories: flavouring substances, flavouring preparations, thermal process flavourings, smoke flavourings, flavour precursors or other flavourings or mixtures thereof.

b) *'flavouring substance'* shall mean a defined chemical substance with flavouring properties.

c) *'natural flavouring substance'* shall mean a flavouring substance obtained by appropriate physical, enzymatic or microbiological processes from material of vegetable, animal or microbiological origin either in the raw state or after processing for human consumption by one or more of the traditional food preparation processes listed in Annex II of Regulation (EC) No 1334/2008. Natural flavouring substances correspond to substances that are naturally present and have been identified in nature.

d) *'flavouring preparation'* shall mean a product, other than a flavouring substance, obtained from:

   i) food by appropriate physical, enzymatic or microbiological processes either in the raw state of the material or after processing for human consumption by one or more of the traditional food preparation processes listed in Annex II of Regulation (EC) No 1334/2008 and/or

   ii) material of vegetable, 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 Regulation (EC) No 1334/2008.

e) *'thermal process flavouring'* shall mean a product obtained after heat treatment from a mixture of ingredients not necessarily having flavouring properties themselves, of which at least one contains nitrogen (amino) and another is a reducing sugar; the ingredients for the production of thermal process flavourings may be (i) food and/or (ii) source material other than food.

f) *'smoke flavouring'* shall mean a product obtained by fractionation and purification of a condensed smoke yielding primary smoke condensates, primary tar fractions and/or derived smoke flavourings as defined in points (1), (2) and (4) of Article 3 of Regulation (EC) No 2065/2003[9]. As explained in the paragraph 'Background and Terms of Reference as provided by the requestor' of the present guidance document, this type of flavourings is excluded from the scope of this guidance.

g) *'flavour precursor'* shall mean 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; it may be obtained from (i) food and/or (ii) source material other than food.

h) *'other flavouring'* shall mean a flavouring added or intended to be added to food in order to impart odour and/or taste and which does not fall under definitions (b) to (g).

i) *'food ingredient with flavouring properties'* shall mean a food ingredient other than flavourings which may be added to food for the main purpose of adding flavour to it or modifying its flavour and which contributes significantly to the presence in food of certain naturally occurring undesirable substances.

j) *'source material'* shall mean material of vegetable, animal, microbiological or mineral origin from which flavourings or food ingredients with flavouring properties are produced; it may be (i) food and/or (ii) source material other than food.

[9] Regulation (EC) No 2065/2003 of the European Parliament and of the Council of 10 November 2003 on smoke flavourings used or intended for use in or on foods. OJ L 309, 26.11.2003, p. 1–8.

Data required for the evaluation of a food flavouring

1. Characterisation

The following sections include the information that is required for the characterisation of a food flavouring, which may vary depending on the type of flavouring to be evaluated.

1.1. Flavouring substances

According to Article 3 of Regulation (EC) No 1334/2008, a *flavouring substance* shall mean a defined chemical substance with flavouring properties.

1.1.1. Identity

- Chemical name, when appropriate, according to IUPAC nomenclature rules.
- CAS-, E-, EINECS-, CoE-, JECFA-, FLAVIS- and FEMA-numbers (if attributed), and other identification numbers.
- Synonyms, trade names, abbreviations.
- Molecular and structural formulae, including SMILES linear notations, molecular weight.
- Spectroscopic data, e.g. MS, IR and NMR spectra or other data.
- Chromatographic data, e.g. capillary gas chromatography (including retention indices), high-performance liquid chromatography.
- Stereochemistry: for *flavouring substances* for which stereoisomers may exist, information must be provided on their configuration, i.e. whether it is one of the geometrical/optical isomers, or a defined mixture of stereoisomers. *Flavouring substances* with different configurations should have individual chemical names and codes (CAS number, FLAVIS number, etc.). In case individual registry numbers are not available, the name of the flavouring substance must provide an unequivocal assignment of the configuration.
- Physical properties: appearance, boiling point (for liquids), melting point (for solids), refractive index (for liquids), specific gravity (for liquids), solubility in water and other solvents relevant for use of the *flavouring substance* in foods and in toxicity/genotoxicity tests; influence of pH on solubility; octanol–water partition coefficient (Ko/w), vapour pressure. Study reports or other sources from which these data were taken should be included in the dossier.
  In case the *flavouring substance* consists of solid particles, please refer to Section 4.2 of the present guidance which outlines the technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles, in accordance with the EFSA Scientific Committee guidance (EFSA Scientific Committee, 2021a).
- Sensory properties: qualitative (odour/taste) and quantitative (odour/ taste thresholds or intensity/frequency descriptions of the sensory properties); or provision of data substantiating the function of the flavouring substance as modifier of odour and/or taste (e.g. concentration ranges needed).

1.1.2. Manufacturing process

Information on manufacturing should focus on the potential of the applied procedure to result in the presence of by-products (e.g. substances formed in the course of chemical synthesis), impurities (e.g. co-extracted substances) or contaminants (e.g. heavy metals) in the final *flavouring substance*. Therefore, for each manufacturing process, a detailed description should be provided covering the following information requirements.

1.1.2.1. *Flavouring substances* obtained by synthesis

Chemical synthesis

- Starting reagents; reaction sequence; side reactions; side products.
- Reaction conditions, e.g. time, temperature, pressure, solvents, catalysts; special precautions to the reaction conditions (if applicable).
- Physical and/or chemical purification steps employed to obtain the *flavouring substance*.
- Steps to prepare the material of commerce of the *flavouring substance*.

Enzyme-catalysed synthesis

Should the complete synthesis of the *flavouring substance* or certain steps of the reaction sequence be catalysed by (an) enzyme(s), the following information should be provided:

- Identity, function and source of the enzyme.
- CAS-, EC-number, if attributed.
- Starting substrate(s); enzyme-catalysed reaction step(s); side reactions; side products.
- Confirmation that the involved enzyme(s) has/have been assessed or is/are being assessed by EFSA in the framework of Regulation (EC) No 1332/2008[10] on food enzymes, the relevant EFSA question number(s) linked to the corresponding application for the food enzyme and the respective EFSA scientific opinion, if available, should be submitted.
- Demonstration of the inactivation and/or removal of the enzyme.

Microorganism-catalysed synthesis

Should the complete synthesis of the *flavouring substance* or certain steps of the reaction sequence be catalysed by a microorganism (e.g. bacteria, yeasts, filamentous fungi), the information should be provided according to Section 1 of the Scientific Guidance for the submission of dossiers on Food Enzymes (EFSA CEP Panel, 2021). In particular:

- The production microorganism should be characterised according to Section 1.1 of the Scientific Guidance for the submission of dossiers on Food Enzymes (EFSA CEP Panel, 2021).
- Information on the fermentation stage of the production of the *flavouring substance* should specify the type of the fermentation system used (e.g. continuous, (fed-) batch or solid state). A list of the raw materials contributing to the medium and a compilation of the reagents used for process control is required. These should be the actual materials used; an indicative list will not be accepted. For the raw materials which typically provide the nitrogen and carbon sources, which are included to meet mineral and vitamin requirements or used in pH control, only qualitative data are needed. Quantitative data may be required for medium ingredients of potential concern.
- The specific methods used to kill, disrupt and remove microbial biomass after completion of fermentation, to purify, concentrate and to remove microorganisms from the *flavouring substance* should be described, when applicable. For all substances used during downstream processing, the chemical identity, the CAS or any other unique identification number (if available) and the function should be provided. These should be the actual materials used; an indicative list will not be accepted.
- The absence of viable cells of the production strain in the *flavouring substanc*e should be demonstrated following section 1.3.4.1 of the Guidance for the submission of dossiers on Food Enzymes (EFSA CEP Panel, 2021). This applies to all food flavourings except those obtained using a non-genetically modified qualified presumption of safety (QPS) production strain.
- When the production strain has been genetically modified or contains acquired antimicrobial resistance genes, the absence of DNA from the production strain in the *flavouring substance* should be demonstrated following section 1.3.4.2 of the Guidance for the submission of dossiers on Food Enzymes (EFSA CEP Panel, 2021).
- In case a *flavouring substance* is produced from genetically modified organisms (GMOs), these have to be authorised in accordance with the provisions of Commission Regulation (EC) No 1829/2003[11] in order to prepare an application for the evaluation of the flavouring substance under Regulation (EC) No 1334/2008. The provisions for products of category 3 and 4 of the 'Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use' (EFSA GMO Panel, 2011) should be followed.
- Information regarding the possible production of toxic secondary metabolites, e.g. mycotoxins from the production strain.

[10] Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on food enzymes and amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, p. 7–15.

[11] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.

1.1.2.2. *Flavouring substances* obtained from material of vegetable, animal or microbiological origin

For this type of *flavouring substances*, information on the starting source material as well as information on the production process employed to obtain the *flavouring substance* from this source is required.

1.1.2.2.1. Source material

Plants:

In agreement with section 2.1.1.1 of the EFSA Guidance on the safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA Scientific Committee, 2009), the following information on the identity of the source material of plant-derived flavouring *substances* should be provided:

- Scientific (Latin) name (botanical family, genus, species, subspecies, variety with author's name, chemotype, if applicable) according to the international codes of nomenclature.
- Synonyms (botanical name) that may be used interchangeably with the preferred scientific name.
- Common names (if a trivial or a common name is used, it should be linked to the scientific name and part used).
- Part(s) used (e.g. root, leaf, seed, etc.).
- Geographical origin (continent, country, region).
- Growth and harvesting conditions (wild or cultivated, cultivation practices, time of harvest in relation to both season and stage of the plant growth).

Animals:

- Scientific (Latin) name (zoological family, genus, species, subspecies, breed, if applicable).
- Synonyms that may be used interchangeably with the preferred scientific name.
- Common names (if a trivial or a common name is used, it should be linked to the scientific name and part used).
- Part(s) used.
- Geographical origin (continent, country, region).

Microorganisms:

Information as described in Section 1.1.2.1 for *flavouring substances* obtained by microorganism-catalysed synthesis should be provided.

Mineral origin:

Information allowing unequivocal assignment of identity and authenticity of the material should be provided.

1.1.2.2.2. Production process

Physical process:

- Type of process, e.g. extraction, distillation.
- Key operational parameters, e.g. solvent, time, temperature, pressure; special precautions (if applicable).
- Physical and/or chemical purification steps.

Enzymatic process:

Information as described in Section 1.1.2.1 for *flavouring substances* obtained by enzyme-catalysed synthesis should be provided.

Microbiological process:

Information as described in Section 1.1.2.1 for *flavouring substances* obtained by microorganism-catalysed synthesis should be provided.

In addition, for all manufacturing processes mentioned in Section 1.1.2, a description of the measures implemented for production control and quality and safety assurance should be provided

(e.g. Hazard Analysis and Critical Control Points (HACCP), good manufacturing practices (GMP), International Organization for Standardization (ISO)).

1.1.3. Compositional data

- Purity assay value of the *flavouring substance*. Normally, the minimum purity should be at least 95%.
- Identification and quantification of chemical and biological impurities. The analysis should particularly focus on those impurities to be expected in the light of the employed manufacturing process. For the identification and quantification of the impurities, state-of-the-art techniques should be applied. Examples could be capillary gas chromatography coupled with flame ionisation detection and mass spectrometry or HPLC coupled with dedicated UV/MS detectors. Limits of detection and limits of quantification generally established and accepted for these techniques should apply.
- Unequivocal chemical identifications (names and, if available, CAS numbers) of the individual impurities should be provided. The criteria underlying the identifications should be clearly listed (e.g. which analytical methods used, use of authentic reference substances or use of tabulated chromatographic and mass spectral data of reference standards extracted from databases).
- The approach used for the quantification of the impurities should be described (e.g. response factors determined with authentic reference substances, GC area proportions, limits of quantification).
- Demonstration of batch-to-batch variability. Compositional data should be provided for at least five batches of the *flavouring substance* produced from different production runs. Information on how these batches were selected should be provided. In case, the intervals between production runs are long, data on fewer batches (at least three) might be considered acceptable; however, this would have to be justified on a case-by-case basis.

1.1.4. Stability

- Demonstration of the physicochemical and chemical stability of the *flavouring substance* upon storage of the material of commerce under conditions reflecting the intended shelf-life, i.e. assessment of the loss of the *flavouring substance* and identification and quantification of degradation products; investigation of the effect of storage conditions, such as temperature and environment (e.g. light, oxygen, moisture).
- Stability experiments may be performed under real-time conditions or under respective experimental, accelerated conditions ('forced ageing').

1.1.5. Reaction and fate in foods

- A method should be provided for the qualitative and quantitative analyses of the *flavouring substance* in the intended foods.
- Demonstration of the physicochemical and chemical stability of the *flavouring substance* upon storage of foods to which the *flavouring substance* is intended to be added; investigation of the effect of parameters such as storage temperature and light or pH and moisture content of the food.
- Demonstration of the physicochemical and chemical stability of the *flavouring substance* upon subjecting the foods to which the *flavouring substance* has been added to typically applied processing steps, e.g. heating.
- Information on the nature of interactions and reactions of the *flavouring substance* with constituents of the foods to which the *flavouring substance* has been added. Such information may encompass new experimental data with the flavouring substance, as well as existing literature data on structurally related substances.
- Stability experiments may be performed with the intended final foods under real-time conditions or in model systems mimicking the foods; justifications for the suitability of such model systems must be given.

1.1.6. Specifications

Applicants should provide specifications for the *flavouring substance* according to the format shown in Table 1, Appendix A. For all analytical parameters, the applied methods have to be included; if applicable, the respective limits of detection and limits of quantification have to be reported.

1.2. Flavouring preparations

According to Articles 3 and 9, respectively, of Regulation (EC) 1,334/2008, a *flavouring preparation* for which an evaluation and approval is required shall mean a product, other than a *flavouring substance*, obtained from material of vegetable, 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.

1.2.1. Identity

- Chemical name, when appropriate, according to IUPAC nomenclature rules.
- CAS-, E-, EINECS-, CoE-, JECFA-, FLAVIS- and FEMA numbers (if assigned), and other identification numbers.
- Synonyms, trade names, abbreviations.
- For a *flavouring preparation* of which individual components are identified the identity parameters listed under the first seven indents in Section 1.1.1 should be provided for each identified component.
- Physical properties: appearance, boiling point (for liquids), melting point (for solids), refractive index (for liquids), specific gravity (for liquids).
- In case the *flavouring preparation* consists of or contains solid particles, please refer to Section 4.2 of the present guidance which outlines the technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles, in accordance with the EFSA Scientific Committee guidance (EFSA Scientific Committee, 2021a).
- Sensory properties: qualitative (odour or taste) and quantitative (e.g. odour/taste thresholds or intensity/frequency descriptions of the sensory properties) or provision of data substantiating the function of the *flavouring preparation* as modifier of odour and/or taste (e.g. concentration ranges needed).
- Solubility in water and other solvents relevant for use of the *flavouring preparation* in foods and in toxicity/genotoxicity tests; influence of pH on solubility.

1.2.2. Manufacturing process

1.2.2.1. Source material

The information as described in Section 1.1.2.2.1 should be provided for the material of vegetable, animal or microbiological origin, other than food, used to obtain the *flavouring preparation*.

In addition, information has to be provided whether the material was used as such or whether one or more of the traditional food preparation processes listed in Annex II of Regulation (EC) No 1334/2008 have been applied.

1.2.2.2. Production process

The information as described in Section 1.1.2.2.2 for physical, enzymatic or microbiological production processes, respectively, has to be provided.

