COVER PAGE

Output category	Guidance
Date endorsed by the Panel	21 September 2020
Author list	Maged Younes, Gabriele Aquilina, Laurence Castle, Paul Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Ursula Gundert-Remy, Rainer Gürtler, Trine Husøy, Melania Manco, Wim Mennes, Peter Moldeus, Sabina Passamonti, Romina Shah, Ine Waalkens-Berendsen, Detlef Wölfle, Matthew Wright, Romualdo Benigni, Claudia Bolognesi, Polly Boon, Kevin Chipman, Joop De Knecht, Ullrika Sahlin, Stefania Barmaz, Maria Carfì, Carla Martino, Alexandra Tard, Giorgia Vianello and Karl-Heinz Engel
DOI	10.2903/j.efsa.201Y.xxxx
Requestor	European Commission
Output number	ONxxx
Question number	EFSA-Q-2019-00687
Short title	Scientific guidance on smoke flavouring primary products
Panel members	Maged Younes, Gabriele Aquilina, Laurence Castle, Karl-Heinz Engel, Paul Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Ursula Gundert-Remy, Rainer Gürtler, Trine Husøy, Melania Manco, Wim Mennes, Peter Moldeus, Sabina Passamonti, Romina Shah, Ine Waalkens-Berendsen, Detlef Wölfle and Matthew Wright
Acknowledgments	The FAF Panel wishes to thank the following for the support provided to this scientific output: Andrew Hart and Caroline Merten

Scientific Guidance for the preparation of applications on smoke flavouring primary products

EFSA Panel on Food Additives and Flavourings (FAF),
Maged Younes, Gabriele Aquilina, Laurence Castle, Paul Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Ursula Gundert-Remy, Rainer Gürtler, Trine Husøy, Melania Manco, Wim Mennes, Peter Moldeus, Sabina Passamonti, Romina Shah, Ine Waalkens-Berendsen, Detlef Wölfle, Matthew Wright, Romualdo Benigni, Claudia Bolognesi, Polly Boon, Kevin Chipman, Joop De Knecht, Ullrika Sahlin, Stefania Barmaz, Maria Carfì, Carla Martino, Alexandra Tard, Giorgia Vianello and Karl-Heinz Engel

Abstract

Following a request from the European Commission, EFSA developed updated scientific guidance to assist applicants in the preparation of applications on smoke flavouring primary products. This guidance describes the scientific data to be included the applications for the authorisation of new smoke flavouring primary products, as well as for the modification or for the renewal of existing authorisations, submitted under Articles 7, 11 and 12 of Regulation (EC) No 2065/2003. Information to be provided in all applications relates to: the characterisation of the primary product, including the description of the source materials, manufacturing process, chemical composition, specifications and stability; the proposed uses and use levels and the assessment of the dietary exposure; the safety data, including information on the genotoxic potential of the identified components and of the uncharacterised fraction of the primary product, toxicological data other than genotoxicity and information on the safety for the environment. For the toxicological studies, a tiered approach is devised in the guidance consisting of two tiers, for which the testing requirements, key issues and triggers are described. A description of the standard uncertainties relevant for the evaluation of primary products and how these are considered in the standardised risk assessment procedure is also included. The applicant should generate the data requested in each section to support the safety assessment of the smoke flavouring primary product. On the basis of the submitted data, EFSA will assess the safety of the primary product and conclude whether or not it presents risks to human health and to the environment under the proposed conditions of use.

Keywords

Smoke flavourings, primary products, guidance, renewal, mixtures.

Summary

The European Commission asked the European Food Safety Authority (EFSA) to develop updated consolidated guidance for submission of applications on smoke flavouring primary products under Regulations (EC) No 2065/2003 and No 1321/2013.

This document provides guidance to applicants on the data to be included in applications for the authorisation of new smoke flavouring primary product, as well asfor the modification or for the renewal of existing authorisations, submitted respectively under Articles 7, 11 and 12 of Regulation (EC) No 2065/2003.

This document is also intended to outline the type and quality of information required by EFSA to carry out the evaluation of a smoke flavouring primary product and to conclude whether it is safe under the proposed conditions of use.

Chapters 1–3 of the guidance document reflect the structure that should be followed by applicants when preparing the dossier to support such an application:

- Chapter 1 – *Characterisation of the Primary Product*, containing the information specific to the production process, compositional data, specification and stability of the smoke flavouring primary product.
- Chapter 2 – *Proposed uses and exposure assessment*, including the information specific to the proposed uses and use levels and the anticipated intake of the primary product.
- Chapter 3 – *Safety data*, describing the type of toxicity studies needed to demonstrate the safety of the primary product for human health and for the environment. It includes the data requirements needed to assess the genotoxic and toxicity potential of the primary product and the potential impact of its use on the environment.

Chapter 4 on *Uncertainty* includes the characterisation of the standard uncertainties relevant to the safety assessment of smoke flavouring primary products together with a description of how they are expected to influence the outcome of the risk assessment.

The applicant should generate the data requested in each section to support the safety assessment of the smoke flavouring primary product. Based on the submitted data, EFSA will assess the safety of the primary product and conclude whether or not it presents risks to human health and to the environment under the proposed conditions of use.

Table of contents

Introduction

Background as provided by the requestor

Smoke flavourings are a specific category of flavourings and are subject to the general Regulation (EC) No 1334/2008[1] on flavourings and certain food ingredients with flavouring properties for use in/on foods. This Regulation lays down the general requirements for safe use of flavourings, provides definitions for different types of flavourings and sets out flavouring substances for which an evaluation and approval is required.

Smoke flavourings are specifically regulated by Regulation (EC) No 2065/2003[2] of the European Parliament and of the Council on smoke flavourings used or intended for use in or on foods. This Regulation establishes a Community procedure for the safety assessment and the authorisation of smoke flavourings intended for use in or on foods on the basis of a high level of protection of human health and protection of consumers' interests, as well as to ensure fair trade practices.

Regulation (EU) No 1321/2013[3] establishing the Union list of authorised smoke flavouring primary products for use as such in or on foods and/or for the production of derived smoke flavourings, was published on 12 December 2013. This Regulation lists the 10 authorised smoke flavouring primary products for use in or on foods and their conditions of use. This list was established on the basis of the applications submitted under Article 20 of the Regulation (EC) No 2065/2003[2] and after evaluation by EFSA.

As provided for under Article 7, paragraph 4 of Regulation (EC) No 2065/2003[2], EFSA developed the existing current guidance for the submissions of applications intended to establish the list of authorised smoke flavourings in view of their evaluation under the same Regulation.

The guidance is applicable to new applications on smoke flavourings primary products and for the renewal of the existing authorisations.

The current guidance is essentially based on a set of EFSA documents mentioned below:

- Guidance on the submission of a dossier on a smoke flavouring primary product (EFSA AFC Panel, 2005)

This lays down the information required by applicants to be included in the application. It lays down requirements in terms of administrative, technical and toxicological data necessary to enable EFSA to carry out the safety assessment of a smoke flavouring primary product.

This document is supplemented by the following additional documents:

[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 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.

[3] Commission Implementing Regulation (EU) No 1321/2013 of 10 December 2013 establishing the Union list of authorised smoke flavouring primary products for use as such in or on foods and/or for the production of derived smoke flavourings. OJ L 333, 12.12.2013, p. 54–67.

- Dietary exposure assessment methods for smoke flavouring primary products (EFSA CEF Panel, 2009)

Dietary exposure for smoke flavourings is assessed using specifically developed methods, the SMK-TAMDI and SMK-EPIC methods.

- Statement on the interpretation of the Margin of Safety for Smoke Flavourings Primary Products (EFSA, 2010)

This statement clarifies the use of the margin of safety for smoke flavouring primary products on the basis of the available toxicological data.

EFSA is asked to update the above-mentioned documents and compile them in a single comprehensive document taking into account cross-sectional guidance documents, such as:

- Opinion on genotoxicity testing strategies applicable to food and feed safety assessment (EFSA Scientific Committee, 2011);
- Opinion on the clarification of some aspects related to genotoxicity assessment (EFSA Scientific Committee, 2017);
- Statement on the genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019);
- Harmonised methodologies for human and animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA Scientific Committee, 2019);
- Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment (EFSA, Scientific Committee, 2019).

In addition, in the preparation of the new guidance, EFSA should also consider the latest updated version of the relevant OECD Test Guidelines (TG), such as:

- OECD TG 488 (OECD, 2020) Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays;
- OECD TG 474 (OECD, 2016a) *In vivo* mammalian erythrocyte micronucleus test OECD TG 489 (OECD, 2016b) *In vivo* Mammalian Alkaline Comet Assay.

As regards the exposure assessment, EFSA should take into account that the food categories used for regulatory purposes in flavourings are the food categories mentioned in Part D of Annex II of Regulation (EC) No 1333/2008[4] on food additives. A more refined exposure assessment could also be considered, based on actual use levels and on 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 be considered, where appropriate.

[4] 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.

Furthermore, the relevant provisions arising from the recently published transparency Regulation[5] should also be taken into account in the preparation of this updated guidance and consistency should be ensured with other sectors where similar updates will be done.

While recognizing a connection with the general guidance and requirements for flavourings which may need also to be revised, the Commission considers that it is desirable, in view of the specific conditions of smoke flavourings, to consider this update of the guidance on smoke flavouring primary products separately.

Terms of Reference as provided by the requestor

The Commission requests EFSA to prepare an updated consolidated guidance for the submission of applications on smoke flavouring primary products under Regulations (EC) No 2065/2003[2] and No 1321/2013[3], taking into account the experience gained with the assessment and the regulation of the currently authorised and assessed smoke flavouring products in the EU and, notably, the numerous other relevant scientific and technical documents published by EFSA since the adoption of the current guidance related to the safety of smoke flavourings.

The guidance should be updated taking into account applications on new smoke flavourings and the renewals of the existing authorisations.

EFSA should take into account the relevant provisions of Regulation (EU) 2019/1381[5] of the European Parliament and of the Council on the transparency and sustainability of the EU risk assessment in the food chain in the preparation of this updated guidance and should ensure consistency with other sectors where similar updates will be done.

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

Interpretation of the Terms of Reference

In accordance with the Terms of Reference as provided by the European Commission, the comparison between smoke flavouring primary products (see 'Definitions') and conventional methods of smoking with respect to their respective impact on human health and the environment is not considered in this guidance document, as it is outside the scope of the request.

All administrative information related to the preparation and submission of an application for a new authorisation, or for a modification, or a renewal of an existing authorisation of smoke flavouring primary products is addressed in a separate EFSA document, 'Administrative guidance for the preparation of applications on smoke flavouring primary products' (EFSA, 2020), which is applicable to applications submitted as of 27 March 2021.

As indicated in the Terms of Reference, this document is mainly intended to provide guidance to applicants for the preparation of applications:

- for the authorisations of new smoke flavouring primary products submitted under Article 7 of Regulation (EC) No 2065/2003 and

[5] Regulation (EU) 2019/1381 of the European Parliament and of the Council on the transparency and sustainability of the EU risk assessment in the food chain. OJ L 231 of 6/9/2019 p.1

- for renewals of the existing authorisations of smoke flavouring primary products submitted under Article 12 of Regulation (EC) No 2065/2003 and Regulation (EU) No 1321/2013.

