

Guidance for submission for food additive evaluations¹

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)

European Food Safety Authority (EFSA), Parma, Italy

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The present guidance has been revised and it is republished with editorial changes: the sections containing "Administrative requirements" and the "Procedure" in Appendix B – were deleted as presented in the "Administrative guidance on the preparation of applications on food improvement agents (food enzymes, food additives and food flavourings)" (EFSA, 2021) following the new provisions defined by Regulation (EC) 178/2002, as amended by Regulation (EU) 2019/1381 of the European Parliament and of the Council of 20 June 2019 on the transparency and sustainability of the EU risk assessment in the food chain, applicable as from 27 March 2021. The scientific content has been left unchanged. For application submitted until 26 March 2021, the former version of this guidance applies.

Abstract

This guidance document refers to the applications for authorisation of a new food additive or to a modification of an already authorised food additive, combining in a single document the description of the data requirements and their context, and also a description of the risk assessment paradigm applied. The document is arranged in four main sections: chemistry and specifications, existing authorisations and evaluations, proposed uses and exposure assessment, and toxicological studies. Assessment of the exposure to food additives is based on information on known or anticipated human exposure to the proposed additive or toxicologically relevant components of the additive from food, and any other potential dietary sources. For the toxicological studies, this guidance describes a tiered approach which balances data requirements against the risk, taking into consideration animal welfare by adopting animal testing strategies in line with the 3-Rs (replacement, refinement, reduction). This tiered approach for toxicological studies consists of 3 tiers, for which the testing requirements, key issues and triggers are described. According to this tiered approach, a minimal dataset applicable to all compounds has been developed under Tier 1, while Tier 2 testing, generating more extensive data, will be required for compounds which are absorbed and/or demonstrate (geno)toxicity in Tier 1 tests. Tier 3 should be performed on a case-by-case basis taking into consideration all the available data, to elucidate specific endpoints needing further investigation of findings in Tier 2 tests. This guidance document has been

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Summary

The Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked by the European Food Safety Authority (EFSA) to develop a guidance on the scientific data required to be submitted for food additive evaluations, in order to reflect the current thinking in risk assessment.

The present document provides guidance on data requirements for applications supporting the authorisation of a new food additive or modifications to an already authorised food additive. The document is arranged in four main sections: the **Chemistry and specifications** section seeks to identify the food additive, potential hazards (e.g. impurities, residuals) from its manufacture, and, through the specifications, to define the material tested; the **Existing authorisations and evaluation** section seeks to give an overview of previous risk assessments on the additive and their conclusions; the **Proposed uses and exposure assessment** section seeks to estimate dietary exposure based on the proposed uses and use levels and the consumption of the proposed foods for various age groups in the population of EU Member States; the **Toxicological studies** section seeks to describe the methods which can be used to identify (in conjunction with data on manufacture and composition) and characterise hazards. The document also describes the risk assessment paradigm (including hazard identification, hazard characterisation, exposure assessment and risk characterisation) utilised by the Panel in undertaking risk assessments. Consequently, it identifies relevant data and information that should be made available to permit an adequate risk assessment. The Panel stresses that applicants should base their dossier on sound science and state-of-the art principles of risk assessment.

Assessment of the exposure to food additives is performed taking into account dietary sources, based on information on known or anticipated human exposure to the proposed additive from food or toxicologically relevant components of the additive, and any other potential dietary sources (e.g. natural occurrence in food, non-additive use in food supplements, use as a nutrient, use as flavouring, use as food contact material, use in pharmaceuticals or cosmetic products). For the purpose of carrying out an exposure estimation in accordance with this guidance document, it is recommended that data for a new food additive or for a modification of the proposed uses or use levels of an already authorised food additive is provided in an exposure assessment tool made available by EFSA.

For the toxicological studies, this guidance describes a tiered approach which balances data requirements against the risk. The tiered approach initially uses less complex tests to obtain hazard data; these are then evaluated to determine if they are sufficient for risk assessment or, if not, to design studies at higher tiers. The tiered approach for toxicological studies consists of 3 tiers, for which the testing requirements, key issues and triggers are described. According to this tiered approach, a minimal dataset applicable to all compounds has been developed under Tier 1, while Tier 2 testing will be required for compounds which are absorbed, demonstrate toxicity or genotoxicity in Tier 1 tests, in order to generate more extensive data. Tier 3 testing should be performed on a case-by-case basis taking into consideration all the available data, to elucidate specific endpoints needing further investigation of findings in Tier 2 tests.

In particular, the tiered approach is designed to evaluate the following core areas: toxicokinetics, genotoxicity, toxicity (encompassing subchronic toxicity, chronic toxicity and carcinogenicity), and reproductive and developmental toxicity. In each of these core areas for evaluation, the general considerations and tiered approach to testing are outlined. In addition to the core areas for evaluation, the Panel noted that other tests may be required to allow an adequate risk assessment. Other studies that may be relevant and useful for assessing the risk and establishing the safety of an additive include immunotoxicity, hypersensitivity and food intolerance, studies on neurotoxicity, endocrine activity and mechanisms and modes of action. A number of issues related to the design, conduct and interpretation of all toxicological studies, are addressed in the document.

Applicants are advised to design the actual testing on a case-by-case basis taking into account physicochemical data on the compound, toxicity data on structurally related compounds and any available information on structure activity relationships. Inherent in the rationale of a tiered approach is the concept that results of studies at higher tiers will in principle supersede results at lower tiers. The intention is that in developing their dossier, applicants will be able to more readily identify relevant data needs, which will allow adequate assessment of risks to humans from the intended use, whilst strengthening the scientific basis for the assessment. In addition, this approach takes into consideration

animal welfare by adopting animal testing strategies in line with the 3-Rs (replacement, refinement, reduction). The Panel recommends that an integrated testing strategy, which may include alternative approaches, should be used to further support the risk assessment.

This guidance was originally adopted by the Food Additives and Nutrient Sources added to Food (ANS) Panel on 7 June 2012; the present revision was endorsed by the Food Additives and Flavourings (FAF) Panel on 2 July 2020.

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Introduction

This guidance document refers to the applications for authorisation of a new food additive or to an extension of the authorisation of an already authorised food additive. It describes the scientific data required for the evaluation of a food additive which allow its safety in proposed uses to be evaluated within the established framework for risk assessment as well as the risk assessment paradigm used by the Panel. A description of the risk assessment paradigm is given, followed by guidance arranged in the following four main sections:

1. The **Chemistry and specifications** section seeks to identify the food additive, potential hazards (e.g. impurities, residuals) from its manufacture, and, through the specifications, to define the material tested.
2. The **Existing authorisations and evaluation** section seeks to give an overview of previous risk assessments on the additive and their conclusions.
3. The **Proposed uses and exposure assessment** section seeks to estimate dietary exposure based on the proposed uses and use levels and the consumption of the proposed foods for various age groups in the population of EU Member States.
4. The **Toxicological studies** section seeks to describe the methods which can be used to identify (in conjunction with data on manufacture and composition) and characterise hazards.