1.2.3. Compositional data

The components of the *flavouring preparation* should be characterised as fully as possible. This information is particularly required as basis for the component-based approach employed in the course of the genotoxicity assessment of flavouring preparations.

1.2.3.1. Identification and quantification of individual volatile components

For the identification and quantification of volatile constituents of *flavouring preparations* suitable state-of-the-art techniques should be used, e.g. capillary gas chromatography coupled with mass spectrometry (for identification) and with flame ionisation detection (for quantification). Unequivocal chemical identifications (names and CAS numbers) of the individual components of the volatile fraction should be provided. The criteria underlying the identifications should be clearly listed. In general, the identification of a component requires a comparison of at least two criteria, i.e. chromatographic (retention times or retention indices) and mass spectral data of the individual components with those

of authentic reference substances. The identification of a component must be considered as 'tentative' if authentic reference substances are not available and the identification is solely based on the comparison of mass spectral data of the components to those of a fragmentation mass spectral library.

'Tentatively' identified components should be considered as part of the unidentified fraction (see Section 1.2.3.3). However, the information gained in the course of the tentative identification of components may assist in the assessment of the unidentified fraction, by taking into account the structural elements and possible similarities to identified constituents. To this end, the criteria underlying the tentative identifications of the components should be clearly described. For example, it should be stated if the tentative identifications are based on the comparison of the chromatographic (retention times/indices, specifying the type(s) of stationary phase(s) used) and mass spectral data of the components to the corresponding tabulated data for the reference compounds (extracted from databases) or just based on the comparison of the mass spectrometry fragmentation pattern of homologous compounds. The analytical data supporting the tentative identifications performed should be provided.

Information on the concentrations of the individual components of the volatile fraction should be provided, as well as information on the principles underlying the quantification. For example, it should be stated whether internal standards or response factors have been used. Validation data for the limits of detection, limits of quantification, repeatability and reproducibility of the employed methods should be given.

If components of the volatile fraction remain unidentified, information on their quantitative contribution to the total volatile fraction should be provided, e.g. using peak areas determined by gas chromatography-flame ionisation detector (GC-FID) analysis to estimate the proportions of unidentified components.

1.2.3.2. Characterisation of the non-volatile fraction

Flavouring preparations may not only consist of volatile constituents but may also contain a non-volatile fraction. The Panel recognises the difficulties in identifying and quantifying individual components in the non-volatile fraction of *flavouring preparations*. However, applicants should make use of meanwhile routinely available analytical approaches, e.g. gel permeation chromatography (GPC) or high-performance liquid chromatography (HPLC) coupled with dedicated mass spectrometers. This should allow, for example, different classes to be characterised, and to get more detailed information on the non-volatile fraction.

1.2.3.3. Unidentified fraction

In case the components of the *flavouring preparation* could not be fully characterised, the proportion of the unidentified fraction (% m/m) in the flavouring preparation should be provided, encompassing unidentified volatile as well as non-volatile constituents, but excluding solvents present in the flavouring preparation. Any analytical information available to characterise the type and to estimate the proportions of chemical classes of components constituting the unidentified fraction should be presented. Explanations should be provided as to why the unidentified fraction could not be reduced via manufacturing steps and why no higher proportion of the product could be identified.

1.2.3.4. Batch-to-batch-variability

To demonstrate batch-to-batch variability, compositional data should be provided for at least five independent batches of the *flavouring preparation* produced in different production runs. Information on how these batches were selected should be provided. In case the intervals between production runs are long, data on fewer batches (at least three) might be considered acceptable; however, this would have to be justified on a case-by-case basis. The reproducibility of the batches should be judged based on the relative standard deviations of the data determined on individual components in the different batches. The similarity of the batches should be tested using appropriate statistical methods. The sole provision of GC chromatogram overlays is not sufficient to properly judge the batch-to-batch variability of a flavouring preparation.

1.2.4. Stability

— Demonstration of the physicochemical and chemical stability of the *flavouring preparation* upon storage of the material of commerce under conditions reflecting the intended shelf-life, i.e. assessment of the loss of individual constituents of the flavouring preparation and

identification and quantification of degradation products; investigation of the effect of storage conditions, such as temperature and environment (e.g. light, oxygen, moisture).
- The stability should be judged based on the data determined for individual constituents of the *flavouring preparation* at the different time points of storage. There is no fixed number of constituents which have to be assessed to demonstrate the stability of the flavouring preparation. However, the spectrum of the constituents selected should be representative of the chemical classes identified.
- Stability experiments may be performed under real-time conditions or under respective experimental, accelerated conditions (´forced ageing´).

1.2.5. Reaction and fate in foods

- The Panel is aware that a qualitative and quantitative analysis of *flavouring preparations* in food matrices is challenging. Therefore, a method for the analysis of representative, individual components of the *flavouring preparation* in the proposed food categories could be provided along with a justification for the selection of the components. The stability of the resulting analytical profile over time should then be followed.
- Stability studies may be performed with the respective foods under real-time conditions or in model systems; justifications for the suitability of the employed model systems must be given.

1.2.6. Specifications

Applicants should provide specifications of the *flavouring* preparation according to the format shown in Table 2, Appendix A. For all analytical parameters, the applied methods have to be included; if applicable, the respective limits of detection and limits of quantification have to be reported.

1.3. Thermal process flavourings

According to Article 3 of Regulation (EC) 1,334/2008, a *thermal process flavouring* shall mean a product obtained after heat treatment from a mixture of ingredients not necessarily having flavouring properties themselves, of which at least one contains nitrogen (amino) and another is a reducing sugar. According to Article 9 of Regulation (EC) No 1334/2008, an evaluation and approval is required for *thermal process flavourings* obtained by heating ingredients which are partially or totally source materials other than food and/or for which the conditions for the production of *thermal process flavourings* and/or the maximum levels for certain undesirable substances set out in Annex V of the Regulation are not met.

1.3.1. Identity

Thermal process flavourings are generally expected to be chemical mixtures. Accordingly, information regarding their identity as described in Section 1.2.1 for *flavouring preparations* has to be provided.

1.3.2. Manufacturing

Regarding the manufacturing of *thermal process flavourings*, the following information on the composition of the mixture subjected to thermal treatment has to be provided:

- Identities, purities and proportions of the nitrogen (amino)-containing ingredient(s).
- Identities, purities and proportions of the reducing sugar(s).
- Identities and proportions of other ingredients of the mixture subjected to heat treatment to obtain the *thermal process flavouring*. In case of plant-based, animal-based or microorganism-based ingredients, information as described in Section 1.1.2.2.1 for source materials used to obtain *flavouring substances* should be provided. In case chemically synthetised ingredients are used, information on their identities, purities and proportions should be provided.

In addition, the conditions of the process applied to obtain the *thermal process flavouring* have to be described. Information on key operational parameters, e.g. temperature, time and pH, have to be provided. Any specific conditions, e.g. high pressure, or special treatments (if applicable) should be described.

Physical and/or chemical purification steps employed to purify and/or to alter the composition of the mixture obtained upon the thermal treatment of the starting ingredients should be described.

1.3.3. Compositional data

The information as described in Section 1.2.3 for *flavouring preparations* has to be provided.

In addition, compositional analyses should focus on undesirable substances known to be formed upon thermal treatment of foods. This should include qualitative and quantitative data, for example, on heterocyclic aromatic amines, acrylamide and furan. Regarding the heterocyclic aromatic amines, it must be demonstrated that the maximum levels for 2-amino-3,4,8-trimethylimidazo [4,5-f] quinoxaline (4,8-DIMeIQx) and 2-amino-1-methyl-6-phenylimidazol [4,5-b]pyridine (PhIP), as set out in Annex V of Regulation (EC) No 1334/2008, are not exceeded. Depending on the source materials(s) and the production process, the analysis of other possible undesirable substances should be considered.

The analytical data provided should be supported by adequate certificates of analysis, specifying the methodology(ies) applied for the analytical determinations along with their respective performances (i.e. reporting how the LOD and LOQ values have been established by the laboratories).

1.3.4. Stability

Information as described in Section 1.2.4 for *flavouring preparations* should be provided.

1.3.5. Reaction and fate in foods

Information as described in Section 1.2.5 for *flavouring preparations* should be provided.

1.3.6. Specifications

Applicants should provide specifications of the *thermal process flavouring* according to the format shown in Table 3, Appendix A. For all analytical parameters, the applied methods have to be included; if applicable, the respective limits of detection and limits of quantification have to be reported.

1.4. Flavour precursors

According to Article 3 of Regulation (EC) No 1334/2008, a *flavour precursor* shall mean 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. According to Article 9 of Regulation (EC) No 1334/2008, an evaluation and approval is required for flavour precursors obtained from material other than food.

1.4.1. Identity

If the *flavour precursor* is a single substance, information as described in Section 1.1.1 has to be provided. If the *flavour precursor* is a chemical mixture, the information as described in Section 1.2.1 has to be provided. If the *flavour precursor* is (part of) a plant, animal or microorganism, information as described in Section 1.1.2.2.1 has to be provided. If the *flavour precursor* is of mineral origin information allowing unequivocal assignment of its identity and authenticity should be provided.

1.4.2. Manufacturing

Flavour precursors may be obtained by different manufacturing processes. Depending on the type of procedure employed, the following information has to be provided for flavour precursors:

- Obtained by synthesis (chemical, enzyme-catalysed, microorganism-catalysed): information as described in Section 1.1.2.1;
- Obtained by physical, enzymatic or microbiological processes from source material of vegetable, animal or microbiological origin: information regarding the source material as described in Section 1.1.2.2.1, as well as information regarding the employed production process as described in Section 1.1.2.2.2.

1.4.3. Compositional data

1.4.3.1. Compositional data on the flavour precursor

If the *flavour precursor* is a single substance, respective information as described in Section 1.1.3 should be provided. If the *flavour precursor* is a chemical mixture, information as described in Section 1.2.3 should be provided. If the flavour precursor is (part of) a plant, animal or microorganism, available information on the composition of such material which might be relevant considering the intended use as *flavour precursor* should be provided. At any rate, levels of contaminants (e.g. inherent plant toxins, mycotoxins, heavy metals, pesticide residues, polycyclic aromatic hydrocarbons, polyhalogenated organic chemicals) should be determined.

1.4.3.2. Compositional data on substances formed from the *flavour precursor*

1.4.3.2.1. Substances formed from the *flavour precursor* by breakdown

- Information should be provided on the conditions of use resulting in the intended breakdown of the *flavour precursor*.
- Data should be submitted showing the extent of breakdown (partial/complete) of the *flavour precursor*. The influence of the conditions of the intended applications (e.g. food matrix, temperature, pH) on the extent of breakdown should be described.
- If the *flavour precursor* is a chemically defined substance, information on the identities and proportions of the breakdown products should be provided.
- If the *flavour precursor* is a chemical mixture or is being applied in a complex food matrix, the data available to characterise the breakdown products are expected to vary; they may range from the identification/quantification of single compounds to a mere chromatographic profiling.

1.4.3.2.2. Reaction products of the *flavour precursor* with other components during food processing

- Information should be provided on the type of food and the food processing conditions resulting in the intended reactions of the *flavour precursor* with other components.
- Data should be submitted on the extent of reactions (partial/complete) of the *flavour precursor* with other components under the intended food processing conditions.
- If the *flavour precursor* is a chemically defined substance, information on the identities and proportions of the products resulting from the reaction with other components during food processing should be provided. If the *flavour precursor* is a mixture, it may be difficult to obtain this information.

1.4.4. Stability

If the *flavour precursor* is a single substance, information as described in Section 1.1.4 should be provided. If the *flavour precursor* is a chemical mixture, information as described in Section 1.2.4 should be provided.

1.4.5. Reaction and fate in foods

If applicable, methods able to identify and quantify the (remaining) *flavour precursor* in food should be provided. If the *flavour precursor* is a single substance, the nature of interactions and reactions of the flavour precursor with food constituents, other than those expected for the intended purpose of producing flavour, should be investigated.

1.4.6. Specifications

Applicants should provide specifications of the *flavour precursor* according to the format shown in Table 4, Appendix A. For all analytical parameters, the applied methods have to be included; if applicable, the respective limits of detection and limits of quantification have to be reported.

1.5. Other flavourings

According to Article 3 of Regulation (EC) No 1334/2008, *other flavouring* shall mean a flavouring added or intended to be added to food in order to impart odour and/or taste and which does not fall under the definitions of a *flavouring substance*, a *flavouring preparation*, a *thermal process flavouring* or a *flavour precursor*.

Considering this definition, it remains open what *other flavourings* might consist of, and it is difficult to anticipate what kind of materials will undergo an evaluation as *other flavouring*. This suggests that the standard evaluation template should be flexible.

Accordingly, for some of the requirements listed in this section, only key aspects and general principles of the information to be supplied are presented.

1.5.1. Identity

Other flavourings are chemical mixtures. Accordingly, information regarding their identity as described in Section 1.2.1 for *flavouring preparations* has to be provided.

1.5.2. Manufacturing

A detailed description of the employed procedure to obtain the *other flavouring* should be provided. The data should encompass information on the source material(s) used and on the process applied to obtain the flavouring. The information on the manufacturing should particularly focus on the potential of the applied procedure to result in the presence of by-products, impurities or contaminants in the final flavouring. Depending on the type of source materials used and processes applied to obtain the *other flavouring*, information as described in Sections 1.1.2.2.1 (source materials) and 1.1.2.2.2 (manufacturing) may apply.

1.5.3. Compositional data

Information as described in Section 1.2.3 has to be provided.

The data provided should take into account any peculiarities to be expected from the used source material(s) and the type of production process employed regarding the composition of the *other flavouring* and the presence of undesirable by-products/contaminants.

1.5.4. Stability

Information as described in Section 1.2.4 should be provided.

1.5.5. Reaction and fate in foods

Information as described in Section 1.2.5 has to be provided.

1.5.6. Specifications

Considering that *other flavourings* are chemical mixtures, the specifications to be provided by applicants should generally correspond to the format shown in Table 2, Appendix 1 for *flavouring preparations*. Any further parameters needed to complement the characterisation of the *other flavouring* in terms of identity or purity should be added.

1.6. Source materials

According to Articles 3 and 9 of Regulation (EC) No 1334/2008, *source material* for which an evaluation and approval is required shall mean material of vegetable, animal, microbiological or mineral origin other than food from which flavourings or food ingredients with flavouring properties are produced.

1.6.1. Identity

For material of vegetable, animal, microbiological or mineral origin other than food, information as described in Section 1.1.2.2.1 should be provided.

1.6.2. Manufacturing process

Information has to be provided whether the source material is intended to be used as such for the production of flavourings or food ingredients with flavouring properties or whether one or more of the traditional food preparation processes listed in Annex II of Regulation (EC) No 1334/2008 or any other preparation process is intended to be applied.

1.6.3. Compositional data

Analytical data on the presence of substances listed in Annex III of Regulation (EC) No 1334/2008 in the source material should be provided.

In addition, depending on the source and the intended manufacturing process(es) information on the presence of other undesirable substances, e.g. inherent plant toxins, mycotoxins, should be provided.

At any rate, levels of contaminants (e.g. heavy metals, pesticide residues, polycyclic aromatic hydrocarbons, polyhalogenated organic chemicals) should be determined.

1.6.4. Stability

Depending on the type of source material, data supporting the physicochemical, chemical and microbiological stability upon storage of the material under conditions reflecting the intended shelf-life should be provided.

1.6.5. Specifications

Applicants should provide specifications of the *source material* according to the format shown in Table 5, Appendix A. For all analytical parameters, the applied methods have to be included; if applicable, the respective limits of detection and limits of quantification have to be reported.