It also applies to applications for modifications of existing authorisations of smoke flavouring primary products submitted under Article 11 of Regulation (EC) No 2065/2003. Such modifications may involve changes in the conditions of use, production processes or in the specifications.

Scope of the guidance

This guidance provides information on the type and quality of the data that EFSA needs to conclude whether a smoke flavouring primary product 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 for applications for new smoke flavouring primary products, as well as for the renewal and modification of existing applications, is to demonstrate that in the light of the current knowledge, smoke flavouring primary products do not present risks to human health or to the environment, under the conditions of use, in line with Article 4 of Regulation (EC) No 2065/2003 and Article 1 of Regulation (EC) No 1334/2008.

This guidance has four main sections. Chapters 1–3 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 new smoke flavouring primary products and/or for the renewal or modification of an existing authorisation.

- Chapter 1 contains the information specific to the production process, compositional data, specification and stability of the primary product.
- Chapter 2 contains the information specific to the proposed uses and use levels and anticipated intake of the primary product.
- Chapter 3 contains the information related to the safety of the primary product, including data on its genotoxic potential, toxicological information and information on the safety for the environment.
- Chapter 4 contains a characterisation of the standard uncertainties relevant to the safety assessment of primary products together with a description how they are expected to influence the outcome of the risk assessment.

General principles

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

- Regulation (EC) 178/2002[6], 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;

[6] 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.

- Commission Implementing Regulation (EU) No 1321/2013, establishing the EU list of authorised smoke flavouring primary products for use as such in or on food and/or for the production of derived smoke flavourings;
- Regulation (EC) 1334/2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods;
- Commission Regulation (EC) No 627/2006[7], implementing Regulation (EC) No 2065/2003 as regards quality criteria for validated analytical methods for sampling, identification and characterisation of primary smoke products;
- Commission Regulation (EC) No 2065/2003 of the European Parliament and of the Council, on smoke flavourings used or intended for use in or on food, as amended.

In addition, the following guidance documents should be also considered:

- Administrative guidance on the preparation and presentation of applications for new authorisation and for renewal of authorisation of smoke flavourings primary products (EFSA, 2020 under preparation).
- All the relevant cross-sectional EFSA guidance documents cited throughout this guidance document should also be considered for the preparation of applications on smoke flavouring primary products. 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.

In this guidance document the principles described in the Scientific Committee Guidance on Uncertainty Analysis (EFSA Scientific Committee, 2018) have been considered (see Chapter 4) and will be applied to the assessment of smoke flavouring primary products.

Definitions

As per Article 3 of Regulation (EC) No 2065/2003, the following definitions apply:

- 'primary smoke condensate' refers to the purified water-based part of condensed smoke and falls within the definition of 'smoke flavourings';
- 'primary tar fraction' refers to the purified fraction of the water-insoluble high-density tar phase of condensed smoke and falls within the definition of 'smoke flavourings';
- 'primary products' refers to primary smoke condensates and primary tar fractions;
- 'derived smoke flavourings' refers to flavourings produced as a result of the further processing of primary products and which are used or intended to be used in or on foods in order to impart smoke flavour to those foods.

[7] Commission Regulation (EC) No 627/2006 of 21 April 2006 implementing Regulation (EC) No 2065/2003 of the European Parliament and of the Council as regards quality criteria for validated analytical methods for sampling, identification and characterisation of primary smoke products. OJ L 109, 22.4.2006, p. 3–6.

Content of the technical dossier

1 Characterisation of smoke flavouring primary products

The data requirements for the characterisation of the primary product described in the following sections apply to the assessment of new smoke flavouring primary products as well as to renewals and modifications of existing authorisations. Any proposed modification of the production process has to be assessed for a potential impact on the composition of the primary product and should be reflected in the specifications.

1.1 Manufacturing process

1.1.1 Source materials for the primary product

All source materials used for the production of the primary product must be listed. They should comply with the provisions of Article 5 and Annex I of Regulation (EC) No 2065/2003. Full botanical names should be provided; in particular, the species of trees (woods) used for the production of the primary product must be specified. If more than one species of wood or other ingredients form the basis of the primary product, the proportions and ranges should be indicated. If primary products are produced from different species of trees as source materials they are considered as different primary products.

1.1.2 Method of manufacture of the primary product

The process by which the raw materials are converted into the primary product should be described. The description should be detailed enough to allow the evaluators to understand the key steps involved in the production of the primary product. In particular, the fractions of the smoke condensate used to obtain the primary product, i.e. the water-soluble phase (primary smoke condensate) and/or the water-insoluble tar phase (primary tar fraction), and the employed purification steps should be described in detail. A flow chart diagram showing the most important steps in the process should accompany the description; it should clearly indicate the primary product. Description of the operational limits and how key parameters such as moisture of feedstock, oxygen content of pyrolysis atmosphere, residence time, condensation temperature and cooling time are controlled should be given. Measures implemented for production control and quality and safety assurance should be described (e.g. HACCP, GMP, ISO).

1.2 Identity of the primary product

1.2.1 Trade names of the primary product.

All trade names used for the primary product should be provided.

1.2.2 Description of physical state

Physicochemical parameters, e.g. solubility characteristics, specific gravity, staining index, and pH of the primary product should be provided.

1.2.3 Chemical composition

1.2.3.1 General requirements

The analytical methods for sampling, identification and characterisation of the primary product should comply with the quality criteria laid down in Commission Regulation (EC) No 627/2006.

Analyses should be performed in an accredited laboratory. Quality systems in place for control and documentation should be indicated. Information on the accreditation of the facilities involved and certificates of analyses should be provided.

The proportion of solvent-free mass (% m/m) in the primary product, as defined in Commission Regulation (EC) No 627/2006, should be provided with an explanation of how it was determined.

The proportion of volatile fraction (% m/m) in the primary product, as defined in Commission Regulation (EC) No 627/2006, should be provided with an explanation of how it was determined.

1.2.3.2 Chemical characterisation

Information on the primary product should be provided via chemical sum parameters, i.e. parameters determining the content (% m/m) of major classes of components with common structural aspects (e.g. acids, carbonyls or phenols).

1.2.3.3 Identification and quantification of individual components

Since the previous assessments of the currently authorised smoke flavouring primary products, as listed in Regulation (EU) No 1321/2013, there has been considerable analytical progress allowing improved qualitative and quantitative analyses of both volatile and non-volatile target compounds. This offers applicants the opportunity and the obligation to minimise the unidentified fraction of smoke flavouring primary products. Therefore, without prejudice to the provisions in Commission Regulation (EC) No 627/2006 on the minimum proportions of the solvent-free mass and the volatile fraction that should be identified and quantified, the components of the primary products should be characterised as fully as possible. This information is particularly required as the basis for the component-based approach employed in the course of the genotoxicity assessment of primary products (see Section 3.2).

1.2.3.3.1 Identification and quantification of the volatile fraction

Capillary gas chromatography coupled with mass spectrometry (for identification) and with flame ionisation detection (for quantification) are state-of-the-art techniques suitable for the analysis of the volatile fraction.

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.

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 GC-FID analysis to estimate the proportions of unidentified components.

1.2.3.3.2 Characterisation of the non-volatile fraction

The Panel recognises the difficulties in identifying and quantifying individual components in the non-volatile fraction of smoke flavouring primary products. However, the applicant 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, e.g. lignin-derived polymers, and to get more detailed information on the non-volatile fraction

1.2.3.4 Unidentified fraction

The proportion of the unidentified fraction (% m/m) in the primary product should be provided, encompassing unidentified volatile as well as non-volatile constituents.

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.

1.2.3.5 Polycyclic aromatic hydrocarbons

The concentrations of the 16 polycyclic aromatic hydrocarbons (PAHs) listed in Appendix A should be provided. The method applied must fulfil the performance criteria of Commission Regulation (EC) No 627/2006. However, with the analytical techniques currently available, it is expected that PAHs are now determined at lower limits of detection (LOD) and limits of quantification (LOQ) than those reported in the Annex of Regulation (EC) No 627/2006. Besides the concentrations of the 15 PAHs reported by Regulation (EC) No 627/2006, the concentration of benzo[c]fluorene should also be determined (JECFA, 2005). 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).

1.2.3.6 Batch-to-batch variability

Batch-to-batch variability should be investigated in at least five batches from different production runs. Information on how these batches were selected should be provided. The proportions of source materials (e.g. woods) used to produce the analysed batches should be described; the batches analysed should cover the range and the different proportions of the source materials subjected to the pyrolysis step, as described in the specifications. In addition,

the range of conditions used in the pyrolysis step (such as time and temperature ranges, gas flow rates, etc) should be represented by the tested samples, if relevant.

Information on batch-to-batch variability for the measured chemical sum parameters (see Section 1.2.3.2) as well as for individual identified and non-identified components of the primary product should be provided. The variability should be judged based on the relative standard deviations of the data determined on individual components in the different batches. The similarity of the different batches should be tested using appropriate statistical methods.

Analytical data should be given demonstrating that the sample(s) tested toxicologically fall within the range expected from the determined batch-to-batch variability and are considered to be representative of the primary product.

1.3 Specifications

Specifications of the primary product that include identity parameters (e.g. source materials used, proportions of the major classes of components and the 20 principal constituents of the volatile fraction) and purity criteria (e.g. maximum levels for PAHs and toxic elements) should be provided. Any proposed specifications of the primary product should be supported by adequate analytical data in order to demonstrate that the primary product is consistently manufactured within its proposed specifications. The proposed specifications should be submitted in line with the format presented in Appendix B.

1.4 Stability and fate in food

Information on storage stability from chemical analysis of the primary product (e.g. compounds representative for each chemical class; minimum 25 substances) should be provided from experimental conditions reflecting the intended shelf-life of the product, either in real time settings or under forced, accelerated ageing.

If available, a method for the analysis of characteristic components of the primary product in commercial formulations, derived smoke flavourings, as well as in the proposed food categories should be provided. The stability of the resulting analytical profile over time should then be followed.

2 Proposed uses and exposure assessment

2.1 Data needed for exposure assessment

As described above, this guidance deals with the authorisation of new smoke flavouring primary products and with the renewal or modification of existing authorisations of primary products. Data needed to assess the (potential) exposure to smoke flavouring primary products are described below.

2.1.1 Data to be provided for new smoke flavouring primary products

For assessing exposure to new smoke flavouring primary products, the applicant should provide:

- proposed maximum use levels for foods within a food category; and

- expected typical use levels, i.e. the most common use levels of the primary products proposed for foods in a food category.

Proposed maximum and expected typical use levels should be provided for all food categories for which authorisation of the smoke flavouring primary product is requested. The food categories should be coded according to the food categories in Annex II, Part D, of Regulation (EC) No 1333/2008 and the FoodEx2 nomenclature[8]. FoodEx2 is a standardised food classification and description system developed by EFSA.

As the food categories authorised to contain smoke flavouring primary products can be very broad, use levels should preferably be provided for specific foods in a food category in which the primary product(s) is (are) or may be used. For this level of detail, FoodEx2 nomenclature should be used. The more detailed the information is on foods in which the primary product(s) is (are) or may be used, the less conservative the exposure estimate will be.

