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SCIENTIFIC GUIDANCE

Guidance on the scientific data requirements for an application for authorisation of a food additive submitted under Regulation (EC) No 1331/2008

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts.

Abstract

This guidance document applies to applications for a new authorisation as well as for a modification of an existing authorisation of a food additive, submitted under Regulation (EC) No 1333/2008. It defines the scientific data required to evaluate if the food additive is safe under the proposed conditions of use, in accordance with Articles 1 and 6 of Regulation (EC) No 1333/2008. The data requirements pertain to the characterisation of the proposed food additive, including the description of its identity, manufacturing process, specifications, stability, reaction and fate in foods and methods of analysis in food; the proposed uses and use levels and the dietary exposure; the safety data, including information on the genotoxic potential of the food additive, toxicological data other than genotoxicity and information on the safety for the environment. For the toxicological studies, a tiered approach is applied, for which the testing requirements, key issues and triggers are described. Applicants should provide data in accordance with this guidance document to support the safety assessment of the proposed food additive. Based on the submitted data, EFSA will assess the safety of the food additive in line with the risk assessment principles described in this document and conclude whether or not it presents risks to human health and to the environment, if applicable, under the proposed conditions of use.

KEYWORDS

authorisation, EFSA guidance, food additives, risk assessment, tiered approach

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1 | INTRODUCTION

1.1 | Background as provided by EFSA

Following the adoption of Regulation (EC) No 1331/2008[1] of the European Parliament and Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings, the European Commission requested EFSA to develop a guidance addressing the evaluation of dossiers on submission for authorisations of food additives.

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) adopted the "Food Additive Guidance for submission for food additive evaluations" in June 2012 (EFSA ANS Panel, 2012) (FA Guidance).

As of 1st July 2018, the ANS Panel has been replaced by the newly established EFSA Panel on Food Additives and Flavourings (FAF), tasked with the evaluation of food additives and flavourings.

In June 2020, the FA Guidance was subject to an editorial revision following the new provisions defined by Regulation (EC) 178/2002[2] ('GFL Regulation'), as amended by Regulation (EU) 2019/1381[3] 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, applicable as from 27 March 2021, however the scientific content remained unchanged.

Since the publication of the FA Guidance, considerable experience has been gained by the FAF Panel in assessing different types of applications on new food additives and modifications of already authorised food additives. The assessments performed so far highlighted the need for this sectoral guidance to be updated to account for the latest developments in the risk assessment methodologies and to include horizontal and cross-cutting guidance documents, where applicable as well as the latest "Scientific Guidance on the data required for the risk assessment of flavourings to be used in or on foods" adopted by the FAF Panel in November 2022 (EFSA FAF Panel, 2022).

1.2 | Terms of Reference as provided by EFSA

In accordance with Article 29(1) of Regulation (EC) No 178/2002, the European Food Safety Authority requests its scientific Panel on Food Additives and Flavourings (FAF) to revise and update the "Food Additive Guidance for submission for food additive evaluations", issued by the EFSA Panel on Food Additives and Nutrient Sources (ANS) in 2012.

The update of the guidance should account for the experience gained with the practical implementation of the 2012 ANS Panel guidance in the assessment of food additives applications submitted under Regulation (EC) No 1331/2008. Where possible, the FAF Panel should ensure consistency with applicable horizontal, cross-cutting guidance documents as well as the latest guidance for the risk assessment of food flavourings.

1.3 | Interpretation of the Terms of Reference

When updating the FA Guidance, the Panel has taken into account the scientific and technical developments since 2012, in the light of the latest experience gained with the assessment of food additive applications and considering updated horizontal and sector-specific guidance documents frequently used in the assessment of food additives.

Special attention has been given to the parts of the risk assessment that have been subject to additional data requests issued by EFSA during the evaluation of the food additive applications, based on the practical experience accrued over the years.

The Panel is aware of other scientific issues which may be relevant for the safety evaluation of food additives, but at this moment, the regulatory science is not mature enough to include specific recommendations on how to address those issues (e.g. the impact that the food additive may have on the gut microbiota composition and function (microbiome), see Section 5.4.5.5).

In particular, the following information has been updated or introduced in this revision of the guidance:

- Developments in the techniques/approaches applied in the manufacturing of food additives and improvements in the performances of the analytical methods, which allow an in-depth characterisation of the final product, and its source materials. These developments and improvements also allow defining more accurately the specifications for the food additive. Regarding the use of microorganisms and/or enzymes in the manufacturing process, the Scientific Committee 'Guidance on microorganisms' (EFSA Scientific Committee, 2025a) and the Scientific Guidance for the submission of dossiers on Food Enzymes (EFSA CEP Panel, 2021) has been considered.

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

[2]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 031 1.2.2002, p. 1.

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

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- Depending on the nature of the food additive, emphasis should be given to the potential exposure of consumers to small particles including nanoparticles, if present in the food additive. To this purpose, reference to the 'Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles' (EFSA Scientific Committee, 2021a) and to the 'Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: human and animal health' (EFSA Scientific Committee, 2021b) have been included.
- For the dietary exposure assessment, the most recent versions of the Food Additives Intake Model (FAIM) tool and the Dietary Exposure (DietEx) tool should be used in the risk assessment of the food additive.
- In the dietary exposure assessment, specific considerations should be given to infants and young children, representing a vulnerable part of the population. Where relevant, not only foods intended for infants and young children defined in Regulation (EU) 609/2013[4] should be considered, but also foods typically consumed by adults that may also be consumed by infants and young children from a certain age. The updated guidance also takes into consideration the Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age (EFSA Scientific Committee, 2017a).
- In the updated guidance, the Panel has considered all the cross-cutting guidance documents relevant for the genotoxicity and toxicological assessment of food additives, published after 2012, such as:

  - Clarification of some aspects related to genotoxicity assessment (EFSA Scientific Committee, 2017b),
  - Genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019a),
  - Guidance on aneugenicity assessment (EFSA Scientific Committee, 2021c).
  - Guidance on the use of the benchmark dose approach in risk assessment (EFSA Scientific Committee, 2022),
  - Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment (EFSA Scientific Committee, 2019b),
  - Guidance on allergenicity assessment of genetically modified plants (EFSA GMO Panel, 2017).

- Reference to the latest versions of the relevant Organisation for Economic and Co-operation and Development Test Guidelines (OECD TG) have been included.
- The protection of the environment has been considered in this update, where appropriate, in accordance with Article 1 of Regulation (EC) 1333/2008.[5] Experience has shown that occurrence in the environment may be a relevant issue for some food additives (EFSA FAF Panel, 2024a; Lewis & Tzilivakis, 2021).

1.4 | Scope

The present document provides guidance to applicants on the data requirements for applications supporting the authorisation of a new food additive or modifications of the conditions of use of an already authorised food additive (which may be related to changes in its manufacturing process, its specifications, or its conditions of use) in accordance with the provisions of Regulation (EC) No 1331/2008[1] and Regulation (EC) No 234/2011.[6]

This guidance is intended to explain the type and quality of scientific information needed to assess whether or not the food additive for which an application is submitted (referred to in this document as '**proposed food additive**') is safe under the proposed conditions of use, in accordance with Articles 1 and 6 of Regulation (EC) No 1333/2008.[5]

The evaluation of the proposed food additive (i.e. a new food additive or modifications of the conditions of use of an already authorised food additive) is conducted by EFSA in line with the established framework for risk assessment which is described further below under section 'Risk assessment process'.

This guidance is organised in six main sections and two Appendices:

The '**Introduction**' section lists the general principles applicable to this guidance and provides an overview of the risk assessment process followed by EFSA when evaluating the data submitted for the proposed food additive.

The **'Information on existing authorisations and evaluations'** section provides an overview of existing evaluations of the proposed food additive and their conclusions.

The **'Characterisation and specifications'** section seeks to identify the proposed food additive, potential hazards (e.g. impurities) from its manufacture, and, through the proposed specifications, to characterise the material submitted for toxicity testing and the material intended to be placed on the market.

The '**Proposed uses and exposure assessment'** section describes the dietary exposure to the proposed food additive for various age groups in the population of EU Member States based on the proposed uses and use levels and the consumption of the foods to which the proposed food additive is intended to be added.

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

[5]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.

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

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The '**Toxicological data'** section describes the methods which can be used to identify (in conjunction with data on manufacture and composition) and characterise biological and toxicological hazards of the proposed food additive. It describes the type of toxicity studies needed to demonstrate the safety of the food additive, including data on its genotoxic potential and other toxicological information.

The '**Safety for the environment**' section describes the information needed to ensure that the proposed food additive does not present a risk for the environment.

Appendix A includes the format for the submission of the specifications of the proposed food additive.

Appendix B includes the list of the parameters to be investigated in the modified OECD TG 422 (OECD, 2025a) as compared to standard OECD Test Guidelines (TGs).

Procedural aspects linked to the submission of an application for authorisation of a new food additive or for modifications of the conditions of use of an already authorised food additive, in the context of Regulation (EU) 1331/2008[1], are not in the scope of this guidance document. Instead, applicants are advised to consult the EFSA Administrative guidance for the preparation of applications on food improvement agents (food enzymes, food additives and food flavourings) (EFSA, 2021a), the EFSA Administrative guidance for the processing of applications for regulated products (EFSA, 2021b) and the EFSA Catalogue of support initiatives during the life-cycle of applications for regulated products (EFSA, 2021c).

This guidance will be further updated as appropriate in the light of experience gained from the evaluation of food additive applications.

1.5 | General principles

1. This guidance should be read in conjunction with the following EFSA guidance documents:

   – EFSA Administrative guidance for the preparation of applications on food improvement agents (food enzymes, food additives and food flavourings) (EFSA, 2021a), for information on the procedural aspects related to the preparation of food additive applications, submitted under Regulation (EU) 1331/2008[1];
   – All relevant horizontal EFSA scientific guidance documents cited throughout this guidance document, especially those of the EFSA Scientific Committee.

   Over time, these guidance documents may be updated by EFSA, or new guidance documents may be developed which could be of relevance for the current guidance. Therefore, when preparing a food additive application, applicants should follow the most recent version of the EFSA guidance documents cited in this guidance and of any newly available guidance documents, tools and online resources, relevant for the evaluation of food additives, including the OECD TGs.
2. Applications concerning modification of already authorised food additives may be related to changes in the manufacturing process, the specifications, or the conditions of use (e.g. requests to widen the use to include new food categories and/or increase of maximum use levels). In such cases, not only the changes as such should be described in detail, but also consequences regarding the identity and specifications of the food additive (if a manufacturing process is changed), the exposure (if uses are added and/or maximum use levels increased) and the safety of the food additive must be addressed. For these types of applications, the applicant needs to provide a scientific justification to substantiate why new data might not be required for certain sections of the present guidance.
3. Data on the identity of the proposed food additive, manufacturing process, specifications, proposed uses and use levels and dietary exposure (as described in Sections 3 and 4 of the present guidance document) represent the minimum data requirements which must be fulfilled in all applications for marketing authorisation of a food additive. Data should also be provided on its absorption, distribution, metabolism and excretion, as well as on its toxicity (as described in Section 5), unless the applicant can provide scientific justification and argumentation as to why new data are not needed for one or more of these endpoints to support the safety of the proposed food additive (see Sections 5.3 and 5.4 for more details).
4. The applicant is responsible for providing all the available confidential and non-confidential scientific data that are pertinent to the safety assessment of the proposed food additive.
   Such data should be identified and documented in order to demonstrate that the application covers all available information on the proposed food additive. Full study reports should be provided. For existing non-proprietary information, the search strategy used to retrieve relevant scientific data or published literature, including the description of the search terms, the databases and the criteria used to perform the search should be provided (see Section 5.4.1 for further details).
5. The applicant should provide a description and justification of the testing strategy chosen to prove the safety of the proposed food additive, including the rationale for choice of specific in vitro/in vivo toxicity studies. In addition, in case the proposed food additive consists of or contains a fraction of small particles including nanoparticles, the applicant should provide evidence to substantiate whether conducting a conventional risk assessment, in line with the requirements of the present guidance, is sufficient, or whether the assessment needs to be complemented with nano-specific considerations (for more details on this issue please refer to Section 5.2.2).

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6. Although the re-evaluation of food additives, according to Regulation (EU) No 257/2010,[7] falls outside the scope of this guidance, the principles outlined in this guidance should be considered by interested business operators also in the context of the re-evaluation process when submitting the requested information from the relevant calls for data.
7. The applicant should justify deviations from the requirements specified in the respective sections of this guidance, if applicable.
8. The data requirements described in this guidance will become applicable 6 months after the date of its publication in the EFSA Journal.

1.6 | Risk assessment process

The risk assessment process comprises four steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation. The four steps of the risk assessment process of a proposed food additive are described in detail below.

Hazard identification and characterisation

The chemical and technological assessment identifies the hazards of the food additive, which are then further characterised via its biological and toxicological dose–response relationships. Traditionally, the Panel has sought to identify the most sensitive endpoint from a range of toxicological hazards and their dose–response relationships, for identification of a reference point (RP), which is used to derive a health-based guidance value (HBGV), typically an acceptable daily intake (ADI) (IPCS, 2004) by applying uncertainty factors to account for toxicokinetic and toxicodynamic differences between experimental animals and humans (interspecies differences) and interhuman variability. Typical RPs are the lower confidence bound of the benchmark dose (BMDL) value (EFSA Scientific Committee, 2022) or, if a BMDL cannot be derived, the no observed adverse effect level (NOAEL). The EFSA Scientific Committee (EFSA Scientific Committee, 2022) has recommended benchmark dose (BMD) modelling to derive a BMDL. This requires consideration of the appropriate dose selection for toxicity testing.

If the RP is based on toxicity tests in experimental animals, the default uncertainty factors used by the Panel are a factor of 10 for toxicokinetic and toxicodynamic interspecies differences and an additional factor of 10 for toxicokinetic and toxicodynamic variability between humans resulting in an overall default uncertainty factor of 100. Other or additional uncertainty factors may be applied case-by-case to take into account any deficiency in the available data (EFSA Scientific Committee, 2012a). On the other hand, if chemical-specific data are available, a quantitative analysis of toxicokinetics and toxicodynamics can be used to derive chemical-specific adjustment factors (CSAFs) (Bhat et al., 2017; IPCS, 2005, 2020), which can replace default uncertainty factors, following the extended approach as described in WHO/IPCS (2018). In the context of the EFSA evaluation of food additives, there are examples where CSAFs have replaced default uncertainty factors, e.g. EFSA FAF Panel (2019, 2020); EFSA FAF Panel (2024b).

When two or more food additives have a common chemical structure and/or mode of action, a group ADI may be set, (e.g. EFSA FAF Panel, 2019, 2020). In the absence of potency data for individual members within such a group, the group ADI will be conservatively based on the lowest RP identified among the toxicity testing performed on the food additives of the group.

An ADI is established for compounds for which a threshold mechanism of toxicity can either be demonstrated or reasonably be expected based on the available toxicity data.

The ADI does not apply to infants below 16 weeks of age; assessing the use of food additives in this population group represents a special case for which recommendations were given by the EFSA Scientific Committee (EFSA Scientific Committee, 2017a) (for more details see Section 5.5).

When during the re-evaluation of a food additive, in accordance with Regulation (EU) No 257/20107, the need for additional data is identified, a temporary ADI (tADI) may be set. The Panel will not set a tADI for a new food additive to allow its use whilst addressing data gaps.

If the derivation of an ADI for the proposed food additive is considered not appropriate based on the available information, e.g. in case there are uncertainties about its effects, the identified RP can be used to calculate a margin of exposure (MOE), which is the ratio between a RP (BMDL or NOAEL) and the estimated exposure to the proposed food additive (EFSA Scientific Committee, 2025b).

Exposure Assessment

Assessing the dietary exposure to the proposed food additive involves the quantitative evaluation of its likely exposure in the EU population, taking into account all relevant dietary sources. These data are essential to determine whether the use of a food additive may pose a risk to the EU population. Typically, data on actual food consumption from national surveys in Europe are combined with the proposed use levels of the proposed food additive to estimate its exposure. This exposure

[7]Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additive. OJ L 080 26.3.2010, p. 19.

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assessment is intended to cover the population of all EU Member States taking into account the variation in exposure due to differences in food consumption across the Member States and different population groups.

Dietary exposure to a proposed food additive is determined by summing the exposure per food in which the food additive is intended to be used, at the level of the individual. This is achieved by multiplying the concentration of the food additive in a food by the average daily consumption of this food per individual in the dietary survey. The concentration of the food additive in a food is the proposed maximum/typical use level as indicated by the applicant or the maximum permitted level (MPL) as laid down in legislation. The exposure estimates per individual are then divided by the corresponding body weight and number of days providing individual mean exposure estimates expressed per kg body weight/day. This is carried out for all individuals resulting in distributions of individual exposure. On the basis of these distributions, the mean and 95th percentile (P95) of exposure to the proposed food additive is calculated per population group and dietary survey.

The exposure estimates of the proposed food additive are initially based on the proposed maximum/typical use levels or MPLs for high-level consumers (i.e. P95). These high exposure estimates may be refined if a possible health risk (see 'Risk characterisation') cannot be excluded (see also Section 4.2.1).

Risk characterisation

The overall evaluation of the proposed food additive for potential human risk is done by comparing the high exposure estimates (i.e. P95) with the ADI. If relevant, this comparison may also include exposure from other sources (e.g. if the proposed food additive is also naturally present in food or added to food as a food flavouring or as a food supplement or used in non-dietary sources such as medicines or cosmetics. For more details, see Sections 4.3 and 4.4 of this guidance document).

When using the margin of MOE approach, the Panel will consider the magnitude of the MOE on a case-by-case basis to determine whether the proposed food additive does not raise a safety concern. The value of the MOE corresponding to no safety concern is directly dependent on the data available on the proposed food additive. As explained above, the availability of chemical-specific data on toxicokinetic and/or toxicodynamic would allow the derivation of CSAFs, which can be lower than the default uncertainty factors for interspecies variability and intrahuman variability. In case of deficiencies in the available data set and additional data cannot be obtained, additional uncertainty factors may be applied on a case-by-case basis (EFSA Scientific Committee, 2012a).