2. Information on existing evaluation from other regulatory bodies

Information on any existing evaluations and authorisations should be provided for the food flavouring. This should include details of the body which carried out the evaluation and when this was undertaken. Any relevant data/studies generated/conducted in the context of other regulatory frameworks should be provided in full, including the details of the evaluation in which reference point (s) and/or health-based guidance value(s) may be derived.

3. Proposed uses and exposure assessment

3.1. Data needed for the assessment of the dietary exposure to food flavourings

As described in the Terms of Reference, this guidance deals with applications for new food flavourings (i.e. *flavouring substances*, *flavouring preparations*, *thermal process flavourings*, *flavour precursors* and *other flavourings*) and for source materials, as well as modifications of already authorised food flavourings. Data needed to assess the (potential) dietary exposure to all types of flavourings are described below.

For assessing the dietary exposure to a new food flavouring, applicants should provide proposed maximum use levels[12] for each food category for which authorisation is requested. The food categories should be selected from those listed in Annex II, Part D, of Regulation (EC) No 1333/2008 as foreseen in Regulation No 1334/2008. In addition, applicants are encouraged to provide typical use levels for each food category. Typical use levels are the expected use levels of a food flavouring in foods.

Applicants are also encouraged to use the food categories of the FoodEx2 food classification system[13] for all use levels provided. FoodEx2 is a standardised food classification and description system developed by EFSA, which facilitates a better mapping of use levels to the relevant foods than based on the (broad) food categories in Annex II, Part D, of Regulation (EC) No 1333/2008.

The provision of typical use levels and the use of food categories of the FoodEx2 food classification system are not mandatory; however, this information will give EFSA the possibility to refine the exposure estimates. The link between the food categories in Annex II, Part D, to Regulation (EC) No 1333/2008 and the base terms of FoodEx2 is available.[14] FoodEx2 base terms are sometimes not sufficiently specific to link them with the food categories in Annex II, Part D of Regulation (EC)

[12] The Panel emphasises that maximum is the highest level of a food flavouring proposed in food and not the 95th percentile as referred to in e.g. Appendix D, FGE 5 revision 3.

[13] More information here: https://www.efsa.europa.eu/en/data/data-standardisation

[14] Mapping of FoodEx2 Exposure Hierarchy with the food categories of Annex II (part D) of Regulation (EC) No 1333/2008 on food additives: https://zenodo.org/record/4461577#.YBAaPuhKiUl

No 1333/2008, and therefore, additional information present in FoodEx2 (e.g. facets and original food descriptors, not shown in the abovementioned link) may be used by EFSA in the exposure assessment.

Food categories in which flavourings are authorised are usually very broad. In order to reduce possible overestimation of the dietary exposure, proposed maximum and typical use levels should preferably be provided for the specific food(s) in a food category in which the flavouring is expected to be used. For this, the FoodEx2 classification system should be used. The more detailed the information is on foods in which the flavouring may be used, the more accurate the dietary exposure estimate will be.

For compound foods, i.e. processed foods belonging to food category 18 in Annex II, Part D, of Regulation (EC) No 1333/2008, with ingredients in which the use of the flavouring is intended, the use levels should be provided per ingredient (at food name level).[15] It would be beneficial for the dietary exposure assessment if the quantities of the ingredients in the compound foods containing the flavouring are also specified.

In case of modifications of existing authorisations that would imply changes in the conditions of use of already authorised food flavourings, i.e. those for which the use is currently restricted, applicants should provide the same information as described above.

3.2. Information to be provided in case food flavourings are used for purposes other than use as a flavouring

Apart from being added to food as food flavouring, flavourings can also, e.g. be (i) naturally present in food, (ii) present because they are added to food as food additive or food ingredient or (iii) present due to their use in food contact materials or plant protection products. If relevant, applicants should provide qualitative and, if possible, quantitative information on the different dietary sources of the flavouring for which authorisation is requested. For this, data from literature (i.e. primary references as well as available databases, e.g. VCF[16]) could be considered.

Furthermore, flavourings may also be used in non-food sources such as cosmetics or tobacco products/tobacco replacement products ('electronic cigarettes'), etc. Qualitative and, if possible, quantitative information about this route of exposure should also be provided when relevant.

3.3. Exposure assessment

3.3.1. Dietary exposure assessment

The safety evaluation of substances intentionally added to food is based on food consumption data from the EFSA Comprehensive European Food Consumption Database[17] (Comprehensive Database). These data cover many EU countries and the following population groups: infants (from 16 weeks of age), toddlers (1–2 years), children (3–9 years), adolescents (10–17 years), adults (18–64 years) and the elderly (65 years and older).

If authorisation is requested in infant formulae, dietary exposure should be estimated for infants below 16 weeks of age following the recommendation of the EFSA Scientific Committee (EFSA Scientific Committee, 2017a).

- For the general population, including infants from 16 weeks of age and young children.

Applicants should provide dietary exposure estimates of a food flavouring by means of the Food Additive Intake Model (FAIM).[18] This model uses food consumption data from the Comprehensive Database to estimate the dietary exposure based on maximum or typical use levels. Consumption data are categorised according to the food categories in Annex II, Part D, of Regulation (EC) No 1333/2008. This tool is expected to overestimate the actual dietary exposure to food flavourings, which will be particularly pronounced when the flavouring is only used in specific foods within a food category as defined in Annex II, Part D, of Regulation (EC) No 1333/2008.

[15] See Note 4 in the Annex to Commission Regulation (EU) No 1321/2013, i.e. 'the presence of a smoke flavouring shall be permitted: (a) in a compound food other than as referred to in the Annex, where the primary product is permitted in one of the ingredients of the compound food; (b) in a food which is to be used solely in the preparation of a compound food and provided that the compound food complies with this Regulation'.

[16] https://www.vcf-online.nl/VcfHome.cfm

[17] https://www.efsa.europa.eu/en/data-report/food-consumption-data

[18] FAIM tool is described here: https://www.efsa.europa.eu/en/applications/food-improvement-agents/tools and can be accessed from here: https://dwh.efsa.europa.eu/MicroStrategy/servlet/mstrWeb

A second tool to estimate the dietary exposure, the DietEx tool,[19] is also available to applicants. This tool uses the same food consumption data as FAIM, but the data are categorised according to the FoodEx2 food classification system. As FoodEx2 includes more information on the foods coded in the food consumption data, this tool can potentially result in more accurate estimates of dietary exposure. Applicants are therefore encouraged to also use this tool to estimate the dietary exposure, but this is not mandatory.

Both dietary exposure tools calculate the exposure to a food flavouring by combining consumed amounts of foods recorded in the Comprehensive Database with use levels inserted by applicants. Applicants should perform separate calculations with the maximum and, if available, with the typical use levels, using FAIM (mandatory) and DietEx (optional). The tools provide mean and 95th percentile dietary exposure estimates and information on the contribution of the food categories to the mean dietary exposure to the food flavouring, for different population groups and EU countries.

If applicants require a use level for a food category that is not available in FAIM or DietEx, they should refer to its parent food category, i.e. the next higher level according to the food hierarchy. Furthermore, the level of detail of foods which could contain the food flavouring will often not be specific in these tools and consequently maximum or typical use levels will be assigned to parent food categories. Due to this, dietary exposure estimates provided by both tools are expected to overestimate the dietary exposure to the food flavouring.

Dietary exposure results obtained with the tools should be included in the dossier submitted by applicants. EFSA may refine the exposure assessment when the estimates provided by applicants result in an insufficient margin of exposure (MOE) (see Section 4.5.1.5). Such a refined exposure assessment will consider all submitted use levels (both maximum and typical levels (EFSA ANS Panel, 2017)) and aims at estimating the dietary exposure as realistically as possible based on the provided data. The refined dietary exposure assessment will be performed using the food categories in Annex II, Part D, of Regulation (EC) No 1333/2008, or FoodEx2 if the level of detail is sufficient. EFSA may use additional information, such as from the facets within FoodEx2[20] or from Mintel's GNPD,[21] to further refine the dietary exposure assessment. EFSA will consider also any additional information (such as market share data) provided by applicants to refine the dietary exposure assessment; however, the Panel does not consider it mandatory to submit this information.

If after such refinement steps, the MOE is still insufficient (see Section 4.5.1.5), applicants may submit proposals for use that would reduce the dietary exposure to the food flavouring.

Dietary exposure will be estimated for the population groups listed above if considered relevant. Consideration will also be given to the possibility that some consumers may be more highly exposed than the general population.

The risk assessment will be based on the dietary exposure estimates for high consumers (95th percentile estimated exposures) across relevant population groups and EU countries, based on the proposed maximum use levels either calculated with one or both exposure tools or using a refined exposure assessment.

In case of flavour precursors, the starting point of the exposure assessment is the use levels provided for the *flavour precursors* as such. Taking into account the information provided on the degree of breakdown and/or reaction products of the *flavour precursor* and on the qualitative and quantitative information of the formed substances (see Sections 1.4.3.2.1 and 1.4.3.2.2), the dietary exposure assessment to the remaining precursor and to the newly formed substances should be performed by applicants. In case the flavour precursor and/or its breakdown products react with food constituents, the information available on the resulting reaction products (see Sections 1.4.3.2.1 and 1.4.3.2.2) should be taken into account in the exposure assessment. If such information is not available, at least an assessment of the exposure to the remaining flavour precursor should be performed.

— For infants below 16 weeks of age

Until 16 weeks of age, infants have a diet that mainly consists of breastmilk or infant formulae. To assess the safety of foods consumed by young infants, EFSA issued a guidance on the risk assessment

[19] Described here: https://www.efsa.europa.eu/en/science/tools-and-resources and accessible from: https://www.efsa.europa.eu/en/science/tools-and-resources/dietex

[20] See the food classification and description system FoodEx2 (EFSA, 2015) and EFSA Catalogue browser User Guide, (EFSA, 2019).

[21] The Mintel's GNPD is an online database providing information available on the packaging of foods and drinks products.

of substances present in foods intended for infants below 16 weeks of age (EFSA Scientific Committee, 2017a). This guidance provides mean and high level consumption amounts of infant formulae (in mg/kg body weight (bw) per day) for assessing the dietary exposure to substances. Values of 200 and 260 mL/kg bw per day as conservative mean and high level consumption are recommended for substances that do not accumulate in the body. These values are derived from data for infants aged 2–4 weeks, when formula consumption is highest, expressed on a body weight basis. According to the guidance, for substances for which toxicokinetic studies indicate a long half-life and accumulation in the body, consumption values for infants of around 2 months of age (56–83 days) are proposed, i.e. around 170 (P50) or 210 (P95) mL/kg bw per day. At present, food consumption data for infants present in the Comprehensive Database do not allow to perform a risk assessment of substances present in food during the first 16 weeks of age.

Applicants should use the proposed consumption levels in the EFSA guidance for calculating the dietary exposure to a flavouring for infants below 16 weeks of age if it is intended for use in infant formulae.

3.3.2. Acute exposure assessment

EFSA may perform an acute dietary exposure assessment if needed based on the toxicity data. Acute exposure will be assessed for each reporting day in the Comprehensive Database by multiplying the total daily consumption amount for each relevant food by the maximum use level available for that food. Respective exposures for each relevant food consumed on that day (by the considered subject) will be summed and divided by the individual's body weight to provide an estimate of the exposure on that specific day. By doing this for all consumption days in the database, a distribution of daily acute exposure estimates is generated. From these distributions, a high (P95) acute intake will be calculated and used in the risk characterisation.

This assessment will be performed for the relevant population groups and EU countries present in the Comprehensive Database.

For infants below 16 weeks of age, considering the unique food in their diet being infant formulae, the 95th percentile of infant formulae consumption per kg body weight should be considered as maximum daily amount of that unique food consumed. This consumption amount will be multiplied by the maximum use level available for infant formulae to estimate the acute exposure in this population group.

3.3.3. Exposure assessment to the food flavouring coming from other sources

Depending on the available data and when relevant, applicants should provide exposure estimates of the food flavouring for each individual dietary source other than resulting from the addition as food flavouring and for each individual non-dietary source.

3.3.3.1. Exposure assessment from dietary sources other than food flavouring

If, based on the information provided by applicants (see Section 3.2), there is evidence that the flavouring occurs in food due to natural presence, addition as food additive or food ingredient and/or its use as (component of) food contact material and/or plant protection product, applicants should estimate dietary exposure from these sources, as described in Section 3.3.1.

3.3.3.2. Exposure assessment from non-dietary sources

Applicants should provide an exposure estimate of the food flavouring for each non-dietary source reported to contain the flavouring, e.g. cosmetics and 'e-cigarettes' (Section 3.2). The international agreed methodologies used by ECHA and the Scientific Committee for Consumers Safety should be considered to assess the exposure via these sources, as summarised in EFSA (2016). If information is available on exposure assessments resulting from non-oral sources (e.g. 'e-cigarettes') performed by other bodies, this may also be provided.

Based on the exposure estimates provided by applicants, EFSA will perform an aggregate exposure assessment based on the exposure via the different oral sources on a case-by-case basis. The resulting aggregate exposure estimate will be included in the risk characterisation. Non-oral sources will not be included in this aggregate exposure estimate, because this would require route to route extrapolation which is connected to very high scientific uncertainty.

4. Safety data

4.1. General considerations

Toxicological studies should be carried out with the food flavouring as intended to be marketed. Thus, depending on the type of flavouring submitted for evaluation applicants should submit data to demonstrate that (i) the test material has been manufactured according to (a) production process(es) as described in Sections 1.1.2, 1.2.2, 1.3.2, 1.4.2, 1.5.2, 1.6.2, respectively; (ii) it meets the compositional data as presented in Sections 1.1.3, 1.2.3, 1.3.3, 1.4.3, 1.5.3, 1.6.3, respectively; and (iii) it complies with the specifications proposed in Sections 1.1.6, 1.2.6, 1.3.6, 1.4.6, 1.5.6, 1.6.5, respectively. Since adequate human data on toxicity are unlikely to be available, *in vivo* studies using experimental animals are needed in order to assess possible risks to humans derived from the consumption of food flavourings. Toxicity studies should generally be conducted in accordance with OECD TGs. If a testing method is considered necessary or useful for which there is no OECD TG, this may be acceptable on a case-by-case basis under the condition that the method is based on an internationally validated experimental protocol. In any case, a statement of good laboratory practices (GLPs)[22] compliance is required.

4.2. Safety evaluation strategy regarding the presence of small particles including nanoparticles

The EFSA Scientific Committee published a Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles (EFSA Scientific Committee, 2021a).

This guidance is applicable to all chemical materials, including food flavourings, marketed or to be marketed as substances or mixtures, to be assessed by EFSA, including mixtures and products marketed as liquid formulations unless the information confirms that they are true liquids and do not contain small particles in suspension. In this document, the Scientific Committee establishes information requirements for conventional materials which do not meet the definition of engineered nanomaterial set out in the Novel Food Regulation (EU) No 2015/2283.[23] The guidance outlines appraisal routes (e.g. solubility/dissolution/degradation in water rate; particle size distribution; appropriateness of safety studies) to confirm that an assessment of the fraction of small particles including nanoparticles is not needed for the proposed food flavouring, or that this is already covered in the safety assessment process following the conventional sectorial guidance (i.e. the present guidance on food flavourings). In accordance with these technical requirements, scientific evidence supported by data should be provided confirming that:

a) the food flavouring meets the solubility or the dissolution rate criteria indicated in Section 2 of EFSA Scientific Committee (2021a), or
b) the food flavouring meets the screening or the quantitative criteria for particle size distribution indicated in Section 3 of EFSA Scientific Committee (2021a), or
c) the safety studies provided for the food flavouring are adequate for addressing the safety of the fraction of small particles, including nanoparticles, according to the principles indicated in Section 4 of EFSA Scientific Committee (2021a).