For composite dishes with ingredients containing smoke flavouring primary products, the proposed maximum and expected typical use levels for the respective primary products should be provided per ingredient (at food name level). It may be beneficial for the exposure assessment if the quantities of the primary products-containing ingredients in the composite dishes are also specified.

2.1.2 Data to be provided for renewals of authorisations of smoke flavouring primary products included in Regulation (EU) No 1321/2013

For already authorised smoke flavouring primary products, maximum permitted levels (MPLs)[9] are established for broad food categories, which are specified in Annex II, Part D, of Regulation (EC) No 1333/2008. However, these primary products may be used at a lower level than the MPL or only for some foods within a food category. Therefore, use levels actually used in food products available on the market are required to perform a more realistic exposure assessment.

For assessing exposure to already authorised smoke flavouring primary products, the applicant should provide:

- proposed maximum use levels for foods within a food category; and
- typical use levels, i.e. the most common use levels of the primary products for foods in a food category.

Proposed maximum and typical use levels should be provided for all food categories for which a renewal of the authorisation is requested. Food categories should be coded according to the food categories in Annex II, Part D, of Regulation (EC) No 1333/2008 and the FoodEx2 nomenclature.

As the food categories authorised to contain smoke flavouring primary products can be very broad, use levels should preferably be provided for specific foods in a food category in which the primary product(s) is (are) or may be used. For this level of detail, FoodEx2 nomenclature should be used. The more detailed the information is on foods in which the primary product(s) is (are) or may be used, the less conservative the exposure estimate will be.

For composite dishes with ingredients containing smoke flavouring primary products, the proposed maximum and typical use levels for the respective primary products should be

[8] https://www.efsa.europa.eu/en/data/data-standardisation

[9] Maximum permitted levels in this document correspond to the maximum levels included in Regulation (EU) No 1321/2013.

provided per ingredient (at food name level). It may be beneficial for the exposure assessment if the quantities of the primary products-containing ingredients in the composite dishes are also specified.

2.1.3 Data to be provided in case of modifications of existing authorisations of smoke flavouring primary products

For assessing exposure in case of modifications of existing authorisations that would imply changes in the conditions of use of authorised smoke flavouring primary products, the applicant should provide:

- proposed maximum use levels for foods within a food category; and
- (expected) typical use levels, i.e. the most common use levels of the primary products used for foods in a food category.

Equivalent data as described in Sections 2.1.1. and 2.1.2 should be provided *mutatis mutandis*.

2.2 Exposure assessment

The applicant should provide dietary exposure estimates of smoke flavouring primary products by means of two exposure assessment tools developed by EFSA based on the proposed maximum and typical use levels:

- FAIM (Food Additive Intake Model)[10]
- 'EFSA exposure' tool[11]

Both tools use consumption data from the EFSA Comprehensive European Food Consumption Database[12] to estimate the exposure based on these use levels. Consumption data are categorised according to the food categories in Annex II of Regulation (EC) No 1333/2008, Part D for FAIM and FoodEx2 for the EFSA exposure tool.

These exposure assessment tools calculate the exposure by combining consumed amounts of foods recorded in the EFSA Comprehensive Database with use levels inserted by the user of the tool. The applicant should perform separate calculations with the maximum and with the typical use levels using both tools resulting in four exposure assessments. The tools provide mean and 95th percentile exposure estimates and information on contribution of the food categories to the mean exposure, for different age groups and countries.

If the applicant wants to enter a use level for a food category that is not available in FAIM or the 'EFSA exposure tool', the applicant should refer to the parent food category. Furthermore, the level of detail of foods which may contain the smoke flavouring primary product will often not be specific in these tools and consequently maximum or typical use levels will be assigned to whole food categories. Due to this, exposure estimates provided by both tools are expected to overestimate the dietary exposure to smoke flavouring primary products.

[10] FAIM tool is described here: http://www.efsa.europa.eu/en/applications/foodingredients/tools and can be accessed here: https://dwh.efsa.europa.eu/bi/asp/Main.aspx?rwtrep=FAIM

[11] Future link to the EFSA exposure tool

[12] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

Exposure results obtained from the tools should be included in the dossier submitted by the applicant, as well as possible uncertainties of the exposure estimates observed by the applicant.

EFSA will consider these exposure estimates submitted by the applicant and will refine them, if necessary. Such a refined exposure assessment will consider all use levels submitted in the dossier and aims to estimate the exposure as realistically as possible based on the available data. The refined exposure assessment will be performed using the food category nomenclature in Annex II, Part D, of Regulation (EC) No 1333/2008 or FoodEx2, if the level of detail is sufficient. Additional information, such as from the facets within the FoodEx2 nomenclature or from Mintel's GNPD[13], may be used to refine the exposure assessment. Exposure will be estimated for the population groups that are considered relevant. In the EFSA Comprehensive Database, consumption data are available for infants, toddlers, children, adolescents, adults and the elderly. 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 exposure estimates for high consumers (95th percentile estimated exposures) across relevant population groups and countries, based on the proposed maximum use levels either calculated with the two exposure assessment tools described above or using a refined exposure assessment. Typical use levels can be considered in the refined exposure assessments. The variability in the exposure due to differences in food consumption between individuals will be taken into account.

3 Safety data

3.1 General considerations

Toxicological studies should be carried out with the smoke flavouring primary product as intended to be marketed, i.e. (i) the test material should be manufactured according to the production process as described in Section 1.1, (ii) it should meet the compositional data as presented in Section 1.2, and (iii) it should comply with the specification proposed in Section 1.3. Since adequate human data 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 smoke flavourings.

Toxicity studies should generally be conducted in accordance with OECD TGs. If a testing method for which there is no OECD TG is considered necessary or useful, 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 GLP[14] compliance is required. Alternative validated testing methods for different toxicological endpoints may be considered on a case-by-case basis. Such methods must provide the same level of re-assurance as the methods they aim to replace.

Smoke flavouring primary products are complex mixtures. Accordingly, the principles outlined in the guidance document from the EFSA Scientific Committee on harmonised methodologies for risk assessment of combined exposure to multiple chemicals (EFSA Scientific Committee,

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

[14] Directive 2004/10/EC of the European Parliament and of the Council of 11 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.

2019a) should be applied. This EFSA guidance document differentiates between component-based and whole mixture approaches. If a mixture is judged to be fully chemically defined, the preferred approach is generally component-based, i.e. the risk is assessed based on data for exposure and effects of its individual components. However, smoke flavouring primary products may contain substantial portions of unidentified constituents. It is acknowledged that in many cases, toxicity data on multiple individual components of smoke flavourings will be lacking and difficult to obtain. According to the EFSA guidance document, for such insufficiently chemically defined mixtures it may only be feasible to apply a whole mixture approach, i.e. the mixture is treated as a single entity, similar to the approach used for single chemicals. The testing of the whole mixture of components for toxicity has the advantage of not only including individual components but could also reflect interactive effects of multiple components. Toxicity testing of the whole mixture therefore would be appropriate for the derivation of a reference value (see Section 3.3.3).

For the genotoxicity assessment of mixtures containing a substantial fraction of unidentified components, the respective statement of the EFSA Scientific Committee (EFSA Scientific Committee, 2019b) requires a combination of a component-based and a whole mixture approach, since genotoxicity of individual components may not be detected in a whole mixture testing approach, e.g. as a result of dilution.

In accordance with Directive 2010/63/EU[15] on the protection of animals used for experimental and other scientific purposes, the unnecessary use of animals in toxicological studies should be avoided. The studies to be carried out should be those necessary to demonstrate the safety of a smoke flavouring primary product and planned in accordance with the principles of replacement, reduction and refinement of animal studies. Therefore characterisation of individual components and an assessment of their genotoxic potential, as well as the assessment of the genotoxic potential of the unidentified constituents in a primary product should be carried out before embarking on any *in vivo* toxicity studies, other than to test for genotoxicity. According to the EFSA Scientific Committee (2011), clear evidence of genotoxicity in somatic cells *in vivo* has to be considered as an adverse effect *per se*.

Since the compositions of smoke flavouring primary products may differ from one product to another, and since a significant proportion of a primary product may remain unidentified, read-across of toxicity data from one primary product to another is not considered justified.

3.2 Genotoxicity

Smoke flavouring primary products are complex mixtures that may contain a substantial fraction of unidentified components. The recommended approach for the genotoxicity assessment of such type of mixtures is described by the statement of the EFSA Scientific Committee (EFSA Scientific Committee, 2019b).

In line with this statement, an evaluation scheme describing the recommended approach for the genotoxicity assessment of smoke flavouring primary products is also reported in Appendix C.

[15] Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. OJ L 276, 20.10.2010, p. 33–79.

As a first step, the mixture should be chemically characterised as fully as possible. Concentrations of the identified components in the primary product should be provided (see Section 1.2).

The genotoxic potential of the chemically identified components in a smoke flavouring primary product should be assessed individually, using all available data. Genotoxicity data should be collected and evaluated based on the Scientific Committee guidance on genotoxicity (EFSA Scientific Committee, 2011, 2017, 2020). Conclusions on genotoxicity are required for all identified components.

Structure-activity relationship (SAR) information about the genotoxic potential of an identified component may be considered (for details, see Section 3.2.1) when no other information on genotoxicity is available, e.g. published or unpublished studies (for published studies, see also Section 3.5.1).

If only *in silico* predictions of the genotoxicity endpoints are available for an identified component and for its predicted or reported metabolites, and it is assessed as negative in a combination of independent and scientifically valid (Q)SAR models, (i.e. it is required to run more than one (Q)SAR model for each genotoxicity endpoint), the substance may be considered not to raise a concern for genotoxicity and, accordingly, no experimental genotoxicity testing may be necessary. In fact, the combination of different (Q)SAR models increases the overall sensitivity, and the occurrence of fully negative patterns of predictions reduces the probability of false negatives. However, EFSA will closely consider the information and in specific cases additional data may be requested.

For more details on the conditions needed to consider the results of (Q)SAR analyses as reliable for risk assessment, please refer to Section 3.2.1.

A positive *in silico* prediction sets the chemical as potentially genotoxic and it must be further investigated with appropriate experimental testing, to be conducted in line with the Scientific Committee guidance on genotoxicity (EFSA Scientific Committee, 2011, 2017, 2020). Negative results in *in vitro* genotoxicity tests, would rule out the positive *in silico* prediction. On the other hand, a positive result in *in vitro* genotoxicity tests requires an appropriate *in vivo* follow-up test to complete the assessment (steps A.1 and A.1.1 of the evaluation scheme reported in Appendix C) (EFSA SC, 2011, 2017, 2020).

If genotoxicity tests are required on identified components that are structurally related, read across principles for the selection of a representative chemical substance may be considered. In this case, the selected representative substance should then be tested with respect to genotoxicity and used as an indicator substance for all structurally related components that it represents. This assessment on genotoxicity should be carried out in line with the Scientific Committee guidance on genotoxicity (EFSA Scientific Committee, 2011, 2017, 2020).