1.7 | Assessment of impurities present in the proposed food additive

For impurities for which a RP or an HBGV is available based on relevant toxicological data, the potential exposure to these impurities from the use of the proposed food additive are calculated by assuming they are present at a certain value (that can be the proposed limit for such impurities) and then by calculating pro-rata to the estimates of exposure to the proposed food additive (see Section 4.5). The exposure to impurities estimated from the most relevant scenario chosen by EFSA for the risk assessment of the proposed food additive, using both the highest mean and 95th percentile exposure estimates, are used. The resulting values are then compared with the available RP or HBGV for each impurity.

For impurities which are both genotoxic and carcinogenic, irrespective of their origin, the Scientific Committee states that the MOE approach can be applied (EFSA Scientific Committee, 2005, 2012b, 2025b), which means that limits of such impurities in the specifications should be as low as reasonably achievable and should result in an MOE of at least 10,000.

For impurities[8] for which toxicity data are not available, non-testing methods may be used for a preliminary assessment of their toxicological potential. Relevant methods include grouping and 'read-across' (see Section 5.1), computational methods (structure–activity relationships (SAR), quantitative structure–activity relationships (QSARs)) and the Threshold of Toxicological Concern (TTC) approach (EFSA Scientific Committee, 2019b).

For non-genotoxic impurities for which no toxicity data are available, the TTC approach is applied. In this case, the Panel checks if the high exposure estimates based on the most relevant scenario used for the risk assessment of the proposed food additive, using both the highest mean and 95th percentile exposure estimates, are below the relevant Cramer Class TTC values (i.e. 1800, 540, 90 μg/person/day for Cramer I, II, III substances, respectively (Cramer et al., 1978; Munro et al., 1996)).

For genotoxic impurities for which no carcinogenicity data are available, the TTC approach is also applied. In this case, the Panel checks if the high exposure estimates based on the most relevant scenario used for the risk assessment of the proposed food additive, using both the highest mean and 95th percentile exposure estimates, are below the TTC for genotoxic compounds of 0.15 μg/person per day (i.e. 0.0025 μg/kg body weight (bw) per day; EFSA Scientific Committee, 2019b).

2 | INFORMATION ON EXISTING AUTHORISATIONS AND EVALUATIONS

Information on any existing evaluations and authorisations of the proposed food additive should be provided. This should include details of the body which carried out the evaluation and when this was undertaken. Any relevant data/studies

[8]For the purposes of this guidance, the word 'impurities' covers residuals and by-products resulting from a manufacturing process.

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generated/conducted in the context of other regulatory frameworks should be provided in full, including the details of the evaluation in which RPs and/or HBGVs may have been derived.

3 | CHARACTERISATION AND SPECIFICATIONS

This section outlines the main parameters needed to characterise and specify the proposed food additive. This list of parameters is not exhaustive and may be complemented with additional data based on the specific nature of the proposed food additive.

3.1 | General information on the requested data to support the identity and specifications

The applicant is requested to provide information and analytical data to support the identity and specifications of the proposed food additive. To fulfil this requirement, attention should be given to the following considerations:

- In order to ensure that the specifications are representative and accurately reflect the proposed food additive, the analytical data supporting the specifications should be based on batches of the proposed food additive that have been independently produced (i.e. with different lots of raw materials of potential compositional variations (e.g. seasonal) and produced on different dates) with the same manufacturing process. To demonstrate batch-to-batch consistency, analytical data on at least five independently produced batches should be provided in order to show that the proposed food additive can be consistently manufactured within the proposed specifications. Preferably, the same individual batches should be used across the different analyses for which data are reported in the dossier. Information describing the criteria used to select the batches tested and on any relevant existing legally defined or standard sampling protocols should be considered and provided.
- Analytical data on the identity and content of different components and impurities should be generated using appropriate analytical methods applying state-of-the-art techniques (the highest sensitivity for each analytical method should be achieved, especially for impurities and components of potential toxicological concern for which a specification limit is proposed or may be necessary). Validated methods, preferably nationally or internationally recognised, should be used for the analyses. If in-house methods or modified standard methods are employed, the analytical protocols implemented should be fully described and the results of the respective method validation procedures should be provided. Detailed information on the analytical methods should be provided to demonstrate the quality of the data provided, such as the sensitivity and specificity (e.g. limit of detection (LOD) and limit of quantification (LOQ)), recovery and variability and other validation parameters of the methods.
- All analytical data should be reported as numerical values, when they are above the LOQ, and should be supported by certificates of analysis or internal test reports. Results should not be reported as below an arbitrary reporting limit, that is different from the respective LOD/LOQ. In addition, wordings such as 'not detected' and/or 'not quantified' should always be supported by the respective LOD and/or LOQ.

3.2 | Identity

Information on the identity of the proposed food additive should be provided considering the requirements outlined in the subsections listed below.

There may be cases where two or more of these subsections could be of relevance to the proposed food additive. In those circumstances, the respective information from all relevant subsections should be provided.

The provided analytical data should be given for the proposed food additive itself (e.g. excluding any optional added ingredients, other food additives or diluent solvents). This ensures that the characterisation is based solely on the proposed food additive as such, without carriers or diluents, and on a solvent-free basis. If the proposed food additive must be produced and used as a preparation/formulation for technological reasons (e.g. instability), this should be fully justified and the analytical data should be provided accordingly. In such cases, the composition of the preparation/formulation should also be provided and described in detail.

Where applicable, the European Chemicals Agency (ECHA) guidance for identification and naming of substances under REACH[9] and CLP Regulations[10] (ECHA, 2023) could be followed.

[9]Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. OJ L 396, 30.12.2006, p. 1–849.

[10]Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, p. 1–1355.

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3.2.1 | Single substances, simple mixtures and polymers

The following information, where applicable, should be provided for the proposed food additive consisting of a single substance, or of a simple mixture,[11] i.e. a mixture whose components can be fully characterised, or of a polymer, along with other data that the applicant considers useful to support the identity of the proposed food additive.

In the case of **single substances**, the following information should be provided:

a. purity assay value (expressed as the quantitative amount in weight percentage (% w/w)) of the substance,
b. chemical name, according to IUPAC nomenclature rules,
c. CAS number (if attributed), European Community (EC) number – ECHA (if attributed), EINECS number (if attributed), E number (where appropriate) and other identification numbers,
d. synonyms or common names, trade names and abbreviations,
e. InChI (International Chemical Identifier) and InChIkey (digital representation of the InChI),
f. molecular and structural formulae with stereochemistry, including canonical and isomeric SMILES linear notations,
g. molecular weight (g/mol) or atomic weight (in case of elements),
h. spectroscopic data such as nuclear magnetic resonance (NMR), infrared (IR), ultraviolet–visible (UV–Vis) spectra and/or spectrometric data such as mass spectrometry (MS) spectra,
i. concentrations of potential impurities, taking into account the manufacturing process (e.g. solvent residues, toxic elements, mycotoxins, dioxins and polychlorinated biphenyls (PCBs), pesticides, polycyclic aromatic hydrocarbons (PAHs), microbiological criteria, catalysts),
j. physicochemical properties:
    - appearance,
    - melting point (for solids),
    - boiling point (for liquids),
    - solubility in water or in a non-aqueous matrix, or in a solvent relevant for the use of the proposed food additive in foods and in toxicity/genotoxicity tests (see Section 5.2 and EFSA Scientific Committee, 2021a),
    - pH, when applicable,
    - octanol–water partition coefficient (K_{ow}), when applicable,
    - particle size, shape and distribution, if applicable (see Section 5.2). The recommendations of the EFSA Scientific Committee Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles (EFSA Scientific Committee, 2021a) should be followed.

In the case of **simple mixtures**, i.e. mixtures whose components can be fully characterised (EFSA Scientific Committee, 2019a), information on the quantitative amount in weight percentage (% w/w)) of each component of the mixture should be provided. In addition, information of points b to g listed above should be provided for each component of the mixture, when applicable. For the points h to j, information on the mixture itself should be provided, when applicable. The functional component(s) (i.e. the component(s) responsible for the technological function of the proposed food additive) should be clearly identified.

In the case of **polymers** obtained from natural sources or through chemical synthesis or modification, in addition to the information described above for single substances and simple mixtures, the following information should be provided:

a. molecular and structural formulae of monomer(s),
b. degree of polymerisation, number average molecular weight (Mn) and weight average molecular weight (Mw) and, when applicable, viscosity average molecular weight (Mv),
c. degree of substitution and percentages of substituted groups, where appropriate,
d. if the polymer is obtained by chemical synthesis, structural formulae of starting materials and other agents involved in the polymerisation process,
e. in the case of chemical modification of the polymer, the nature and degree of modification of the polymer (e.g. degree of substitution and percentages of substituted groups).

3.2.2 | Complex mixtures

Complex mixtures,[12] i.e. mixtures containing a substantial fraction of unidentified components (for which not all the components have been chemically, fully identified) (EFSA Scientific Committee, 2019a), may be derived for example from plants, macroscopic fungi, macroalgae, animals, or their parts.

[11]"Simple mixtures" refers to chemically fully defined mixtures (EFSA Scientific Committee, 2019a), excluding optional added ingredients, other food additives, or diluent solvents (see Section 3.2).

[12]'Complex mixtures' refers to mixtures containing a substantial fraction of unidentified components (EFSA Scientific Committee, 2019a) (for which not all the components have been chemically, fully identified), excluding optional added ingredients, other food additives, or diluent solvents (see Section 3.2).

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The components of complex mixtures should be characterised as fully as possible. This information is particularly important for the component-based approach, which is employed in the genotoxicity and toxicity assessments (see Section 5) and, where relevant, the environmental safety assessment (see Section 6). In case that the components of the complex mixtures cannot be fully characterised, the proportion of the unidentified fraction (quantitative amount in weight percentage (% w/w)) in the proposed food additive should be provided. Any analytical information available to characterise the type and to estimate the proportions of the components in the unidentified fraction should be presented.

The following non-exhaustive list of tools can help identifying the possible substances of concern in complex mixtures including those derived from botanical materials:

- the EFSA Compendium of Botanicals,[13] which provides information on naturally occurring substances that may be of concern for human health,
- the EFSA Chemical Hazard Database (OpenFoodTox).[14]

In the case of **complex mixtures**, the following information should be provided along with other data that the applicant considers useful to support the identity of the proposed food additive. Information on the quantitative amount in weight percentage (% w/w)) of each identified component of the mixture should be provided. In addition, the information of points b to g listed above for single substances should be provided for each identified component of the mixture. For the points h to j, listed under single substances information on the mixture itself should be provided, when applicable. The functional component(s) (i.e. the component(s) responsible for the technological function of the proposed food additive) should be also clearly identified. In addition, the following information should be also provided on the complex mixtures:

a. the proportion of the unidentified fraction (quantitative amount in weight percentage (% w/w)),
b. concentration of the major classes of components present (e.g. carbohydrates, proteins, lipids) and concentration of characteristic components of the proposed food additive (e.g. polyphenols) and concentration of other identified components of potential concern (e.g. substance listed in the EFSA Compendium of Botanicals[13]), along with a justification of the choice of the analysed compounds.

In addition to the information requested above, further information is needed for plant-derived proposed food additives and for proposed food additives isolated from or produced from macroscopic fungi or macroalgae or animals.

For **plant-derived** proposed food additives, and in agreement with section 2.1.1.1 of the EFSA Guidance on Safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA Scientific Committee, 2009), the additional following information should be provided, along with other data that the applicant considers useful to support the identity of the proposed food additive:

a. scientific (Latin) name (botanical family, genus, species, subspecies, variety with author's name, if applicable) according to the international codes of nomenclature,[15]
b. synonyms (botanical name) that may be used interchangeably with the preferred scientific name,
c. common names (if a trivial or a common name is used, it should be linked to the scientific name and part used),
d. part(s) used (e.g. root, leaf, seed, fruit),
e. history of consumption of the plant or the part(s) used, when available,
f. geographical origin,
g. growth and harvesting conditions (e.g. wild or cultivated, stage of the plant growth and harvest – ripeness of the fruit, if relevant),
h. indication whether the plant material is compliant with the EU food law regarding the use of plant protection products.[16]

The additional following information should be provided in the case of food additives isolated from or produced from **macroscopic fungi or macroalgae**, along with other data that the applicant considers useful to support the identity of the proposed food additive:

[13] https://www.efsa.europa.eu/en/data/compendium-botanicals.

[14] https://www.efsa.europa.eu/en/microstrategy/openfoodtox.

[15] Plants of the World Online (https://powo.science.kew.org/) facilitated by the Royal Botanic Gardens, Kew and The World Flora Online (https://www.worldfloraonline.org/); The USDA-ARS Germplasm Resources Information Network (GRIN) database (https://npgsweb.ars-grin.gov/ gringlobal/taxon/taxonomysimple.aspx) in case the two above sources do not provide the required information; The International Plant Names Index (https://www.ipni.org/) in case the three above sources do not provide the required information.

[16] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC and Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC Text with EEA relevance.

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a. scientific (Latin) name and taxonomy (family, genus, species, and if applicable subspecies, strain) according to the international codes of nomenclature for macroscopic fungi and macroalgae,[17]
b. synonyms that may be used interchangeably with the preferred scientific name,
c. common names (if a trivial or a common name is used, it should be linked to the scientific name and part used),
d. part(s) used,
e. history of consumption of the macroscopic fungi or macroalgae or the part(s) used, when available,
f. geographical origin,
g. growth and harvesting conditions (e.g. wild or cultivated, stage of the macroscopic fungi or macroalgae growth).

The additional following information should be provided in the case of food additives isolated from or produced from **animals**, along with other data that the applicant considers useful to support the identity of the proposed food additive:

a. scientific (Latin) name (zoological family, genus, species, subspecies, breed, if applicable),
b. synonyms that may be used interchangeably with the scientific name,
c. common names (if a trivial or a common name is used, it should be linked to the scientific name and part used),
d. part(s) used (e.g. organ(s) or tissue(s)),
e. history of consumption of the animal or the part(s) used, when available,
f. geographical origin,
g. information on the suitability of the animal sources for human consumption according to Regulation (EU) No 2015/1162,[18]
h. data on the absence of any risk of infectivity from viruses or other zoonotic agents that may be present in the source materials.

3.2.3 | Food additive containing microorganisms or prepared/obtained from/with microorganisms

Information on the proposed food additive containing microorganisms or prepared/obtained from/with microorganisms, including genetically modified microorganisms (GMMs), should be provided in line with the EFSA Scientific Committee 'Guidance on the characterisation of microorganisms in support of the risk assessment of products used in the food chain' (EFSA Scientific Committee, 2025a, 2025b, 2025c).

3.2.4 | Engineered nanomaterials

Information on the characterisation of engineered nanomaterials (ENM) should be provided in line with Section 5 of the 'Guidance on risk assessment of nanomaterials to be applied in the food and feed chain' (EFSA Scientific Committee, 2021b).

3.3 | Manufacturing process

The information on the manufacturing process should focus on the possible presence of any residual substance/element which may remain in the proposed food additive as an impurity, e.g. toxic elements, residual solvents/reagents and reaction intermediates of potential toxicological concern that require further considerations.

A detailed description of the main steps of the manufacturing process should be provided. Details on the identity of raw materials and if they are suitable for human consumption, identity of catalysts (both chemical and enzymes), identity of solvents and processing conditions that may affect the safety of the proposed food additive (e.g. temperature and pressure ranges) should be provided. Information on purification steps and, in the case of powders, the drying methods applied should also be included. A production flow chart should accompany this information to offer visual support.

Measures implemented for production control and quality assurance should also be described. The proposed food additive should be manufactured according to the Food Hygiene Regulation (EC) No 852/2004[19] with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP), and in accordance with current Good Manufacturing Practice (GMP).

For a proposed food additive **manufactured by chemical synthesis**, the following information should be provided, along with other data that the applicant considers useful to support its manufacturing:

a. identity of starting materials, reagents, solvents and other chemicals involved in the manufacturing process,
b. reaction sequence, side reactions and side products,

[17]Mycobank (www.mycobank.org), Index fungorum (https://www.indexfungorum.org), Catalogue of Life (CoL), Integrated Taxonomic Information System (ITIS), Global Biodiversity Information Facility (GBIF), Encyclopedia of Life (EOL).

[18]Commission Regulation (EU) 2015/1162 of 15 July 2015 amending Annex V to Regulation (EC) No 999/2001 of the European Parliament and of the Council laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. OJ L 188 16.7.2015, p. 3.

[19]Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139 30.4.2004, p. 1.

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c. reaction conditions (e.g. duration of the reaction, temperature, pressure, solvents and catalysts),
d. purification steps employed to obtain the proposed food additive (e.g. solvent extraction, crystallisation, filtration).

For a proposed food additive **manufactured by fermentation**, the following information should be provided in line with Section 1 of the Scientific Guidance for the submission of dossiers on Food Enzymes (EFSA CEP Panel, 2021), along with other data that the applicant considers useful to support its manufacturing:

a. identity of the microorganism. Information on the microorganism to be submitted according to the EFSA Scientific Committee (2025a, 2025b, 2025c), if relevant.
b. information on the fermentation step(s) of the production of the proposed food additive specifying the type of the fermentation system used (e.g. continuous, (fed-) batch or solid state). A list of the raw materials contributing to the medium and a compilation of the reagents used for process control is required. These should be the actual materials used; an indicative list will not be accepted. For the raw materials which typically provide the nitrogen and carbon sources, which are included to meet mineral and vitamin requirements, or used in pH control, only qualitative data are needed. Quantitative data may be required for medium ingredients of potential concern.
c. the specific methods used to kill, disrupt and remove microbial biomass after completion of fermentation, to purify, concentrate and remove microorganisms from the proposed food additive, when applicable.
d. for all substances used during the processing (e.g. processing aids, antifoam agents), the chemical identity, the CAS or any other unique identification number (if available) and the function. These should be the actual materials used; an indicative list will not be accepted.