These information requirements cover complementary appraisal routes and it is sufficient to demonstrate that the food flavouring meets at least one of the decision criteria listed in Table 1 of the EFSA Scientific Committee guidance. Nevertheless, applicants may submit information on more than one appraisal route (EFSA Scientific Committee, 2021a).

If after retrieving the information, it cannot be demonstrated that the food flavouring meets at least one of the decision criteria listed in Table 1 of the EFSA Scientific Committee guidance (EFSA Scientific Committee, 2021a), data should be generated taking into account the requirements

[22] Directive 2004/10/EC of the European Parliament and of the Council of 1 February 2004 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances. OJ L 50, 20.2.2004, p. 44–59.

[23] Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. OJ L 327, 11.12.2015, p. 1–22.

established in the EFSA Scientific Committee Guidance on risk assessment of nanomaterials (EFSA Scientific Committee, 2021b).

4.3. Read-across

The principle of read-across is that toxicological information for one or more substances (source substance(s)) is used to predict the toxicological properties for other substances (target substance(s)), the latter being considered to be similar by scientific justification. Read-across may provide a possibility to avoid unnecessary toxicity testing in experimental animals.

In the past, grouping of flavouring substances in FGEs and application of read-across of toxicity and genotoxicity data has been extensively applied. In nearly all cases, this grouping or read-across has been done on the basis of simple comparison of two-dimensional representations of the chemical structures of the candidate and supporting flavouring substances. However, it is recognised that read-across on this basis alone may not be sufficiently robust (Patlewicz et al., 2013; ECHA, 2015).

The fundamental tenet of read-across is that structurally similar chemicals are expected to elicit similar effects. Hence, knowledge of one chemical (or a group of chemicals) can be used to predict the characteristics of similar chemicals. Since the intrinsic properties, potential interactions and ultimate effects of a chemical are encoded within its molecular structure, knowledge and comparison of chemical structures is central to read-across. At the same time, limitations to this approach should be carefully considered, e.g. absence of the same mechanisms of action or situations in which a change in structure (e.g. the presence/absence of a reactive substituent) leads to a substantial change in biological response.

Whilst structural similarity is the key tenet in developing a read-across grouping, a mechanistic justification and in particular toxicokinetic similarity are critical factors in ensuring acceptance. ADME studies are important to support or preclude read-across. These studies may demonstrate (dis) similarity of absorption and elimination routes, and (dis)similarities in metabolism. Therefore, the submission should include toxicokinetic studies (OECD TG 417 (OECD, 2010)) that address at least extent of absorption, Cmax, Tmax and T1/2 of the substance in blood or plasma, identification of tissues in which the substance or its metabolites may accumulate, identification and quantification (up to at least 90% of an oral dose) of urinary, faecal and exhaled metabolites. The studies should address the relationship between magnitude of exposure and toxicokinetic characteristics (dose-proportionality). To be useful to support read-across, data on the selected source substance (i.e. a 'data-provider' or 'supporting substance') should also be available to allow for a comparison of kinetic and metabolic profiles preferably in the same species. If read-across is applied using several source substances, such kinetic profiling should be provided for each source substance. This may be required to address different endpoints of toxicity, in cases where a different data package is available for each source substance. If read-across can only partially cover the toxicological data requirements, for those endpoints for which no data are available, additional toxicity testing will be necessary.

The rationale used to determine what characteristics a chemical should have in order to belong to a category or group, and hence be suitable for read-across, should be scientifically justified and transparently reported. Justification may be based on more than one criterion, e.g. structural and physicochemical parameters and ADME data/metabolic pathway. Multiple justifications increase the confidence in the category.

A case that deserves special attention is when read-across does not indicate a hazard. Such a read-across is more reliable if the target substance is part of a tested negative structural domain (i.e. populated by known and well-studied 'non-toxic'[24] substances, for which toxicological information is available on the endpoints for which read-across is intended). This means that similarity with well-known 'non-toxicants'[14] gives a robust indication of lack of toxicity, whereas lack of similarity with proven toxicants would not justify to waive a concern for toxicity.

When a read-across or category definition is accepted, some estimate should be generated with respect to toxic potency of the target substance. Read-across includes intrinsic uncertainty, since the target substance has not been tested. The observation of a quantitative trend in the experimental data for a given endpoint (e.g. increasing, decreasing or constant BMDL or NOAEL) across chemicals in a category can also be used as the basis for interpolation or extrapolation (i.e. trend analysis), thereby reducing this uncertainty. The inevitable uncertainty in read-across should be accounted for in the

[24] Placed between quotation marks because non-toxic/non-toxicants do not exist, as toxicity always depends on the dose.

evaluation of the adequacy of the calculated margins of exposure (MOE). This point has been recognised in REACH guidance document R.8 (ECHA, 2012a).

In case read-across analysis is applied by applicants, the general provisions outlined in ECHA guidance documents (ECHA, 2008, 2012b, 2013) should be followed.

An important requirement is that the scientific rationale and justification for the read-across are elaborated and documented thoroughly. A data matrix must be part of the documentation, in which it is indicated which are the reliable key study results for both source and target chemicals and what are the data gaps. Any applied read-across should be documented using the format as prescribed by ECHA (2008).

The endpoints covered by read-across should be compliant with the data requirements as prescribed in this guidance document (see Sections 4.3 and 4.4). The Panel will decide on the validity of any applied read-across on a case-by-case basis.

It should be noted that read-across will not be accepted to waive the provision of experimental genotoxicity data for new *flavouring substances* (EFSA Scientific Committee, 2011). If the new flavouring is a chemical mixture, the EFSA Scientific Committee guidance documents on mixtures will apply (EFSA Scientific Committee, 2019a, 2019b). Thus, read-across for genotoxicity and for endpoints other than genotoxicity will not be accepted for flavourings that consist of mixtures. However, for identified individual components in such mixtures, read-across for genotoxicity and for other toxicological endpoints could be applied, if experimental data are not available, in order to avoid a need for extensive toxicological testing (EFSA Scientific Committee, 2019a).

4.4. Genotoxicity

The assessment of the genotoxic potential of a new food flavouring should be carried out before embarking on any *in vivo* toxicity studies, other than to test for genotoxicity or to study toxicokinetics (ADME).

The approach to be followed for the generation and evaluation of the data on the genotoxic potential of food flavourings is described in the guidance documents of the EFSA Scientific Committee (EFSA Scientific Committee, 2011, 2017b, 2021c).

For food flavourings that consist of mixtures also the EFSA SC statement from 2019 is applicable (EFSA Scientific Committee, 2019a).

The different types of flavourings do require specific considerations that are described in the sections below.

4.4.1. Assessment of the genotoxic potential of *flavouring substances*

The first step is to test the *flavouring substance* in *in vitro* tests, covering all three genetic endpoints, i.e. gene mutations, structural chromosomal aberrations (clastogenicity) and numerical chromosomal aberrations (aneuploidy). As no individual test can provide information on all three endpoints, the Scientific Committee recommends the following two *in vitro* tests:

- a bacterial reverse mutation test, OECD TG 471 (OECD, 2020a),
- an *in vitro* mammalian cell micronucleus test, OECD TG 487 (OECD, 2016c).

The bacterial reverse mutation assay covers gene mutations and the *in vitro* micronucleus (MN) test covers both structural and numerical chromosome aberrations (CA).

The application of hybridisation with centromeric/telomeric probes (fluorescence in situ hybridisation (FISH)) or immunochemical labelling of kinetochores (CREST analysis) in the MN test provides information on the mechanisms of chromosome damage and micronucleus formation (clastogenicity and aneugenicity). In order to reliably differentiate between these mechanisms, the Panel strongly recommends using FISH analysis instead of CREST analysis due to the higher likelihood of false-negative results for aneugenicity by this test, as also reported in the EFSA Scientific Committee guidance on aneugenicity (EFSA Scientific Committee, 2021c).

If all *in vitro* endpoints are clearly negative in adequately conducted tests, it can be concluded with reasonable certainty that the substance has no genotoxic potential.

In the case of inconclusive, contradictory or equivocal results from the *in vitro* tests, it may be appropriate to conduct further testing *in vitro*, e.g. by repetition of a test already conducted, perhaps under different test conditions.

In the case of positive results from the basic battery of tests, it may be that further testing *in vitro* is appropriate to optimise any subsequent *in vivo* testing, or to provide additional useful mechanistic information, e.g. a FISH analysis in case of a positive *in vitro* MN test.

In case of one or more confirmed positive results obtained from an adequately performed set of *in vitro* assays, *in vivo* follow-up testing should be performed to assess whether the genotoxic potential observed *in vitro* is expressed *in vivo*.

The Scientific Committee recommends that *in vivo* tests should be selected based on the genotoxicity endpoint for which positive results were observed in the *in vitro* studies. In addition, the choice of the test should be based also on other relevant data on the test substance, such as information about chemical reactivity (which might predispose to site of contact effects), bioavailability, metabolism, toxicokinetics, and any target organ specificity. Additional useful information may come from structural alerts and read-across from structurally related substances (see section 4.2). The *in vivo* tests recommended by the EFSA Scientific Committee (EFSA Scientific Committee, 2011, 2017b, 2021c) are:

- *In vivo* transgenic rodent somatic and germ cell gene mutation assay, OECD TG 488 (OECD, 2020b), to follow-up *in vitro* positive results for gene mutations,
- *In vivo* mammalian alkaline comet assay, OECD TG 489 (OECD, 2016a) to follow-up *in vitro* positive results for gene mutations and/or structural chromosomal aberrations,
- *In vivo* mammalian erythrocyte micronucleus assay, OECD TG 474 (OECD, 2016b) to follow-up *in vitro* positive results for structural and numerical chromosomal aberrations. If there are any indications for aneugenicity, the EFSA guidance on aneugenicity (EFSA Scientific Committee, 2021c) should be consulted.

Transgenic rodent assays can detect point mutations and small deletions and are without tissue restrictions. The transgenic rodent assay can also be combined with the micronucleus assay. The *in vivo* Comet assay detects primary DNA damage and can be used with many target tissues. The MN assay and the Comet assay can be integrated in a repeated-dose toxicity study in order to fulfil animal welfare requirements, in particular the reduction in animal usage. A combination of an *in vivo* micronucleus and Comet assay, as recommended by the EFSA Scientific Committee (EFSA Scientific Committee, 2011), should be performed as a follow-up to a positive *in vitro* micronucleus assay.

If the *in vivo* testing provides negative results, the relevance of these findings should be evaluated based on the recommendations given by the OECD TG 474 and by the Scientific Committee (EFSA Scientific Committee, 2017b), concerning the demonstration of target tissue exposure. This may include the need for a full ADME study according to OECD TG 417 (OECD, 2010).

Overall, the interpretation of the genotoxicity data of chemically defined *flavouring substances* will be based on the recommendations given by the Scientific Committee in the relevant guidance documents on genotoxicity (EFSA Scientific Committee, 2011, 2017b, 2021c).

4.4.2. Assessment of the genotoxic potential of flavourings consisting of mixtures

4.4.2.1. Assessment of the genotoxic potential of *flavouring preparations*

Flavouring preparations may either be chemically fully defined mixtures or complex chemical mixtures containing a substantial fraction of unidentified components (see Section 1.2.3.3).

The recommended approach for the testing and the evaluation of genotoxic potential of this type of flavourings is described by the EFSA's Scientific Committee statement on genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019a) as well as by the EFSA scientific guidance for the preparation of applications on smoke flavouring primary products (EFSA FAF Panel, 2021). In line with these documents, a step-wise approach should be followed for the generation and assessment of the data, where first the mixture should be chemically characterised as fully as possible. Concentrations of the identified components in the *flavouring preparation* should be provided. The genotoxic potential of the chemically identified components should then be assessed individually, using all available data. Genotoxicity data should be collected and evaluated based on the Scientific Committee guidance documents on genotoxicity (EFSA Scientific Committee, 2011, 2017b, 2021c), as described in Section 4.4.1 for *flavouring substances*. Conclusions on genotoxicity are required for all identified components or at least for representative substances in case of structurally related identified components that could be grouped based on justified criteria (ECHA, 2008; ECHA, 2012b). Structure–activity relationship (SAR) information about the genotoxic potential of an identified component may

be considered when no adequate information on genotoxicity from published or unpublished studies is available. For more details on this aspect, please refer to section 4.2 on read across and to the recommendations described in sections 3.2 and 3.2.1 of the EFSA scientific guidance on smoke flavouring primary products (EFSA FAF Panel, 2021).

If the *flavouring preparation* contains one or more components that have been assessed (i.e. they are already known) to be genotoxic *in vivo* via a relevant route of administration, then the flavouring raises a concern for genotoxicity and the risk to human health related to this identified hazard needs to be taken into account in the risk assessment.

If a component of a *flavouring preparation* is evaluated to be genotoxic *in vivo* via a relevant route of administration and no relevant carcinogenicity data are available, it might be possible to apply the Threshold of Toxicological Concern (TTC) concept (EFSA Scientific Committee, 2019b). There would be no concern for genotoxicity only if the estimated exposure to the identified genotoxic component(s) is very low, i.e. below the TTC value of 0.0025 μg/kg body weight (bw) per day (or 0.15 μg/person per day) for DNA-reactive mutagens and/or carcinogens, and if the(se) component(s) is/are unavoidable from the production process of the *flavouring preparation*.

If none of the identified chemical substances in the *flavouring preparation* raises a concern for genotoxicity, the Scientific Committee recommends evaluating the genotoxic potential of the fraction of unidentified components. This applies only in case the *flavouring preparation* contains a substantial fraction of unidentified components and not in case all the components of the *flavouring preparation* have been fully identified, i.e. chemically fully defined mixtures.

Experimental testing of the fraction of unidentified components should be considered as a first option or, if this is not feasible and a scientific justification can be provided, the whole mixture should be tested following the testing strategy recommended by the Scientific Committee for individual chemical substances as described in Section 4.4.1 (EFSA Scientific Committee, 2019a).

Overall, for the interpretation of the genotoxicity data of *flavouring preparations*, recommendations are described in EFSA's Scientific Committee statement on genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019a) as well as in the EFSA scientific guidance for the preparation of applications on smoke flavouring primary products (EFSA FAF Panel, 2021).

4.4.2.2. Assessment of the genotoxic potential of *thermal process flavourings*

As mentioned in Section 1.3, *thermal process flavourings* are generally expected to be chemical mixtures. Accordingly, the recommendations as described in Section 4.4.2 for *flavouring preparations* should be followed.

4.4.2.3. Assessment of the genotoxic potential of *flavour precursors*

For *flavour precursors*, different scenarios may apply in line with Section 1.4:

A) For a *flavour precursor* that is a chemically defined substance or a mixture of chemically defined substances which have all been identified, it might be possible to demonstrate that the substance or the components in the mixture is/are completely broken down in food or have completely reacted with other components during food processing resulting either in identified substances only (Table 1 – scenario A1) or in identified and/or unidentified substances (Table 1 – scenario A2). Then, no exposure to the *flavour precursor* itself will occur, and therefore, the assessment of the genotoxic potential of the precursor as such does not need to be addressed. However, a genotoxicity assessment of the identified individual break-down and/or reaction products will be required in line with the approach described for *flavouring substances* in Section 4.4.1. In case however there are unidentified breakdown and/or reaction products (Table 1 – scenario A2), the genotoxic potential of these cannot be adequately studied, which would add uncertainty to the outcome of the assessment.