For grouping chemicals and selecting representative substances for testing, the criteria outlined in ECHA guideline R6 (ECHA, 2008) and practical guidance (ECHA, 2012), should be applied. Applicants should provide documentation to substantiate the applicability of the grouping and read-across. There are several software tools available that may be used to identify structurally related substances such as the OECD QSAR Toolbox (see Section 3.2.1).

The choice of a representative substance among the structurally related substances that may be present in the primary product should be justified; for example, based on the presence of

experimental data, or because it is expected to have the highest genotoxic potential based on, for example, DNA or protein reactivity.

If a primary product contains one or more components that are evaluated to be genotoxic *in vivo* via a relevant route of administration (i.e. after oral exposure), then the primary product raises concern for genotoxicity and the risk to human health related to this identified hazard needs to be taken into account in the risk assessment (step A.2 of the evaluation scheme reported in Appendix C).

If none of the identified chemical substances in a primary product raises concern for genotoxicity (step A.3 of the evaluation scheme reported in Appendix C), as a following step, the Scientific Committee recommends evaluating the genotoxic potential of the unidentified fraction of the mixture. Experimental testing of the fraction of unidentified components should be considered as a first option (step B.1 of the evaluation scheme reported in Appendix C) or, if this is not feasible and a scientific justification can be provided, the whole mixture should be tested (step B.2 of the evaluation scheme reported in Appendix C) (EFSA Scientific Committee, 2019a). It is recognised that for primary products unidentified components may be in the volatile as well as in the non-volatile fraction, therefore a clear separation of identified and unidentified components might be difficult. Nevertheless, attempts to fractionate the test material should be made on a case-by-case basis to minimise the dilution of the components of interest or to remove highly cytotoxic components from the tested sample.

The testing strategy for individual components, a whole mixture or its fraction(s) should follow the Scientific Committee's testing strategy guidance for individual chemical substances (EFSA Scientific Committee, 2011), according to which the following two *in vitro* tests are recommended as the first step:

- A bacterial reverse mutation assay, Test No. 471 (OECD, 2020a), and
- An *in vitro* mammalian cell micronucleus test, Test No. 487 (OECD, 2016c).

As recommended in the OECD test guidelines for *in vitro* genotoxicity testing, the maximum test concentration is based on the cytotoxicity. In the Ames test, the recommended maximum test concentration for soluble non-cytotoxic substances is 5 mg/plate. In the *in vitro* micronucleus test, if no precipitate or limiting cytotoxicity is observed, the highest test concentration should correspond to 10 mM or 2 mg/mL. However, if the test substance is not of defined composition, such as in the case of primary products, the recommended top concentration may need to be higher (e.g. 5 mg/mL), in the absence of sufficient cytotoxicity, to increase the concentration of each of the components (OECD, 2016c).

If testing of the whole smoke flavouring mixture or all of its fractions in an adequately performed set of *in vitro* assays, following the Scientific Committee testing strategy (EFSA Scientific Committee, 2011), provides clearly negative results, the primary product could be considered to be of no concern with respect to genotoxicity and no further testing would be required.

If testing the whole mixture or its fraction(s) in an adequately performed set of *in vitro* assays provides one or more positive results, *in vivo* follow-up testing should be conducted to assess the relevance of these findings for risk assessment (step B.3 of the evaluation scheme reported in Appendix C). The follow-up study should be tailored case by case based on the

activity profile/mode of action observed *in vitro*, following the Scientific Committee genotoxicity testing strategy (EFSA Scientific Committee, 2011, 2017, 2020), and taking into account any other relevant information (e.g. on source material, production process and available physicochemical information on the primary product).

The *in vivo* tests recommended by the EFSA Scientific Committee (EFSA Scientific Committee, 2011, 2017, 2020) are:

- *In vivo* transgenic rodent somatic and germ cell gene mutation assay, Test No. 488 (OECD, 2020b), to follow-up *in vitro* positive results for gene mutations,
- *In vivo* mammalian alkaline comet assay, Test No. 489 (OECD, 2016b) to follow-up *in vitro* positive results for gene mutations and/or structural chromosomal aberrations,
- *In vivo* mammalian erythrocyte micronucleus assay, Test No. 474 (OECD, 2016a) to follow-up *in vitro* positive results for structural and numerical chromosomal aberrations.

A combination of an *in vivo* micronucleus and a 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 of a primary product or its components provides negative results, the relevance of these findings will be evaluated based on the recommendations given by the Scientific Committee in the guidance documents on genotoxicity (EFSA Scientific Committee 2011, 2017) and in the statement on genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019b) (steps B.4 and B.4.1 of the evaluation scheme reported in Appendix C).
If positive results are observed in the *in vivo* test(s), the primary product raises a concern for genotoxicity (step B.5 of the evaluation scheme reported in Appendix C).

If a component of a primary product 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, 2019c), if its estimated exposure is very low, i.e. below the TTC value of 0.0025 µg/kg bw per day (or 0.15 µg/person per day) for DNA-reactive mutagens and/or carcinogens. In such circumstances, it can be concluded that there is a low probability of adverse health effects (EFSA Scientific Committee, 2012, 2019c) (step A.4 of the evaluation scheme reported in Appendix C).

3.2.1 *In silico* methods for the prediction of genotoxicity

In silico predictive methods include: a) structure–activity relationships (SAR) and quantitative structure–activity relationships (QSAR) models – collectively referred to as (Q)SAR – that qualitatively or quantitatively predict the toxicological endpoint from the knowledge of their chemical structure; and b) read-across, that uses data on one or more analogues (the 'source') to make a prediction about a query compound or compounds (the 'target') recognised to be 'similar' to the analogues.

These methods can only be applied to individual chemicals and not to mixtures. Therefore, when used in the context of an application for a primary product, they may be applied to the chemical structure of an identified component. Chemical identifiers such as CAS numbers and SMILES codes should be provided by the applicant for all identified components of the primary

product. Applicants should submit this information as part of the application dossier in an appropriate electronic format (either an Excel sheet or a text file) that allows for direct *in silico* analyses.

Whenever *in silico* methods are used, the general provisions outlined in ECHA Guidance R6 should be followed (ECHA, 2008) both for (Q)SAR and for read-across analyses. Further practical guidance is provided in (ECHA, 2016) for (Q)SAR, and in (ECHA, 2012) for read-across.

(Q)SAR models are implemented in a wide range of commercial and public software tools. Table 1 in Appendix D provides some examples of available software tools for predicting the various genotoxicity endpoints. Many of these software platforms also support read-across.

The software tools usually include separate (and sometimes multiple) (Q)SAR for predicting the genotoxicity endpoints of interest (i.e. *in vitro/in vivo* gene mutations, chromosomal aberrations): if the application of (Q)SAR were considered necessary, it is recommended that the whole spectrum of genotoxicity endpoints is predicted by the applicant.

As already mentioned in Section 3.2, it is required to run more than one (Q)SAR model for each genotoxicity endpoint. The models should be independent from each other (i.e. the algorithms are based on different descriptors, structural alerts or training sets). As an example, when employing the OECD QSAR Toolbox the following combination of profilers (i.e. (Q)SAR models) may be used: 1) DNA binding by OASIS; 2) DNA binding by OECD; 3) DNA alerts for AMES, chromosomal aberrations (CA) and micronucleus test (MNT) by OASIS; 4) *in vitro* mutagenicity (Ames test) alerts by ISS; 5) *in vivo* mutagenicity (micronucleus) alerts by ISS; 6) protein binding alerts for chromosomal aberrations by OASIS.

The results of (Q)SAR methods may be considered as sufficient in the risk assessment provided that the following conditions are met:

(i) (Q)SAR models for which scientific validity has been established are used. In particular the models should comply with the five OECD principles for (Q)SAR validation (OECD, 2007)[16];
(ii) the substance falls within the applicability domain of the (Q)SAR models;
(iii) the predictions are relevant for the regulatory purpose; and
(iv) the information on the models and the predictions are well documented.

More detailed information on the above conditions is available from ECHA (2008, 2016).

[16] A (Q)SAR model intended to be used for regulatory purposes should be associated with the following information: (1) a defined endpoint: the model must predict the same endpoint that would be measured to fulfil the regulatory requirements; (2) an unambiguous algorithm: the algorithm underlying the model must be available and documented to ensure transparency and reproducibility of the calculation; (3) a defined domain of applicability: the applicability domain (in terms of, e.g. physicochemical parameters or molecular sub-structures) and the limitations of the model have to be described to allow the assessment of the applicability domain for the specific prediction; (4) appropriate measures of goodness-of–fit, robustness and predictivity: provide appropriate measure of the internal performance of a model (as represented by goodness-of-fit and robustness) and the predictivity of a model (as determined by external validation); (5) a mechanistic interpretation, if possible: reasoning on, and documenting the causal link between the descriptors used in the model and the predicted endpoint add confidence in the reliability of the predictions.

3.3 Toxicity other than genotoxicity

Since smoke flavouring primary products are mixtures, the principles outlined by the EFSA Scientific Committee on the testing of combined exposures (EFSA Scientific Committee, 2019a) are to be used for the assessment of potential toxicity. As explained above (see Section 3.1), toxicity testing of primary products should be based on the assessment of the whole mixture for derivation of the reference point. Applicants are reminded that before conducting any *in vivo* toxicity testing, any concern for genotoxicity should be ruled out.

Diagrams outlining the recommended tiered toxicity testing for primary products, as described in this chapter, are given in Appendix E.

3.3.1 Acute toxicity

In general, from past experience obtained from sub-chronic toxicity studies, there were no indications that primary products are potent acute toxicants. Therefore, there is no default requirement for acute toxicity data. If, however, the 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.

3.3.2 Toxicokinetics (absorption distribution metabolism excretion (ADME))

ADME studies can only address the kinetics of identified individual constituents. However, smoke flavouring primary products are complex mixtures of components belonging to many different chemical classes, for which significant differences in toxicokinetics may be anticipated. In addition, a substantial part of the primary products may be unidentified, so a full prediction of their toxicokinetic behaviour is difficult. Considering these limitations, the Panel does not ask for ADME studies with primary products.

Based on the information available from previous evaluations, it can be assumed that many primary products will contain constituents that will be absorbed in the gastrointestinal tract, and given the molecular structures and molecular weights of the constituents identified up to now, the absorption in the gastrointestinal tract can be anticipated to be substantial. It can therefore be concluded that toxicity data are needed for the safety assessment of these primary products (see Section 3.3.3).

3.3.3 Testing for repeated dose, reproductive and developmental toxicity

For primary products an individual evaluation should be performed, since they are complex mixtures for which read-across is not applicable. Further, due to the presence of a fraction of unidentified substances in these primary products, an evaluation according to the Threshold of Toxicological Concern (TTC) principles is not applicable.

From previous evaluations it has become clear that exposure levels of smoke flavouring primary products approach those observed for food additives. Consequently, toxicity data are needed in line with the data requirements for food additives. Comparable to food additives, the toxicity data required for smoke flavouring primary products are set following a tiered approach. 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). In agreement with this approach, at Tier I of the safety assessment of

smoke flavouring primary products, subchronic oral toxicity data are needed. Based on already available knowledge on primary products as presented in previous Opinions, it can be assumed that at least part of any orally administered primary product will be absorbed and systemically available. As a result of this anticipated absorption of constituents of a smoke flavouring primary product, data on developmental and reproductive toxicity will also be needed and are included as a requirement in Tier I.