In contrast to the Scientific Committee for Food (SCF) guidance document published in 2001 (SCF, 2001), which describes core and supplementary toxicological studies, this guidance describes a tiered approach which balances data requirements against other considerations such as use and animal welfare. The tiered approach initially uses less complex tests to obtain hazard data; these are then evaluated to determine if they are sufficient for risk assessment or, if not, to design studies at higher tiers. The intention is that in developing their dossier, applicants will be able to more readily identify relevant data needs which will allow adequate assessment of risks to humans from the intended use whilst strengthening the scientific basis for the assessment. In addition, this approach takes into consideration animal welfare by adopting animal testing strategies in line with the 3 Rs (replacement, refinement, reduction). The Panel recommends that an integrated testing strategy, which may include alternative approaches, should be used to further support the risk assessment.

The Panel has sought to provide an overall concept with clear information on a tiered approach for risk assessment. Using this tiered approach, a minimal dataset applicable to all compounds has been developed under Tier 1. Compounds which are systemically absorbed or for which toxic or genotoxic effects are found in Tier 1 will require Tier 2 testing to generate more extensive data. Tier 3 defines detailed testing for specific endpoints, for which Tier 2 testing results raised concerns, and is performed on a case-by-case basis. A diagram of the tiered approach is presented in Appendix A –.

Applicants are advised to design the actual testing on a case-by-case basis taking into account physicochemical data on the compound, toxicity data on structurally related compounds and any available information on structure activity relationships. Inherent in the rationale of a tiered approach is the concept that results of studies at higher tiers will in principle supersede results at lower tiers.

Applications for modification of the proposed uses or use levels of already authorised additives may only require additional exposure data and information on the existing authorisation.

The guidance document includes the following three appendices: a diagram outlining the tiered toxicity testing for food additives (Appendix A –), the general data requirements as published before (Appendix B –), and the Specifications as required by the Commission (Appendix C –).

Note: The present guidance has been revised and it is republished with editorial changes: the chapter “Administrative requirements” and “Procedure” in Appendix B – were deleted as presented in the “Administrative guidance on the preparation of applications on food improvement agents (food enzymes, food additives and food flavourings)” (EFSA 2021) following the new provisions defined by Regulation (EC) 178/2002 (‘GFL Regulation’), as amended by Regulation (EU) 2019/1381 of the European Parliament and of the Council of 20 June 2019 on the transparency and sustainability of the EU risk assessment in the food chain, applicable as from 27 March 2021. The scientific content has been left unchanged. For application submitted until 26 March 2021, the former version of this guidance

applies. For application submitted as of 27 March 2021, the present scientific guidance applies until further notice.

Background as provided by EFSA in 2010

Regulation (EC) No 1331/2008 of the European Parliament and Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings lays down a common procedure for the assessment and authorisation of food additives, food enzymes and food flavourings in view of updating the Community lists of permitted substances defined in the corresponding sectoral food laws.

According to this procedure, EFSA is requested to carry out a risk assessment of the substance under consideration for inclusion in the relevant Community list following an application or on the initiative of the Commission.

Regulation (EC) No 1333/2008 of the European Parliament and Council on food additives is the sectoral food law for food additives referred to in Regulation (EC) No 1331/2008.

In accordance with the provisions of regulation (EC) No 1331/2008 on implementing measures for the sectoral food laws, the ANS Panel has adopted on 9 July 2009 a statement on data requirements, while suggestions for specific scientific approaches can be found in the guidance for food additives applicable at the time of the application.

During its second plenary meeting in September 2008, the Scientific Panel on Food Additives and Nutrient Sources added to food (ANS) endorsed provisionally the guidance document for food additive evaluations adopted by the Scientific Committee on Food (SCF) in 2001.

In the statement on data requirements for the evaluation of food additive applications, the ANS Panel indicated that it would start a detailed reappraisal of the guidance document of the SCF in order to reflect the current thinking in risk assessment.

Terms of reference as provided by EFSA in 2010

The European Food Safety Authority asks the ANS Panel to develop a guidance on submission for food additives evaluation, considering especially the following aspects:

- Chemistry of the substance and specifications
- Proposed uses and exposure assessment
- Toxicokinetics and toxicity

The ANS Panel will work in close collaboration with the Scientific Committee in order to take into account the ongoing developments on issues related to the guidance and to contribute to them.

Interpretation of the terms of reference by the ANS panel in 2010

The Panel considered that the guidance should not only describe scientific data essential for the risk assessment but also additional information which might help in providing context for the risk assessment and in decreasing uncertainties in the risk assessment². The European legislation does not foresee that EFSA performs an environmental risk assessment for food additives. The guidance document should combine in a single document the description of the data requirements and their context and also a description of the risk assessment paradigm applied. The latter will enable stakeholders to understand the use and interpretation of the data. The Panel stresses that applicants should base their dossier on sound science and evolving principles of risk assessment, in order to provide a high level of public health protection whilst avoiding unnecessary animal experiments. To this end, this technical guidance on data requirements should also indicate possible flexibility in the data requirements compatible with this aim.

² For administrative and other requirements, readers should refer to the Scientific Statement of the Panel on Food Additives and Nutrient Sources added to Food on data requirements for the evaluation of food additives applications following a request from the European Commission (EFSA Journal 1188, 1-7, 2009) and the Practical guidance for applicants for addresses, contact points and the relevant documents for risk assessment available at the DG SANCO website: http://ec.europa.eu/food/food/FAEF/authorisation_application_en.htm

Risk assessment paradigm

The risk assessment process comprises four steps; hazard identification, hazard characterisation, exposure assessment and risk characterisation. In carrying out its risk assessments, the Panel seeks to define a health-based guidance value e.g. an Acceptable Daily Intake (ADI) (IPCS, 2004) applicable to the general population.

The ADI is established for compounds for which a threshold mechanism of toxicity can either be demonstrated or reasonably expected based on the available data. The ADI does not apply to infants below 12 weeks (JECFA, 1978; SCF, 1998) and the use of food additives for infant formula represents a special case for which recommendations were given by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 1972; 1978) and by the SCF (SCF, 1996; 1998). The Panel endorses these recommendations. For compounds with (or presumed to have) a common mode of action, group ADIs may be set which apply to any single compound in the group or to the sum of the compounds in the group. The Panel will not routinely set temporary ADIs (tADI) for new additives to allow their use whilst data gaps are addressed but may apply this status during re-evaluations which identify the need for additional data.