B) The *flavour precursor* is a chemically defined substance or a mixture of chemically defined components which have all been identified but for which, under the intended conditions of application, it cannot be demonstrated that the substance or the components in the mixture are completely broken down or that they have completely reacted with other components during food processing, resulting either in identified substances only (Table 1 – scenario B1) or in identified and/or unidentified substances (Table 1 – scenario B2). In such cases, the genotoxicity assessment of the *flavour precursor* and of the identified individual break-down and/or reaction products should be carried out according to the principles as described for

flavouring substances in Section 4.4.1. In case however there are unidentified breakdown and/or reaction products (Table 1 – scenario B2), the genotoxic potential of these cannot be adequately studied, which would add uncertainty to the outcome of the assessment.

C) If the flavour precursor is a chemical mixture containing a substantial fraction of unidentified components, it will be virtually impossible to demonstrate that these are completely broken down or that they have completely reacted with other components during food processing. In addition, it will also not be possible to fully identify all the breakdown and/or reaction products. In such cases, the genotoxicity assessment should follow the same strategy as described for scenario B2 in Table 1. The uncertainty related to the unidentified breakdown and/or reaction products will be larger than for scenario B2 (Table 1 – scenario C).

Table 1: Differentiation of safety assessment scenarios depending on the type of flavour precursor

Flavour precursor: chemically defined single substance or mixture in which all components have been identified			
A: 100% breakdown and/or reaction with other components during food processing		**B: < 100% breakdown and/or reaction with other components during food processing**	
SCENARIO A1	**SCENARIO A2**	**SCENARIO B1**	**SCENARIO B2**
All breakdown and/or reaction products identified	Not all breakdown and/or reaction products identified	All breakdown and/or reaction products identified	Not all breakdown and/or reaction products identified
Component-based approach for all identified products, according to *flavouring substances* + dose addition[a)]	Component-based approach for all identified products, according to *flavouring substances* + dose addition[(a)]; uncertainty for the unidentified breakdown and/or reaction products will remain	Component-based approach for remaining flavour precursor (constituents) and all identified products, according to *flavouring substances* + dose addition[(a)]	Component-based approach for remaining flavour precursor (constituents) and all identified products, according to *flavouring substances* + dose addition[(a)]; uncertainty for the unidentified breakdown and/or reaction products will remain
Flavour precursor: mixture containing a substantial fraction of unidentified components			
SCENARIO C: The assessment should follow the same strategy as described for scenario B2. The uncertainty related to the unidentified flavour precursor constituents and the unidentified breakdown and/or reaction products will be larger than for scenario B2.			

(a): Dose addition only applies to the evaluation of toxicity other than genotoxicity, as described in Section 4.5.2.2.

4.4.2.4. Assessment of the genotoxic potential of *other flavourings*

In general, the approach for genotoxicity assessment as described in Section 4.4.2.1 for *flavouring preparations* should be followed. However, due to the highly variable nature of *other flavourings* in specific cases, a different approach may need to be followed.

4.4.2.5. Assessment of the genotoxic potential of *source materials*

The Panel considers that potential genotoxicity of source materials will be covered by the genotoxicity assessment of the flavouring obtained from the source material. This flavouring will be subject to a comprehensive genotoxicity evaluation as described in the above-mentioned sections.

4.5. Toxicity other than genotoxicity

Applicants are reminded that, before conducting any testing to address toxicity other than genotoxicity, any concern for genotoxicity should be ruled out. Studies on ADME could be crucial for the interpretation of the results of genotoxicity studies *in vivo*.

4.5.1. Flavouring substances

4.5.1.1. Initial considerations for the toxicity data requirements

Article 10 of Commission Regulation (EU) No 234/2011 lists the data required for risk assessment of food flavourings. However, the Regulation does not explicitly specify which type of toxicity data are needed to evaluate the safety of flavouring substances. It only states that endpoints such as (sub) chronic toxicity, developmental toxicity and carcinogenicity should be covered *'where applicable'*.

From previous evaluations, it has become clear that for certain *flavouring substances* use levels and the related exposure estimates approached those observed for food additives. Therefore, it was considered appropriate to align the toxicological data requirements for flavouring substances as much as possible with those for food additives. Previously, the evaluation of food flavourings was based on application of the concept of thresholds of toxicological concern (TTC). This concept is based on the paradigm that when exposure to a substance is below a certain threshold (based on existing toxicological data of a variety of substances), no health risk to consumers is anticipated. It has been demonstrated that when exposure to a substance is below the TTC[25] of its corresponding structural class (Cramer I, II or III; for explanations, see Section 4.5.1.2.2), it can be assumed that the toxicity of the substance is sufficiently captured (WHO/EFSA, 2016; EFSA Scientific Committee, 2019b). However, when the exposure to a *flavouring substance* under the proposed conditions of use exceeds the TTC for its structural class additional toxicity data are needed in line with the data requirements for food additives. Similar to food additives, the toxicity data required for *flavouring substances* are set following a tiered approach. For *flavouring substances*, data requirements may be covered either by toxicity testing or by application of read-across (see Section 4.2).

The requested minimum purity of 95% ensures that at the highest intake at which the TTC principle would be applicable, i.e. 1800 μg/person per day for *flavouring substances* from Cramer class I, the maximum intake of (an) impurity(ies) would not be higher than 90 μg/person per day and thus not exceed the TTC for the(se) impurity(ies) even if they belonged to Cramer class III. In case exposure to the *flavouring substance* is higher than its TTC, additional toxicity data for the substance will be needed and this would implicitly encompass the toxicity of these impurities. For impurities for which the TTC concept does not apply (e.g. heavy metals), a separate assessment may be necessary.

The tiered procedure that will be followed is based on the previously applied Procedures for the evaluation of *flavouring substances* (EFSA CEF Panel, 2010) and the recently published guidance for the safety evaluation of smoke flavouring primary products (EFSA FAF Panel, 2021). The underlying rationale and detailed considerations for the toxicological requirements were set out in the guidance for submission for food additive evaluations (EFSA ANS Panel, 2012).

A flowchart outlining the recommended tiered toxicity testing for *flavouring substances*, as described in the following sections, is given in Appendix B.

In this guidance for the safety evaluation of *flavouring substances*, the toxicological data which are required depend on the magnitude of margins of exposure (MOE). Generally, for flavouring substances, an acceptable daily intake (ADI) will be derived (see Section 4.5.1.7). The safety evaluation of

[25] Based on its chemical structure a substance can be allocated to one of several structural classes for which different TTCs have been derived; for further information, see Section 4.5.1.2.2.

flavouring substances may also make use of toxicity data for structurally related substances following the procedures for read-across laid down in Section 4.3.

The sections below provide additional information and considerations on the respective steps and decisions to be made. The schemes by which it will be decided whether there is a need for additional toxicity testing are described in Appendix C – Figure C.1.

The steps and data requirements with respect to genotoxicity assessment have been discussed extensively in Section 4.4. As previously mentioned, *in vivo* studies should only be performed if there is no concern for genotoxicity. Exempt from this are studies to investigate genotoxicity *in vivo* and, if needed for that purpose, studies on toxicokinetics.

4.5.1.2. Data requirements at Tier I

Acute toxicity

Evaluation of acute toxicity is part of the safety assessment. However, in general, from past experience obtained from subchronic toxicity studies, there were no indications that chemically defined *flavouring substances* are acute toxicants. Therefore, there is no requirement to submit acute toxicity data, and evaluation of acute toxicity and related risk is not a part of the assessment. If applicants consider it appropriate, the WHO EHC 240 Section 5.2.9 (WHO/IPCS, 2009) could be consulted for derivation of an acute reference dose.

Assignment to Structural Class and application of the TTC approach

The initial step in the procedure is the assignment of a *flavouring substance* to a structural class according to Cramer, Ford and Hall, (1978). According to the Guidance on TTC (EFSA, 2019) and following the approach of Munro et al. (1996), the TTC that is applicable to that substance depends on the assigned structural class. In the risk assessment, it is decided that the proposed use of the respective *flavouring substance* is considered to raise no safety concern when the exposure(s) as estimated according to section 3.2 is (are) lower than this TTC.

In Cramer et al. (1978), three structural classes were identified:

- Structural class I which includes substances '*with structures and related data suggesting a low order of toxicity*',
- Structural class II which is 'intermediate' between class I and III; '*these substances are clearly less innocuous those of class I, but do not offer the basis either of the positive indication of toxicity or of the lack of knowledge characteristic of those in class III*', and
- Structural class III substances '*are those that permit no strong initial presumptions of safety, or that may even suggest significant toxicity*'.

Munro et al. (1996) derived TTC values of 1800, 540 or 90 μg/person per day were for structural classes I, II and III, respectively, taking up the proposal by Cramer for classifying substances. Further work extensively reported and discussed in the EFSA SC guidances of 2012 and 2019 and in the EFSA/WHO, 2016 report have endorsed the use of these values (expressed as 30, 9 or 1.5 μg/kg bw per day, on the basis of an individual body weight of 60 kg).

The evaluation of the exposure to a *flavouring substance* on the basis of the TTC approach follows the same procedural steps as those used by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in their updated procedure in 2016 (JECFA, 2016). This updated procedure was developed following a workshop on application of TTCs organised by EFSA and WHO (EFSA/WHO, 2016). It does no longer encompass the evaluation of the possible noxious/innocuous character of putative/anticipated metabolites. This step was considered superfluous, since, amongst other arguments, it is implicitly included in the assignment of a substance to a structural class. The need for this change has also been expressed in the Guidance document from the EFSA Scientific Committee in, 2019b.

The EFSA/WHO workshop also recognised that the allocation of a substance to a structural class is not always reproducible, since some of the steps in the Cramer et al. (1978) decision tree are ambiguous, difficult to interpret or not based on toxicological considerations. Therefore, as a starting point in future the Panel will use the OECD (Q)SAR Toolbox[26] as the standard tool for the allocation. However, an additional evaluation according to the tool as developed by Cramer Ford and Hall, as implemented in the TOXTREE tool may be useful to get an indication of the uncertainty in the allocation. When different software tools or expert judgement result in a different (in particular lower)

[26] https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm

Cramer classification than that based on the Toolbox, a justification should be provided. A reasoned comparison of outputs should be provided.

The EFSA/WHO (2016) workshop report and EFSA SC guidance documents (2012; 2019b) also indicated that a TTC of 0.3 μg/kg bw per day for organophosphates and carbamates could be applied. However, because – to date – no such substances have been used or notified as *flavouring substances*, this TTC is not included in the TTC evaluation process in this document, but it can be applied if an application for such a substance were submitted. The EFSA, 2019 Guidance also mentions a TTC of 0.0025 μg/kg bw per day for DNA-reactive genotoxic substances. This TTC will not be applied for the evaluation of *flavouring substances* but, in line with the EFSA SC guidance document (EFSA SC, 2011), it may be applicable for the evaluation of unavoidable impurities or of individual components of flavourings constituting mixtures (see Section 4.4.2).

Allocation of a substance to a structural class and thus application of the TTC criterion in the evaluation of a *flavouring substance* is not acceptable if that substance belongs to one of the exclusion categories as identified already in the publication by Cramer, Ford and Hall in 1978 and supplemented by a number of additional categories in the EFSA/WHO workshop report and the EFSA SC guidance documents (EFSA Scientific Committee, 2012; EFSA/WHO, 2016; EFSA Scientific Committee, 2019b). Amongst these categories are inorganic substances, proteins, nanomaterials, radioactive substances, organosilicon substances and metals in elemental, ionic or organic form.[27] When a substance belongs to a TTC exclusion category, Tier I is not applicable. For such a substance, the safety evaluation would start with Tier II.

If in Tier I, it is concluded that the exposure to the *flavouring substance* is above the class-specific TTC and reduction of exposure to the substance by limiting uses and use levels and/or by refining the exposure assessment (see Section 3.3.1) is not feasible, the safety assessment proceeds to Tier II. On the other hand, if the exposure is below its class-specific TTC and if the assessment of genotoxicity data does not raise a concern for genotoxicity, it can be concluded that the use of the flavouring substance is not of safety concern for the consumer.

4.5.1.3. Data requirements at Tier II

The subsequent text addresses the requirements for toxicity testing in Tier II. The data requirements given here would apply for any *flavouring substance* for which application of the TTC approach is not possible or for which exposure is above the TTC for its structural class.

4.5.1.3.1. Toxicokinetics (absorption, distribution, metabolism, excretion (ADME))

The requirement for ADME data is a new element in the assessment, compared to the previous guidance for the evaluation of flavouring substances (EFSA CEF Panel 2010). Note that this requirement is already a standard element of the safety evaluation of food additives. Note that ADME is not sufficiently covered by the TTC principle, since allocation of a substance to a structural class is limited to predictive qualitative metabolism based on the functional groups present in the molecule.

The requirement of ADME data is included for several purposes:

- ADME data may demonstrate the extent of absorption from the gastro-intestinal tract. If absorption is negligible, this may reduce the need for extensive toxicity testing (see Section 4.5.1.3.2). Regarding criteria to decide whether absorption is negligible, the guidance on food additives should be consulted (EFSA ANS Panel, 2012). The assessment of negligible absorption has to consider both the anticipated exposure and the extent of absorption, and therefore, this would have to be considered on a case-by-case basis and no generic cut-off value for negligible absorption can be given. An option to judge whether the condition of negligible absorption is fulfilled could be to compare internal exposures from the use as flavouring with the internal TTCs as suggested by Partosch et al. (2015). It should be noted that these internal TTCs cover both pre-systemic and systemic exposure. Application of *in silico* modelling approaches, including read-across, that estimate toxic potential based on anticipated internal exposure could also be considered on a case-by-case basis and should then be well documented.
- ADME data can inform on the extent of internal exposure and, in particular, on the extent of exposure of tissues relevant for genotoxicity testing, if needed.

[27] In the case of organic salts, where the counter ion is an essential metal (e.g. sodium), the Scientific Committee recommends that the TTC approach could be applied to the organic ion.

- ADME data will inform about the extent of metabolism and nature of metabolites, which may be helpful in the interpretation of observations on toxicity and genotoxicity and are important for the evaluation of environmental risk.
- ADME data will inform on the extent and rate of elimination from the circulation and the body, which could lead to a request for further studies (e.g. of longer duration than a 90-day oral toxicity study).
- ADME data are supportive for read-across, in particular when it is applied to predict *in vivo* endpoints. This applies especially when for a data-providing, structurally related substance also ADME data are available.

ADME studies should be performed according to OECD TG 417 (OECD, 2010) and should cover all aspects of kinetics (absorption, distribution, metabolism, excretion) *in vivo* (for an extensive listing, see also Section 4.3). When the safety evaluation of a substance will be limited to an evaluation through Tier I only (i.e. comparison of the exposure estimates with TTC leads to a conclusion of no safety concern), most aspects of ADME studies are of limited relevance. However, for the environmental risk assessment, knowledge on biotransformation products in animals or humans and/or biodegradability is essential and may therefore be requested. Also, when proof of target tissue exposure is needed for substances that have been found to be genotoxic *in vitro*, but non-genotoxic *in vivo*, ADME studies, and in particular studies on the distribution in target tissues of the parent compound and metabolites, are essential.