It is recognised that all the data needed at Tier I can be obtained from an Extended One Generation Reproductive Toxicity study (EOGRT), according to OECD TG 443 (OECD, 2018a). In the EOGRT study, testing should be in both male and female animals covering a defined pre-mating period (minimum of two weeks) and a two-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 EOGRT study will provide information evaluating specific life stages not covered by the other toxicity studies: on fertility and reproductive function, and on short- to long-term developmental effects from exposure during pregnancy, lactation and pre-pubertal phases, as well as effects on juveniles and adult offspring. In addition, an EOGRT study will provide information on immunotoxicity and neurotoxicity.

The toxicity studies that are to be used in the assessment should be designed in such a way that they provide reliable and useful BMDL–BMDU intervals[17] in accordance with the EFSA Guidance on Dose Response Modelling (EFSA Scientific Committee, 2017b) or with the most recent version of it. For all parameters studied, the data should be submitted in an appropriate electronic format (either spreadsheet or text tables) that allows for direct evaluation of the data.

Data requirements for new applications at Tier I

In the light of the likely absorption of the constituents for a smoke flavouring primary product, an EOGRT study (OECD TG 443) is mandatory. This study should comprise the full arms of the parental cohorts as well as cohorts 1A, 1B, 2A, 2B and 3. For new applications 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. A scheme outlining the tiered toxicity testing for new authorisations of smoke flavouring primary products is presented in Appendix E (1).

Data requirements for renewal applications at Tier I

For all currently authorised primary products, i.e. those already listed in Regulation (EU) No 1321/2013 for which renewal applications are submitted, subchronic studies are already available. However, these 90-day subchronic toxicity studies have been carried out according to OECD guidance, which did not include requirements for examination of the endocrine systems. Therefore, for renewal applications, an EOGRT study (OECD TG 443) is also mandatory. The 90-day sub-chronic toxicity studies which are already available may be used as a dose-range finding study before conducting the EOGRT. The parental animals in the

[17] The EFSA BMDL calculation tool is available at: https://shiny-efsa.openanalytics.eu/

EOGRT study will have to be examined, and this study should further comprise the cohorts 1A, 1B, 2A, 2B and 3 as prescribed by OECD TG 443. A scheme outlining the tiered toxicity testing for renewal applications of authorized smoke flavouring primary products is presented in Appendix E (2).

Data requirements at Tier II

Depending on the results of the toxicity studies in Tier I, additional toxicity data may be required in Tier II. In Tier II there is no difference between new and renewal applications.

A scheme by which it will be decided whether there is a need for additional toxicity testing in Tier II is given in Figure 1 in Appendix F. The decision is based on the outcome of the Tier I testing for (subchronic) repeated dose toxicity and reproductive–developmental toxicity testing in combination with the outcome of the exposure assessment. For both aspects of toxicity, sufficiently large Margins of Safety (MOS) must be calculated to conclude that no additional toxicity testing is needed.

For repeated dose toxicity, conventionally, an MOS of at least 300 is required (EFSA CEF Panel, 2010) if the reference point originates from a 90-day sub-chronic oral toxicity study. An MOS of less than 300 would indicate that a combined chronic oral toxicity/carcinogenicity study, Test No. 453 (OECD, 2018b) would be required in Tier II testing. A need for further testing in Tier II for chronic toxicity/carcinogenicity may also emerge from histological changes that could be indicative of potential pre-carcinogenic lesions. Alternatively, the applicant may lower the exposure by limiting the number of food categories for the use and/or the maximum use levels applied. An MOS which is lower than 100 would raise a safety concern, even if this is based on the results of a study according to OECD TG 453. In this case the only option is to reduce exposure.

In addition, a need for Tier II 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 MOS criterion of 300 mentioned above may not apply. The minimal MOS 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 II. Nevertheless, similar to what has been described above for repeated dose toxicity, the applicant may try to mitigate the need for testing in Tier II by limiting the number of food categories for use of the smoke flavouring primary product and/or the maximum use levels applied.

It is further noted that the primary product should be evaluated according to both legs of the scheme in Figure 1 and that it is not enough to consider only the endpoint for which the lowest MOS is calculated. The following example may demonstrate this: assume that a MOS for subchronic toxicity of 200 were calculated and also an MOS for reproductive–developmental toxicity of 125. It may well be that in such a case the MOS for reproductive–developmental toxicity is considered sufficient. However, it would be inappropriate to conclude that the primary product is not of safety concern, since the MOS for subchronic toxicity would be too low and would indicate a need for further testing in Tier II.

3.3.4 Additional studies

Apart from data specifically required in this guidance document, there may be additional toxicity studies that could be supportive for the safety assessment. For instance, toxicity studies that are not required for evaluation of the primary products, but which may have been conducted for other purposes (e.g. acute toxicity (see Section 3.3.1), irritation and sensitisation studies). If such studies are available, they should be submitted as they may provide useful background information.

3.4 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.

Regarding the potential impact of the use of smoke flavourings on the environment, the Panel noted the following:

1. Smoke flavouring primary products are produced by pyrolysis of defined types of woods, i.e. naturally occurring source materials. The type of compounds generated by this step are expected to be similar to those formed upon conventional burning of wood.
2. Smoke flavouring primary products are produced under controlled conditions and the manufacturing process involves, in most cases, the extraction into an aqueous phase. Constituents with high lipophilicity will, therefore, be absent or present in very low concentrations.
3. Since primary products are added to foods, their constituents would be subject to human consumption and metabolism in the body and degradation in the sewage treatment plant before their release into the environment. It is expected that most of the constituents present in the primary products are extensively metabolised and/or readily biodegraded in a sewage treatment plant, and therefore they are of low concern for the environment.

Based on these considerations, an environmental risk assessment is not required by default.

There may, however, be primary products for which these considerations may be less applicable or not applicable at all, e.g. primary products which are obtained by manufacturing processes resulting in an increased proportion of the water-insoluble high-density phase of condensed smoke (primary tar fraction), see also Section 1.1. In those cases, the applicant should investigate whether the primary product contains constituents that are not extensively metabolised and/or readily biodegradable. For these constituents the applicant should provide evidence to demonstrate absence of concern for the environment. The testing strategy and risk assessment schemes already described for substances with a similar emission pattern and/or exposure routes such as biocides (ECHA, 2017) or medicinal products for human use (EMA, 2019) could be followed. In this respect the generation of data using non-testing approaches, such as (Q)SAR (ECHA, 2008), could also be considered provided they are relevant, reliable and adequate for the purpose and are documented in an appropriate manner (see also ECHA, 2008). Applicants are reminded that, before conducting any testing

addressing environmental safety, where applicable, any concern for genotoxicity should be ruled out.

It is noted that smoke flavourings are complex mixtures in which a fraction of components may not be fully characterised. 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 (see Section 1.2.3.4). In this respect, it might be relevant to assess whether the unidentified constituents might share similar properties of the constituents in the characterised fraction. On a case-by-case basis, further data might be needed. As already described above, in some cases (e.g. for primary products which are obtained by manufacturing processes resulting in an increased proportion of primary tar fraction) further data on the primary product as a whole, including both the characterised and uncharacterised fraction, may need to be generated. Further guidance can be found in the OECD guidance document on aquatic toxicity testing of difficult substances and mixtures (OECD, 2019).

3.5 Other scientific data

This section is intended to provide a description of other types of scientific data that may be used to complement and to support the studies required by this guidance document, as indicated in the above sections.

3.5.1 Published literature

Applicants should provide all the information needed to enable a conclusion on the safety assessment of a smoke flavouring primary product. This also includes the review of the published literature on both the smoke flavouring primary product and its characterised components. This may be particularly relevant for the hazard identification related to genotoxicity potential and environmental safety of the characterised components (see Sections 3.2 and 3.4).

The methods used to identify relevant scientific data, including the scope and criteria for literature searches, should be described in line with the principles of the systematic review methodology which aims to systematically identify, evaluate and synthesise evidence for a specific question. 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.
This would promote a more structured and transparent use of the body of evidence, reducing bias in the selection of the studies by the extensiveness and reproducibility of the entire process. For more detailed instruction on how to identify and select scientific literature according to the principles of the systematic literature review, applicants should refer to the EFSA guidance on application of systematic review methodology to food and feed safety assessments to support decision making (EFSA, 2010).

3.5.2 Information on existing evaluation from other regulatory bodies

Information on any existing evaluations and authorisations should be provided for the smoke flavouring primary product. This should include details of the body which carried out the

995 evaluation and when this was undertaken. Any relevant data/studies generated/conducted in
996 the context of other regulatory frameworks should be provided in full.

997

998 4 Uncertainty

999 4.1 Introduction to uncertainty analysis

1000 Uncertainty is any limitation in knowledge. Uncertainty analysis is part of the risk assessment
1001 performed by EFSA. In line with the principles described in the EFSA Scientific Committee's
1002 Guidance on Uncertainty Analysis (EFSA Scientific Committee, 2018), each step of the risk
1003 assessment performed by EFSA should clearly and unambiguously document what sources of
1004 uncertainty have been identified and evaluate their impact on certainty in the assessment
1005 conclusion. This applies to all EFSA's areas of work, all types of scientific assessment and all
1006 types of uncertainty affecting assessment. An uncertainty analysis is usually planned as a
1007 process in which individual sources are identified, characterised and combined with the aim
1008 of evaluating overall uncertainty in the output of the assessment.

1009 4.2 Approach to treat uncertainties in the risk assessment of smoke
1010 flavouring primary products

1011 The risk assessment of a smoke flavouring primary product aims to evaluate whether a
1012 primary product is a concern for human health and the environment. As described in detail in
1013 the previous sections of this guidance, the risk assessment is a step-wise approach and each
1014 step requires specific considerations with respect to uncertainties.

1015 Identification and characterisation of uncertainties in the standardised procedure for the risk
1016 assessment for smoke flavouring primary products has been described in parallel to the
1017 specification of data requirements in this guidance document. The standardised procedure
1018 covers every step of the risk assessment and is accepted by the assessors and decision-makers
1019 as providing an appropriate basis for decision-making (see Section 6 of the EFSA Guidance for
1020 uncertainty analysis (EFSA Scientific Committee, 2018)).
1021 Specifying a standardised procedure and its associated uncertainty analysis is efficient
1022 because it helps the assessors to identify and plan for uncertainties in each assessment of a
1023 smoke flavouring primary product.

1024 The list of standard uncertainties affecting the assessment of smoke flavourings and how
1025 these are treated in the standardised procedure for smoke flavouring primary products is
1026 described in Table 1 in Appendix G. EFSA considers that the approaches taken to address
1027 these uncertainties are sufficient to meet the protection goals as specified in Regulation (EC)
1028 No 2065/2003. The presence of non-standard uncertainties in a risk assessment is a trigger
1029 for a more detailed uncertainty analysis when a standardised procedure may not be sufficient.
1030 Table 1 in Appendix G includes criteria to aid EFSA in determining whether non-standard
1031 uncertainties are present, i.e. additional uncertainties that go beyond the standard
1032 uncertainties covered by the standardised procedure.