For a proposed food additive **manufactured by enzyme-catalysed synthesis**, the following information should be provided in line with Section 1 of the Scientific Guidance for the submission of dossiers on Food Enzymes (EFSA CEP Panel, 2021), along with other data that the applicant considers useful to support its manufacturing:

a. identity, function and source of each enzyme,
b. CAS-, EC-number, if attributed,
c. starting substrate(s); enzyme-catalysed reaction step(s); side reactions; side products,
d. indication if the food enzyme(s) used is/are commercially available or produced in-house,
e. information whether the involved food enzyme(s) has/have been assessed or is/are being assessed by EFSA in the framework of Regulation (EC) No 1332/2008[20] on food enzymes, the relevant EFSA question number(s) linked to the corresponding application for the food enzyme(s) and the respective EFSA scientific opinion, if available. In case the food enzyme(s) fall(s) outside the scope of Regulation (EC) No 1332/2008 as it is when enzymes are used exclusively in the production of the proposed food additive, data related to the safety evaluation of the enzyme(s) should be provided and assessed in the context of the food additive application and following the same scientific principles, outlined in the relevant EFSA guidance document (EFSA CEP Panel, 2021).
f. information on whether the food enzyme(s) used is/are present or removed from the proposed food additive. If the food enzyme(s) is/are present in the proposed food additive, the enzymatic activity (in enzyme activity units (U) per unit weight) and potential residual activity should be reported in at least three representative batches of the proposed food additive that have been independently produced (EFSA CEP Panel, 2021). If the food enzyme(s) has/have been removed, experimental data demonstrating the removal should be provided in at least three representative batches of the proposed food additive that have been independently produced (EFSA CEP Panel, 2021).

For a proposed food additive derived from for example **plants, macroscopic fungi, macroalgae and animal sources**, the following information should be provided, along with other data that the applicant considers useful to support its manufacturing:

a. information on the specific procedure employed, e.g. extraction, distillation, fractionation and fermentation, or on any other relevant procedure(s) employed,
b. information on the extraction and used reagents and solvents, along with their concentration,
c. in case components of potential concern may be present in the raw material(s), the amount of raw materials expressed in weight units needed to produce a certain quantity of the proposed food additive expressed in weight units.

In the case of an application submitted for the assessment of a new manufacturing process for an already authorised food additive according to Regulation (EC) No 1331/20081, the differences between the existing manufacturing process and the new manufacturing process should be described, in line with information described in this section.

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

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3.4 | Specifications

The specifications define the requirements concerning the identity, the source, the purity and the limits of any impurity present in the proposed food additive (see Section 3.1).

Regulation (EU) No 231/2012 lays down specifications for authorised food additives listed in Annexes II and III to Regulation (EC) No 1333/2008.[21]

A clear rationale for the proposed specifications should be provided, ensuring alignment and consistency with the analytical provided data. The specification parameters must be selected considering the identity of the proposed food additive, its manufacturing process and any other relevant factors and the selection of each parameter should be justified. Additionally, the specifications should reflect the variability observed with the applied method of manufacture to guarantee accuracy and reliability. If appropriate, for each parameter, minimum, and/or maximum limits should be provided. The proposed specification limits for impurities should be as low as possible and justified.

The proposed specifications should be given for the proposed food additive itself (e.g. excluding any optional added ingredients, other food additives or diluent solvents). This ensures that the characterisation is based solely on the proposed food additive as such, without carriers or diluents, and on a solvent-free basis. If the proposed food additive must be produced and used as a preparation/formulation for technological reasons (e.g. instability), this should be fully justified. In such cases, the composition of the preparation/formulation should also be provided and described in detail.

The proposed specifications should be submitted according to the format of Appendix A. In line with this Appendix, among other relevant to the proposed food additive information, the following information should be provided for the specifications of a proposed food additive, when applicable:

a. the definition of the proposed food additive, including relevant information on the manufacturing process applied (e.g. raw materials, different manufacturing steps, enzymes, microorganisms, solvents and catalysts used),
b. relevant parameters such as chemical name, chemical formula, molecular weight, solubility and pH,
c. the purity assay value (expressed as the quantitative amount in weight percentage (% w/w)) of the substance or of the functional component(s) (i.e. the component(s) responsible for the technological function of the proposed food additive) in the case of mixtures,
d. in case of mixtures, the concentration of major classes of components present (e.g. carbohydrates, proteins, lipids) and concentration of characteristic components of the proposed food additive (e.g. polyphenols) and concentration of other identified components of potential concern (e.g. substance listed in the EFSA Compendium of Botanicals13),
e. limits for impurities (e.g. solvent residues, toxic elements) of potential toxicological concern,
f. microbiological criteria.

In the case that the purity assay value refers to a wide range on the content of the functional component (based on which the proposed maximum permitted levels are to be expressed, see Section 4.1.2), the limits for impurities should be expressed based on the lowest (worst-case scenario) content of the functional component (i.e. mg of impurity per kg of the functional component) (e.g. for more details see EFSA FAF Panel, 2024c, 2024d).

Information on compliance with recent EU or other internationally accepted specifications (e.g. JECFA, pharmacopoeia) where appropriate should also be provided. When the proposed specifications differ from those already existing specifications, these specifications should be set out alongside the proposed new specifications, and any differences should be pointed out.

In the case of an application submitted for the assessment of an amendment of the specifications for an already authorised food additive according to Regulation (EC) No 1331/2008[1], the differences between the existing specifications and the new proposed specifications should be described, in line with information to support the amendment.

3.5 | Stability, reaction and fate in food

The stability of the proposed food additive, as produced (e.g. solid and/or liquid) and in food during storage, should be evaluated and described. This information is used to identify hazards, which might arise from reactions leading to transformation and/or degradation products.

Appropriate information should be provided on:

a. the stability of the proposed food additive under the different conditions of storage (temperature, environment (light exposure, oxygen, relative humidity), duration or any other factor supporting the proposed shelf-life and conditions of storage);
b. the stability of the proposed food additive in food or in relevant model systems (mimicking the food matrix and the respective processing conditions), representing the intended conditions of use (uses and use levels);
c. the identity and concentration of any transformation and/or degradation product;

[21]Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83 22.3.2012, p. 1.

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d. the stability and reactivity of any transformation and/or degradation product, if applicable for those cases when the resulting transformation and/or degradation products are not stable and are expected to transform/degrade further;
e. the nature of interactions and reactions of the proposed food additive or its functional component(s) with constituents of the foods to which it is intended to be added. This includes investigation whether such interactions or reactions could lead to the formation of compounds of toxicological concern. Such information may encompass new experimental data on the proposed food additive, and/or existing literature data on structurally related substances.

Stability experiments should be performed under storage conditions reflecting the intended shelf-life and/or under accelerated conditions ('forced ageing'). The extrapolation of the results from accelerated conditions to intended conditions of storage should be justified. Recommendations on the conditions and duration of the studies to be performed and extrapolation of the results can be found in, e.g. ICH Q1A–Q1F stability guidelines.[22]

3.6 | Methods of analysis in food

A detailed description of the analytical method(s), along with the LOD, LOQ, repeatability and reproducibility, used to determine the proposed food additive (based on the analysis of, i.e. the single substance or the functional component(s) in case of mixtures) in food to which the proposed food additive is intended to be added (see Section 4.1) should be provided. The analytical method(s) should be specific and fit-for-purpose.

4 | PROPOSED USES AND EXPOSURE ASSESSMENT

4.1 | Data needed for the assessment of the dietary exposure to a proposed food additive

This guidance deals with applications for a proposed food additive. Information needed to assess the (potential) dietary exposure to such food additives is described below. The data requested should at least indicate in which foods/food categories the food additive is proposed to be used/added, and at which intended use level.

4.1.1 | Information on the foods/food categories in which the proposed food additive is requested to be used

Each food or food category in which the food additive is proposed to be used should be defined at the highest level of detail possible according to the two following food classification systems:

- Food classification system defined in Annex II, Part D, of Regulation (EC) No 1333/2008.
- FoodEx2 classification system as used in the EFSA Comprehensive European Database[23] (Comprehensive Database). FoodEx2 is a standardised food classification and description system developed by EFSA, which facilitates improved mapping of use levels to the relevant foods compared to the (broad) food categories in Annex II, Part D, of Regulation (EC) No 1333/2008.

The link between the food categories in Annex II, Part D, of Regulation (EC) No 1333/2008 and the base terms of FoodEx2 is available[24] and the applicant can use this link to categorise the foods according to FoodEx2. Additional information present in FoodEx2 (e.g. facets[25] and original food descriptors, not shown in the abovementioned link) was used by EFSA for making this linkage.

An additional description of the proposed foods/food categories can be provided as supporting information. This information may be used by EFSA to refine the exposure assessment (see Section 4.2.1).

4.1.2 | Information on the use levels requested for the proposed food additive

The applicant is requested to provide information on the proposed maximum[26] and typical use levels of the proposed food additive for each food or food category in which the food additive is proposed to be used. The proposed maximum level

[22] https://www.ich.org/page/quality-guidelines.

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

[24] Mapping of FoodEx2 Exposure Hierarchy with the food categories of Annex II (part D) of Regulation (EC) No 1333/2008 on food additives: https://zenodo.org/records/4461577#.Y1EQF3ZByHs.

[25] In FoodEx2, facets are used to add further descriptions of the foods consumed in relation to different properties and aspects of the foods (e.g. related to its ingredients, its packaging). https://www.efsa.europa.eu/en/data/data-standardisation.

[26] *Quantum satis* cannot be requested, a numerical maximum should also be proposed.

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is the maximum level at which the additive is expected to be used in foods within a food category, whereas a typical use level is the common use level at which the additive is expected to be used in foods within a food category. If available, detailed information on the different use levels within the same food category should be provided to allow for a refinement of the exposure estimates.

The proposed use levels should be expressed in the food additive itself in the case of a single substance or in the functional component(s)[27] if the food additive consists of a mixture (see Sections 3.2.1 and 3.2.2). If the proposed food additive is (likely to be) part of a group of existing authorised food additives that have a group restriction (e.g. saccharins with MPLs expressed as the free imide, steviol glycosides as steviol equivalents, phosphoric acid and phosphates as P_2O_5, or sorbic acid and sorbates as free acid) then its proposed use levels (e.g. a new steviol glycoside) should be expressed on that group basis (e.g. proposed maximum and typical use levels expressed as steviol equivalents).

In addition, if carry over of the proposed food additive is expected (i.e. when the applicant proposes to modify Annex III of Regulation (EC) No 1333/2008), information is required on the resulting concentration of the proposed food additive in the final food product due to carry-over (i.e. from its proposed use in food additive preparations, flavourings, enzymes and/or nutrients). Similarly, carry-over estimates of concentrations in final food products should be provided when the food additive is to be used in foods which can be used as ingredients, e.g. tomato sauce containing the proposed food additive used in composite dishes. Information on the amount of ingredients in a composite dish could be obtained from recipe data. The concentration of the additive in the composite dish can then be calculated pro-rata to the concentration of the additive in the ingredient.

Information on the proposed foods/food categories and the use levels of the proposed food additive should be provided using a specific template. Instructions for completing this template are available.[28]

4.2 | Dietary exposure assessment

4.2.1 | Chronic exposure assessment

The applicant is requested to assess the dietary exposure to the proposed food additive with the EFSA dietary exposure tool(s), as explained below. These tools are based on food consumption data from the Comprehensive Database.[29] These data cover many EU Member States and the following population groups: infants (from 16 weeks of age), toddlers (1–2 years), children (3–9 years), adolescents (10–17 years), adults (18–64 years) and the elderly (65 years and older). With both dietary exposure tools, the exposure to a food additive is assessed by combining consumed amounts of foods with the proposed use levels in these foods. The applicant is requested to assess the dietary exposure using the EFSA tool(s) for the general population including infants at and above 16 weeks of age to the elderly. For infants below 16 weeks of age, the applicant is requested to provide dietary exposure estimates obtained via another approach. See Section 4.2.1.2 for further information.

The applicant is also requested to perform a 'consumers only scenario' for proposed food additives requested to be added in food supplements (FCs 17, 17.1 and 17.2 of Annex II, Part D, of Regulation (EC) No 1333/2008), and/or in dietary foods for infants and young children for special medical purposes (FCs 13.1.5.1 and 13.1.5.2). How to perform such a scenario is described in Section 4.2.1.3 of this Guidance. Further explanation of this scenario is available in EFSA (2020), and examples can be seen in EFSA FAF Panel (2023e); EFSA FAF Panel (2024e). For additives to be used as sweeteners, the 'consumers only scenario' approach is required but the current EFSA tools do not allow to perform such scenario at the time of the publication of this guidance document; see end of Section 4.2.1.3.

4.2.1.1 | *For the general population, including infants from 16 weeks of age and young children*

The mandatory EFSA tool to be used by the applicant to assess the exposure to the proposed food additive is FAIM.[30] This tool estimates the dietary exposure by combining the proposed maximum or typical use levels with consumption data from the Comprehensive Database that is categorised according to the food categories in Annex II, Part D, of Regulation (EC) No 1333/2008. In the case that the maximum and typical proposed use levels for foods within a food category cover a broad range, the maximum of the maximum levels and the mean of the typical levels should be considered for the food categories. If these levels are determined by food(s) that are hardly consumed (so-called niche products), then the levels to be considered should be based on the generally most consumed foods within the food category. Exposure estimates obtained with this tool are expected to overestimate the actual dietary exposure to the proposed food additive, which will be

[27] See Section 3.2.1 for the definition of functional component(s): i.e. the component(s) responsible for the technological function of the proposed food additive.
[28] The template for the submission of use levels and the related instructions are available under the Supporting Information section of the online version of the EFSA Administrative guidance for the preparation of applications on food improvement agents (food enzymes, food additives and food flavourings) (EFSA, 2021a).
[29] https://www.efsa.europa.eu/en/data-report/food-consumption-data.
[30] FAIM tool (version 3.0) is described here: https://www.efsa.europa.eu/en/applications/food-improvement-agents/tools and can be accessed from here: https://r4eu.efsa.europa.eu/ by signing up to get an account.

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particularly pronounced when the additive is only used in specific food(s) within a food category. This is because the tool cannot consider the restrictions/exceptions indicated in Annex II, Part D, of Regulation (EC) No 1333/2008.

A second EFSA tool to estimate the dietary exposure is DietEx.[31] Consumption data in this tool are categorised according to the FoodEx2 food classification system. As FoodEx2 includes more information on the foods coded in the food consumption data compared to the food classification of Annex II, Part D, of Regulation (EC) No 1333/2008, potentially more accurate estimates of dietary exposure can be obtained with this tool. The applicant is encouraged to also use this tool to estimate the dietary exposure, but this is not mandatory. Also, this tool cannot consider (but to a lesser extent than for FAIM) all restrictions/exceptions indicated in Annex II, Part D, of Regulation (EC) No 1333/2008, and is expected to also overestimate the actual exposure.

If the food additive is proposed to be used in a food/food category that is not available in FAIM or DietEx, the applicant is requested to select its parent food category, i.e. the next higher level according to the food categorisation hierarchy, unless this parent food category would result in a large overestimation of the exposure via the relevant food/food category. In any case, the applicant should justify why a parent food category was considered or not. The applicant may also select another relevant food category.[32]

Scenarios to be performed

The way to calculate the exposure with FAIM, and optionally with DietEx, differs between a new food additive and an already authorised food additive for which an extension/modification of use is requested.

For a new food additive, the applicant should calculate the dietary exposure with the proposed maximum use levels and with the proposed typical use levels, considering all relevant foods/food categories except food supplements (FCs 17, 17.1 and 17.2) and dietary foods for infants and young children for special medical purposes (FCs 13.1.5.1 and 13.1.5.2) which will be addressed each using a 'consumers only scenario', this is also the case for additives to be used as sweeteners (see Section 4.2.1.3). This results in two dietary exposure estimates when using FAIM and four if also DietEx is used.

For an already authorised food additive (whether or not this food additive was already re-evaluated by EFSA) for which an extension/modification of use is requested, the applicant should calculate the exposure following four scenarios depending on the concentrations[33] used, as explained below and summarised in Table 1. These scenarios should be calculated with FAIM and optionally with DietEx. As for a new additive, exposure via food supplements (FCs 17, 17.1 and 17.2) and dietary foods for infants and young children for special medical purposes (FCs 13.1.5.1 and 13.1.5.2) should be addressed using a consumer only approach (see Section 4.2.1.3), which is also the case for additives to be used as sweeteners.

TABLE 1 Concentrations to be used in the dietary exposure assessment scenarios for an already authorised food additive for which an extension/modification of use is requested.

	Concentrations of the food additive to be used for the:	
Scenarios[a]	**New FC(s) or authorised FC(s) requested to be modified**	**Already authorised FCs for which no modification of use is requested**
Maximum 1	Proposed maximum use levels	– Numerical MPLs – In case of QS, highest concentration in the maximum level exposure assessment scenario from the latest EFSA opinion (i.e. re-evaluated FA with data for that FC); if not available, FC cannot be considered
Typical 1	Proposed typical use levels	
Maximum 2*	Proposed maximum use levels	Mean use/analytical levels from last EFSA opinion (i.e. re-evaluated FA with data for that FC); if not available, FC cannot be considered
Typical 2*	Proposed typical use levels	

Abbreviations: FA, food additive; FC, food category; MPL: maximum permitted level; QS, *quantum satis*.

*These scenarios can only be calculated when there is a published EFSA opinion reporting concentrations.

[a]Food supplements and dietary foods for infants and young children for special medical purposes should not be considered within any of these scenarios. Also, food additives to be used as sweeteners (including those that may have other function(s) in foods) should not be considered by these scenarios.

Scenario Maximum 1: The applicant should use the proposed maximum use levels for the food categories for which an extension/modification of use is requested and assign the MPL(s) as laid down in the relevant Regulation,[34] if relevant, to the currently authorised food categories for which no modification of use is requested. In the case current MPL(s) are at *quantum satis* (QS), the highest concentration in the maximum level exposure assessment scenario from the latest EFSA opinion on a (re-)evaluation of the additive by EFSA should be used. If such a (re-)evaluation is not available or no concentrations have been reported in the opinion, the food category cannot be considered, which should be clearly indicated in the dossier explaining why.

[31]DietEx tool is described here: https://www.efsa.europa.eu/en/science/tools-and-resources/dietex and can be accessed https://analytics.efsa.europa.eu/, by signing up to get an account.

[32]Instructions of use of FAIM: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2025.EN-9583.

[33]Use levels or analytical data.

[34]That is mainly in Annex II, Part D, of Regulation (EC) No 1333/2008, but some MPLs may be defined in specific regulations like for instance on wine.