4.5.1.3.2. Testing for repeated dose, reproductive and developmental toxicity

For substances for which absorption cannot be considered to be negligible, data on subchronic oral toxicity (OECD TG 408 (OECD, 2018a)) and developmental and reproductive toxicity (OECD TG 443 (OECD, 2018b) should be submitted and their assessment proceeds immediately to Tier II scheme B/Tier III (see Appendix C).

In contrast, if, the absorption of the *flavouring substance* is considered negligible, in first instance, Tier II scheme A (Appendix C) is applicable, where initially a subchronic toxicity study is requested. If in this subchronic oral toxicity study no effects or only local effects are observed (i.e. in the gastrointestinal tract), or when systemic effects are secondary to such local effects (e.g. weight loss as a result of malabsorption of nutrients from the gastrointestinal tract or dehydration), the assessment can proceed further via Tier II scheme A. Based on the reference point from the subchronic oral toxicity study and the exposure estimates, an MOE can be calculated. This MOE should be sufficiently large to conclude that there is no safety concern. However, if in scheme A in Appendix C, the MOE is not large enough and there are no possibilities to (further) reduce the exposure estimate, then it will be concluded that the proposed uses are of safety concern. Since there is hardly any absorption in this leg of the approach, there will only be local effects. A chronic study would not contribute further to the risk assessment. Alternatively, an ADI could be calculated, and exposure should not exceed this ADI. For local effects in the gastrointestinal tract modified uncertainty factors may be applicable.

If, on the other hand, in this oral 90-day toxicity study, despite negligible absorption, still systemic effects (i.e. other than in the gastrointestinal tract) are observed, then the Tier II scheme A is no longer applicable, and the assessment should then proceed according to Tier II scheme B/Tier III. Accordingly, more extensive toxicity data should be generated by conducting an Extended One-Generation Reproductive Toxicity study (EOGRTS), according to OECD TG 443 (OECD, 2018b) (see Tier II scheme B/Tier III). Alternatively, data on all endpoints covered by the EOGRTS could be made available from other studies.

In the EOGRTS, testing should be in both male and female animals covering a defined pre-mating period (minimum of 2 weeks) and a 2-week mating period, with parental males being treated until at least the weaning of the F1, for a minimum of 10 weeks, and parental females during pregnancy and lactation until weaning of the F1. Dosing of the F1 offspring should begin at weaning and continue until scheduled necropsy in adulthood. The EOGRTS will provide information evaluating specific life stages not covered by other toxicity studies, i.e. on fertility and reproductive function, and on short- to long-term developmental effects from exposure during pregnancy, lactation and prepubertal phases, as well as effects on juveniles and adult offspring. In addition, an EOGRTS will provide information on immunotoxicity and neurotoxicity. This EOGRTS should always comprise the full arms of the parental cohorts as well as cohorts 1A, 1B, 2A, 2B and 3. It is recommended to perform a dose range-finding study, e.g. according to OECD TG 422 (Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening, Test No. 422 (OECD, 2016d), as also recommended

by OECD TG 443. It is not mandatory to perform such a study (OECD TG 422), if data are already available that would make a range-finding study superfluous.

The toxicity studies that are to be used in the assessment should be designed in such a way that they provide a reliable and useful lower confidence limit of the benchmark dose (BMDL)–upper confidence limit of the benchmark dose (BMDU) intervals[27] in accordance with the EFSA Guidance on Dose Response Modelling (EFSA Scientific Committee, 2022) or with the most recent version thereof. For all parameters studied, as specified in the respective OECD TGs, the data should be submitted in an appropriate electronic format (i.e. excel spreadsheet) that can be added to the study report and used as input file for dose–response modelling software, allowing a direct evaluation of the data included in the study report. On the basis of these data, applicants should perform a dose–response analysis for all relevant parameters (i.e. parameters for which a dose-related effect is observed, including those for which effects are observed only at the top dose) because the results of this analysis are part of the evaluation in Tier II. The reporting of the dose–response modelling should include its results and the information as specified in Appendix E of the EFSA Guidance on Dose Response Modelling (EFSA Scientific Committee, 2022).

The intention is to enhance reliability through the use of benchmark dose analysis. Therefore, by default for new studies dose–response modelling is required. However, for previous assessments in which NOAELs have been used, these may remain applicable.

4.5.1.4. Data requirements at Tier III

The decision to proceed to Tier III is based on the outcome of the Tier II testing for subchronic repeated dose toxicity and reproductive–developmental toxicity in combination with the outcome of the exposure assessment. A need for further data in a third Tier may emerge in the following situations:

(1) Observations from the EOGRTs (or alternatives to that, see section 4.5.1.3.2) may raise additional concerns so that based on this information, an appropriate reference point for the assessment cannot be derived, and thus, an MOE cannot be calculated. This would apply to the study legs that address repeated dose toxicity as well as the study legs that address reproductive and/or developmental toxicity. Such studies could be necessary to clarify the relevance of an observed effect for human health (e.g. prove that kidney effects in males are related to accumulation of α2-microglobulin) or to provide more insight to evaluate that an observed change is really a substance-related effect or just a chance finding.

(2) The following considerations apply in case an adequate reference point can be derived, but the MOE is too small. In such a case as in the first option, a reduction of exposure to the substance may be achieved by limiting uses and use levels and/or by refining the exposure assessment (see Section 3.3.1) which would increase the MOE. If reduction of exposure is not possible, as a second option, additional toxicity testing in Tier III will be needed.

For both aspects of toxicity (subchronic repeated dose toxicity and reproductive–developmental toxicity), sufficiently large MOE must be calculated to conclude that no additional toxicity testing or modification of proposed uses and/or use levels is needed.

4.5.1.5. Considerations with respect to the magnitude of the MOE

For repeated dose toxicity, conventionally in the case of smoke flavourings, an MOE of at least 300 is required (EFSA CEF Panel, 2010; EFSA FAF Panel, 2021) if the reference point originates from a 90-day subchronic oral toxicity study. The same cut-off value will be applied for *flavouring substances*. This criterion would not only apply to an MOE based on a no-observed-adverse-effect level (NOAEL) as reference point, but also to an MOE which is calculated from a BMDL, provided that the benchmark response (BMR) on which this BMDL is based, can be considered of toxicological significance (EFSA Scientific Committee, 2022).

An MOE of less than 300 (irrespective of whether it is based on a NOAEL or on a BMDL) would normally indicate that a combined chronic oral toxicity/carcinogenicity study, Test No. 453 (OECD, 2018d) would be required in Tier III testing.

A need for further testing in Tier III for chronic toxicity and/or carcinogenicity may also emerge from histological changes that could be indicative of potential pre-carcinogenic lesions, considering also their biological relevance (EFSA Scientific Committee, 2017c). An MOE which is lower than 100 obtained after Tier III testing for chronic toxicity/carcinogenicity would usually raise a safety concern.

In addition, a need for Tier III testing may emerge from toxicity observed in the EOGRTS on reproductive (including possible endocrine effects) and developmental toxicity parameters and/or

neuro- or immunotoxic effects in the different cohorts. In that case, the MOE criterion of 300 mentioned above may not apply. The minimal MOE requirement which is applicable for effects observed in the reproductive–developmental toxicity leg in the EOGRTS may well be less than 300, depending on the nature of the effects observed. However, no general strategy has been developed yet to give a precise cut-off value here and a case-by-case assessment will be needed to decide on the need for a follow-up in Tier III. Nevertheless, similar to what has been described above for repeated dose toxicity, applicants may try to eliminate the need for testing in Tier III by limiting the number of food categories for use of the *flavouring substance* and/or the maximum use levels applied. An adequate MOE should be available for all endpoints.

When use is made of read-across from one substance (the data provider) to another substance (the target substance), intrinsically additional uncertainty will be included. In such cases, an additional uncertainty factor needs to be considered when evaluating the adequacy of the MOE. All the toxicological endpoints that need to be covered (see the text in Section 4.5.1.3.2) should also be covered when read-across is used. The toxicity data do not need to come from only one data provider *per se*, as long as per data provider, the conditions for an appropriate read-across have been met (see Section 4.3). Nevertheless, the quality of the studies (in terms of compliance with GLP and OECD guidelines) underlying the read-across should be sufficient and the full study reports should be made available to EFSA for evaluation.

4.5.1.6. Derivation of an ADI

With the data generated in Tier II and/or Tier III, it is possible to decide whether a numerical ADI is needed for the *flavouring substance* and, if this is the case, to derive such a health-based guidance value. Conventionally for the derivation of an ADI uncertainty factors are applied to take into account toxicokinetic and toxicodynamic differences between species and between individuals. In addition, also uncertainty factors for study duration can be applied. For the determination of the magnitude of these uncertainty factors, the same reasoning may be applied as for the evaluation of the adequacy of the MOE (see above). When a numerical ADI is derived for a *flavouring substance*, exposure estimates should remain below this ADI in order to conclude that there is no safety concern for the *flavouring substance*, when used as proposed.

In case a numerical ADI is not needed, it can be concluded that the use of the *flavouring substance* is of no safety concern.

4.5.1.7. Application for authorisations for use in foods for infants and young children

The toxicity tests described above or the application of TTCs are generally considered not to be sufficient for the safety assessment of dietary exposure to chemical substances for infants below 16 weeks of age. For such applications, additional toxicity data are needed as recommended by the EFSA Scientific Committee Guidance (EFSA Scientific Committee Guidance, 2017a; EFSA Committee Guidance, 2019b).

Following these guidance documents, in principle no additional data would be needed if the evaluation of a substance proceeds to Tier II B. When the evaluation of a substance remains in Tier I or Tier II A, then studies in neonatal animals will be necessary.

The use of food flavourings in foods for young children (over the age of 16 weeks) is covered by the standard studies described above, in particular by the EOGRTS.

4.5.2. Flavourings that consist of mixtures

4.5.2.1. Flavouring preparations, thermal process flavourings, other flavourings

For the food flavourings covered in this section, the principles outlined by the EFSA Guidance on smoke flavourings primary products (EFSA FAF Panel, 2021) are to be followed for the assessment of potential toxicity. Basically, these principles are also reflected in the Tier II scheme B and Tier III data requirements (see above in Section 4.5.1.3.2 and in Appendix C) and considerations as outlined for *flavouring substances*. Data on acute toxicity and ADME will not be requested by default. In addition, similar to smoke flavouring primary products, for these materials read-across is not feasible. For these food flavourings, the toxicity testing should be based on the assessment of the whole mixture for derivation of the reference point and, if appropriate, of an ADI. For mixtures of which the individual constituents have been identified and quantified also a component-based approach may be followed, e.g. as applied by EFSA in a previous assessment (EFSA CEF Panel, 2017). Applicants are reminded

that, before conducting tests for *in vivo* toxicity, other than genotoxicity, any concern for genotoxicity should be ruled out.

4.5.2.2. *Flavour precursors* for which breakdown and/or reactions with other food constituents are intended

For *flavour precursors*, different scenarios may apply:

A) For a *flavour precursor* that is a chemically defined substance or a mixture of chemically defined substances which have all been identified, it might be possible to demonstrate that the substance or the components in the mixture is/are completely broken down in food or have completely reacted with other components during food processing resulting either in identified substances only (Table 1 – scenario A1) or in identified and/or unidentified substances (Table 1 – scenario A2). Then, no exposure to the *flavour precursor* itself will occur and therefore the toxicity of the precursor as such does not need to be addressed. However, a toxicity and safety assessment of the identified individual break-down and/or reaction products will be required in line with the approach described for *flavouring substances* in Section 4.5.1. Data should be made available to match with that approach, including ADME data. Subsequently, a safety assessment of the total of the identified breakdown and/or reaction products is required, based on the principle of dose addition (EFSA Scientific Committee, 2019a). In case, however, there are unidentified breakdown and/or reaction products (Table 1 – scenario A2), the safety of these cannot be adequately studied. This would add uncertainty to the outcome of the assessment. A possible option to reduce this uncertainty is given below scenario C in this section.

B) The *flavour precursor* is a chemically defined substance or a mixture of chemically defined components which have all been identified but for which, under the intended conditions of application, it cannot be demonstrated that the substance or the components in the mixture are completely broken down or that they have completely reacted with other components during food processing, resulting either in identified substances only (Table 1 – scenario B1) or in identified and/or unidentified substances (Table 1 – scenario B2). In such cases, the toxicity and safety assessment of the *flavour precursor* and of the identified individual breakdown and/or reaction products should be carried out according to the principles as described for *flavouring substances* in Section 4.5.1. Data should be made available to match with that approach, including ADME data. Subsequently, a safety assessment of the total of the identified breakdown and/or reaction products and of the remaining flavour precursor is required, based on the principle of dose addition (EFSA Scientific Committee, 2019a). In case however there are unidentified breakdown and/or reaction products (Table 1 – scenario B2), the safety of these cannot be adequately studied. This would add uncertainty to the outcome of the assessment. A possible option to reduce this uncertainty is given below scenario C in this section.

C) If the *flavour precursor* is a chemical mixture containing a substantial fraction of unidentified components, it will be virtually impossible to demonstrate that these are completely broken down or that they have completely reacted with other components during food processing. In addition, it will also not be possible to fully identify all the breakdown and/or reaction products. In such cases, the toxicity and safety assessment should follow the same strategy as described for scenario B2 in Table 1. The uncertainty related to the unidentified breakdown and/or reaction products will be larger than for scenario B2 (Table 1 – scenario C).

For scenario A, preference should be given to the component-based approach described above (Table 1 – scenarios A1 and A2). For scenarios B and C, in particular if a multitude of constituents and breakdown and/or reaction products (whether identified or not) are present, an alternative option would be to perform toxicological feeding studies, encompassing subchronic toxicity and reproductive and developmental toxicity on the mixture. In such studies, the precursors should be added in increasing amounts to animal feed, including a control group, which then has to undergo the same processing steps as human food. It has to be ensured that the same substances that are expected to serve as reaction partners for the flavour precursor in human food are also present in the animal feed. In addition, the breakdown and/or reaction products, as far as they can be identified, should be formed in approximately the same proportions as in human food.

Another method that could be applied is to add the *flavour precursor* to human food which is then treated as required to produce the ultimate flavour and subsequently to feed animals with this treated human food. Also, here a range of doses should be studied, including a control group.

For both options, care should be taken that the toxicity of the flavouring is investigated rather than results of nutritional imbalance or feed rejection. This may require pairwise feeding with feeding restriction. The levels of dietary exposure that are studied should be such that they allow the application of uncertainty factors. In both cases, the concentrations in animal feed should be substantially higher than those in human foods.

The same feeding studies testing strategy could be applied to reduce the above-described uncertainties related to the scenarios A2, B2 and C. If no such testing is included in the dossier, this may negatively affect the outcome of the assessment.

The suitability of the chosen approach to reflect all intended uses of the *flavour precursor* will be judged case by case.

4.5.3. Source materials

The Panel considers that the potential toxicity of the source materials will be covered by the toxicity assessment of the flavouring obtained from the source material. This flavouring will be subject to a comprehensive toxicity evaluation as described in the above-mentioned sections, as applicable.

4.6. Safety for the environment

Regulation (EC) No 1334/2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods lays down rules to ensure protection, where appropriate, of the environment.

It should be noted that flavourings are defined as products 'not intended to be consumed as such, which are added to food in order to impart or modify odour and/or taste'. Prior to their potential release into the environment, food flavourings (i) are subject to human consumption, (ii) are anticipated to be (partly) metabolised in the body and (iii) flavouring substances as such as well their metabolites are possibly subject to degradation in sewage water treatment plants. Thus, the physicochemical properties of a flavouring substance and/or its metabolites, the extent of metabolism in the human body and the extent of degradation in the sewage treatment plant determine the amount and type of these substances that will finally reach the environment. The main environmental compartments into which flavourings or their metabolites might be expected to enter are surface water, sediment, soil and groundwater. As mentioned in the section on the interpretation of ToR, industrial emission of food flavourings is out of scope of the present guidance as it is covered in other regulatory frameworks.