4.3 What is required by the applicants

Applicants do not need to describe or assess the uncertainties themselves. After submission, the uncertainty in e.g. manufacturing, composition, exposure estimates and toxicity is characterised by EFSA. When assessing submitted applications for smoke flavouring primary products, EFSA will check whether there are any uncertainties beyond those listed as standard uncertainties. If so, EFSA will evaluate their impact on the results from the standardised procedure (Section 3 in EFSA Scientific Committee, 2018) or, if non-standard uncertainties are substantial, perform a case-specific assessment (Section 4 in EFSA Scientific Committee, 2018). Applicants should be aware that a case-specific assessment may require more data, expert judgement or modelling to alleviate uncertainty concerns related to the non-standard character of the application.

References

ECHA (European Chemicals Agency), 2008. Guidance for the implementation of REACH, Chapter R.6: QSARs and grouping of chemicals. ECHA, Helsinki. 134 pp. Available online: https://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf/77f49f81-b76d-40ab-8513-4f3a533b6ac9

ECHA (European Chemicals Agency), 2012. Practical Guide 6. How to report read-across and categories. Version 2.0, December 2012. Available online: https://echa.europa.eu/documents/6362380/7127661/pg_report_readacross_en.pdf/69860e5b-c669-4a0d-b868-72f5dba5b560

ECHA (European Chemicals Agency), 2016. Practical guide. How to use and report (Q)SARs. Version 3.1, July 2016. Available online: https://echa.europa.eu/documents/10162/13655/pg_report_qsars_en.pdf

ECHA (European Chemicals Agency), 2017. Guidance on Biocidal Products Regulation: Volume IV Environment - Assessment and Evaluation (Parts B+C). Available online: https://echa.europa.eu/documents/10162/23036412/bpr_guidance_ra_vol_iv_part_b-c_en.pdf/e2622aea-0b93-493f-85a3-f9cb42be16ae

EFSA (European Food Safety Authority), 2010. Application of systematic review methodology to food and feed safety assessments to support decision making. EFSA Journal 2010; 8(6):1637, 90 pp. doi:10.2903/j.efsa.2010.1637

EFSA (European Food Safety Authority), 2020. Administrative guidance on the preparation and presentation of applications for new authorisation and for renewal of authorisation of smoke flavourings primary products. *Under preparation*

EFSA ANS Panel (Panel on Food Additives and Nutrient Sources added to Food), 2012. Guidance for submission for food additive evaluations. EFSA Journal 2012; 10(7):2760, 60 pp. doi:10.2903/j.efsa.2012.2760

EFSA AFC Panel (Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food), 2005. Guidance on submission of a dossier on a Smoke Flavouring Primary Product for evaluation by EFSA. EFSA Journal 2005; 3(4):492, 8 pp. doi:10.2903/j.efsa.2005.492

EFSA CEF Panel (Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2009. Scientific Opinion on Dietary exposure assessment methods for smoke flavouring Primary Products. EFSA Journal (2009) RN-284, 1–30.

EFSA CEF Panel (Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids), 2010. Statement on the Safety Evaluation of Smoke Flavourings Primary Products: Interpretation of the Margin of Safety. EFSA Journal 2010; 8(1):1325, 7 pp. doi:10.2903/j.efsa.2009.1325

EFSA Scientific Committee, 2011. Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011; 9(9):2379, 69 pp. doi:10.2903/j.efsa.2011.2379

EFSA Scientific Committee, 2012. Scientific Opinion on Exploring options for providing advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC). EFSA Journal 2012; 10(7):2750, 103 pp. doi:10.2903/j.efsa.2012.2750

EFSA Scientific Committee, 2017. Scientific Opinion on the clarification of some aspects related to genotoxicity assessment. EFSA Journal 2017;15(12):5113, 25 pp. doi:10.2903/j.efsa.2017.5113

EFSA Scientific Committee, 2017b. Update: Guidance on the use of the benchmark dose approach in risk assessment. EFSA Journal 2017; 15(1):4658, 41 pp. doi:10.2903/j.efsa.2017.4658

EFSA Scientific Committee, 2018. Guidance on Uncertainty Analysis in Scientific Assessments. EFSA Journal 2018;16(1):5123, 39 pp. doi:10.2903/j.efsa.2018.5123

EFSA Scientific Committee, 2019a. Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. EFSA Journal 2019;17(3):5634, 77 pp. doi:10.2903/j.efsa.2019.5634

EFSA Scientific Committee, 2019b. Statement on the genotoxicity assessment of chemical mixtures. EFSA Journal 2019;17(1):5519, 11 pp. doi:10.2903/j.efsa.2019.5519

EFSA Scientific Committee, 2019c. Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment. EFSA Journal 2019;17(6):5708, 17 pp. doi:10.2903/j.efsa.2019.5708

EFSA Scientific Committee, 2020. Draft Guidance on aneugenicity assessment. EFSA Journal 2020; volume(issue):NNNN, XXX 30 pp. doi:10.2903/j.efsa.2020.eNNNN

EMA (European Medicines Agency), 2019. Draft guideline on the environmental risk assessment of medicinal products for human use. Draft available online: https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-environmental-risk-assessment-medicinal-products-human-use-revision-1_en.pdf

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2005. Sixty-fourth meeting, Rome, 8–17 February 2005. Summary and Conclusions. Available online: http://www.who.int/ipcs/food/jecfa/summaries/summary_report_64_final.pdf

OECD (Organisation for Economic Co-operation and Development), 2007. Guidance document on the validity of (Quantitative) Structure-activity relation ships ((Q)SAR) models. OECD Environment Health and Safety Publications Series on Testing and Assessment No.69 ENV/JM/MONO (2007), OECD Publishing, Paris. Available online: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2007)2&doclanguage=en

OECD (Organisation for Economic Co-operation and Development), 2016a. Test No. 474: Mammalian erythrocyte micronucleus test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. https://doi.org/10.1787/9789264264762-en

OECD (Organisation for Economic Co-operation and Development), 2016b. Test No. 489: *In vivo* mammalian alkaline comet assay, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. https://doi.org/10.1787/9789264264885-en

OECD (Organisation for Economic Co-operation and Development), 2016c. Test No. 487: *In Vitro* Mammalian Cell Micronucleus Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. https://doi.org/10.1787/9789264264861-en

OECD (Organisation for Economic Co-operation and Development), 2016d. Test No. 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. https://doi.org/10.1787/9789264264403-en

OECD (Organisation for Economic Co-operation and Development), 2018a. Test No. 443: Extended One-Generation Reproductive Toxicity Study, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. doi:10.1787/9789264185371-en

OECD (Organisation for Economic Co-operation and Development), 2018b. Test No. 453: Combined Chronic Toxicity/Carcinogenicity Studies, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. doi:10.1787/9789264071223-en

OECD (Organisation for Economic Co-operation and Development), 2019. Guidance document on aquatic toxicity testing of difficult substances and mixtures, OECD Series on Testing and Assessment, OECD Publishing, Paris. doi:10.1787/0ed2f88e-en

OECD (Organisation for Economic Co-operation and Development), 2020a. Test No. 471: Bacterial Reverse Mutation Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. doi:10.1787/9789264071247-en

OECD (Organisation for Economic Co-operation and Development), 2020b. Test No. 488: Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, doi:10.1787/9789264203907-en

WHO/IPCS, 2009. Environmental Health Criteria 240. Principles and methods for the risk assessment of chemicals in food. Avaialble online: https://apps.who.int/iris/bitstream/handle/10665/44065/WHO_EHC_240_eng.pdf

Abbreviations

ADME - absorption, distribution, metabolism and excretion

BMD - benchmark dose

BMDL - benchmark dose lower confidence limit

EOGRT - Extended One-Generation Reproduction Toxicity study

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 Organization for Standardization

LOD - limit of detection

LOQ - limit of quantification

MOS - margin of safety

MPL - maximum permitted level

OECD - Organisation for Economic Co-operation and Development

PAHs - polycyclic aromatic hydrocarbons

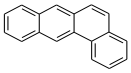
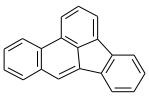
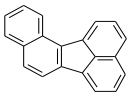
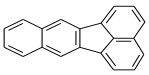
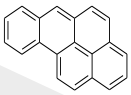
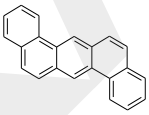
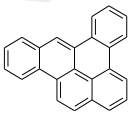
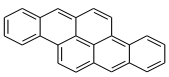
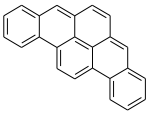
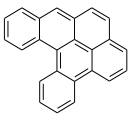
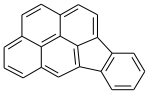
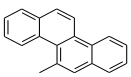
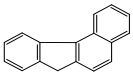
(Q)SAR - quantitative structure-activity relationship

SAR - structure-activity relationship

TG - test guideline

TTC - Threshold of Toxicological Concern

Appendix A – Priority group of PAHs to be analysed

	Compounds	Structure	Molecular weight
1	Benz[a]anthracene		228 AMU
2	Benzo[b]fluoranthene		252 AMU
3	Benzo[j]fluoranthene		252 AMU
4	Benzo[k]fluoranthene		252 AMU
5	Benzo[ghi]perylene		276 AMU
6	Benzo[a]pyrene		252 AMU
7	Chrysene		228 AMU
8	Cyclopenta[cd]pyrene		226 AMU
9	Dibenz[a,h]anthracene		278 AMU
10	Dibenzo[a,e]pyrene		302 AMU
11	Dibenzo[a,h]pyrene		302 AMU
12	Dibenzo[a,i]pyrene		302 AMU
13	Dibenzo[a,l]pyrene		302 AMU
14	Indeno[1,2,3-cd]pyrene		276 AMU
15	5-Methylchrysene		242 AMU
16	Benzo[c]fluorene		216 AMU

Appendix B – Format for the submission of the proposed specifications of a smoke flavouring primary product

Name of smoke flavouring primary product	
Source materials:	
• woods	
• other ingredients	
Identity parameters:	
• Physicochemical parameters:	
- pH	
- density	
- refraction index	
- staining index	
Chemical composition:	
• Chemical classes:	
- acids	
- carbonyls	
- phenols	
• 20 principal constituents of the volatile fraction	
Purity:	
• Polycyclic aromatic hydrocarbons (PAHs)	
• Heavy metals:	
- Lead	
- Arsenic	
- Cadmium	
- Mercury	