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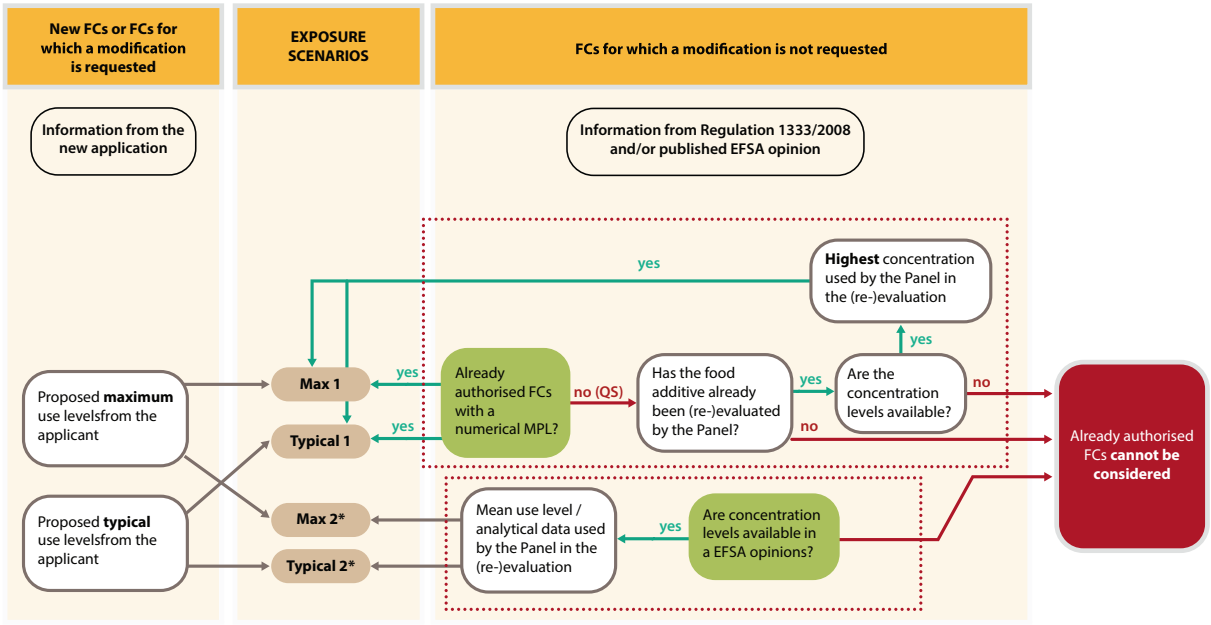
Scenario Typical 1: The applicant should use the proposed typical use levels for the food categories for which an extension/modification of use is requested and assign the MPL(s) as laid down in the relevant Regulation, if relevant, to the currently authorised food categories for which no modification of use is requested. In the case current MPL(s) are at *quantum satis* (QS), the highest concentration in the maximum level exposure assessment scenario from the latest EFSA opinion on a (re-)evaluation of the additive by EFSA should be used. If such a (re-)evaluation is not available or no concentrations have been reported in the opinion, the food category cannot be considered, which should be clearly indicated in the dossier explaining why.

Scenario Maximum 2: The applicant should use the proposed maximum use levels for the food categories for which an extension/modification of use is requested and assign the mean use level used in the non-brand loyal refined scenario from its re-evaluation, if relevant, to the currently authorised food categories for which no modification of use is requested. If such a (re-)evaluation is not available or no concentrations have been reported in the opinion, the food category cannot be considered, which should be clearly indicated in the dossier explaining why.

Scenario Typical 2: The applicant should use the proposed typical use levels for the food categories for which an extension/modification of use is requested and assign the mean use level used in the non-brand loyal refined scenario from its re-evaluation, if relevant, to the currently authorised food categories for which no modification of use is requested. If such a (re-)evaluation is not available or no concentrations have been reported in the opinion, the food category cannot be considered, which should be clearly indicated in the dossier explaining why.

Please note that the **Maximum 2** and **Typical 2** scenarios can only be performed when an EFSA (re-)evaluation for the authorised food additive with use levels/analytical data has been performed. Also note that the Maximum 1 and 2 scenarios are the same if modifications of use are requested for all already authorised food categories. Same is true for the Typical 1 and 2 scenarios.

The scenarios have also been described in a flow chart shown in Figure 1.



FC(s): Food category(ies); those related to food supplements (FCs 17, 17.1 and 17.2) and to food for special medical purposes FCs 13.1.5.1 and 13.1.5.2 should not be considered in the exposure scenarios described here. Food additives to be used as sweeteners should also not be considered by these scenarios.
MPL: Maximum permitted levels
QS: Quantum Satis
...: Process to be repeated for each FC already authorised
*: These scenarios are only possible when there is a published EFSA opinion reporting concentration levels

FIGURE 1 Flow chart describing the dietary exposure scenarios of an already authorised food additive for which an extension/modification of use is requested.

4.2.1.2 | *For infants below 16 weeks of age*

If authorisation of a proposed food additive is requested in infant formulae for infants below 16 weeks of age (FCs 13.1.1 and 13.1.5.1), the applicant should estimate the dietary exposure for this age group following the recommendations of the EFSA Scientific Committee (EFSA Scientific Committee, 2017a).

Until 16 weeks of age, infants have a diet that consists exclusively of breastmilk or infant formulae. To assess the safety of infant formulae, EFSA issued a guidance document on the risk assessment of substances present in these foods (EFSA Scientific Committee, 2017a). This guidance document provides mean and high-level consumption amounts of infant formulae (in mg/kg bw per day) for assessing the dietary exposure to substances. Values of 200 and 260 mL/kg bw per day as conservative mean and high-level consumption amounts are recommended to be used for the risk assessment of substances that do not accumulate in the body (as is generally the case for food additives).

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The applicant should use these conservative consumption amounts of infant formulae combined with the proposed maximum and typical use levels in infant formulae to calculate the dietary exposure to the proposed food additive in infants below 16 weeks of age.

4.2.1.3 | *Consumers only scenario*

A 'consumers only scenario' estimates the exposure to a food additive in a sub-group of a population that consists of consumers of a specific food (e.g. food supplements) and possibly also considering their whole diet (e.g. food supplements and all other food categories that could contain the food additive).

FAIM (version 3.0) has recently been updated to also estimate the exposure to a food additive according to the 'consumers only scenario' (see FAIM user manual32; an example of a 'consumers only scenario' performed by EFSA can be seen for instance in EFSA FAF Panel (2023e); EFSA FAF Panel (2024e)). Currently, the DietEx tool only provides the possibility to estimate dietary exposure for 'consumers only' of one single FoodEx2 code without considering possible additional exposure via other food categories that could contain the proposed food additive.

A 'consumers only scenario' should be calculated when the proposed food additive is requested to be used in the food categories food supplements (FCs 17, 17.1 and 17.2) and/or dietary foods for infants and young children for special medical purposes (FCs 13.1.5.1 and 13.1.5.2). The 'consumers only scenario' should be performed by assigning the proposed maximum use levels/numerical MPLs or maximum use/analytical levels to these food categories. If relevant, the proposed typical use levels or mean use/analytical levels from the last EFSA opinion should be assigned to the other food categories in which the additive may be added.

When the proposed food additive is only to be used in food supplements, it is possible for the applicant to estimate the dietary exposure using DietEx as food supplements are identified in DietEx via one unique FoodEx2 code.

If an applicant requests the use of the additive in FCs 13.1.5.1 and/or 13.1.5.2, the FCs 13.1.1. and/or 13.1.2 respectively should be selected in FAIM. This is based on the assumption that the consumption of FSMP formulae is similar to that of the 'standard' formulae. In FAIM, FC 13.1.5.1 is not available and consumption amounts of foods belonging to FC 13.1.5.2 are not reliable due to very few eating occasions reported in the Comprehensive Database (see Section 4.2.1.1).

For food additives to be used as a sweetener, the applicant is not requested to perform an exposure assessment, because the current available EFSA tools do not allow to perform it at the time of the publication of this guidance document. Therefore, in that case EFSA will perform a 'consumers only scenario' according to the sweeteners exposure protocol (EFSA FAF Panel, 2024f), i.e. selecting relevant foods that may contain a sweetener based on facets25 (see Section 4.2.1). Indeed, this scenario cannot be calculated with the EFSA dietary exposure tool(s), because these tools do not contain facets. If the application is on an already evaluated sweetener, the applicant could refer to the dietary exposure estimates available in the relevant EFSA opinion. Please note that this applies to food additives that may also have other function(s) than as a sweetener in the foods (e.g. polyols).

How to provide the results of the exposure assessment

Both FAIM and DietEx provide mean and 95th percentile estimates of dietary exposure to the proposed food additive and information on the contribution of the food categories to the mean dietary exposure for the different population groups and EU Member States. The tools also provide a spreadsheet with the foods/food categories and the concentrations used in the exposure assessment. All inputs and outputs should be provided in the dossier (in the form of an Excel file).

The 95th percentile estimates (high consumers), for the different scenarios, will generally be used for the risk assessment (see section 'Risk assessment process'). EFSA may also decide to refine the exposure assessment[35] (e.g. when the risk assessment shows a concern and the data available allow a refinement of the assessment). Such a refinement will be performed using the food categories in Annex II, Part D, of Regulation (EC) No 1333/2008, or FoodEx2 if the level of detail is sufficient in the latest version of the Comprehensive Database. For this, EFSA may use additional information (e.g. from the facets within FoodEx2) to improve the selection of the foods that may contain the proposed food additive and thus the linkage between the proposed use levels and foods recorded in the Comprehensive Database.

4.2.2 | Acute exposure assessment

EFSA may perform an acute dietary exposure assessment (short-term exposure) if acute effects of the proposed food additive are relevant (see, e.g. erythritol (E 968) (EFSA FAF Panel, 2023g)). Such an exposure assessment is not requested from the applicant.

For information, if relevant, acute exposure will be calculated by the Panel by multiplying the total consumption amount per day or per meal for each relevant food by the relevant concentration available for that food. Respective exposures per relevant food consumed on that day/meal will be summed per individual and divided by the individual's body weight

[35]It is highlighted that the brand-loyal refined exposure assessment scenario, as defined in EFSA ANS Panel (2017), cannot be performed using FAIM and DietEx as this would require access to food consumption data at the individual level.

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to provide an estimate of the exposure on that specific day/meal. By doing this for all consumption days/meals in the Comprehensive Database, a distribution of acute exposure estimates per day or per meal is generated. From these distributions, a high (highest reliable percentile) acute intake will be calculated and used in the risk assessment.

This assessment will be performed for the relevant population groups and EU Member States available in the Comprehensive Database. As indicated in the revised version of the protocol for assessing the exposure to sweeteners (EFSA FAF Panel, 2024f), an acute exposure assessment will be based on the maximum use level/highest reliable percentile of analytical levels for the two food categories contributing most to the exposure for a single day/meal, and the mean/median use or analytical levels for the remaining food categories. Which concentration data to be used in the assessment will be decided on a case-by-case basis.

For infants below 16 weeks of age, the 95th percentile of infant formulae consumption per kg body weight is to be considered as a maximum daily consumption amount of that food. EFSA will use this amount to assess the acute exposure by multiplying this amount by the proposed maximum use level for infant formulae.

4.3 | Exposure assessment from dietary sources other than food additive

Apart from being added to food as a food additive, a substance can also be, e.g. (i) naturally present in food, (ii) present because it is added to food as a food flavouring or food ingredient (e.g. food fortification and supplements) or (iii) present due to its use in food contact materials or plant protection products.

In such cases, the applicant is requested to provide quantitative information, i.e. concentrations, consumption amounts of the relevant foods and estimates of exposure (average and high-level exposure for the relevant dietary sources). If not possible, qualitative information on this source should be provided. For providing such information, data from literature (i.e. primary references as well as available databases, e.g. food composition databases) could be considered.

If applicable, the applicant may also estimate the dietary exposure from these sources for the same population groups, as described in Section 4.4. For this, FAIM and/or DietEx could be used.

4.4 | Exposure assessment from non-dietary sources

Furthermore, a proposed food additive may also be used in non-dietary sources such as medicines, cosmetics, tobacco products and/or tobacco replacement products ('electronic cigarettes').

If applicable, quantitative information, i.e. concentrations, intake amounts of the relevant sources and estimates of exposure (average and high-level exposure for the relevant non-dietary sources) should be submitted. If not possible, qualitative information via these routes should be provided. For this, data from literature could be considered.

The international agreed methodologies used by ECHA and the Scientific Committee for Consumers Safety could be considered for estimating exposure, as summarised in EFSA (2016). Approaches on exposure assessments performed by other bodies may also be used.

4.5 | Estimates of exposure to impurities or to components of toxicological concern from the use of the proposed food additive

Dietary exposure to any toxicologically relevant impurities, e.g. toxic elements, present in the proposed food additive should be provided taking into account purity criteria proposed by the applicant or specific legislative purity criteria as applicable. The same applies to components of toxicological concern identified in the proposed food additive. Exposure to these impurities and/or components should be estimated pro-rata from the exposure estimates of the proposed food additive. In this way, an estimate of the anticipated exposure (mean and 95th percentile) to these toxicologically relevant impurities and/or components will be obtained for the population groups (information can be found on the presentation made at the March 2024 info session: (Re-)Evaluating Food Additives[36]). Both the highest mean and 95th percentile exposure estimates of the impurities, for the most relevant scenario, will generally be used in the risk assessment.

If it is not possible to estimate the dietary exposure to the additive as described in Section 4.2.1 or if there are no existing European estimates of exposure, a potential exposure to the impurities should be provided based on the information an applicant should have of the proposed food additive (e.g. levels in food categories in which the additive is authorised). This may be the case for example if a new manufacturing process is used for an additive that is authorised at QS and this process generates new impurities. A conservative estimate would be sufficient (e.g. using FAIM or DietEx). It will also be possible to liaise with EFSA about the exposure assessment needed.

[36] https://www.efsa.europa.eu/sites/default/files/2024-03/06-emerging-changes-in-eu-specifications-authorised-food-additives.pdf.

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5 | TOXICOLOGICAL DATA

5.1 | Issues to be considered before or when conducting toxicological studies

The following aspects should be considered in the design, conduct and interpretation of toxicological studies on the proposed food additive.

- Toxicological studies should be carried out with the **test material** as manufactured according to the specified manufacturing process (Section 3.3) and accompanied by data showing that it complies with the proposed specifications of the food additive (Section 3.4). In the absence of such information, the applicant should provide a scientific justification as to why the data generated with the test material can be used to evaluate the safety of the proposed food additive.
- According to the EFSA Scientific Committee's 'Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles' (EFSA Scientific Committee, 2021a) (Guidance on Particle-TR), applicants must determine whether a conventional risk assessment is sufficient or if it must be complemented with nano-specific aspects (see Section 5.2.2 for further details). If such case, appropriate adaptations of conventional test methods would be needed, according to the 'Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: human and animal health' (EFSA Scientific Committee, 2021b).
- All toxicokinetic and toxicity studies should be carried out in compliance with the principles of **Good Laboratory Practice (GLP)** as described in Directive 2004/10/EC[37] of the European Parliament and of the Council. In addition, except for modifications described in this guidance, they should be conducted in accordance with current OECD TGs.[38] The use of any testing methods differing from internationally agreed test guidelines, amendments of these as described in this guidance, and/or not carried out according to GLP, should be duly justified by the applicant.
- Where available, **historical control data** shall be provided routinely. The data submitted shall be for endpoints that could represent critical adverse effects and shall be strain-specific and from the laboratory which carried out the toxicity study under investigation. The principles of the EFSA scientific opinion on the 'Use and reporting of historical control data for regulatory studies' should be considered by the applicant (EFSA PPR Panel, 2025).
- In animal studies, substances should normally be **administered using the oral route**. For additives that are to be added to solid foods, or added to both solid foods and beverages, administration should normally be through the diet. In case of reduced palatability following incorporation of high concentrations of the food additive in foods, administration by oral gavage or the use of additional pair feeding control groups should be considered. For additives that are only to be used in beverages, administration to animals via drinking water may not adequately reflect the fact that humans can consume beverages such as soft drinks in significant quantities over a short time period. Therefore, in such cases, bolus administration, such as by oral gavage, should be used. The same applies to substances such as an additive exclusively used in food supplements which are marketed in liquid forms or as tablets with rapid release. An intravenous administration is necessary in case the absolute bioavailability of the proposed food additive is of interest. This is determined by comparing the toxicokinetic endpoints (e.g. AUCs and/or urinary excretion) following intravenous administration with those obtained following an oral administration.
- The toxicity studies should be designed in such a way that they provide data allowing **Benchmark Dose analysis** (EFSA Scientific Committee, 2022), particularly concerning the selection of the doses to be tested. For all relevant endpoints, as specified in the relevant OECD TGs, the data should be submitted in an appropriate electronic format (e.g. spreadsheet) in order that these data can be used as input file for dose–response modelling, allowing a direct evaluation of the data included in the study report. The applicant should perform a dose–response analysis for all relevant endpoints in line with the EFSA Guidance on Benchmark Dose analysis (EFSA Scientific Committee, 2022) in order to derive a reference point. If the data do not allow to perform a BMD analysis, a NOAEL/LOAEL can be used.
- For **food additives consisting of mixtures**, relevant safety data on the functional components of the proposed food additives as well as of the components of toxicological concerns, if applicable, are required when toxicological data are not available for the whole mixture. In relation to the genotoxicity assessment, the recommendations of the EFSA Scientific Committee (2011, 2017b, 2021c) and EFSA (2023) should be followed (see Section 5.5.1).
- In general, there is no requirement to submit **acute toxicity data** on a proposed food additive, since experience from previous assessments of food additives indicates that in most cases food additives are not acute toxicants. In case existing data on acute toxicity are available these should be submitted and will be considered in the assessment. If applicants consider it relevant to derive an acute reference dose, WHO EHC 240 section 5.2.9 (WHO/IPCS, 2020) should be consulted.
- In line with the EFSA 'Guidance on Safety assessment of **botanicals** and botanical preparations intended for use as ingredients in food supplements' (EFSA Scientific Committee, 2009), a 'presumption of safety' may be applied to botanicals and botanical preparations intended to be used as food additives when they are derived from conventional food sources with a long-term history of use, and that oral exposure to known levels of the botanical ingredient has occurred in large population groups without reported adverse effects (e.g. reliable data derived from the European Member States' mean

[37]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.
[38]https://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm.