In the case of an additive which is neither genotoxic nor genotoxic and carcinogenic, but where the available data are considered to have certain deficiencies which nonetheless do not prevent the Panel reaching a conclusion regarding safety, the Panel will consider a Margin of Safety (MOS) approach to conclude whether or not there would be a risk at the proposed use and use levels. For compounds for which no safe level of exposure can be anticipated, for example genotoxic carcinogens, an ADI would not be established. In assessing the risk from levels of unavoidable contaminants or residuals in the additive which are genotoxic and carcinogenic, the Panel generally uses the Margin of Exposure (MOE) approach described in the European Food Safety Authority (EFSA) Scientific Committee opinion (EFSA, 2005; EFSA, 2012a).

Hazard identification and characterisation

The chemical and technological assessment identifies the hazards of an additive, which are then further characterised via their 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 so-called Reference Point or "Point of Departure" (POD)³. This POD is used to establish an ADI, by application of uncertainty factors to account for toxicokinetic and toxicodynamic differences between individuals and species. Typical PODs include the No Observed Adverse Effect Level (NOAEL) or a BMDL value (the lower confidence bound of the benchmark dose (BMD)). The EFSA Scientific Committee has recently endorsed the benchmark dose procedure and the use of the BMDL05 for continuous data or the BMDL10 for quantal data as a preferred approach to the NOAEL, to define the POD for deriving health-based guidance values (EFSA, 2005; 2009a). The Panel in line with the Scientific Committee expects to increasingly use BMDL values rather than the NOAEL for deriving an ADI, and this should be considered when designing toxicology studies. In the absence of potency data allowing definition of individual toxic equivalents, the group ADI will be based on the lowest NOAEL and assumes all members of the group are equipotent.

For the rat and mice, the default uncertainty factors used by the Panel are a factor of 10 for toxicokinetic and toxicodynamic differences between individuals, and an additional factor of 10 for toxicokinetic and toxicodynamic differences between species. Other or additional uncertainty factors may be applied depending on the entire database and/or the species (EFSA, 2012b). Furthermore, where data are available, they can potentially be used in risk assessment to derive chemical-specific adjustment factors (CSAFs) (Meek et al., 2003; IPCS, 2005; 2009). CSAFs may replace and be higher or lower than default uncertainty factors.

The International Programme on Chemical Safety (IPCS) has published guidance on the use of quantitative toxicokinetic and toxicodynamic data for the derivation of CSAFs as part of its project on the Harmonisation of Approaches to the Assessment of Risk from Exposure to Chemicals (IPCS, 2005). Toxicokinetic data can also be of value in developing adjustment factors for groups of related chemicals

³ The Panel considers that Reference Point and Point of Departure (POD) are to be essentially identical for the purposes of this document.

that share common physical or chemical characteristics or toxicokinetic or toxicodynamic pathways (Bokkers and Slob, 2007; Dorne and Renwick, 2005; IPCS, 2005; Naumann et al., 2001).

Exposure Assessment

Assessment of the exposure to food additives is the qualitative and/or quantitative evaluation of their likely intake by the European population, taking into account all dietary sources as appropriate. Exposure assessment is an essential component for quantifying risk and for determining whether a food additive poses a risk to the European population. Typically, data on actual food consumption from national or international surveys in Europe are combined with the intended use levels of the food additive to estimate the exposure to a food additive. This exposure assessment is intended to cover the population of all European Member States taking into account the variation of exposure due to differences in food consumption across the Member States and between various age groups of the population, in particular toddlers, children, adolescents, adults and elderly. The aim is to ensure that set safety levels (e.g. ADI, etc) would not be exceeded by consumers, including the high consumers.

Dietary exposure to a food additive is determined by summing the contribution made by each food in which the food additive is intended to be used. This in turn is achieved by multiplying the concentration of the food additive in a given food or food category by the consumption of this food or food category. The concentration of the food additive would be derived from the proposed use levels or the maximum permitted levels laid down in legislation, and if appropriate, from normal use levels as determined analytically or as indicated by industry. This result is divided by the corresponding body weight of the individuals within the population affected, to give the exposure on a kg body weight and day basis.

The risk assessment is initially based on the exposure estimates resulting from the proposed use levels or the maximum permitted levels for high level consumers, covering 95% of the European population.

Exposure assessment and outcome of the risk assessment

The overall evaluation of the additive for potential human risk should be made in the context of the known or likely human exposure in comparison with the ADI derived from the POD, with application of an appropriate uncertainty factor. In a further evaluation the ADI is compared with the human exposure estimate resulting from use of the additive at the proposed uses and use levels, and in comparison, also includes exposure from other sources, where relevant. When using the MOS approach, the Panel considers that a MOS of 100 or more between a NOAEL or BMDL and the anticipated exposure would be sufficient to account for uncertainty factors for extrapolating between individuals and species. However, the Panel considers each MOS on a case-by case basis to determine whether the magnitude of the MOS between the anticipated exposure from the proposed uses and use levels and the NOAEL or BMDL are sufficient to conclude that there would be no safety concern given the uncertainties identified in the database as a whole.

Unavoidable genotoxic and carcinogenic impurities

The Scientific Committee is of the opinion that the MOE approach can be applied to impurities⁴ which are both genotoxic and carcinogenic, irrespective of their origin. The Scientific Committee initially described that for contaminants "a MOE of 10,000 or higher, if it is based on the BMDL10 from an animal study, and taking into account overall uncertainties in the interpretation, would be of low concern from a public health point of view and might be reasonably considered as a low priority for risk management actions" (EFSA, 2005). "When using the MOE approach for assessing impurities, EFSA Scientific Committee and Panels should describe the derivation of the MOE, its magnitude, and the associated uncertainties regarding its derivation. They should also give their view on whether the MOE is of high concern, low concern, or unlikely to be of safety concern. It will then be the role of the risk managers to decide whether the substance containing the impurities should be authorised" (EFSA, 2012a). Whenever possible, it would be prudent to establish levels of this type of residuals in the specifications as low as reasonably practicable. The Panel would expect that any proposed specification for unavoidable genotoxic and carcinogenic residuals would result in a MOE of at least 10,000 and preferably as large as possible using exposure estimates for high level consumers at the proposed maximum permitted levels, and that this should be reflected in the specifications.

⁴ For the purposes of this description, the word impurities also cover residuals and metabolites.

The Panel noted that for the unavoidable genotoxic residuals, for which carcinogenicity data are not available, the TTC approach would be considered. The Panel would expect exposures for high level consumers at the proposed maximum use levels to be below the TTC for genotoxic compounds of 0.15 µg/person/day (EFSA, 2012c).