Taking these aspects and experiences from previous evaluations into account, EFSA does not anticipate a need to perform an environmental safety assessment on a regular basis for each new food flavouring.

However, there may be cases in which an environmental risk assessment is required. For this to be the case, the following three conditions must be fulfilled altogether:

i) the food flavouring is synthesised and has not been reported to occur in nature, and
ii) the intended production volume of such food flavouring, as declared by the applicant, is above the tonnage band as specified in Article 14 of REACH Regulation (EC) No 1907/2006 (currently 10 t per year), and
iii) the structural and physical chemical properties of such flavouring or its metabolites[28] indicate that they meet the criteria to be classified according to Annex I Part 4 (Environmental hazards), Section 4.1.2 (Classification criteria for substances) of the Classification, Labelling and Packaging (CLP) Regulation.[29]

[28] ADME studies according to OECD TG 417 (OECD, 2010) will not be available for flavouring substances for which the exposure is < TTC (see Appendix C – Scheme A Tier I). In such a case, it may be necessary to examine the formation of putative metabolites via *in silico/in vitro/in vivo* methods.

[29] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, p. 1–1,355.

In addition, in case the flavouring and/or its metabolites are identified as persistent, bioaccumulative and toxic substances (PBT substances), and/or very persistent and very bioaccumulative substances (vPvB substances), as per Annex XIII of the REACH Regulation (EC) No 1907/2006[30], they would raise a concern for the environment, irrespective of their tonnage band, as no safe concentration in the environment can be established with sufficient reliability for an acceptable risk to be determined in a quantitative way.

When appropriate, the generation of data using non-testing approaches, such as (Q)SAR, could also be considered provided they are relevant, reliable and adequate for the purpose and are documented in an appropriate manner (ECHA, 2008 and Appendix D of EFSA FAF Panel, 2019).

In case an environmental safety assessment is needed for the flavouring and/or its metabolites, it will be based on the same principles as mentioned in other existing guidances on environmental risk assessment for substances with similar release patterns and/or exposure routes such as medicinal products for human use (EMA, 2019). In addition, the ERA guidances developed for biocides (ECHA, 2017) or industrial chemicals (ECHA, 2016a, 2017) could be considered. Such principles and the data requirements connected to that may need to be reconsidered if, in the future, an EFSA cross-cutting guidance document on environmental risk assessment becomes available.

In the case of complex flavouring mixtures with proportions of unidentified constituents, the approach described above for chemically defined substances may not be fully applicable as information on the complex mixtures might not be available and hazard and exposure assessment, on the basis of constituents or fractions of similar constituents exhibiting similar properties, may need to be applied (see EFSA Scientific Committee, 2019c). For those constituents that have been chemically identified, applicants should apply the same considerations and approach as described above. For the fractions which have not been chemically fully characterised, it is expected that a qualitative characterisation of the main constituents is available, and that the percentage of unidentified constituents is indicated and is as low as possible. In this respect, it might be relevant to assess whether the unidentified constituents might share similar properties of the constituents in the characterised fraction. Further guidance can be found in the OECD guidance document dealing with 'aquatic toxicity testing of difficult substances and mixtures' (OECD, 2019).

4.7. Other scientific data

Applicants should provide any other available information that could have an impact on the safety assessment of the food flavouring.

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
BMD	benchmark dose
BMDL	lower confidence limit of the benchmark dose
BMDU	upper confidence limit of the benchmark dose
BMR	benchmark response
CRP	C-reactive protein
EOGRTS	Extended One-Generation Reproduction Toxicity
FAIM	Food Additive Intake Model
FID	flame ionisation detector
GC	gas chromatography
GLP	good laboratory practices
GMP	good manufacturing practices
GPC	gel permeation chromatography
GNPD	global new products database GPC gel permeation chromatography
HACCP	hazard analysis and critical control points
HPLC	high performance liquid chromatography
ISO	International Organisation for Standardisation
ISS	Istituto Superiore di Sanità
LOD	limit of detection
LOQ	limit of quantification
MOE	margin of exposure
MN	Micronucleus
MPL	maximum permitted level
NK	natural killer
NOAEL	no-observed-adverse-effect level
OECD TG	Organisation for Economic Co-operation and Development Test Guideline
OASIS-LMC	OASIS-Laboratory of Mathematical Chemistry)
PAHs	polycyclic aromatic hydrocarbons
PBT	Persistent Bioaccumulative and Toxic
(Q)SAR	quantitative structure–activity relationship
SAR	structure–activity relationship
SMILES	simplified molecular-input line-entry system
TTC	Threshold of Toxicological Concern
vPvB	very Persistent and very Bioaccumulative

Appendix A – Format for the submission of the proposed specifications of a food flavouring

Table A.1: Specifications to be provided for *flavouring substances*[(a)]

Description/Definition
• Source material and process used to obtain the *flavouring substance* (e.g. synthesis or production from material of vegetable, animal or microbiological origin)
Identity
• Chemical name (according to IUPAC nomenclature, when appropriate) • Synonyms, trade names, abbreviations • CAS-, E-, EINECS-, CoE-, JECFA-, FLAVIS- and FEMA numbers (if assigned), and other identification numbers
• Molecular formula, structural formula • SMILES linear notation • Molecular weight
• ID tests (spectroscopic data, e.g. MS, IR and NMR spectra, or other data)
• Chromatographic data (GC, HPLC)
• Stereochemistry
• Physical properties: – Appearance – Boiling point (for liquids) – Refractive index (for liquids) – Specific gravity (for liquids) – Melting point (for solids) – Solubility – Octanol–water partition coefficient – Vapour pressure
• Sensory properties
• Particle size, shape and distribution (for material consisting of solid particles, if applicable)
Composition
• Purity/minimum assay value
• Identities/quantities of by-products (e.g. substances formed in the course of chemical synthesis), impurities (e.g. co-extracted substances) or contaminants (e.g. heavy metals)

(a): For details regarding the listed parameters, the respective sections of Section 1.1 should be consulted.

Table A.2: Specifications to be provided for *flavouring preparations*[a]

Description/Definition
• Source material of plant, animal or microbiological origin, other than food, used to obtain the *flavouring preparation* • Process(es) used to prepare the source material, if applicable • Process(es) used to obtain the *flavouring preparation*
Identity[b]
• Chemical name (when appropriate) • Trade names, synonyms, abbreviations • CAS-, E-, EINECS-, CoE-, JECFA-, FLAVIS- and FEMA numbers (if assigned), and other identification numbers
• Physical properties: – Appearance – Boiling point (for liquids) – Refractive index (for liquids) – Specific gravity (for liquids) – Melting point (for solids) – Solubility
• Sensory properties
• Particle size, shape and distribution (for material consisting of or containing solid particles, if applicable)
Composition
• Proportions of volatile and non-volatile fractions • Identities and concentrations of the 20 principal constituents of the volatile fraction, related to the solvent-free mass • Proportions of major chemical classes of the non-volatile fraction (e.g. proteins, lipids, carbohydrates) • Depending on the source material and the process(es) used to obtain the *flavouring preparation*, levels of contaminants (e.g. microorganisms, mycotoxins, heavy metals, pesticide residues, polycyclic aromatic hydrocarbons)

(a): For details regarding the listed parameters, the respective sections of Section 1.2 should be consulted.
(b): For a *flavouring preparation* of which individual components are identified, the complete list of identity parameters listed under the first seven indents in Section 1.1.1 should be provided for each component.

Table A.3: Specifications to be provided for *thermal process flavourings*[a]

Description/Definition
• Composition of the mixture subjected to heat treatment to obtain the *thermal process flavouring*: – Identities and proportions of the nitrogen (amino)-containing ingredient(s) – Identities and proportions of the reducing sugar(s) – Identities and proportions of other ingredients • Conditions of heat treatment (temperature, time, pH)
Identity[b]
• Chemical name (when appropriate) • Synonyms, trade names, abbreviations • CAS-, E-, EINECS-, CoE-, JECFA-, FLAVIS- and FEMA numbers (if assigned), and other identification numbers
• Physical properties: – Appearance – Boiling point (for liquids) – Refractive index (for liquids) – Specific gravity (for liquids) – Melting point (for solids) – Solubility
• Sensory properties
• Particle size, shape and distribution (for material consisting of or containing solid particles, if applicable)
Composition
• Proportions of volatile and non-volatile fractions • Identities and proportions of the 20 principal constituents of the volatile fraction, related to the solvent-free mass • Proportions of major chemical classes of the non-volatile fraction (e.g. proteins, lipids, carbohydrates) • Levels of heterocyclic aromatic amines, in particular – 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine (PhIP) – 2-amino-3,4,8-trimethylimidazo [4,5-*f*] quinoxaline (4,8-DIMeIQx) • Levels of other heat-induced contaminants (e.g. acrylamide, acrolein, furan) • Depending on the ingredients of the mixture subjected to heat treatment, levels of contaminants (e.g. mycotoxins, heavy metals, pesticide residues, polycyclic aromatic hydrocarbons)

(a): For details regarding the listed parameters, the respective sections of Section 1.3 should be consulted.
(b): For a *thermal process flavouring* of which individual components are identified, the complete list of identity parameters listed under the first seven indents in Section 1.1.1 should be provided for each component.

Table A.4: Specifications to be provided for *flavour precursors*[(a)]

Description/Definition
• Product intended to be added to food for the purpose of producing flavour: – defined chemical substance obtained from material other than food – chemical mixture obtained from material other than food – material other than food. • Conditions of use resulting in the intended breakdown and/or reaction products of the *flavour precursor* • Type of food and food processing conditions resulting in the intended breakdown and/or reaction products of the *flavour precursor* with other food components.
Identity
• Defined chemical substance – Chemical name (according to IUPAC nomenclature, when appropriate) – Synonyms, trade names, abbreviations – CAS-, E-, EINECS-, CoE-, JECFA-, FLAVIS- and FEMA numbers (if assigned), and other identification numbers • Chemical mixture obtained from material other than food – Chemical name (when appropriate) – Synonyms, trade names, abbreviations – CAS-, E-, EINECS-, CoE-, JECFA-, FLAVIS- and FEMA numbers (if assigned), and other identification numbers • Material other than food – Plants: Scientific (Latin) name, synonyms, common names; part(s) used; geographical origin; growth and harvesting conditions – Animals: Scientific (Latin) name, synonyms, common names; part(s) used; geographical origin – Microorganisms: Information according to section 1.1 of the Scientific Guidance for the submission of dossiers on Food Enzymes (EFSA CEP Panel, 2021) – Mineral origin: information allowing unequivocal assignment of identity and authenticity • Sensory properties, if applicable • Particle size, shape and distribution (for material consisting of or containing solid particles, if applicable)
Composition
• If the *flavour precursor* is a single substance: information as described in Table 1 for *flavouring substances* • If the *flavour precursor* is a chemical mixture: information as described in Table 2 for *flavouring preparations* • If the *flavour precursor* is material other than food: levels of contaminants (e.g. microorganisms, mycotoxins, heavy metals, pesticide residues, polycyclic aromatic hydrocarbons), depending on the type of material

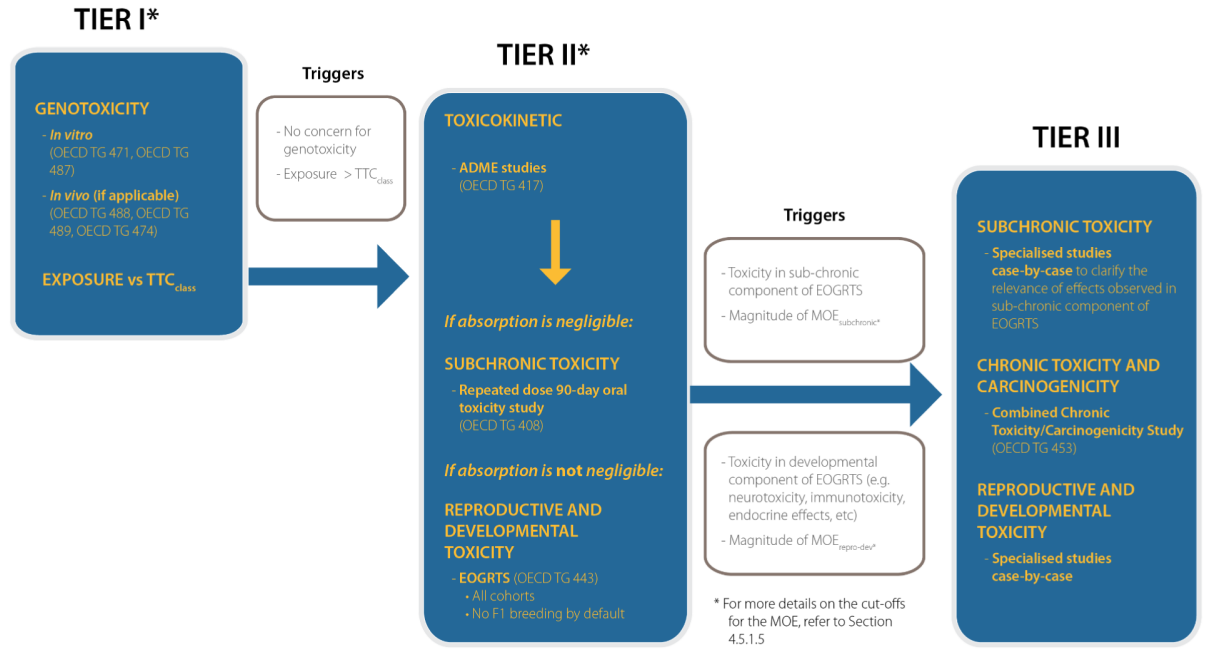
(a): For details regarding the listed parameters, the respective sections of Section 1.4 should be consulted.

Table A.5: Specifications to be provided for *source materials*[(a)]

Description/Definition
• Material intended to be used for the production of flavourings or food ingredients with flavouring properties • Process(es) intended to prepare the source material, if applicable
Identity
• Material of plant origin, other than food: Scientific (Latin) name, synonyms, common names; part(s) used; geographical origin; growth and harvesting conditions • Material of animal origin, other than food: Scientific (Latin) name, synonyms, common names; part(s) used; geographical origin • Material of microbiological origin, other than food: Information according to section 1.1 of the Scientific Guidance for the submission of dossiers on Food Enzymes (EFSA CEP Panel, 2021) • Material of mineral origin, other than food:information allowing unequivocal assignment of identity and authenticity
Composition
• Analytical data on the presence of substances listed in Annex III of Regulation (EC) No 1334/2008 in the source material should be provided. • In addition, depending on the source and the intended manufacturing process(es) information on the presence of other undesirable substances, e.g. inherent plant toxins, mycotoxins, should be provided. • At any rate, levels of contaminants (e.g. heavy metals, pesticide residues, polycyclic aromatic hydrocarbons, polyhalogenated organic chemicals) should be determined.