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diets or from studies on specific subgroups). The Panel noted that the Guidance on botanicals states that 'an important requirement is that the technical data, the data on exposure and the available toxicological data are provided, and that no significant increase of intake compared to historical levels is to be expected due to the intended levels of use'. In this regard, the Panel further considers that:

a. the definition of what is considered a significant increase of intake, compared to historical dietary intake levels, has to be judged on a case-by-case basis. This implies that not only use levels but also chemotypes of botanicals and the chemical composition of the botanical preparations should be in line with historically used ones.
b. methods of extraction/manufacturing of the botanical preparation used as food additive should be considered, since processes differing from the traditional methods of food preparation may lead to compositional differences and concentrate undesirable components.
c. for botanical preparations with a potential to contain toxic, addictive, psychotropic or other substances that may be of concern, the presumption of safety approach can only be applied if there is convincing evidence that these undesirable substances in the specific plant parts or preparations are either absent in the source material, or significantly reduced if not excluded, or inactivated during processing. The EFSA Compendium of Botanicals database[13], including information on botanicals that are reported to contain toxic, addictive, psychotropic or other substances that may be of concern, should be consulted as it facilitates the identification of substances of concern that may be present in the botanical or botanical preparation intended to be used as food additive.

5.2 | Safety evaluation and corresponding testing strategy

5.2.1 | Justification of the testing strategy applied to the proposed food additive

A description and justification of the testing strategy chosen by the applicant to demonstrate the safety of the proposed food additive, in line with the data requirements of the present guidance document, should be provided in the technical dossier. This should include the rationale for inclusion and exclusion of specific types of in vitro/in vivo toxicity studies, developed in line with the tiered approach described in this guidance document (see Sections 5.3, 5.4, 5.5, 5.6, 5.7, 5.8 and 5.9), taking into account existing relevant data from the literature (see Section 5.4.1).

5.2.2 | Strategy to establish whether the conventional risk assessment is sufficient

Applicants should provide evidence whether the proposed food additive can be assessed following the conventional risk assessment as described in the present guidance document or whether nano-specific aspects should be addressed.

For this purpose, the EFSA Scientific Committee 'Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles' (Guidance on Particle-TR) explains the process to decide whether or not the material, or a fraction of it, does require specific assessment of properties at the nanoscale (EFSA Scientific Committee, 2021a[39]). The applicants may select, according to their knowledge and available information, the best appraisal route or combination of appraisal routes when preparing the application/dossier (Figure 1 and Table 1 of EFSA Scientific Committee (2021a)).

If it cannot be demonstrated that the food additive meets at least one of the decision criteria listed in Table 1 of the EFSA Scientific Committee guidance, data should be provided taking into account the requirements established in the EFSA Scientific Committee Guidance on risk assessment of nanomaterials (EFSA Scientific Committee, 2021b).

5.2.3 | Food additive consisting of nanomaterials

Toxicological data on engineered nanomaterials should be provided in line with section 7 of the 'Guidance on risk assessment of nanomaterials to be applied in the food and feed chain' (EFSA Scientific Committee, 2021b).

5.3 | Tiered approach for conducting toxicokinetic and toxicological studies

The purpose of conducting toxicokinetic and toxicological studies on the proposed food additive is to identify and characterise its potential hazards, and to study the kinetics of the proposed food additive at toxicologically relevant concentrations, as the basis for establishing safe intake levels for humans.

This guidance document describes a tiered approach for conducting toxicokinetic and toxicity studies, designed to evaluate the following core areas of the safety assessment of the proposed food additive:

[39]Complementary information to the Guidance available at https://www.efsa.europa.eu/en/topics/topic/nanotechnology.

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- genotoxicity
- toxicokinetics
- toxicity other than genotoxicity, to identify toxicity in target organs following repeated dosing, including reproductive and developmental toxicity and carcinogenicity.

The relevant information is obtained by in vitro and in vivo experimental studies in animals. Data from studies in humans, when existing, are considered in the weight of evidence together with the data from the in vivo experimental animal studies. Information shall be obtained by non-animal testing if feasible, in accordance with the requirements described in Section 5.4 and in line with the 3R (replacement, reduction and refinement) principles (Directive 2010/63/EU[40]).

The tiered approach for toxicokinetic and toxicity testing recommends animal testing until the information available is sufficient to reach a conclusion in the risk assessment. It consists of four tiers, for which a description of the data requirements for the different tiers, and the triggers prompting the need to move to a higher tier, are described in Sections 5.4, 5.5, 5.6, 5.7 and 5.8. A flow chart of the tiered approach is shown in Figure 2. For food additives intended for use in infants below 16 weeks of age, the recommendations of the EFSA Scientific Committee Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age (EFSA Scientific Committee, 2017a) should be followed (see paragraph 5.9).

According to this tiered approach, the first tier, referred as Tier I, aims at making the use of the existing information and/or to produce a set of non-animal data which inform on the safety of the proposed food additive, before moving to in vivo animal testing, in line with the principle of minimising studies in animals. The data provided in Tier I may already allow to conclude on the safety of the proposed food additive, provide guidance for the preferred follow-up testing strategy or support the weight of evidence.

The in vitro genotoxicity assessment of the proposed food additive is required at Tier I and, if positive results are observed in vitro, in vivo follow-up genotoxicity testing are also required at Tier II(A), to assess whether the genotoxic potential observed in vitro is expressed in vivo. to assess whether the genotoxic potential observed in vitro is expressed in vivo.

The assessment of the genotoxic potential is a basic component of chemical risk assessment in view of the adverse consequences of genetic damage to human health. As pointed out by the EFSA Scientific Committee (EFSA Scientific Committee, 2011) clear evidence of genotoxicity in vivo is considered an adverse effect of toxicological concern, independent of the outcome of carcinogenicity studies, as the accumulation of DNA damage in somatic tissues may play a role in degenerative conditions different from cancer, i.e. accelerated aging, immune dysfunction, cardiovascular and neurodegenerative diseases, which are not covered in carcinogenicity studies. Moreover, not all genotoxicity endpoints are clearly related to carcinogenicity (e.g. aneuploidy) (EFSA Scientific Committee, 2011, 2021c).

Therefore, if the proposed food additive is concluded to be genotoxic in vivo via a relevant route of administration, the safety of the food additive cannot be established, and the applicant should not perform any further toxicological testing. However, if the genotoxicity is restricted to aneugenicity, the recommendations provided in the EFSA Guidance on aneugenicity (EFSA Scientific Committee, 2021c) should be considered.

For proposed food additives that do not raise a concern for genotoxicity, no further systemic toxicity testing is necessary if (i) there is no evidence that the proposed food additive is absorbed in the intestinal tract, based on the results from in vitro absorption studies in Tier I (Section 5.4.4) and (ii) following an in vivo study in Tier II (A) (Section 5.5.2) to assess its potential local toxicity at sites of contact along the gastrointestinal tract, and to confirm lack of absorption.

For proposed food additives that do not raise a concern for genotoxicity and that are absorbed following oral exposure, Tier II(B) testing is needed.

Tier III and IV testing should be performed on a case-by-case basis, to elucidate specific endpoints needing further investigation of findings in Tier II(B) testing or concern raised in Tier I.

The Panel highlights that the testing strategy for toxicokinetics and toxicity allows for more flexibility compared to the approach for the genotoxicity testing, due to the variety of potential datasets available for the proposed food additive. Accordingly, applicants can decide to enter any Tier (from Tier II(B) to Tier IV) on a case-by-case basis, depending on the available data. For example, (i) if relevant and reliable scientific evidence is available from the literature (Tier I) showing that the proposed food additive has an endocrine disruptor (ED) activity, it is recommended to perform directly an OECD TG 443 (OECD, 2025b) in Tier III to conclude on the ED-mediated hazard (ECHA and EFSA, 2018); (ii) if the proposed food additive has a long half-life (greater than about 2 weeks), the applicant can consider performing directly Tier IV toxicity testing without conducting any Tier II or Tier III testing, because in this case steady state condition will not be reached in the toxicity studies recommended in Tier II and/or Tier III.

[40]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 Text with EEA relevance. https://eur-lex.europa.eu/eli/dir/2010/63/oj/eng.

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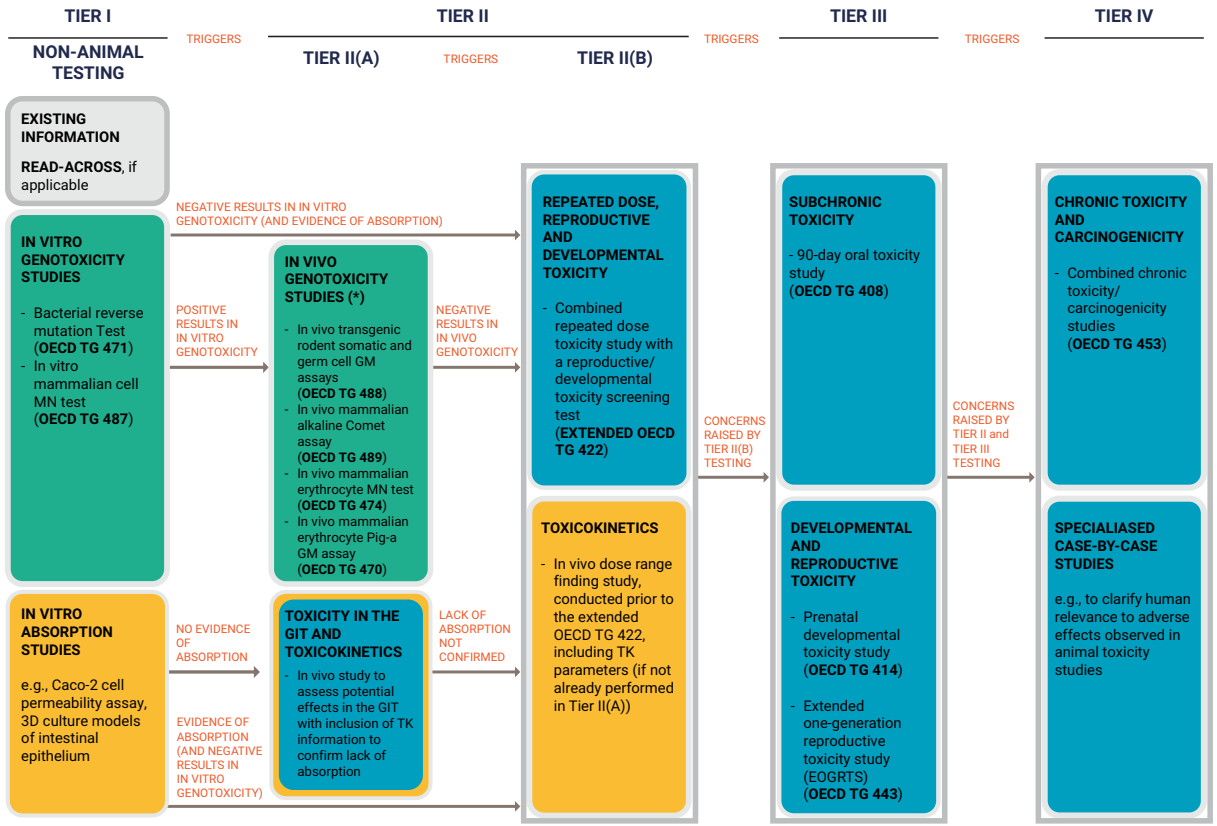

FIGURE 2 Tiered approach for toxicokinetic and toxicity testing of food additives. Supportive data as described in Section 5.4.5 of the guidance (Tier I) are not included in the flow chart. For food additives intended for use in infants below 16 weeks of age this flow chart is not entirely applicable, considering that toxicity data should be provided in line with the recommended by the (EFSA Scientific Committee, 2017a) (for more details see paragraph 5.9). GIT: gastrointestinal tract; GM: gene mutation; MN: micronucleus; TK: toxicokinetic. The following colour code is used to indicate different types of data: grey = non-testing methods; green = genotoxicity studies; yellow = toxicokinetic studies; blue = general toxicity studies, including reproductive/developmental, chronic toxicity and carcinogenicity.
(*) If the proposed food additive is concluded to be genotoxic in vivo via a relevant route of administration, the safety of the food additive cannot be established, and the applicant should not perform any further toxicological testing. If the genotoxicity is restricted to aneugenicity, the EFSA Guidance on aneugenicity (EFSA Scientific Committee, 2021c) should be consulted. If there is no evidence of absorption in Tier I, the integration of in vivo genotoxicity assays into the in vivo toxicity study in Tier II (A) should be envisaged where possible, in line with the 3Rs principle.

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5.4 | Tier I – Non-animal testing

As outlined in Section 5.3, Tier I is designed to maximise the use of existing information and/or generate relevant non-animal data to inform on the safety of the proposed food additive prior to initiating any new in vivo study. The data provided at this stage:

(i) may be sufficient to reach a conclusion on the safety of the substance, without the need for further testing (this may apply to Sections 5.4.1, 5.4.2, 5.4.3 (specific only to genotoxicity endpoints)); or
(ii) can support decision making in the risk assessment process. For example, they can inform on the most appropriate testing strategy to be followed in higher tiers, including providing a scientific rationale for waiving specific toxicological endpoints in in vivo studies recommended in higher Tiers and/or for triggering directly Tier III or Tier IV toxicity studies, without conducting Tier II toxicity testing (this may apply to Sections 5.4.1, 5.4.2, 5.4.4 and 5.4.5); or
(iii) may contribute to the overall weight-of-evidence in the risk assessment process (this may apply to Sections 5.4.1, 5.4.2 and 5.4.5).

5.4.1 | Existing information

Before performing any toxicological studies, the applicant should conduct a comprehensive literature search for relevant data, concerning the safety of the proposed food additive, including its components (if the food additive is a mixture).

The search should address all the relevant endpoints that are needed for the safety assessment of the proposed food additive, as described in the present guidance document, i.e. genotoxicity, toxicokinetics, toxicity other than genotoxicity, including also the safety for the environment (see Section 6).

As mentioned in Section 1.5. 'General principles' (bullet 4) of this guidance, the search strategy used to retrieve the relevant literature data should be documented, including the description of the search terms, the databases and the criteria used to perform the search (e.g. inclusion and exclusion criteria, time-period covered, publication dates, publication types). Where applicable, the published literature should be reviewed taking into account systematic review principles (EFSA, 2010).

5.4.2 | Read-across

In case a read-across approach is applied by the applicant, it has to be justified based on the principles outlined in the EFSA Scientific Committee 'Guidance on the use of read-across in food and feed risk assessment' (EFSA Scientific Committee, 2025c).

The endpoints covered by read-across should be compliant with the data requirements as prescribed in this guidance (see Sections 5.4, 5.5, 5.6, 5.7, 5.8 and 5.9).

If the proposed food additive is a chemical mixture, the EFSA Scientific Committee guidance documents on mixtures will apply (EFSA Scientific Committee, 2019a, 2019b). Thus, read-across for any toxicity endpoint will not be accepted for the whole mixture. However, for identified individual components in such mixtures, read-across for genotoxicity and for other toxicological endpoints could be applied, if experimental data are not available (EFSA Scientific Committee, 2019a). Examples where read-across for genotoxicity has been applied to the identified components of chemical mixtures are available in the EFSA opinions (e.g. EFSA FAF Panel, 2023a, 2023b, 2023c, 2023d).

5.4.3 | In vitro genotoxicity testing

A stepwise approach should be followed for the generation and evaluation of the data on the genotoxic potential of food additives, as described in the guidance documents of the EFSA Scientific Committee (EFSA, 2023; EFSA Scientific Committee, 2011, 2017b, 2021c). For food additives that consist of mixtures, the EFSA Scientific Committee statement from 2019 is also applicable (EFSA Scientific Committee, 2019a). For the assessment of the genotoxic potential of food additive consisting of mixtures, please refer to Section 5.5.1.

The following considerations are relevant to the assessment of the genotoxic potential of food additives consisting of a single substance.

The first step is to test the substance using in vitro tests, covering all three genetic endpoints, i.e. gene mutations, structural chromosomal aberrations (CAs) (clastogenicity) and numerical CAs (aneuploidy). As no individual test can provide information on all three endpoints, the Scientific Committee (EFSA Scientific Committee, 2011) currently recommends the following two in vitro tests:

– a bacterial reverse mutation test (Ames test), OECD TG 471 (OECD, 2020),
– an in vitro mammalian cell micronucleus test, OECD TG 487 (OECD, 2023).

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The bacterial reverse mutation assay allows the detection of gene mutations, and the in vitro micronucleus (MN) test reveals both structural and numerical CAs. The application of hybridisation with centromeric/telomeric probes (fluorescence in situ hybridisation (FISH)) or immunochemical labelling of kinetochores (CREST analysis) in the MN test provides information on the mechanisms of chromosomal damage and MN formation (clastogenicity and aneugenicity). In order to reliably differentiate between these mechanisms, the Panel recommends using FISH analysis, although the CREST analysis could also be applied, as indicated in the OECD TG 487 (OECD, 2023).

If all endpoints are clearly negative in adequately conducted in vitro tests, it can be concluded that the substance has no genotoxic potential. In the case of inconclusive or equivocal results from the in vitro tests, it may be appropriate to conduct further testing in vitro before conducting in vivo genotoxicity studies (EFSA, 2023; EFSA Scientific Committee, 2011).

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

In case the food additive is a protein or provides a source of amino acids, in order to overcome potential problems with histidine or tryptophan present in the tested food additive when performing a bacterial reverse mutation (Ames) test (OECD TG 471), it is recommended to expose the *Salmonella* and *E. coli* strains to the tested food additive in the liquid culture ('treat and wash assay'), instead of the traditionally 'plate incorporation assay'. A recommended protocol incorporating treat and wash is given in Annex C of the EFSA Scientific Guidance for the submission of dossiers on food enzymes (EFSA CEP Panel, 2021). If the Ames test is not applicable, alternatively a test for induction of gene mutations in mammalian cells, i.e. OECD TG 476 (OECD, 2016a) or OECD TG 490 (OECD, 2016b), could be performed.

In case the proposed food additive is known to be biotransformed by the human gut microbiota in a different way compared to the S9-mix, the genotoxicity of the resulting breakdown products/metabolites may also be considered. The Panel is aware of ongoing research in this respect (see Section 5.5.2).

In case the proposed food additive contains a fraction of small particles including nanoparticles, special considerations are needed as recommended by the Scientific Committee (EFSA Scientific Committee, 2021a). For example, an in vitro mammalian gene mutation assay should be performed instead of a bacterial reverse mutation assay, and necessary modifications should be applied to the MN assay.