1. Chemistry and specifications

The chemistry and specifications of a substance (or mixture of substances), in terms of chemical structure(s) and physico-chemical properties, is critical information required for risk assessment and subsequent risk management. The purity of a single substance needs to be defined by specifications, and adequate chemical characterisation of simple mixtures needs to be performed. It may not always be possible to fully characterize more complex mixtures, but as much information as possible is required to understand the extent to which variability in composition is controlled during manufacture. The information required with respect to identity is set out in detail in subsections 1.1.1 to 1.1.7 and the complementary information on Specifications in Section 1.2, Section 1.3 describes information requirements for the manufacturing process. Information on the manufacturing process is used in the risk assessment to identify impurities, residuals, reaction intermediates, precursors and reagents that could have an influence in the toxicological evaluation. Hazards that might need to be controlled in the material of commerce need to be identified and specified (e.g. genotoxic compounds, heavy metals). Section 1.4 describes the information requirements for analytical methods to detect and measure the additive in food. Section 1.5 describes information requirements for evaluating the stability of the additive during storage and over time, when used in different food types. The identification of degradation products might trigger toxicological evaluation of one or more degradation products to characterise any additional hazards and risks. Validation criteria, information on the analytical techniques and/or methods should be provided to demonstrate their sensitivity and specificity (e.g. LOD, LOQ, range) and associated uncertainty.

1.1. Identity of the substance

1.1.1. Single substances (e.g. sorbic acid, sodium ascorbate, propyl gallate, glycerol, etc)

- Chemical name, when appropriate, according to IUPAC nomenclature rules.
- CAS number (if this has been attributed) from the ChemIDplus database, E number (where appropriate), EINECS number (where appropriate), and other identification numbers.
- Synonyms, trade names, abbreviations.
- Molecular and structural formulae.
- Molecular weight (g/mol) or atomic weight (for elements).
- Spectroscopic data (printout) such as NMR or MS spectra or other data.
- Description of physical and chemical properties: appearance, melting point, boiling point, specific gravity, stereochemistry (if any).
- Solubility (reference e.g. JECFA, 2006 - general method for solubility) in water and other common solvents.
- Influence of pH on solubility - ionisation constant(s).
- Octanol: water partition ratio.
- Particle size, shape and distribution, if applicable.
- Other data that the applicant considers may be useful to support the identity of the substance.

1.1.2. Simple mixtures (e.g. sorbitol syrup, lecithins, etc)

These are mixtures whose components can be fully chemically characterised.

- Chemical name, when appropriate, according to IUPAC nomenclature rules.

- Chemical composition-identity of the components of the mixture as required in point 1.1.1.
- CAS number (if this has been attributed) from the ChemIDplus database, E number (where appropriate), EINECS number (where appropriate), and other identification numbers.
- Synonyms, trade names, abbreviations.
- Proportion of each component of the mixture.
- Molecular and structural formulae of each component of the mixture.
- Molecular weight (g/mol) of each component of the mixture.
- Spectroscopic and chromatographic data (printout of spectra/chromatogram) which allow the identification of the components of the mixture.
- Description of physical and chemical properties: appearance, stereochemistry of each component (unless not applicable).
- Solubility (reference e.g. JECFA general method for solubility (JECFA, 2006)) in water and other common solvents.
- Particle size, shape and distribution, if applicable.
- Other data that the applicant considers may be useful to identify the mixture and its components.

1.1.3. Complex mixtures not derived from botanical sources (e.g. mineral hydrocarbons, beeswax, shellac, etc)

These are mixtures whose components cannot be always fully chemically characterised. The level of chemical characterisation required depends on the proposed use and use levels.

- Starting materials or source materials
- Species, in case of animal origin
- Chemical name, when appropriate, according to IUPAC nomenclature rules.
- CAS number (if this has been attributed) from the ChemIDplus database, E number (where appropriate) EINECS number (where appropriate) and other identification numbers.
- Synonyms, trade names, abbreviations.
- Chemical description, the level of principal components in so far as these are known and level of unidentified components.
- Description of physical and chemical properties.
- Solubility (reference e.g. JECFA general method for solubility (JECFA, 2006)) in water and other common solvents.
- Particle size, shape and distribution, if applicable.
- Other data that the applicant considers may be useful to identify the mixture and its components.
- In the special case of food additives consisting of, containing, or produced from genetically modified microorganisms (GMMs), these have to be authorised in accordance with both Regulation (EC) No 1829/2003⁵ and Regulation (EC) No 1333/2008⁶ in order to prepare an

⁵ Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Official Journal of the European Union L268/1, EN, 18.10.2003. Available at: http://eurlex.europa.eu/pri/en/oj/dat/2003/l_268/l_26820031018en00010023.pdf

⁶ Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on Food additives. *Official Journal of the European Union* L354/16, EN, 31.12.2008. Available at: <http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:354:0016:01:EN:HTML>

application for the evaluation under Regulation (EC) No 1333/2008. The Guidance of the GMO Panel on the risk assessment of products from GMMs should be followed (EFSA, 2011b).

1.1.4. Polymers (e.g. anionic methacrylate, agar, alginate and xanthan gums, pectins, modified starches, celluloses, polyvinylpyrrolidone, etc)

- Chemical name, when appropriate, according to IUPAC nomenclature rules.
- CAS number (if this has been attributed) from the ChemIDplus database, E number (where appropriate), EINECS number (where appropriate), and other identification numbers.
- Synonyms, trade names, abbreviations.
- Chemical and structural formula and molecular weight or number average molecular weight and weight average molecular weight (if feasible).
- Structural formulae of monomers and starting materials, other agents involved in the polymerisation.
- Degree of substitution, percentages of substituted groups (where appropriate).
- Description of physical and chemical properties.
- Solubility (reference e.g. JECFA general method for solubility (JECFA, 2006)) in water and other common solvents.
- Particle size, shape and distribution, if applicable.
- Other data that the applicant considers may be useful to identify the polymer and its constituents.

1.1.5. Additives derived from botanical sources (such as steviol glycosides from Stevia, or rosemary extracts)

In agreement with the EFSA Guidance on Safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA, 2009b), the following information for plant-derived additives is required in addition to the chemical information listed in sections 1.1.1 – 1.1.4.

Concerning the plant being the source of the additive, this includes:

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

Furthermore, data on the chemical composition of the plant-derived food additive should be provided with emphasis on the concentrations of constituents of relevance; this includes the concentrations of the following:

- Compounds classified according to their chemical structure (e.g. flavonoids, terpenoids, alkaloids, etc.).
- Constituents being characteristic for the food additive (chemical fingerprint, markers).
- Constituents that provide reasons for concern due to their chemical, pharmacological or toxicological properties.

Information on maximum levels for microorganisms and possible contaminants, including e.g. heavy metals, mycotoxins, pesticide residues and polycyclic aromatic hydrocarbon (PAH) residues, should be provided (EFSA, 2009b).

In the special case of food additives consisting of, containing, or produced from genetically modified microorganisms (GMMs), these have to be authorised in accordance with both Regulation (EC) No 1829/2003 and Regulation (EC) No 1333/2008 in order to prepare an application for the evaluation under Regulation (EC) No 1333/2008. The Guidance of the GMO Panel on the risk assessment of products from GMMs should be followed (EFSA, 2011b).