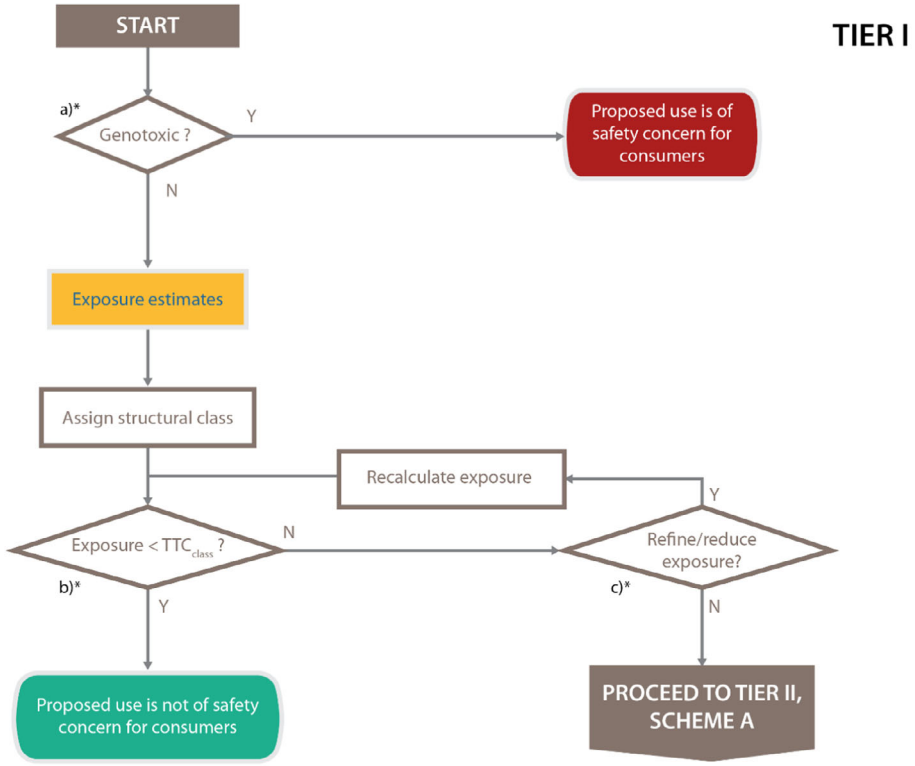
(a): For details regarding the listed parameters, the respective sections of Section 1.6 should be consulted.

Appendix B – Tiered toxicity testing of *flavouring substances*

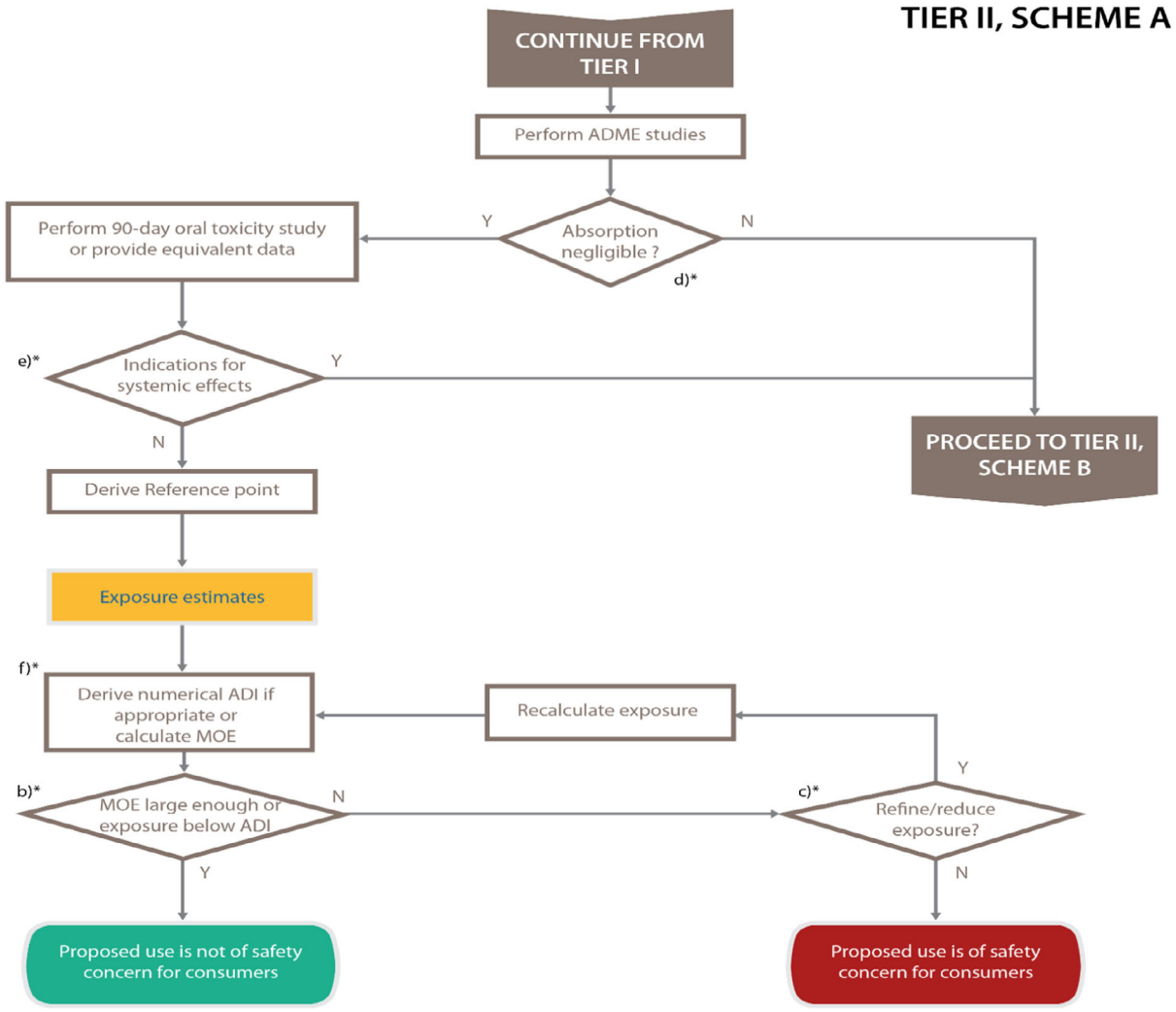


* For *flavouring substances* intended to be used in food for infants and young children and for which the evaluation remains in Tier I or Tier II, Scheme A (see Appendix C) studies in neonatal animals will be necessary, in line with the EFSA Scientific Committee (EFSA Scientific Committee, 2017a).

Appendix C – Decision schemes for the toxicity testing of *flavouring substances*



* For an explanation of these indices, see the text below the schemes in this Appendix



* For an explanation of these indices, see the text below the schemes in this Appendix

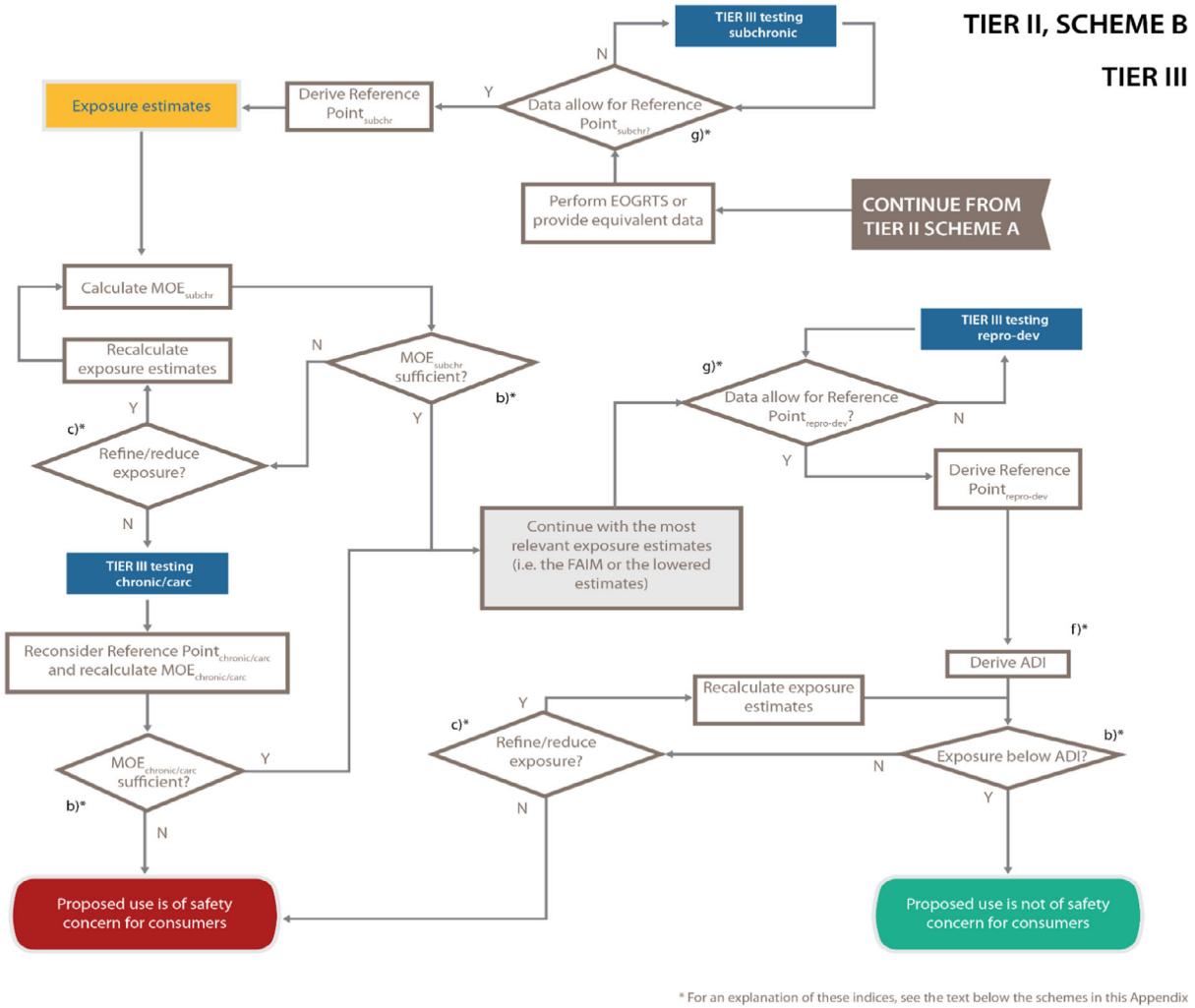


Figure C.1: Flow charts applicable to the evaluation strategy for *flavouring substances*

The chart consists of three decision schemes, in which exposure estimates are compared with TTC (Tier I) or with data on repeated dose toxicity only (Tier II, Scheme A) or with data on repeated dose toxicity as well as reproductive and developmental toxicity (Tier II, Scheme B; Tier III). When needed, additional toxicity data should be generated in Tier III (see Tier II, scheme B; bright blue boxes). For all tiers, initially, the exposure estimate as provided by the applicants (yellow shading) is the starting exposure estimate, but if needed, a refined exposure estimate (done by EFSA during the risk assessment) can also be used, or the applicant may be requested to submit revised data on uses and use levels to lower the exposure estimates. Already after Tier I, a conclusion may be reached that a substance is not of safety concern under the intended conditions of use if the assessment of genotoxicity data does not raise a concern. In addition, the exposure has to be below the class-specific TTC for the substance. If the latter criterion is not met, further testing in Tier II and possibly in Tier III will be necessary.

The scheme for Tier II (Scheme A) starts with the requirement to perform ADME studies and, depending on the results obtained, continues with the decision, whether only a 90-day oral toxicity study would suffice or whether also other toxicological endpoints (e.g. developmental and reproductive toxicity) should be addressed (Tier II, Scheme B). When, based on ADME data, the absorption of the substance is considered negligible and when only local effects are observed (i.e. in the gastrointestinal (G.I.) tract) or when systemic effects are the direct result of such local effects, an MOE could be calculated based on the reference point from the 90-day study and the exposure estimates (those submitted by the applicant or the refined/revised estimates). This MOE should be sufficiently large to conclude that there is no safety concern. For more details on the numerical cut-offs for the MOE, refer to Section 4.5.1.5. Alternatively, an ADI could be calculated, and the exposure should not exceed this ADI. Data on repeated dose toxicity can be provided on the substance itself (OECD TG 408) or on structurally similar substances (read-across).

It is noted that in case the authorisation of the substance is requested in foods for infants and young children and if its evaluation remains in Tier I or Tier II scheme A, studies in neonatal animals will be necessary in line with the EFSA Scientific Committee (EFSA Scientific Committee, 2017).

On the other hand, when ADME data indicate that there will be a relevant absorption of the substance, or when despite negligible absorption still other than local effects (i.e. other than in (or resulting from effects in) the G.I. tract) are observed, more extensive toxicity testing is required (Tier II, Scheme B; TIER III). In this case, the initial exposure estimate (yellow shading) is needed for the calculation of the MOE for subchronic repeated dose toxicity (MOEsubchr) in combination with the reference point for repeated dose toxicity. When the results of the Tier II testing indicate a need for further clarification before reference points for subchronic and/or reproductive or developmental toxicity can be derived additional testing in Tier III may be requested. A request for Tier III testing could also follow when there are no (further) options for reduction of exposure *and* when the calculated MOEs are not large enough. When it is decided that the MOEsubchr (or the $MOE_{chronic/carc}$) is sufficiently large, the reference point for reproductive–developmental toxicity should be derived. Based on both the final reference point for repeated dose toxicity (obtained after either Tier II or Tier III testing) and the final reference point for reproductive/developmental toxicity, an ADI can be calculated, if needed, and the exposure estimates should be below this ADI to reach a conclusion that the substance is not of safety concern. Data on repeated dose toxicity and reproductive and developmental toxicity can be provided on the substance itself (OECD TG 443), on structurally similar substances (read-across), or with a set of studies providing equivalent information equivalent to that obtained from an OECD TG 443 study.

The diamonds in the decision scheme include seven types of questions:

a) Do the data on genotoxicity raise a concern? To answer this question, additional information from ADME studies may be needed already at this stage of the assessment.
b) Are the MOEsubchr or the MOEchronic/carc sufficiently low or are exposures below the TTC or ADI to conclude that the substance can be considered to be of no safety concern? (see Section 4.5.1.5) (Tier I, Tier II Schemes A and B and/or Tier III).
c) Is it possible to reduce the exposure estimates? This could be achieved by refining the exposure estimates (done by EFSA during the risk assessment) or by lowering the (proposed) use levels and/or by reducing the uses (to be done by the applicant) (Tier I, Tier II Schemes A and B and/or Tier III).
d) Is the absorption so low that it can be anticipated that effects (if any) will only be local in the gastrointestinal tract?
e) Are there indications that despite negligible absorption there are effects, which are not the direct result of local effects in the G.I. tract? If the answer is yes, then that indicates a need for further testing (Tier II, Scheme B; Tier III). If the answer is no and there are only local effects in the G.I. tract (or, when there are systemic effects and these are secondary to the effects in the G.I. tract), then proceed with the derivation of a Reference Point from the subchronic toxicity study (Tier II, Scheme A). For further clarification, see also Section 4.5.1.3.2.
f) It should be possible at this stage of the evaluation to derive an ADI. If there are still open issues with respect to toxicity, further testing may still be needed (Tier II Scheme B and/or Tier III). For flavourings that consist of mixtures (see Section 4.5.2), derivation of an ADI might not be appropriate. In that case, their evaluation can only be finalised by calculation and interpretation of MOEs.
g) Are the results/data from the EOGRT study sufficient to derive reference points for subchronic and reproductive/developmental toxicity, respectively? If there are unclarities, f it will be necessary to do additional studies in Tier III. These may focus on specific aspects of the various cohorts in the EOGRT study (including those addressing repeated dose toxicity) and the nature of such additional Tier III studies will be decided on a case-by-case basis (Tier II, Scheme B; Tier III).