5.4.4 | In vitro absorption studies

The absorption from the intestinal tract of the proposed food additive or its breakdown products may be assessed by using human-relevant in vitro models representative of intestinal epithelium.

Although not officially recognised as a validated alternative method, filter-grown Caco-2 cells have been most widely proposed as representing a cell culture model of human enterocytes for screening permeation of test materials through the intestinal epithelium. If the study is well conducted,[41] it could be accepted to indicate that there is no evidence of absorption of the proposed food additive in the gut. To this aim, in line with the DB-ALM protocol n. 142[42], the apparent permeability coefficient (Papp) value of $< 5 \times 10^{-7}$ cm s^{-1} for mannitol should be used as an indication of lack of absorption. The study of the integrity of the epithelial barrier should be included as part of the assay.

Human small intestinal organotypic 3D culture models, which encompass all cell types constitutive of the intestinal epithelium, have been also recently made available. Advantages and limitations of the various models have been described in the literature (e.g. Fedi et al., 2021; Xu, 2021). Other generally accepted models could be considered based on the most up-to-date technical and scientific knowledge.

The selected in vitro model should be justified, and the conduct of the study should follow a well-defined and standardised protocol which should made available. For the qualification of the in vitro models, the use of at least one reference substance per type of permeability (low, mid and high) is needed. In addition, the use of reference substances showing zero absorption is also needed (e.g. for the selection of the reference substances please refer to ICH M9 guideline (ICH, 2020)). It is of key importance to work under well-documented and standardised conditions (e.g. Krebs et al., 2019; OECD, 2018a), and to report in detail the experimental setup and the quality control measures. The most sensitive analytical methods should be used to investigate the potential absorption of a food additive. The sensitivity and specificity of the methods and the detection and quantification limits should be reported. It may be necessary to use radiolabelled compounds to increase the sensitivity.

The Panel also recognises that absorption from the human buccal cavity should also be considered as a possible contributor to the systemic availability of food additives. Human buccal TR146 cells grown on filters have been frequently used for oromucosal permeability studies (Jacobsen et al., 1999), but they have not yet been validated or standardised by an official body (e.g. OECD, JRC).

[41]Examples for the application of permeability assay are given in: (i) ICH M9 guideline (ICH, 2020); (ii) DB-ALM Protocol no 142 on Permeability Assay on Caco-2 Cells Intestinal Absorption, Mechanistic Studies, Systemic Toxicity (https://jeodpp.jrc.ec.europa.eu/ftp/jrc-opendata/EURL-ECVAM/datasets/DBALM/LATEST/online/DBALM_docs/142_P_Permeability%20Assay%20on%20Caco2%20Cells.pdf); (iii) Kus et al. (2023).

[42]DB-ALM Protocol no 142 on Permeability Assay on Caco-2 Cells Intestinal Absorption, Mechanistic Studies, Systemic Toxicity (https://jeodpp.jrc.ec.europa.eu/ftp/jrc-opendata/EURL-ECVAM/datasets/DBALM/LATEST/online/DBALM_docs/142_P_Permeability%20Assay%20on%20Caco2%20Cells.pdf).

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Even when the in vitro data provides no evidence of absorption, the tissues lining the intestine (i.e. intestinal epithelium and lamina propria) have contact with the substance, hence local effects in the gastrointestinal tract (i.e. from oesophagus to intestine) have to be assessed in a study of limited duration (e.g. two weeks). Furthermore, this in vivo study could serve to confirm lack of absorption (i.e. not detectable) of the proposed food additive and/or of its breakdown products (see Section 5.5.2).

5.4.5 | Supportive data

The data presented in this section are intended to support decision making in the risk assessment process. They generally cannot replace the need for experimental in vivo data on the proposed food additive. Applicants can submit such data as part of the application dossier and explain how they could be used to support the overall weight of evidence to assess the safety of the proposed food additive.

5.4.5.1 | *Physicochemical data*

The physicochemical properties, as described in Section 3.2 of this guidance, may predict the dissociation characteristics of the food additive or of its components (for food additives consisting of mixtures) in the gastrointestinal tract, which may have an impact on its intestinal absorption and on its further distribution in the tissues. The following parameters are particularly informative:

- molecular weight
- octanol–water partition coefficient (K_{ow}), when applicable,
- solubility in water or in a non-aqueous matrix, or in a solvent relevant for the use of the proposed food additive in foods and in toxicity/genotoxicity tests (EFSA Scientific Committee, 2021a),
- particle size, shape and distribution, if applicable (see Section 5.2 for further details). The recommendations of the EFSA Scientific Committee Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles (EFSA Scientific Committee, 2021a) should be followed.
- melting point (for solids),
- boiling point (for liquids),
- pKa, when applicable.

5.4.5.2 | *New approach methodologies (NAMs)*

Directive 2010/63/EU emphasises that tests on animals should be replaced, reduced or refined (3Rs), wherever possible. The goal to reduce animal studies and to develop and integrate new scientific developments focusing on NAM[43]-based methods is also in line with the EU's chemicals strategy for sustainability,[44] the European Commission's roadmap towards phasing out animal testing (European Commission, 2024) and the EFSA's Strategy 2027.[45]

Therefore, applicants are encouraged to make use of such methodologies to support the safety of the proposed food additive. This is particularly relevant when these alternative approaches provide mechanistic insights into the mode of action (MoA) of the proposed food additive, which may help to address data gaps and inform the need for further testing. NAMs may also offer valuable information on early biological changes that precede adverse effects observed in traditional apical endpoints. When using NAMs, applicants should assess whether the in vitro concentrations tested are appropriate to support the safety evaluation of the proposed food additive and how the results can be interpreted in the context of human exposure and risk assessment.

Validated and standardised NAM-based methodologies endorsed by an official body (e.g. OECD, JRC) should be preferred, when available.

When using non-validated methods, applicants should at least ensure their relevance, reliability and standardisation as defined in OECD GD 34 (OECD, 2005) on the validation of new test methods and/or following available guidance such as Good In Vitro Method Practices (GIVIMPs) (OECD, 2018a) or OECD GD on PBK models (OECD, 2021). Furthermore, a qualification system, based on an expert opinion, may enable an efficient use of adequate NAMs for specific context-of-use, as proposed for example for nanomaterial risk assessment (EFSA, 2024).

The Panel acknowledges that this revision of the guidance occurs at a time of scientific progress in the development and application of NAMs. While many of these methodologies are still evolving and may not yet fully replace in vivo studies, their progressive integration into the safety assessment of food additives is encouraged. Continued research, case studies and regulatory dialogue are essential to build confidence in these approaches and to support their effective implementation in regulatory decision-making (Cattaneo et al., 2023; European Commission JRC, 2025).

[43] In vitro, in silico and in chemico approaches are often referred to as new approach methodologies or NAMs.

[44] Chemicals strategy - Environment - European Commission.

[45] https://www.efsa.europa.eu/en/corporate-pubs/efsa-strategy-2027-science-safe-food-sustainability.

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5.4.5.3 | *(Q)SAR*

Computational methods such as structure–activity relationships (SARs) and QSAR models may be used as screening tool to predict toxicological endpoints from the knowledge of the chemical structure of the proposed food additives. They cannot be used as stand-alone evidence to make conclusions in the risk assessment, unless they are specifically used to assess the potential genotoxicity of the identified components of those food additives consisting of mixtures (see Section 5.5.1.1) as well as the potential genotoxicity of impurities present in the proposed food additive (see Section 1.7).

Regarding the criteria for their applicability and existing limitations, applicant should refer to the following guidance documents:

- ECHA Practical guide on how to use and report (Q)SARs (ECHA, 2025),
- OECD Guidance for the regulatory assessment of (Quantitative) Structure Activity Relationship models and predictions (OECD, 2024),
- EFSA Scientific Committee 'Guidance on the use of read-across in food and feed risk assessment' (EFSA Scientific Committee, 2025c).

5.4.5.4 | *In vitro comparative metabolism data*

In vitro assessment of the comparative metabolism of the proposed food additive is useful for establishing, where applicable, whether the pattern of metabolites formed in human test systems is comparable to that of the animal species intended for in vivo testing. In this respect, the applicant should refer to the Appendix C of 'Scientific opinion of the Scientific Panel on Plant Protection Products and their Residues on testing and interpretation of comparative in vitro metabolism studies' (EFSA PPR Panel, 2021) to provide a qualitative comparison of metabolites and a quantitative analysis (disproportionate metabolites) between humans and species used in experimental studies. This will inform on the best species to be used in in vivo toxicological studies in higher tiers and on the appropriateness of the genotoxicity testing. The PPR scientific opinion considers human and rodent primary liver cells as preferred test systems and provides specific advice on how to interpret and follow-up any qualitative and quantitative interspecies metabolic differences with respect to the risk assessment.

5.4.5.5 | *In vitro metabolism by gut microbiota*

Gut microbiota and their associated enzymes can have an impact on biotransformation of the proposed food additive, which may lead to the formation of toxicologically active metabolites (Koppel et al., 2017). In vitro studies mimicking the human gut and its microbiota dynamics may be considered by the applicant to identify or quantify relevant metabolites, which could potentially be of safety concern and would deserve further considerations.

Approaches have been published using preparations composed of strains from the main bacterial species commonly described in human gut microbiota, permitting in vitro evaluation of the biotransformation of xenobiotics[46] before intestinal absorption (El Houari et al., 2022).

EFSA recognises the importance of considering the role of gut microbiota in the risk assessment of food additives, taking into account also the possible impact that the proposed food additive may have on host physiology by influencing the gut microbiota composition (Merten et al., 2020). However, a clear understanding of the significance of changes in structure and functionality of the microbiome and their effects on health is not yet elucidated; further research is ongoing and recommendations on how to integrate microbiomes more widely within food and feed safety risk assessment frameworks are being developed (Moreno et al., 2024; Radio et al., 2024). For these reasons, the Panel is not currently in a position to make specific recommendations on how to address this issue. Regardless, as noted in the EFSA SC Guidance on the characterisation of microorganisms in support of the risk assessment of products used in the food chain (EFSA Scientific Committee, 2025a), when an adverse effect can be anticipated based on the body of knowledge, relevant data may still be submitted by the applicant and they will be assessed on a case-by-case basis.

5.5 | Tier II (A)

5.5.1 | In vivo genotoxicity testing

In case of one or more confirmed positive results obtained in Tier I from an adequately performed set of in vitro assays (see Section 5.4.4), or in some cases also when in vitro test results remain equivocal after repeated testing, in vivo follow-up testing should be performed in Tier II (A) to assess whether the genotoxic potential observed in vitro is expressed in vivo.

The Scientific Committee recommends that in vivo tests should be selected based on the genotoxicity endpoint for which positive results were observed in the in vitro studies. In addition, the choice of the test should be based also on other relevant data on the test substance, such as information about chemical reactivity (which might predispose to site

[46]Chemicals which are not natural components of the organism exposed to them.

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of contact effects), bioavailability, metabolism, toxicokinetics, and any target organ toxicity. Additional useful information may come from (Q)SAR considerations and read-across from structurally related substances (see Section 5.4.5.3). The combination of assessing different endpoints in different tissues in the same animal should be considered as well as the possibility to integrate the genotoxicity assessment in toxicity assays using the same animals (EFSA Scientific Committee, 2011). The integration of in vivo genotoxicity assays into repeated-dose toxicity studies should be envisaged, whenever possible, in line with the 3Rs principle to reduce the number of animals for experimental testing. For the purposes of this guidance, the repeated-dose toxicity study in Tier II(A) (see Section 5.5.2) is particularly suitable for possible integration with in vivo genotoxicity studies.

The in vivo tests recommended by the EFSA Scientific Committee (2011, 2017b, 2021c) are:

- in vivo transgenic rodent somatic and germ cell gene mutation assay, OECD TG 488 (OECD, 2022b).
- in vivo mammalian erythrocyte micronucleus assay, OECD TG 474 (OECD, 2016c).
- in vivo mammalian alkaline comet assay, OECD TG 489 (OECD, 2016d).

Transgenic rodent assays can detect point mutations and deletions and are without tissue restrictions. In addition to the above in vivo genotoxicity testing, the Panel considers that an in vivo mammalian erythrocyte Pig-a gene mutation assay, OECD TG 470 (OECD, 2022a) can also be used to follow-up in vitro positive results for gene mutations. The Pig-a gene mutation assay detects point mutations (both base-pair substitutions and frameshift mutations) and small deletions in rodent erythrocytes.

The in vivo MN test covers the endpoints of structural and numerical CAs and is an appropriate follow-up for in vitro clastogens and aneugens. If there are any indications for aneugenicity, the EFSA guidance on aneugenicity (EFSA Scientific Committee, 2021c) should be followed.

The in vivo Comet assay detects primary DNA damage and is considered a useful indicator test in terms of its sensitivity to substances which cause gene mutations and/or structural CAs and can be used with many target tissues.

As a follow-up for in vitro gene mutation positives, both the transgenic rodent gene mutation assay and the comet assay are suitable as recommended by the EFSA Scientific Committee (EFSA Scientific Committee, 2011). As a follow-up to a positive in vitro MN assay, a combination of an in vivo MN and comet assay should be performed (EFSA Scientific Committee, 2011), unless an aneugenic mode of action is demonstrated.

Overall, the genotoxicity data of a proposed food additive consisting in a single substance will be evaluated by the Panel based on the recommendations given by the EFSA Scientific Committee in the relevant guidance documents on genotoxicity (EFSA Scientific Committee, 2011, 2017b, 2021c) and in line with the EFSA harmonised approach for reporting reliability and relevance of genotoxicity studies (EFSA, 2023).

When in vivo testing provides negative results, the relevance of these findings should be further evaluated based on the recommendations given by the EFSA Scientific Committee (EFSA Scientific Committee, 2017b), concerning the evidence of target tissue exposure.

If the results of in vivo genotoxicity tests are positive, no further testing is necessary, and the food additive should be considered as genotoxic in vivo.

In line with the recommendation from the EFSA Scientific Committee, a substance that is positive in tests in somatic tissues in vivo would normally be assumed to reach the germ cells and to be a germ cell mutagen, therefore routine testing in germ cells is not required.

5.5.1.1 | *Assessment of genotoxicity potential of food additives consisting of mixtures*

Food additives consisting of mixtures may either be chemically fully defined mixtures (simple mixtures) or complex mixtures containing a substantial fraction of unidentified components (see Section 3.2.2).

The recommended approach for the testing and the evaluation of genotoxic potential of this type of food additives is described by the EFSA's Scientific Committee statement on genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019a) as well as by the EFSA scientific guidance for the preparation of applications on smoke flavouring primary products (EFSA FAF Panel, 2021a). In line with these documents, a stepwise approach should be followed for the generation and assessment of the data, where first the components of the mixture should be identified and quantified as much as possible.

Concentrations of the identified components in the food additive mixture should be provided. The genotoxic potential of the identified components should then be assessed individually, using all available data. Genotoxicity data should be collected and evaluated based on the Scientific Committee guidance documents on genotoxicity (EFSA Scientific Committee, 2011, 2017b, 2021c), as described above in Section 5.4.1 for food additives consisting of a single substance. Conclusions on genotoxicity are required for all identified components or at least for representative substances in case of structurally related identified components that could be grouped based on justified criteria (ECHA, 2008, 2017a). SAR information about the genotoxic potential of an identified component may be considered when no adequate information on genotoxicity from published or unpublished studies is available (for more details refer to Section 3.2 of (EFSA FAF Panel, 2021a)).

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If the mixture contains one or more components that have been assessed to be genotoxic in vivo via a relevant route of administration, then the food additive raises a concern for genotoxicity and the risk to human health related to this identified hazard needs to be considered in the risk assessment.

If none of the identified chemical substances in the food additive mixture raises a concern for genotoxicity, the EFSA Scientific Committee recommends evaluating the genotoxic potential of the fraction of unidentified components. This applies only in case the food additive contains a substantial fraction of unidentified components and not in case all the components of the food additive mixture have been fully identified, i.e. chemically fully defined mixtures. Experimental testing of the fraction of unidentified components should be considered as a first option or, if this is not feasible and a scientific justification can be provided, the whole mixture should be tested following the testing strategy recommended by the EFSA Scientific Committee for individual chemical substances as described in Section 5.4.1 (EFSA Scientific Committee, 2019a).

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

Additional recommendations regarding the interpretation of the in vivo genotoxicity testing of mixtures containing a substantial fraction of unidentified components are given here below. Similar recommendations were agreed by the FAF Panel at the 24th plenary meeting[47]

– *Maximum dose to be used in* in vivo *genotoxicity studies of mixtures containing a substantial fraction of unidentified components*

For in vivo genotoxicity testing of a chemical mixture with a substantial fraction of unidentified components, the dose levels should be based on a dose-range finding study according to the relevant OECD test guideline. Both OECD TG 474 (OECD, 2016c) and OECD TG 489 (OECD, 2016d) report that 'If the test chemical does not produce toxicity in a range-finding study or based on existing data, the highest dose for an administration period of 14 days or more should be 1000 mg/kg body weight/day, or for administration periods of less than 14 days, 2000 mg/kg /body weight/day'. In addition, OECD TG 488 (OECD, 2022b) reports: 'For an administration period of 28 days (i.e. 28 daily treatments), the limit dose is 1000 mg/kg body weight/day. For administration periods of 14 days or less, the limit dose is 2000 mg/kg/body weight/day'.

In case of chemical mixtures, the Panel is of the view that if no toxicity is observed in an appropriately designed range-finding study, it would be required to test higher doses than the above-mentioned maximum limits, in order to increase the dose of each of the individual components. If this resulted in toxicity, the corresponding dose would be considered sufficiently high.

However, in the absence of any toxicity, the highest dose to be applied is limited by the maximum volume that should be administered to rodents. According to OECD TG 474, 488 and 489 (OECD, 2016c, 2016d, 2022b), the maximum volume of liquid that can be administered by gavage at one time should not normally exceed 1 mL/100 g of body weight except in the case of aqueous solutions where a maximum of 2 mL/100 g of body weight may be used.