1.1.6. Nanomaterials

The following information for nanomaterials, reproduced from Table 1: and its associated footnotes (EFSA, 2011a) of the EFSA Guidance on engineered nanomaterials (ENMs), is required in addition to the chemical information listed in sections 1.1.1–1.1.4.

Table 1: Parameters for characterisation and identification of ENMs (EFSA, 2011a)

Parameter	Requirements	Description
<i>Chemical composition/identity</i>	<i>Essential</i>	<i>Information on chemical composition of the ENM – including purity, nature of any impurities, coatings or surface moieties, encapsulating materials, processing chemicals, dispersing agents and/or other formulants e.g. stabilisers.</i>
<i>Particle size (Primary/Secondary)</i>	<i>Essential (two methods, one being electron microscopy)</i>	<i>Information on primary particle size, size range and number size distribution (indicating batch to batch variation – if any). The same information would be needed for secondary particles (e.g. agglomerates and aggregates), if present.</i>
<i>Physical form and morphology</i>	<i>Essential</i>	<i>Information on the physical form and crystalline phase/shape. The information should indicate whether the ENM is present in a particle-, tube-, rod-/shape, crystal or amorphous form, and whether it is in free particulate form or in an agglomerated/aggregated state, as well as whether the preparation is in the form of a powder, solution, suspension or dispersion.</i>
<i>Particle and mass concentration</i>	<i>Essential for dispersions and dry powders</i>	<i>Information on concentration in terms of particle number and mass per volume when in dispersion, and per mass when as dry powder.</i>
<i>Specific surface area</i>	<i>Essential for dry powders</i>	<i>Information on specific surface area of the ENM.</i>
<i>Surface chemistry</i>	<i>Essential (for ENM with surface modifications)</i>	<i>Information on ENM surface – including any chemical/biochemical modifications that could modify the surface reactivity or add a new functionality.</i>
<i>Surface charge</i>	<i>Essential</i>	<i>Information on zeta potential of the ENM.</i>
<i>Redox potential</i>	<i>Essential for inorganic ENMs</i>	<i>Information on redox potential. Conditions under which redox potential was measured need to be documented.</i>
<i>Solubility and partition properties ^a</i>	<i>Essential</i>	<i>Information on solubility of the ENM in relevant solvents and their partitioning between aqueous and organic phase (e.g. as log K_{ow} if appropriate).</i>
<i>pH</i>	<i>Essential for liquid dispersions</i>	<i>pH of aqueous suspension.</i>
<i>Viscosity</i>	<i>Essential for liquid dispersions</i>	<i>Information on viscosity of liquid dispersions.</i>
<i>Density and pour density</i>	<i>Essential for granular materials</i>	<i>Information on density/porosity of unformulated ENM and pour density.</i>
<i>Dustiness</i>	<i>Essential for dry powders</i>	<i>Information on dustiness of powder products – such as spices, creamers and soup powders.</i>
<i>Chemical reactivity/catalytic activity^b</i>	<i>Essential</i>	<i>Information on relevant chemical reactivity or catalytic activity of the ENM and of any surface coating of the ENM.</i>

<i>Photocatalytic activity</i>	<i>Essential for photocatalytic materials</i>	<i>Information on photocatalytic activity of relevant materials used in food packaging, coatings, and printing inks and internal reactions.</i>
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- a) Dispersion, solution, dissolved: An insoluble ENM introduced to a liquid form a 'dispersion' where the liquid and the ENM coexist. In a true solution the ENM is dissolved (and thus not present) (see OECD ENV/JM/MONO(2010)25).
- b) If an ENM has catalytic properties, it may catalyse a redox or other reaction which may perpetuate resulting in a much larger biological response even with small amounts of the catalytically active ENM. Thus, compared to a conventional biochemical reaction which uses up the substrate, ENM reaction centres may perpetuate catalytic reactions.

The Panel considers that for non-engineered nanomaterials used as food additives, similar characterisation to that required for ENMs should be carried out and provided.

1.1.7. Substances containing microorganisms or derived from microorganisms

The following information is required for additives of microbial origin.

- The microbial origin of food additives produced by fermentation or cultivation, including:
 - Name of the microorganism
 - Taxonomic classification of the microorganism
 - History of modification of the production organism
- Whether the microorganism fulfils the requirements for a Qualified Presumption of Safety (QPS) (EFSA, 2007). In such cases no further data on the microorganism itself are required.
- Information on residual levels of toxins.
- Information on the production process.
- Information on the identity of residual intermediates or microbial metabolites in the final product.
- In the special case of food additives consisting of, containing, or produced from genetically modified microorganisms (GMMs), these have to be authorised in accordance with both Regulation (EC) No 1829/2003 and Regulation (EC) No 1333/2008 in order to prepare an application for the evaluation under Regulation (EC) No 1333/2008. The Guidance of the GMO Panel on the risk assessment of products GMMs should be followed (EFSA, 2011b).

1.2. Specifications

The specifications of an additive define the requirements concerning the identity, the purity and the limits of any impurity present in the additive, indicating also the appropriate methods of analysis. In order to ensure that the specifications are representative of the actual material of commerce, the analytical data supporting the specifications should be obtained on several batches of the additive that have been independently produced (i.e. with independent batches of raw materials and produced on different dates) for a given method of manufacture. In practice, for each method of manufacture, analytical information on preferably at least 5 independently produced batches of the proposed additive, produced according to the method of manufacture and using the analytical methods described, should be provided in order to show that the additive can be consistently manufactured within its proposed specifications. A rationale for the proposed specifications should be provided.

The following information is required about the specifications of an additive.

- The definition of the article of commerce.
- The proposed specifications should include the purity in percentage and the method of determination to allow the identification of the substance (chromatograms, spectra, etc).
- The proposed specifications should include the impurities: nature, limits (including limits for individual heavy metals, and where appropriate, for microorganisms, mycotoxins and solvent residues) and methods of determination and their validation.
- The proposed specifications should be submitted in a format modelled on recent EU (see Appendix C –) or other internationally accepted specifications.