Appendix C – Genotoxicity assessment of primary products

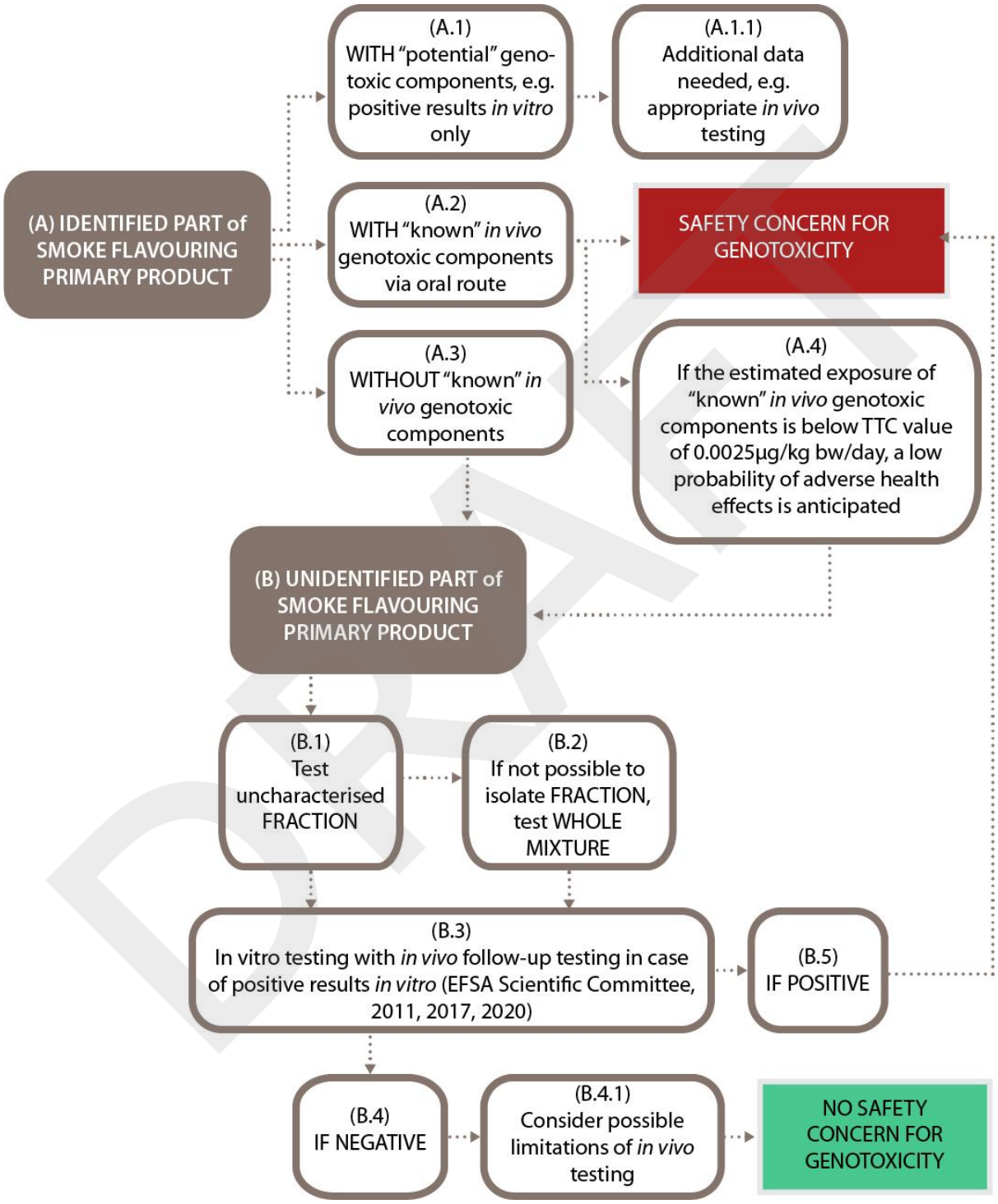


Figure 1: Evaluation scheme for the genotoxicity assessment of smoke flavouring primary products

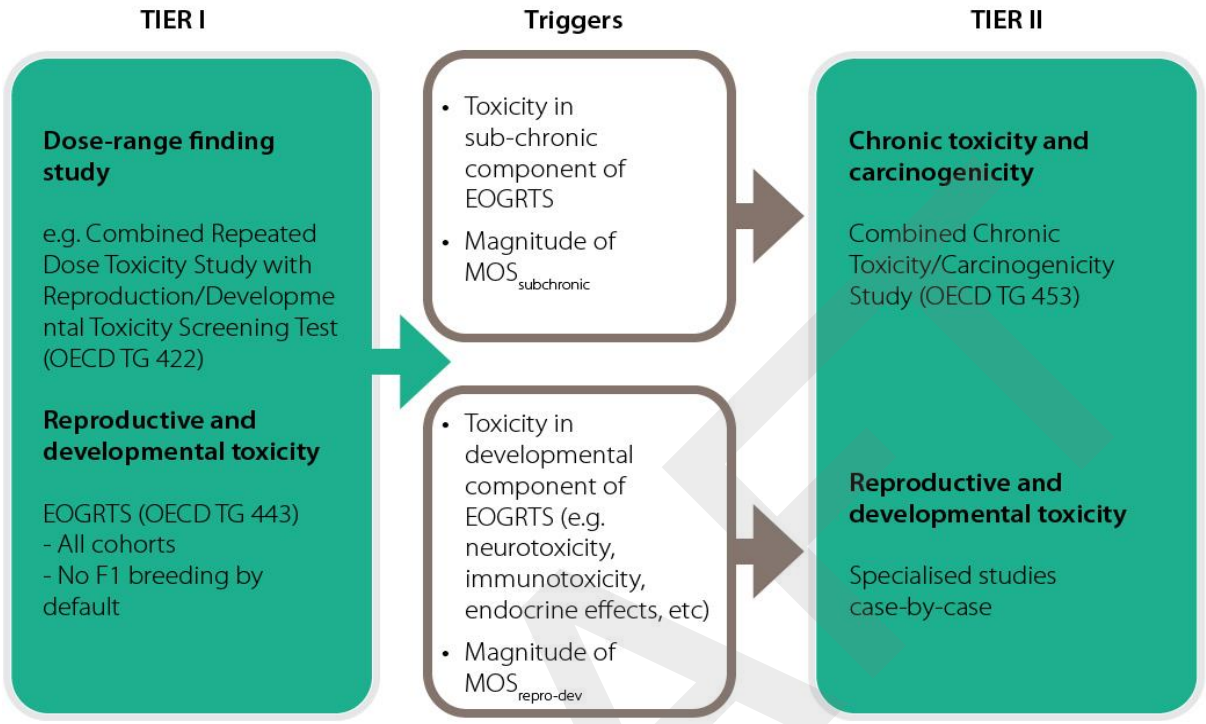
Appendix D – *In silico* computational platforms

Table 1: Examples of *in silico* computational platforms that can be used to estimate the toxic effects or properties of individual chemicals. The table provides references with the full description of the platforms and the methods, together with their status (commercial or public).

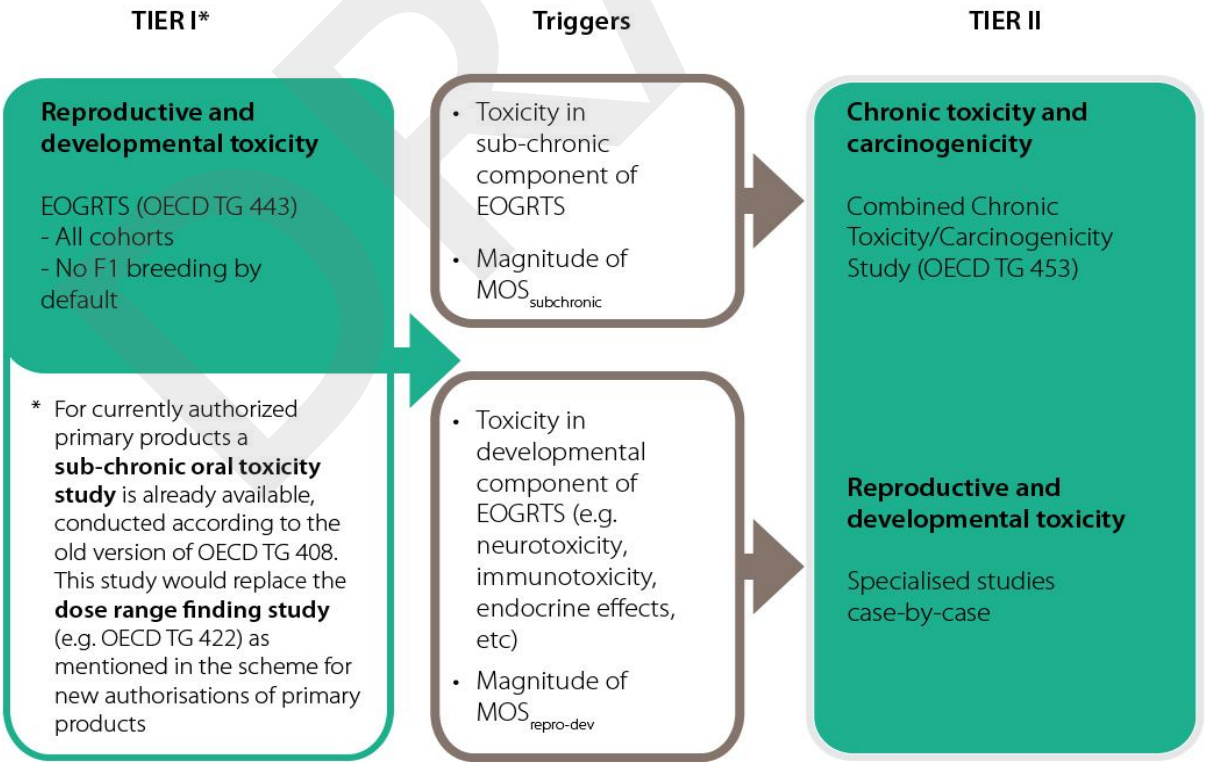
Commercial	
ADMET	https://www.simulations-plus.com/software/admetpredictor/
ACD	https://www.acdlabs.com/
Lhasa	https://www.lhasalimited.org/
CASE	http://www.multicase.com/case-ultra
TIMES	http://oasis-lmc.org/products/software/times.aspx
Public	
Danish QSAR DB	http://qsar.food.dtu.dk/
Lazar	https://www.in-silico.de/
OECD (Q)SAR Toolbox	https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm
ToxTree	http://toxtree.sourceforge.net/
VEGA platform	https://www.vegahub.eu/