– *Demonstration of target tissue exposure to mixtures containing a substantial fraction of unidentified components, in* in vivo *genotoxicity studies*

Concerning the demonstration of target tissue exposure, when negative results are obtained in in vivo follow-up studies, the EFSA Scientific Committee (2019a) stated that the relevance of the findings obtained will depend on the genetic effect assessed, the test protocol applied and expert judgement on the reliability of the results obtained (including considerations of target tissue exposure). Based on the considerations from the Scientific Committee, for the in vivo genotoxicity testing of chemical mixtures containing a fraction of unidentified components, the Panel considers that:

– plasma analysis is not appropriate to demonstrate target tissue exposure to a chemical mixture containing a substantial fraction of unidentified components considering that a substantial part of the mixture is unidentified and therefore the component(s) of the mixture, being responsible for genotoxic effects in vitro, cannot be unequivocally identified in the plasma.
– the range of doses to be applied in in vivo genotoxicity tests should reach the maximum tolerated dose (MTD) in line with the recommendations given in OECD test guidelines. If no toxicity is observed in an adequately designed range-finding study, it would be appropriate to test higher doses than the maximum limits mentioned in the OECD test guidelines, in order to increase the dose of each of the individual components of the mixture (see above).
– in case positive results are observed in an in vitro MN assay, the mechanism (aneugenicity and/or clastogenicity) should be first clarified (see Section 5.4.3).
– if there is an indication of clastogenicity based on in vitro data, a combination of an in vivo MN assay according to OECD TG 474 (2016b) and an in vivo comet assay according to OECD TG 489 (2016d) in the same animals, should be considered

[47] https://www.efsa.europa.eu/sites/default/files/2021-10/24th-plenary-meeting-faf-panel-proposed-be-open-observers-minutes.pdf.

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as a follow-up to a positive in vitro MN assay or CA assay. The comet assay is recommended because the assessment of genotoxic effects in the bone marrow MN assay may be limited by the fact that target tissue exposure cannot be demonstrated, as any toxic effect elicited in the bone marrow by the mixture cannot be unequivocally attributed to the (in vitro) genotoxic component. In this scenario, the conclusion drawn would have a higher uncertainty. Therefore, the Panel would recommend conducting an in vivo Comet assay at least in tissues that are sites of contact after ingestion, e.g. duodenum, and in the liver, where the components of the food additive or their metabolites are expected to be present at the highest concentration.

- for mixtures that are aneugenic but neither clastogenic nor causing gene mutations, the Scientific Committee guidance on aneugenicity (EFSA Scientific Committee, 2021c) should be consulted. In this case, the limitations of the bone marrow exposure in the MN assay conducted on mixtures should be taken into account.
- when performing an in vivo Comet assay (OECD TG 488 (OECD, 2022b) and/or an in vivo transgenic rodent gene mutation assay (OECD TG 489 (OECD, 2016d) in the liver to follow-up genotoxicity testing of mixtures that tested positive in vitro following metabolic activation, there is no need to demonstrate exposure in the liver (where the active metabolites are expected to be present at the highest concentration), provided that the treatment is performed at the MTD or at the limit dose as specified in the OECD TGs. In fact, if a substance or components of a mixture require metabolic activation, the potential genotoxicity in vivo would depend on the ability of the substance to reach the site of metabolism.

5.5.2 | In vivo study to assess potential toxicity in the gastrointestinal tract with toxicokinetic information

If the in vitro absorption data in Tier I provides no evidence of absorption, a repeated dose oral toxicity study of limited duration (for at least 2 weeks) and up to the limit dose has to be performed to evaluate any potential local toxicity in the gastrointestinal tract (i.e. from oesophagus to intestine), with the inclusion of macroscopic and histopathological assessment at study termination. Besides the investigation of local effects, the study should also include toxicokinetic information to confirm lack of absorption. This should be established after verifying the absence of the substance and/or its breakdown products and metabolites in blood and urine using the most sensitive available analytical methods.

5.6 | Tier II (B) – Toxicokinetics, repeated dose toxicity, reproductive and developmental toxicity

For those proposed food additives that do not raise a concern for genotoxicity and which are absorbed via the intestinal tract, Tier II (B) testing is to be conducted.

5.6.1 | In vivo dose range finding study including toxicokinetic aspects

Tier II (B) also includes a dose range finding (DRF) study to be conducted prior to the extended combined repeated dose toxicity study with reproduction and developmental toxicity screening test (OECD TG 422 (OECD, 2025a); see paragraph 5.6.2), including toxicokinetic aspects, if these were not already performed in Tier II (A) (see Section 5.5.2).

The toxicokinetic aspects of the DRF study should include blood sampling to characterise the systemic exposure to parent compound and metabolites (where applicable) and their relationship to dose levels. At least one blood sampling point before dosing and multiple points after dosing are recommended. Area under the curve (AUC) of plasma (or whole blood or serum) concentration, maximum concentration (Cmax), time to reach maximum concentration (Tmax) and elimination half-life (T1/2)) are the most used parameters in assessing systemic exposure and the potential for accumulation.

Sampling of urine at frequent time intervals may be an alternative to blood sampling in cases where urinary excretion is the only/main elimination route. When radiolabelled compounds are used the sum of radiolabelled substances in urine includes parent compound and its metabolites and can be used to calculate the extent of absorption.

5.6.2 | Extended combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422)

A combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422 (OECD, 2025a)) should be conducted in Tier II (B) with the following modifications:

- In order to harmonise the exposure duration between the two sexes, necropsy should be conducted on female as well as on male parental (P) animals around the time of the weaning of the pups, i.e. at PND 13 (noted as optional for males in OECD TG 422).
- Assessment of haematology, clinical chemistry and organ weight should be conducted on all parental animals per dose group (i.e. at least 10 animals per sex per dose group).

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- Gross necropsy should be assessed in parental and in F1 animals, i.e. at least 10 animals per sex per dose group, randomised by litter (at least one animal per sex per litter should be included).
- Histopathological analysis on the full list of organs should be performed in accordance with OECD TG 408 (OECD, 2025c) in all control and high dose parental animals in at least 10 animals per sex to increase the sensitivity of the assays. If treatment-related changes are observed in the high dose group, histopathological assessment should be extended to the lower dose groups (i.e. in at least 10 animals per sex per dose group).
- Assessment of parental oestrus cyclicity should be conducted for 2 weeks pre-exposure and for 2 additional weeks during the pre-mating period.

This extended in-vivo study, while minimising the number of tested animals in line with the 3Rs principle, can provide information on target organ toxicity in adult male and female animals, as well as on reproductive and developmental toxicity and endocrine disruption effects.

Based on the available evidence, the Panel considers that an extended OECD TG 422 is comparable to a 90-day study in its ability to characterise target organ toxicity and to identify a reference point (Monticello et al., 2024, Escher et al., 2020, Lampe et al., 2018, Taylor & Andrew, 2017, Roberts et al., 2015, Beekhuijzen et al., 2014, Taylor et al., 2014).

For more details regarding the endpoints to be investigated in the extended OECD TG 422, please refer to Appendix B, in which the changes as compared to the standard OECD TG 422 are highlighted in blue.

The results from this study can be used for hazard assessment, allowing identification of a reference point, either the BMDL or a NOAEL (in cases where the data do not allow performing a BMD analysis), which may be used for definitive regulatory decisions (e.g. setting of an ADI) or otherwise contribute to decisions with respect to additional testing needed in higher Tiers.

Dose selection for this study may be more complicated than in repeated dose toxicity studies, such as OECD TG 408 (OECD, 2025c), because both systemic organ toxicity, and reproductive/developmental toxicity are evaluated. A rationale for selecting the top dose should be provided, in accordance with OECD TG 422, e.g. the highest dose level should induce toxic effects but not death nor obvious suffering; in the absence of effects, a maximum dose of 1000 mg/kg per day should be used.

5.7 | Tier III – Sub-chronic toxicity and developmental and reproductive toxicity

Triggers leading to Tier III toxicity testing

Normally a reference point (NOAEL/LOAEL or BMDL) can be established from the proposed extended OECD TG 422, and no additional studies are necessary. However, there may be cases where additional in vivo toxicity studies are necessary, e.g.

- Increased post-implantation loss, reduced litter size and/or reduced pup birth weight can be indicative of prenatal toxicity, and may require Tier III testing for embryofetal toxic effects (OECD TG 414 (OECD, 2018b)),
- Target organ toxicity observed in the extended OECD TG 422 (OECD, 2025a) with evidence of a dose relationship in severity and/or incidence from which it is not possible to identify a reference point (BMDL or NOAEL), may require Tier III toxicity testing, i.e. OECD TG 408 (OECD TG, 2025c), for the identification of a reference point.
- Indications of neurotoxicity in the extended OECD TG 422 (OECD, 2025a) may require Tier III testing, i.e. OECD TG 443 (OECD, 2025b), with special attention to developmental neurotoxicity and inclusion of cohorts 2A and 2B.
- Indications of immune suppression or immune stimulation in the extended OECD TG 422 may require Tier III toxicity testing for immune toxicity with case-by-case inclusion of immunological endpoints, for example according to OECD TG 443 (OECD, 2025b) (inclusion of the developmental immunotoxicity cohort) or in OECD TG 408 (OECD, 2025c) (e.g. inclusion of additional endpoints such as immunoglobulin phenotype, C-reactive protein, lymphocyte phenotyping).
- Any indication of effects on endocrine endpoints in the extended OECD TG 422 (OECD, 2025a) may require Tier III toxicity testing, in particular an assessment of endocrine mediated endpoints according to OECD TG 443 (OECD, 2025b) with the inclusion of the second (F2) generation.

Tier III toxicity testing

Toxicity testing in Tier III may include:

- an oral 90-day toxicity study in accordance with OECD TG 408 (OECD, 2025c). The main objective of this study is to identify any adverse effects following repeated and prolonged exposure to the proposed food additive via the oral route.
- A prenatal developmental toxicity study (OECD TG 414 (OECD, 2018b)). This study is designed to provide general information concerning the effects of prenatal exposure on the pregnant test animal and on the developing organism, including assessment of maternal effects as well as death, structural abnormalities, or altered growth in the fetus.
- An extended one-generation reproduction toxicity study (EOGRTS) (OECD TG 443 (OECD, 2025b)). This study provides information on fertility and reproductive function, and short- to long-term developmental effects from exposure during

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pregnancy, lactation and prepubertal phases as well as effects on juveniles and adult offspring, covering the whole reproductive cycle (from gametogenesis through to maturation of the following generation), as well as an assessment of additional more specific endpoints (i.e. developmental neurotoxicity, immunotoxicity and endocrine disruption).

The EOGRTS in Tier III should always comprise cohorts 1A and 1B (without the extension to include a second generation (F2)). Cohorts 2A/2B (developmental neurotoxicity) and/or cohort 3 (developmental immunotoxicity) are required only if the proposed food additive and/or its metabolites are suspected to be neurotoxic, immunotoxic or reprotoxic and/or if the proposed food additive is intended to be used in infants below 16 weeks of age (EFSA Scientific Committee, 2017a, 2019b). In the latter case, direct dosing of the neonatal animals as soon as possible after birth should be also considered (see Section 5.9).

5.8 | Tier IV – Chronic toxicity, carcinogenicity and specialised studies

Triggers leading to Tier IV toxicity testing – chronic toxicity and/or carcinogenicity

- Histological changes that could be indicative of carcinogenic concern observed in a repeated dose study (e.g. the extended OECD TG 422 (OECD TG, 2025a) in Tier II (B) or the sub-chronic 90-day oral toxicity study (OECD TG 408 (OECD TG, 2025c) in Tier III) can trigger Tier IV toxicity testing for carcinogenicity. Such changes include pro-inflammatory alterations, necrosis, signs of regenerative hyperplasia, hyperplasia or preneoplastic changes in one or more tissues and/or organs. A possible carcinogenicity concern should also be considered in case of immune effects or perturbations in hormonal axes and/or endocrine/reproductive organs with potential relevance for humans. However, immunotoxicity and endocrine disruption endpoints are already assessed in Tier III, therefore, triggering or waiving Tier IV studies solely based on these endpoints should be considered on a case-by-case. It should be noted that an integrated analysis of the weight of evidence of factors retrieved from the available data set can also be used to determine whether a 2-year carcinogenicity study can be waived. In the case of, e.g. histopathology findings from the repeat dose toxicity study (extended OECD TG 422 (OECD, 2025a) or OECD TG 408 (OECD, 2025c)) indicative of hyperplastic or other lesions of concern as mentioned above, it can be anticipated that tumour incidence will be increased in a 2-year study and that the substance is a non-genotoxic carcinogen (see also ICH S1B (R1) (ICH, 2022). Therefore, the reference point can be derived from the repeat dose toxicity study (i.e. extended OECD TG 422 (OECD, 2025a) or OECD TG 408 (OECD, 2025c)). In this case, the application of an additional extrapolation factor for dosing duration has to be considered for deriving an HBGV.
- In case no adverse effects are observed in the repeat dose oral toxicity study, careful consideration should be given in the interpretation of the results to the toxicokinetic data available for the proposed food additive. The elimination half-life assessed in Tier II, if greater than about 2 weeks, may be of particular relevance to establish whether the duration of the study is adequate or whether it may be necessary to perform additional toxicity testing, with longer duration, that would allow investigation of potential occurrence of later effects due to substance accumulation.

Tier IV – Chronic toxicity and/or carcinogenicity

The Panel recommends to use the best protocol to investigate the carcinogenicity potential, i.e. carcinogenicity study (according to OECD TG 451) (OECD, 2018c), or other adverse effects expected due to chronic exposure, i.e. chronic toxicity study (according to OECD TG 452) (OECD, 2018d); or to investigate both, i.e. a combined protocol to study chronic toxicity and carcinogenicity in the same experiment (according to OECD TG 453) (OECD, 2018e).

The combined test provides greater efficiency in terms of time and cost compared to conducting two separate studies, without compromising the quality of the data in either the chronic phase or the carcinogenicity process. Careful consideration should however be given to the principles of dose selection when undertaking a combined chronic toxicity and carcinogenicity study (OECD TG 453). In carrying out such a combined study, sufficient satellite animals will normally be included in the design of the study to enable the chronic toxicity aspects of the study to be assessed, without compromising the carcinogenicity part of the study. The OECD Guidance Document (No. 116) on the design and conduct of chronic toxicity and carcinogenicity studies, supporting OECD TGs 451, 452 and 453, provides useful additional information on dose selection and the conduct of such studies (OECD GD, 2014, currently under update).

Tier IV – Specialised case-by-case studies

Tier IV testing may be needed to further characterise adverse effects observed in lower tiers.

Such testing may include specialised studies investigating the modes of action (MoA) that lead to adverse effects. The purpose of investigations into MoA is to determine the relevance for humans of adverse effects observed in the test animal species.

Any useful information from human studies should be submitted, if available. Also, studies performed in a different context (e.g. drug development), such as controlled clinical studies, pharmaco-epidemiological studies or pharmacovigilance data, may provide relevant information on the safety of the proposed food additive; such studies and their results should be submitted.

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5.9 | Studies to evaluate the safety of the proposed food additive in food for infants below 16 weeks of age

In case the proposed food additive is intended for use in food for infants below 16 weeks of age, additional toxicity data compared to the toxicity testing described above for the general population may be needed, as recommended by the EFSA Scientific Committee Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age (EFSA Scientific Committee, 2017a).

Following this Guidance, specific studies in neonatal animals (e.g. piglets) are required if the proposed food additive is not absorbed and is not systemically available. Examples where studies on neonatal piglets were considered pivotal are available in previous EFSA assessment (see, e.g. EFSA FAF Panel, 2021b; EFSA FAF Panel, 2023e, 2023f). If the additive is systemically available, an EOGRTS should be performed. However, the EOGRTS should be conducted according to the recommendations of the 2017 EFSA Scientific Committee Guidance (EFSA Scientific Committee, 2017a), especially with respect to the number of cohorts of F1-animals used and the direct dosing of neonatal animals as soon as possible after birth. In this case, the applicant should complement or adapt the testing strategy developed for the general population to cover also the safety of the use in foods for infants below 16 weeks of age, in line with (EFSA Scientific Committee, 2017a).

For further information, the applicant may wish to consult EFSA opinions addressing the safety of food additives used in food for infants below 16 weeks of age. These opinions could serve as examples on how this part of the assessment could be addressed in line with the recommendations of the EFSA Scientific Committee guidance (EFSA Scientific Committee, 2017a).

Human data, including epidemiological data such as data from clinical trials, could support the assessment and if available they should be submitted (see also Section 5.3).

5.10 | Allergenicity

If the proposed food additive is a potential allergen (e.g. a protein) or contains residues of proteins or other known potential allergenic molecules, the data requirements described in section 10 of the EFSA Guidance on Novel Food (EFSA NDA Panel, 2024) should be followed.

6 | SAFETY FOR THE ENVIRONMENT

Regulation (EC) No 1333/2008 on food additives lays down rules on food additives used in foods with a view to ensure a high level of protection of human health and a consumer protection taking into account, where appropriate, the protection of the environment.

The Panel noted that a guidance on the environmental risk assessment for feed additives was developed by the FEEDAP Panel in 2019 (EFSA FEEDAP Panel, 2019). While there may be substances used both as feed and food additives, the environmental emission routes for food additives differs from that for feed additives.[48] In addition, in the case of feed additives, environmental risk assessment is required according to Regulation (EC) No 1831/2003[49] and its implementing rules (Regulation (EC) No 429/2008[50]). Given that the EFSA FEEDAP Panel guidance (2019) is not fully applicable to the case of food additives, the Panel developed this section to provide guidance to the applicant on the safety for the environment for a proposed food additive.

The emission into the environment of the parent food additive and/or its possible metabolites and/or breakdown products (named hereafter in this section 'related products') can occur following human consumption of food containing food additive and following industrial emission. This section focuses on human consumption as the main route of emission because industrial emission is covered by other regulatory frameworks and thus is not in the remit of this guidance (see 'Terms of Reference as provided by EFSA'). It is acknowledged that another source of environmental contamination would be via food waste (Eurostat, 2024), however, this potential emission route is not considered further here in the absence of exact data and methodologies.

Following human ingestion, excreted food additive and/or its related products reach the sewage treatment plant (STP) where they are possibly subject to (bio)degradation. Non-(bio)degraded food additives and/or their related products can enter surface water (or marine water in some cases) and in sediment (marine or freshwater) via STP effluents. Moreover, STP sludges may be spread in agricultural soil and emission to groundwater (e.g. via leaching from soil) might occur. Thus, the main environmental compartments into which food additives and/or their related products can be expected to enter are surface water, sediment (marine or freshwater), soil and groundwater.