- Where the proposed specifications differ from any already existing EU, JECFA or other internationally recognised specifications, these specifications should be set out alongside the proposed new specification, and any differences pointed out.
- The specifications for additives derived from botanical sources may be based on nutritional or biologically active components or, when these are not known, on selected chemical markers. In agreement with the EFSA Guidance on Botanicals (EFSA, 2009b), specifications for botanical sources should indicate:
 - a) The identity of the article of commerce.
 - b) The purity of the article of commerce in percentage; concentrations of major groups of constituents present in the botanical preparation (e.g. amino acids, lipids, polysaccharides, volatile oil, inorganic ions, polyphenols, alkaloids, terpenes, alkenylbenzenes, lignin, saponins, etc.) as well as the major constituents within these classes. Methods of determination (chromatograms, spectra, etc).
 - c) Limits for specific undesirable/toxic substances known to be present in the plant. Validated methods should be provided for the analysis.
 - d) Information on maximum levels for microorganisms, solvent residues and possible contaminants including e.g. heavy metals. Validated methods should be provided for the analysis of substances considered in the specifications.
 - e) Compliance with recent EU or other internationally accepted specifications (e.g. pharmacopoeia) where appropriate.
 - f) Where the proposed specifications differ from internationally recognised specifications, the latter specifications should be set out alongside the proposed new specifications, and any differences pointed out.
- The specifications should describe the material in full and state the percentage of the material that is not specifically identified in the specifications (calculated as a 100% minus the percentage identified). This percentage of material not identified in the specifications should be minimised.
- Since processing (e.g. extraction solvent, temperature) may influence the composition of the plant-derived food additive, the composition should be characterized for each proposed production process to facilitate read across.

1.3. Manufacturing process

The information on the manufacturing process is used in the risk assessment to identify impurities, reaction intermediates, precursors and reagents that could present a hazard. Where hazardous substances are identified, they might need to be controlled in the material of commerce (e.g. genotoxic compounds, heavy metals). Therefore, in all cases a detailed description of the manufacturing process should be provided covering the following:

- Method of manufacture (e.g. raw materials, the process by which the raw materials are converted to the finished product), production controls and quality assurance.
- For substances synthesised chemically: i) factors such as reaction sequence, side reactions, purification and preparation of the product to be commercialised, which may assist in determining likely impurities and their influence on the toxicological evaluation; ii) information on substances entering the manufacturing process, e.g. identity of the extraction solvent, reagents, special precautions (light and temperature), chemical or physical decontamination methods should be provided.
- For substances derived from botanical, animal, microbiological sources: i) information on the method(s) of manufacture should include the process by which the raw material is converted into a preparation, such as extraction or other procedure(s); ii) information on substances entering the manufacturing process, e.g. identity of the extraction solvent, reagents, special precautions (light and temperature); iii) standardisation criteria (e.g. see European Pharmacopoeia, 2011; for botanicals further guidance can be found in EFSA, 2009b).

In submissions requesting approval of a currently permitted EU additive that is to be manufactured by a new method involving significant change in its production methods or starting materials used, or in which there is a change in form from conventional bulk material to nanoscale dimensions, the main differences between the existing manufacturing method and the new manufacturing method should be highlighted, including information on, or prediction of, any new impurities that may be present as a result.

1.4. Methods of analysis in food

A minimum of a single laboratory validated analytical method should be provided for the determination of the substance and its degradation and reaction products in the food to which the substance is intended to be added. The method(s) provided should be specific and fit-for-purpose. They should be applicable to all the food categories to which the substance may be added. Method(s) should be given in full except where the analytical methods used are well established and may be given by reference only.

In the case of additives made from or containing nanomaterials, the Panel refers to the EFSA opinion on the potential risks arising from nanoscience and nanotechnologies on food and feed safety (EFSA, 2009c; 2011a), which states that "in the absence of exposure data, and where it is not possible to determine the nanoform in the food/feed matrix, it should be assumed that all added ENM is present, ingested and absorbed in the nanoform". The Panel noted that in such cases where the nanoform cannot be determined in food, conventional chemical methods may be used to measure the total amount of the additive present in food.

1.5. Stability of the substance, and reaction and fate in food

The stability of the additive, as produced and in food during storage, should be evaluated and described. This information requirement for establishing the stability of the additive during storage conditions in different food types and over time in food is to identify hazards which might arise from degradation products to characterise any additional hazards and risks. Appropriate information should be provided on:

- The chemical/physico-chemical stability of the food additive in its food additive preparation and under the conditions of storage and effect of storage temperature, environment [light, oxygen, moisture, relative humidity (water activity)] or any other factor that might influence the stability of the food additive preparation.
- The chemical/physico-chemical stability of the additive during storage of the processed food: e.g. effect of the nature of the food to which the substance is added, processing temperature, pH, water activity or any other factor.
- The nature and reactivity of any degradation products and nature of interaction/reaction of degradation products with food components.
- Technologically intended reactions with food constituents and the resulting products in food.

2. Information on existing authorisations and evaluations

Information on existing authorisations and evaluations should be provided. This should include details of the following:

- the body which carried out the evaluation;
- when the evaluation was undertaken;
- details of the evaluation identifying the critical studies and their NOAELs/LOAELs and BMDL values, and
- any uncertainties described, health-based guidance values (e.g. ADIs) and the uncertainty factors used in this evaluation.

3. Proposed uses and exposure assessment

Introduction

Historically, exposure assessment of food additives followed a tiered approach from crude estimates (Tier 1) to more refined estimates (Tiers 2 and 3), as outlined in the report from the Commission on dietary food additive intake in the EU (EC, 2001). Tier 1 started with crude estimates (Budget method), based on theoretical food consumption data and the maximum intended use levels of the food additive (SCOOP report) (EC, 1997). Tier 2 estimates were calculated by using data on actual food consumption and the maximum intended use level of the food additive, thus representing a refined estimate of potential exposure compared to Tier 1. For the re-evaluation of already authorised food additives, Tier 3 estimates (further refinement of exposure estimates at Tier 2) were calculated by using data on actual food consumption and normal⁷ use levels of the food additive. Data on the normal use levels are available from the food industry or post-marketing surveillance by food enforcement authorities in the Member States. The highest normal use levels reported by industry were used for exposure estimation at Tier 3.

Since the concept of the Tier 1 (Budget method) was developed for post-marketing surveillance, Tier 1 calculations are not required for new authorisation of a food additive or a modification of an existing authorisation. Tier 3 estimates are only relevant for already authorised food additives, as no normal use level would exist for applications for the authorisation of a new food additive. Overall, the Panel considered that Tier 1 of this historical tiered approach was no longer appropriate.

Data required for the estimation of exposure in accordance with this guidance document

As already indicated in the introductory section on the risk assessment paradigm, assessment of the exposure to food additives is the qualitative and/or quantitative evaluation of their likely intake by the European population, taking into account all dietary sources as appropriate. To enable this assessment, information should be provided on known or anticipated human exposure (including data from epidemiological or biomonitoring studies) to the proposed additive from food (including natural dietary sources) or toxicologically relevant components of the additive, and any other potential non-dietary sources (e.g. from drinking water, consumer products such as cosmetics, pharmaceuticals, etc.), when information on exposure from these sources is available. When a modification of the conditions of use of an already authorised food additive is requested, the exposure estimates should also take into account all existing authorisations. Exposure estimates are also to be provided on any potential exposure to residues or contaminants present due to the use of the additive.