Appendix E – Tiered toxicity testing of primary products

1) NEW AUTHORISATIONS



2) RENEWALS



Appendix F – Decision scheme for Tier II toxicity testing

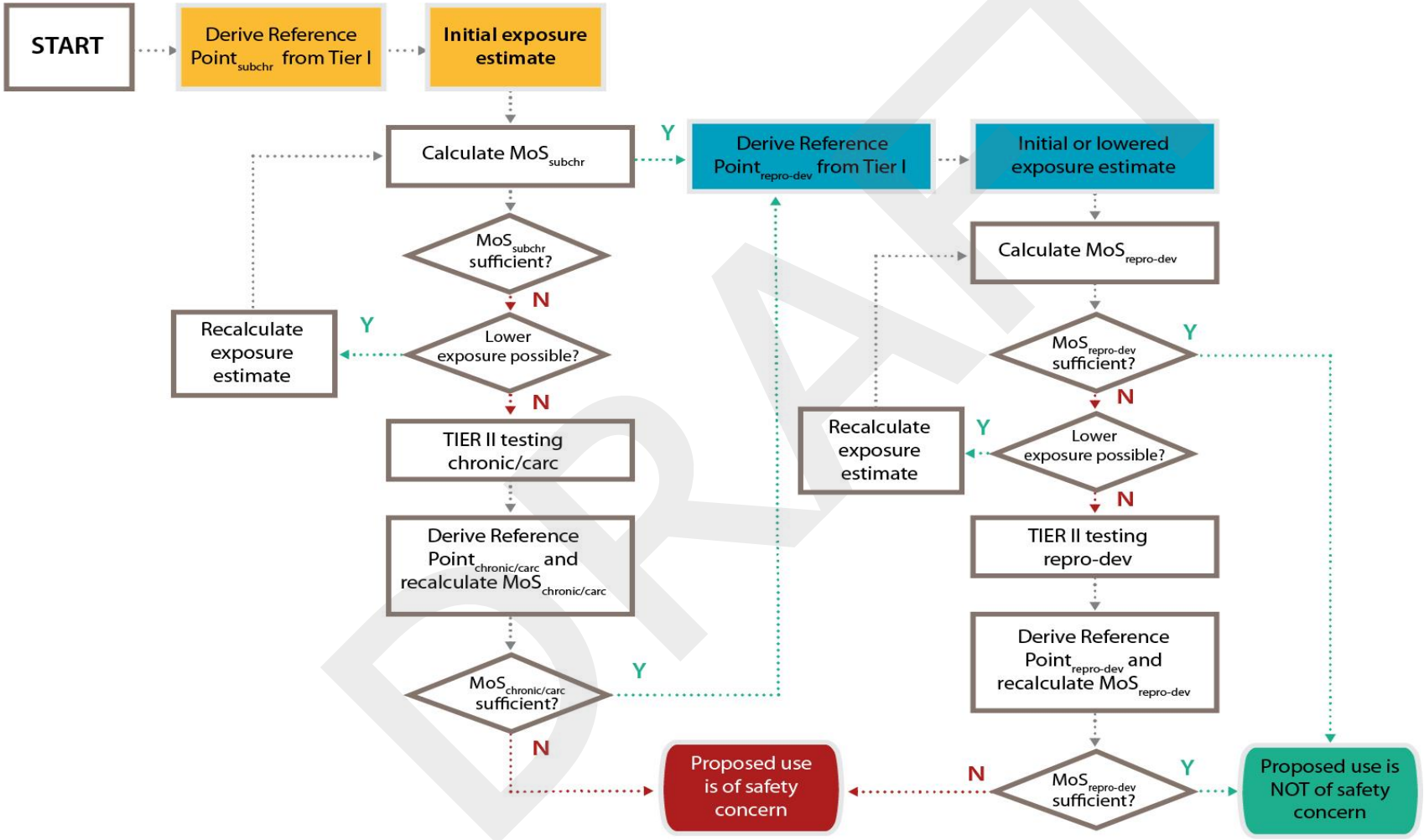


Figure 1: The decision scheme is based on the outcome of the Tier I testing for subchronic repeated dose toxicity and reproductive–developmental toxicity testing in combination with the outcome of the exposure assessment. It is applicable to both new and renewal applications for smoke flavouring primary products. The scheme is the conceptual representation of the considerations leading to either the identification of needs for Tier II testing or to the conclusion of "no concern" on the basis of the data available after Tier I. Following Tier II, a final conclusion will be reached, which could be either "there is a safety concern for the smoke flavouring primary product based on the proposed uses and use levels" or "there is no safety concern for the primary product based on the proposed uses and use levels".

The decision scheme starts at the top with the derivation of the reference point derived for subchronic repeated dose toxicity (subchr) resulting from the Tier I testing (yellow shading). The initial exposure estimate (yellow shading) is also input data that is needed for the calculation of the MOS for subchronic repeated dose toxicity (MOS_{subchr}) at Tier I. The Reference Point for reproductive-developmental toxicity (repro-dev) after Tier I (blue shading) is consecutive input data which should be combined with the exposure estimate as based on the initially submitted information submitted by the applicant or with a lowered exposure estimate following the Tier I assessment of subchronic toxicity (blue shading). From these the MOS for reproductive / developmental toxicity can be calculated ($MOS_{repro\text{-}dev}$).

The diamonds in the decision scheme include two types of questions: a) whether the MOS_{subchr} or $MOS_{repro\text{-}dev}$ or the MOS for chronic toxicity and carcinogenicity study ($MOS_{chronic/carc}$) are sufficient to conclude that the primary product can be considered to be of no safety concern under the proposed conditions of use. For more details on the numerical cut-offs for the MOS, refer to Section 3.3.3; b) whether it is possible to lower the exposure estimates. This could be achieved by refining the exposure estimates (to be done by EFSA during the risk assessment). If this does not result in a sufficient MOS, the exposure can subsequently be lowered by lowering the (proposed) use levels and/or by reducing the uses (to be done by the applicant).

If the answer to the questions in the diamonds is No (N), i.e. if the MOS are too low and if it is not possible to lower the exposure estimate, a need for additional toxicity testing in Tier II is triggered, either for chronic toxicity and carcinogenicity testing (chronic/carc) and/or for additional testing to follow-up toxicity effects observed in the EOGRTS (e.g. endocrine-, neuro- and immuno-toxicity effects). If after the Tier II testing the MOS are still too low, the smoke flavouring primary product is concluded to be of safety concern under the proposed conditions of use. On the other hand, if the answer to the questions in the diamonds is addressing the magnitude of the MOS is Yes (Y), it can be concluded that the smoke flavouring primary product is safe under the proposed conditions of use."

Appendix G – List of standard sources of uncertainty

Table 1: List of sources of uncertainties treated by the standardised assessment of smoke flavouring primary products, how they are treated in the standard procedure, and criteria to support the judgement to be made by EFSA when uncertainties are standard.

ID	Location of standard uncertainty	Treatment in standardised procedure	Criteria to be a standard uncertainty
1	Manufacturing of the primary product (1.1)	Require the applicant to provide a detailed description of the method of manufacturing.	Method of manufacturing is described with enough detail to ensure a consistent production of the primary product complying with the specification.
2	Chemical composition (1.2.3)	Require the applicant to apply appropriate methods to sample and to analyse the volatile and non-volatile parts of the primary product.	Methods are appropriate and comply with the requested performance and quality criteria. A detailed description of the methods applied is included in the dossier.
3	Unidentified fraction (1.2.3.4)	Require the applicant to demonstrate that efforts have been made to reduce the unidentified fraction.	Unidentified fraction is below the limit requested in Regulation (EC) No 627/2006 and, regardless of these limits, sufficient analytical efforts to reduce the fraction of unidentified components have been demonstrated.
4	Reproducibility of the production (1.2.3.6)	Require the applicant to provide analytical data on at least five batches, including a description of how they were selected and ensure that the batches analysed cover the range of different proportions of source materials intended to be used. EFSA will estimate batch-to-batch variability with statistical methods.	The batches are from different production runs. If applicable, for each batch, the proportions of woods are indicated and the batches analysed cover the range of source materials. Batch-to-batch variability per identified compound is acceptable.

5	PAH levels (1.2.3.5)	Require the applicant to show that the methods used to analyse PAHs fulfil the performance criteria of Regulation (EC) No 627/2006. EFSA will estimate average level per PAH and batch-to-batch variability per PAH with statistical methods.	Selection procedure acceptable. The average levels of individual PAHs, the respective relative standard deviations, and the applied limits of detection and quantification are assessed.
6	Proposed use levels (2.1)	EFSA will perform the risk assessment based on the information submitted by the applicant on proposed maximum and (expected) typical use levels. No uncertainty is taken into account in these levels, other than ensuring that the definitions of typical and maximum use levels are unambiguous to avoid different interpretations.	Definitions of use levels are judged as unambiguous by EFSA.
7	Food consumption data for the foods in which the primary product is (proposed to be) used	EFSA will estimate the exposure based on the food consumption data in the EFSA Comprehensive European Food Consumption Database and will consider indications of low reliability in the estimates.	Documentation that there was no indication of low reliability in the exposure estimates due to limitations in the food consumption data.
8	Genotoxicity testing of individual components or of the unidentified fraction (3.2)	Require the applicant to perform the genotoxicity testing of the individual components according to the relevant OECD TGs	Genotoxicity testing performed in line with the criteria reported in relevant OECD TG for genotoxicity testing. Absence of any issues indicating non-standard uncertainties affecting interpretation of the results.

		for genotoxicity assays. Require the applicant to perform genotoxicity testing of the whole mixture containing the unidentified part (only if the identified components are of no concern for genotoxicity and if separation of the unidentified fraction for experimental testing is not feasible).	
9	Lack of experimental data on genotoxicity on individual components (3.2.1)	Require the applicant to apply *in silico* assessment on the individual components for which the experimental data on genotoxicity are missing, when the conditions described in Section 3.2.1 of this guidance are met.	The conditions for the acceptability of the applied (Q)SAR methods are met as described in Section 3.2.1.
10	Acute toxicity (3.3.1)	No default requirement for acute toxicity data.	No criteria needed. Based on previous assessments, it is considered unlikely that primary products of smoke flavourings are potent acute toxicants.
11	Toxicokinetics (ADME) (3.3.2)	No default requirement to assess ADME of the whole primary product.	Manufacturing process and compositional data do not indicate the presence of constituents in the primary product that might trigger the need for specific ADME assessments.
14	Type of toxicity study (3.3.3)	Add uncertainty factors to adjust the requirement for an adequate MOS according to the type of toxicity data available.	See Section 3.3.3

15	Quality of toxicity studies (3.1 and 3.3.3)	Require the applicant to conduct and report toxicity studies in conformity with relevant OECD TG.	Compliance of the toxicity studies with the relevant OECD TG. Absence of any issues indicating non-standard uncertainties affecting interpretation of the results. Consistency of the results if more than one study of the same type is available.
16	Toxicity of unidentified fraction (3.3.3)	Require the applicant to perform toxicity testing with the whole primary product.	Confirmation that the whole primary product was tested in the toxicity studies using a representative batch.
17	Representativeness of the batch selected for toxicity testing (3.1)	EFSA will evaluate whether the tested batch is representative of the material of commerce based on its description, its compositional data and the criteria for its selection.	No indication that batch(es) used in toxicity testing are not representative of the material of commerce.
18	Reference point for toxicity (3.3.3)	EFSA will estimate the benchmark dose corresponding to a specified effect (BMD), considering alternative dose-response functions, and derive a lower bound of a 95% probability or confidence interval on the BMD. To minimise errors in data management, the applicants are asked to provide raw data in a specific format (see Section 3.3.3).	Transparency about the method used to estimate the BMDL. The BMDL estimate should be for the required effect size and should be statistically reliable (see criteria in the Scientific Committee Guidance on Dose Response Modelling (EFSA Scientific Committee, 2017b or later updates thereof).
19	Data on environmental safety (3.4)	No default requirement for environmental risk assessment, provided that the manufacturing process employed does not indicate that	Assessment of the manufacturing process of the primary product does not indicate a potential for the presence of constituents that are not extensively metabolised and/or readily biodegradable.

		the primary product may contain constituents that are not extensively metabolised and/or readily biodegradable.	

1240