[48]Regulation (EC) No 1831/2003 and its implementing rules (Regulation (EC) No 429/2008) describe that an environmental risk assessment (ERA) should be conducted in the case of feed additives for (1) terrestrial compartment (via spreading of animal manure contaminated with feed additives on agricultural soils), (2) the aquatic compartment (via drainage and run-off from agricultural fields to surface water, via direct discharge of waste water from land-based fish farms to surface water, or via excreta from fish farmed in sea cages to marine sediment), and (3) the groundwater compartment (via leaching from soil).

[49]Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268 18.10.2003, p. 29.

[50]Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133 22.5.2008, p. 1.

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The physicochemical properties of the proposed food additive, and/or its possible related products, the level of metabolism in the human body and the extent of (bio)degradation in the STP determine the amount and type of substances that will finally enter the environment. In this respect, the information on the identity (Section 3.2) and the ADME of the proposed food additive requested in line with the present guidance (Section 5) could inform on the fraction of parent substance (non-metabolised food additive) and of the relevant related products that could potentially reach the environmental compartment(s). Taking these aspects into account, the FAF Panel considers that the need to perform a full environmental risk assessment for a proposed food additive and its possible metabolites and/or related products should be considered on a case-by-case basis.

Considering the route of emission to the environment, if a food additive or its components are not xenobiotics[51] and are reported to occur in food and beverages, normally the need to perform an environmental risk assessment is not anticipated. This is because it is not expected that the use of the proposed food additive would significantly alter the concentration of the corresponding natural substance(s) already present in the environment. Therefore, in such cases an assessment of the safety for the environment is in principle not needed. Nevertheless, in line with the 'General principles' and Section 5.1, the applicant should always conduct a comprehensive literature search to retrieve any relevant information on the environmental safety of the proposed food additive and its possible metabolites/breakdown products. Depending on the outcome of the literature search, further assessment may be needed.

In case the proposed food additive contains microorganisms or it is prepared/obtained from/with microorganisms, additional data may be needed in line with the requirements described in Section 5 of the EFSA SC Guidance on the characterisation of microorganisms in support of the risk assessment of products used in the food chain (EFSA Scientific Committee, 2025a).

In the case of food additives that are xenobiotics, further assessment is needed if one or more of the following conditions are met:

(i) The proposed food additive is not extensively metabolised to innocuous products[52] in human. Relevant information may be available, see Section 5. If no information is available, it may be necessary to estimate the metabolism and formation of metabolites and/or breakdown products.
(ii) There is evidence that the proposed food additive and/or its related products are persistent in the environment and expected not to be fully degraded in a STP. Relevant information may be available in the scientific literature or from information on physicochemical characteristics (see Section 3.2) or from an, e.g. OECD TG 301 study in the case of (bio) degradability (OECD, 1992).
(iii) There is evidence that the proposed food additive and/or its related products are expected to bioconcentrate (e.g. log $K_{ow} \geq 3$). Relevant information may be available in the scientific literature or from information on physicochemical characteristics (see Section 3.2).
(iv) Evidence is retrieved from the scientific literature that indicates a potential environmental concern for the proposed food additive and/or its related products (see 'General principles' and Section 5.1).

The generation of data using non-testing approaches, such as (Q)SAR, should be considered, provided that they are relevant, reliable and adequate for the purpose and are documented in an appropriate manner (ECHA, 2008; appendix D of EFSA FEEDAP Panel, 2019).

If an environmental risk assessment is required for the proposed food additive and/or its related products, it should be based on the same principles as mentioned in other existing guidance documents on environmental risk assessment for substances with similar release patterns and/or exposure routes such as medicinal products for human use (EMA, 2024). In addition, the environmental risk assessment guidance documents developed for biocides (ECHA, 2016) or industrial chemicals (ECHA, 2017b) could be considered, where appropriate. Such principles and data requirements may need to be reconsidered if, in the future, an EFSA cross-cutting guidance document on environmental risk assessment becomes available. In line with the 2024 EMA guideline, tailored studies may be needed for a substance with a specific toxicity profile (e.g. endocrine active substances).

If the proposed food additive and/or its related products are identified as persistent, bioaccumulative and toxic substances (PBT substances), and/or very persistent and very bioaccumulative substances (vPvB substances), as per Annex XIII of the REACH Regulation (EC) No 1907/2006[53] and Regulation 2024/2865,[54] they would raise a concern for the environment, because no safe concentration in the environment can be established with sufficient reliability for an acceptable risk to be determined quantitatively.

[51]Chemicals which are not natural components of the organism exposed to them. Physiological substances whose use results in much higher concentrations than usual in the environment may be treated as xenobiotics (adapted from Regulation (EC) No 429/2008 (https://eur-lex.europa.eu/eli/reg/2008/429/oj/eng) and EFSA FEEDAP Panel, 2017)).

[52]Converted into metabolites that do not possess a biological activity of environmental concern, like water, CO2 and common salts.

[53]Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC, OJ L 396, 30.12.2006, pp. 1–849.

[54]Regulation (EU) 2024/2865 of the European Parliament and of the Council of 23 October 2024 amending Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures, OJ L, 2024/2865, 20.11.2024.

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For complex mixtures containing fractions of unidentified constituents, the approach described above for chemically defined substances may not be fully applicable, as complete information on the complex mixtures might not be available. Therefore, the hazard and exposure assessment would need to be based on the constituents or fractions of similar constituents exhibiting similar properties (see EFSA Scientific Committee, 2019c). For those constituents that can be identified chemically, the applicant should apply the same considerations and approach as described above. For the fractions that cannot be fully identified chemically, it is expected that a general characterisation of the main constituents is available, and that the percentage of unidentified constituents is indicated and is as low as possible (see Section 3.2.2). In this respect, the applicant may need to assess whether the unidentified constituents share similar properties with the constituents in the characterised fraction. Further guidance can be found in the OECD guidance document addressing 'aquatic toxicity testing of difficult substances and mixtures' (OECD, 2019).

GLOSSARY

Acceptable daily intake (ADI)	An estimate of the amount of a substance in food or drinking water that can be consumed daily over a lifetime without presenting an appreciable risk to health. It is usually expressed as milligrams of the substance per kilogram of body weight per day and applies to chemical substances such as food additives, pesticide residues and veterinary drugs (EFSA Scientific Committee, 2021d). The ADI is expressed in units of mg of the substance per kilogram of body weight (standard human 70 kg) per day (mg/kg body weight/day).
A **group ADI**	is an ADI established for a group of compounds with (or presumed to have) a common mode of action.
A **temporary ADI** (tADI)	is used when data are sufficient to conclude that use of the substance is safe over the relatively short period of time required to generate and evaluate further safety data but are insufficient to conclude that the use of the substance is safe over a lifetime.
ADI 'not specified'	is a term applicable to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological, and other), the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food does not represent a hazard to health and is generally not relevant since meaningful exposure estimates are often not possible.
Adverse effect	Change in the morphology, physiology, growth, development, reproduction or lifespan of an organism, system or (sub)population that results in an impairment of functional capacity to compensate for additional stress or an increase in susceptibility to other influences (WHO/IPCS, 2009).
Benchmark dose (BMD)	The benchmark dose is a dose level, estimated from the fitted dose–response curve, associated with a specified change in response relative to the control group (background response), the Benchmark Response (BMR) (EFSA Scientific Committee, 2022).
Chemical-specific adjustment factor (CSAF)	A modified default 10-fold uncertainty factor that incorporates appropriate data on species differences or human variability in either toxicokinetics or toxicodynamics.
Health-base guidance value (HBGV)	Umbrella term for values that are established as the result of the risk assessment of chemical substances and provides guidance on the safe consumption of substances, taking into account current safety data, uncertainties in these data, and the likely duration of consumption. Depending on their nature and applications, an HBGV for oral exposure may be termed acceptable daily intake (ADI) (food additives, pesticides), tolerable upper intake level (UL) (nutrients), tolerable daily intake (TDI) (contaminants) or acute reference dose (ARfD) (EFSA Scientific Committee, 2021d).

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Margin of exposure (MOE)	The MOE is the ratio of the Reference Point, e.g. no-observed-adverse-effect level (NOAEL) or benchmark dose lower confidence limit (BMDL) for the critical effect, to the theoretical, predicted, or estimated exposure dose or concentration to a substance for a given population (EFSA Scientific Committee, 2025b).
No-observed-adverse-effect-level (NOAEL)	Greatest concentration or amount of a substance, found by experiment or observation, that causes no adverse alteration of morphology, functional capacity, growth, development or lifespan of the target organism distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure.
Reference point (RP):	The dose of a substance at which a low but measurable adverse effect is observed in a toxicological study.
Threshold of Toxicological Concern (TTC)	The TTC approach is a screening and prioritisation tool for the risk assessment of chemicals when hazard data are incomplete and human exposure can be estimated (EFSA Scientific Committee, 2019b). For substances with exposures below their corresponding TTC values, the probability that they would cause adverse health effects is low. If the estimated exposure to a substance is higher than the relevant TTC value, a non- TTC approach is required to reach a conclusion on potential adverse health effects.
Uncertainty	General term referring to all types of limitations in available knowledge that affect the range and probability of possible answers to an assessment question. Available knowledge refers here to the knowledge (evidence, data, etc.) available to assessors at the time the assessment is conducted and within the time and resources agreed for the assessment (EFSA, 2019).

ABBREVIATIONS

ADI	acceptable daily intake
ADME	absorption distribution metabolism excretion
ANS Panel	Panel on Food Additives and Nutrient Sources added to Food
AUC	area under the curve
BMD	benchmark dose
BMDL	lower confidence bound of the benchmark dose
BW	body weight
CA	chromosomal aberration
CAS	Chemical Abstracts Service
CEP Panel	Panel on Food Contact Materials, Enzymes and Processing Aids
CSAFs	chemical-specific adjustment factors
DRF	dose range finding
ECH	Environmental Health Criteria
EINECS	European Inventory of Existing Commercial Chemical Substance
ENM	engineered nanomaterials
FA	food additive
FAF Panel	Panel on Food Additives and Flavourings
FAIM	Food Additives Intake Model
FC	food category
FISH	fluorescence in situ hybridisation
GALT	gut-associated lymphoid tissue
GIVIMP	Good In Vitro Method Practice
GLP	good laboratory practices
GMP	good manufacturing practice
HACCP	hazard analysis and critical control points
HBGV	health-based guidance value
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
InChI	International Chemical Identifier
IPCS	International Programme on Chemical Safety
IR	infrared
JECFA	Joint FAO/WHO Expert Committee on Food Additives

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JRC	Joint Research Centre
K_{ow}	octanol–water partition coefficient
LOAEL	lowest observed adverse effect level
LOD	limit of detection
LOQ	limit of quantification
MDCK	Madin–Darby canine kidney
MN	micronucleus
MoA	mode of action
MOE	margin of exposure
MPL	maximum permitted level
MS	mass spectrometry
MTD	maximum tolerated dose
NAM	new approach methodologies
NMR	nuclear magnetic resonance
NOAEL	no observed adverse effect level
OECD	Organisation for Economic and Co-operation and Development
P95	95th percentile
Papp	apparent permeability coefficient
PAHs	polycyclic aromatic hydrocarbons
PCBs	polychlorinated biphenyls
QS	*quantum satis*
QSAR	quantitative structure–activity relationships
RP	reference point
SAR	structure–activity relationships
SMILES	Simplified Molecular Input Line Entry System
STP	sewage treatment plant
tADI	temporary ADI
TG	test guidelines
TTC	threshold of toxicological concern
UV–Vis	ultraviolet–visible
WHO	World Health Organization

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REQUESTOR

Self-task mandate

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

Format for the proposed specifications of food additives

Specification parameters, if applicable, should be provided according to the table below, considering the identity of the proposed food additive and the manufacturing process. This table is a template to be considered and it should be adapted to the proposed food additive. Further parameters may also be relevant for the proposed food additive and should be proposed by the applicant.

E number	
Synonyms	
Definition	
CAS No	
EINECS	XX-XX-X
Chemical name	
Chemical formula	
Molecular/ Atomic weight (in case of elements)	
Particle size	
Assay[55]	Not less than XX %
Description	
Appearance of the aqueous solution	
Identification	
Specific identification tests and parameters	
pH	XX-XX (XX% aqueous solution)
Boiling/Melting range or point	XX to XX°C
Solubility	
Purity	
Major classes of components present (e.g. carbohydrates, proteins, lipids) and characteristic components of the proposed food additive (e.g. polyphenols) and other identified components of potential concern	
Potential impurities, taking into account the manufacturing process (e.g. solvent residues, toxic elements)	
Loss on drying	Not more than XX %
Sulfated ash	Not more than XX %
Mercury	Not more than XX mg/kg
Cadmium	Not more than XX mg/kg
Arsenic	Not more than XX mg/kg
Lead	Not more than XX mg/kg
Microbiological criteria	
Total plate count	Not more than XX colonies per gram
Yeasts and moulds	Not more than XX colonies per gram
Salmonella spp.	Absent in XX g
Escherichia Coli	Absent in XX g
Other relevant microbiological criteria	

[55]The following definition of assay in line with JECFA should be considered: A quantitative assay requirement is provided here, where applicable, to indicate the minimum acceptable content, or maximum acceptable content range, of the principal functional component(s) of the additive.

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APPENDIX B

List of the observations and parameters to be investigated in the extended OECD TG 422 as compared to standard OECD TGs

	EXTENDED OECD TG 422 Extended combined repeated dose toxicity study with the reproduction/ developmental toxicity screening test	OECD TG 422 (OECD, 2025a) Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test	OECD TG 443 (OECD, 2025b) Extended one generation reproductive toxicity study	OECD TG 408 (OECD TG, 2025c) Repeated dose 90-day oral toxicity study
Life stages evaluated in the study	**Adult (P) and F1 (male (P) and female (P) treated until time of weaning of the pups (PND13), approximately 63 days)**	**Adult (P) and F1 (male (P) treated for a minimum period of 4 weeks; female (P) treated until time of weaning of the pups (PND13), approximately 63 days)**	**Adult (P), F1 and F2 (optional)**	**Adult (P)**
Window of developmental exposure	**Prenatal and postnatal from conception to termination (PND13)**	**Prenatal and postnatal from conception to termination (PND13)**	**Prenatal and postnatal from conception to termination (at sexual maturation, e.g. 13 weeks)**	**None**
Species and number of animals at start	**Rodents (10 male/dose group and 13–14 female dose group)**	**Rodents (10 male/dose group and 13–14 female /dose group)**	**Rodents (24–30 per sex per dose group)**	**Rodents (10 male and 10 female/ dose group)**
Observations and parameters				
General clinical observations	Y (P, F1)	Y (P, F1)	Y (P, F1)	Y
Detailed clinical observations	Y (P, F1)	Y (P, F1)	Y (P, F1)	Y
Functional observation battery	Y (F1)	Y (F1)	Y (F1)	Y
Oestrus cycle	Y (P) measured during pre-exposure for 2 weeks and during pre-mating for 2 weeks	Y (P) measured pre-exposure for 2 weeks and only optionally during pre-mating for additional 2 weeks	Y (P and F1 if F2 is included) measured during pre-mating for 2 weeks	N (stage of oestrus cycle determined only at necropsy)
Litter size	Y	Y	Y	N
Anogenital distance	Y (F1)	Y (F1)	Y (F1)	N
Nipples/aereolae in male pups	Y (F1)	Y (F1)	Y (F1)	N
Sperm analysis	N	N	Y (P, F1)	Y (optional)
Body weight and food/water consumption	Y (P, F1 if applicable)	Y (P)	Y (P, F1)	Y
Gross necropsy	Y (P, F1)	Y (P)	Y (P, F1)	Y
Organ weight	Y (full list of organ weight in at least 10 (P) animals, in line with OECD TG 408)	Y (in at least 5 (P) animals)	Y (P, F1)	Y

(Continues)

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(Continued)

	EXTENDED OECD TG 422 Extended combined repeated dose toxicity study with the reproduction/ developmental toxicity screening test	OECD TG 422 (OECD, 2025a) Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test	OECD TG 443 (OECD, 2025b) Extended one generation reproductive toxicity study	OECD TG 408 (OECD TG, 2025c) Repeated dose 90-day oral toxicity study
Histopathology	Y (full list of organs in at least 10 (P) animals per sex in control and high dose groups. Examinations should be extended to animals of all other dosage groups, for those tissues where treatment-related changes are observed in the high dose group. Tissues/organs list to be examined in line with OECD TG 408 and with special emphasis on stages of spermatogenesis in the male gonads and histopathology of interstitial testicular cell structure) Histopathology of thyroid glands in F1 animals is optional.	Y (in at least 5 (P) animals sex/in control and high dose groups. Examinations should be extended to animals of all other dosage groups, for those tissues where treatment-related changes are observed in the high dose group. Tissues/organs list to be examined with special emphasis on stages of spermatogenesis in the male gonads and histopathology of interstitial testicular cell structure) Histopathology of thyroid glands in F1 animals is optional.	Y (P, F1; only selected organs unless triggered)	Y (full list of organs in control and high dose groups. These examinations should be extended to animals of all other dosage groups, for those tissues where treatment related changes are observed in the high dose group)
Haematology and clinical chemistry	Y (in at least 10 (P) animals per sex/dose group)	Y (in at least 5 (P) animals per sex/dose group)	Y (P, F1)	Y
T3 and/or T4 level	Y (in at least 10 (P and F1) animals per sex/dose group)	Y (in at least 5 (P and F1) animals per sex/ dose group)	Y (P, F1)	Y
Thyroid-stimulating hormone (TSH) level	Y (in at least 10 (P and F1) animals per sex/dose group)	Y (in at least 5 (P and F1) animals per sex/ dose group)	Y (P, F1)	Y
Urine analysis	Y (in at least 10 (P) animals per sex/dose group)	Y (optional, in at least 5 (P) animals per sex/dose group)	Y (P)	Y (optional)

Differences between the extended OECD TG 422 (second column) and the standard OECD TG 422 (third column) are indicated in blue.

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ANNEX A

Public consultation on draft guidance on the preparation of an application for authorisation of a food additive submitted under Regulation (EC) No 1331/2008

The outcome of the public consultation which was open from 18 December 2024 until 21 February 2025 is presented in Annex A. Annex A is available under the Supporting Information on the online version of the scientific output.

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