For the purpose of carrying out an exposure estimation in accordance with this guidance document, data are required for the relevant one of the two different scenarios:

- i. **Scenario 1** refers to applications for the authorisation of a new food additive;
- ii. **Scenario 2** refers to a modification of the proposed uses or use levels of an already authorised food additive.

To support the calculation of the exposure estimates for the applicable scenario(s), an exposure assessment tool has been developed by the Panel with the support of EFSA: Food Additive Intake Model (FAIM). This exposure assessment tool will provide exposure estimates by combining the data entered by the applicant on the proposed uses and use levels for a new authorisation (**Scenario 1**) with summary statistics data calculated from the EU Comprehensive Food Consumption Database (EFSA, 2011c). In the same way, the FAIM tool will provide the basis of exposure estimates for **Scenario 2** by combining the data on the proposed new uses and use levels for a modification of an existing authorisation and the data of the unmodified normal use levels of the existing authorisation with the EU Comprehensive Food Consumption Database (EFSA, 2011c). The FAIM tool will also provide the opportunity to include data on normal use levels which may be lower than the maximum permitted levels to calculate refined exposure estimates, but the Panel will initially consider only maximum proposed and maximum permitted levels for the safety assessment of the food additive.

⁷ The terms 'typical use level', 'normal use level' and 'actual use levels' represent the same meaning.

3.1. Proposed uses in food and corresponding use levels

The data requested for an authorisation of a new additive should indicate in which foods this additive is proposed to be added/used, and the intended use level of the food additive (Scenario 1). The data requested for a modification of the proposed uses or use levels include the new proposed use levels, and both the maximum permitted levels and the normal use levels of the already authorised uses, if available (Scenario 2). Data on the normal use level are available from the food industry or from post-marketing surveillance by food enforcement authorities in Member States. In principle, a normal use level is the average level of the food additive determined in a number of samples being representative for the food in a given European Member State. It is likely that within the European Member States different levels of food additives are typically found for the same food category. If so, the maximum reported⁸ use levels within the European Member States, or if available sufficiently representative data on the reported use level, should be used for exposure estimation. In most cases, normal use levels are expected to be lower than the maximum permitted use level in a food category. The Panel will not be able to conclude on the safety of a food additive if only quantum satis use is proposed since exposure estimates cannot be calculated in this case.

In order to support the calculation of the most refined possible exposure estimations, each food or food category in which the food additive is used or proposed to be used should be defined at the highest level of detail possible for the two following food classification systems:

- FoodEx classification system (used for the EFSA comprehensive database)⁹
- Food classification system defined in the Annex II of Regulation (EC) No 1333/2008¹⁰.

While it may not be always possible to clearly assign a food to food categories within these two classification systems, foods should still be linked to both of them with clarification provided on any assumptions made.

3.1.1. Authorisation of a new food additive (Scenario 1)

Data required for a new authorisation should be as follows:

- Proposed use and use level of the food additive for each food or food category. For food additives prepared by extraction from natural sources (e.g. rosemary extracts, etc), the use levels provided should be related to i) the additive itself, and ii) the corresponding concentration of the other components (e.g. residues from extraction) in the mixture.
- If the intended use can be achieved by different chemical forms of the food additive (e.g. potassium nitrate/sodium nitrate, lutein/lutein esters), data are required on the proposed use level of each of the chemical forms of the additive and whether they are proposed to be used in combination or as an alternative for each other.

3.1.2. Modification of an existing authorisation (Scenario 2)

Data required for a modification of an existing authorisation should be as follows:

- If applicable, proposed use levels of the food additive for each food or food category for the newly proposed uses. For food additives prepared by extraction from natural sources (e.g. rosemary extracts, etc), the use levels provided should be related to i) the additive itself, and ii) the corresponding concentration of the other components in the mixture.
- If applicable, use level of the food additive proposed to replace the existing maximum permitted level for each food or food category for already authorised uses. For food additives prepared by extraction from natural sources (e.g. rosemary extracts, etc), the use levels provided should

⁸ Maximum reported use level is the highest level reported by the food industry or from post-marketing surveillance by food enforcement authorities in Member States.

⁹ <http://www.efsa.europa.eu/en/datex/datexfooddb.htm>
<http://www.efsa.europa.eu/en/datexfooddb/docs/datexfooddbchronicqday.xls>

¹⁰ Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on Food additives. Official Journal of the European Union L354/16, EN, 31.12.2008. Available at: <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:354:0016:01:EN:HTML>

be related to i) the additive itself, and ii) the corresponding concentration of the other components (e.g. residues from extraction) in the mixture.

- The normal use levels of the food additive for the already authorised uses of the food additive.
- The maximum permitted levels of the food additive as laid down in the relevant regulation for the already authorised uses of the food additive.
- If the intended use can be achieved by different chemical forms of the food additive (e.g. potassium nitrate/sodium nitrate, lutein/lutein esters), data are required on the proposed use level of each of the chemical forms of the additive, and whether they are proposed to be used in combination or replacing each other.

If carry over of the food additive itself or any other toxicological relevant residue may occur, (e.g. when the applicant proposes to modify Annex III of Regulation (EC) No 1333/2008 such as for additives for the stabilisation of vitamin preparations), data are required on the carry over of the food additive and its resulting concentration in the final food product (e.g. the fortified food). Similar carry over estimates should also be made when the additive is to be used in foods which can be used as ingredients, e.g. sugar.

3.2. Exposure data

3.2.1. Assessment of exposure to the food additive

The evaluation of the safety of a food additive is based on the aggregate exposure from all sources. Other potential sources of exposure to the additive or toxicologically relevant components of the additive should therefore be taken into account (e.g. natural occurrence in food, non-additive use in food supplements, use as a nutrient, use as flavouring, use as food contact material, use in pharmaceutical or cosmetic products).

For these sources, the average anticipated exposure and exposure at the 95th percentile are requested for the age groups (toddlers, children, adolescents, adults and elderly) as indicated above. Subsequently, the Panel may decide to request further information (including quantitative data) regarding the exposure resulting from these additional sources, depending on their relevance.

3.2.1.1. Assessment of aggregate exposure to the same compound from different sources

For the estimation of total exposure to the food additive for the age groups (as indicated above), data are requested on aggregate exposure to the food additive from all sources, as outlined above. Aggregate exposure is the sum of:

- average exposure to the food additive from its use as food additive at the proposed use and the corresponding use levels,
- average exposure from its natural sources as appropriate,
- average exposure from food fortification and supplements as appropriate, and
- average exposure from other uses.

Since high percentiles of overall exposure should only be calculated from individual data, in order to avoid gross overestimations, high percentile estimates for each food category or other source should be provided but should not be used for that calculation. The Panel will consider on a case-by-case basis on how to calculate extremes of overall exposure from all or different sources.

The main food groups contributing to the dietary exposure of the additive should be described in the main text of the exposure section of the application.

3.2.1.2. Estimate of exposure to residues or contaminants

Finally, exposure to any toxicologically relevant components coming into foods from the use of the food additive (e.g. potential residues of degradation products, reaction products, or contaminants arising from the use of the additive) should be provided taking into account specific legislative purity criteria as

applicable. It is recommended that the same FAIM tool is used as for the food additive itself, in order to describe the anticipated exposure for average and 95th percentile consumers to this compound for the age groups, as indicated above.

3.2.2. Submission of data

It is recommended that applicants provide also these data through the use of the dedicated exposure assessment tool (FAIM) which will be made available by EFSA.

4. Toxicological studies (toxicokinetics and toxicity)

The tiered approach, described below, is designed to evaluate the following core areas:

- Toxicokinetics
- Genotoxicity
- Toxicity encompassing subchronic, chronic toxicity and carcinogenicity
- Reproductive and Developmental toxicity

These are normally assessed on the basis of toxicological studies performed *in vitro*, and *in vivo* using laboratory animals. Further details of these core areas are given below. Experimental studies (e.g. toxicokinetics data, SARs, data from other toxicity and neurotoxicity studies) and human data (epidemiological studies and case reports, if available) should be included in the evaluation.

For the toxicological studies, this guidance describes a tiered approach which balances data requirements against the risk. The tiered approach initially uses less complex tests to obtain hazard data; these are then evaluated to determine if they are sufficient for risk assessment or, if not, to design studies at higher tiers. The tiered approach for toxicological studies consists of 3 tiers, for which the testing requirements, key issues and triggers are described. According to this tiered approach, a minimal dataset applicable to all compounds has been developed under Tier 1, while Tier 2 testing will be required for compounds which are absorbed, demonstrate toxicity or genotoxicity in Tier 1 tests, in order to generate more extensive data. Tier 3 testing should be performed on a case-by-case basis taking into consideration all the available data, to elucidate specific endpoints needing further investigation of findings in Tier 2 tests. Although higher tier testing may be required based on results in one of the core areas, such testing would only be required in relevant core areas e.g. where results from absorption or the 90-day study require further tier 2 studies but tier 1 *in vitro* genotoxicity is negative, there would be no need for tier 2 genotoxicity.

A number of issues related to the design and conduct of all toxicological studies are addressed in the next section.

ISSUES TO BE CONSIDERED IN THE DESIGN AND PERFORMANCE OF TOXICOLOGICAL STUDIES

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

- Toxicological studies should be carried out with the additive meeting the proposed specifications and manufactured as described in the application, unless there are scientific reasons why this is not appropriate. In such cases the scientific reasons should be clearly and adequately described and justified.
- Ethical approval and welfare standards for animal and human studies should comply with relevant EU standards and regulations on the protection of humans and animals used for scientific purposes.
- Applicants are reminded that Directive 2010/63/EU¹¹, on the protection of animals used for experimental and other scientific purposes, requires that care is taken to avoid unnecessary use of animals. Studies carried out should be those necessary to demonstrate the safety of an

¹¹ 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.

additive and planned in accordance with the principles of replacement, reduction, and refinement. Since adequate human data are unlikely to be available, *in vivo* studies using experimental animals from species relevant to humans are still needed in order to assess possible risks to humans from the ingestion of food additives. There are some exceptions to this (e.g. initial assessment of genotoxic potential by *in vitro* studies), and alternative validated methods for other endpoints in toxicity, involving fewer or no animals, are being developed. Studies submitted using alternative testing methods will be considered by the Panel on a case-by-case basis.

- Studies on toxicokinetics and toxicity of food additives in animals should be conducted using internationally agreed test guidelines. Test methods described in OECD test guidelines (OECD TG) or in Council Regulation (EC) No 440/2008¹² laying down test methods pursuant to Regulation (EC) No 1907/2006¹³ of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) are recommended. The most up-to-date edition of any test guideline should be followed. However, it should be noted that these guidelines provide minimum criteria for acceptance of studies and a specific protocol should be derived for each study which may need additional requirements above these minimum criteria. These may serve as screens for more specialised endpoints and their results may point to the need for additional specialised studies (e.g. neurotoxicity and immunotoxicity). Use of any methods differing from internationally agreed test guidelines, including protocols for special studies, should be justified and their acceptance will be assessed on a case-by-case basis.
- Non-clinical studies should be carried out according to the principles of Good Laboratory Practice (GLP) described in Directive 2004/10/EC¹⁴. Applicants need to be aware that studies that fail to meet the minimum requirements of internationally agreed test guidelines, or which are conducted post 1987 and are not GLP compliant, can be rejected on this basis. The Panel does not generally apply this to historical studies being re-evaluated or mechanistic studies used in support of mode of action analyses.
- Substances should normally be administered via the oral route. Consideration should be given to the choice of mode of administration, bearing in mind the form in which humans are likely to consume the substance and the influence this will have on rate of absorption and subsequent systemic availability. For substances that are to be added to solid foods, or added to both solid foods and beverages, administration should normally be via the diet. In the event of palatability problems following incorporation of high concentrations into the diet, administration by oral gavage or use of additional pair feeding control groups should be considered. For substances that are only to be used in beverages, administration via drinking water may appear to be the mode of choice, but for practical reasons this may limit the maximum amount that can be administered and may not adequately reflect the fact that humans can consume beverages such as soft drinks in significant quantities over a short time period. Thus, alternative modes of bolus administration, such as gavage, could be used for such substances. For other substances that may be consumed by humans as a bolus, such as an additive for use in food supplements marketed in the form of capsules or tablets, administration by oral gavage (or in the case of non-rodents, by capsule) should be considered. The effect of method of administration on toxicokinetics and local effects should be assessed.
- For ENMs, as described in the corresponding EFSA Guidance document, toxicological testing methods may require modifications (e.g. range of organs studied) based on toxicokinetic studies on the ENMs and characterisation of the ENMs tested (EFSA, 2011a). For nanomaterials which

¹² Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

¹³ 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.

¹⁴ Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004, on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances.

exist as a permitted non-nanoform food additive, the limited additional testing on the nanoform establishes whether read across from the non-nanoform is feasible for more complex testing. For novel nanomaterials, all toxicological tests need to incorporate the nanospecific characterisation and additional endpoints described in the EFSA Guidance.

- As a special case, botanical food additives derived from conventional food sources with a long-term history of food use, may benefit from a “presumption of safety” under certain circumstances when an adequate body of knowledge exists. This has to be evaluated on a case-by-case basis. In agreement with the EFSA “Guidance on Safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements” (EFSA, 2009b), a “presumption of safety” could be applied to botanicals and botanical preparations used as food additives when data would allow the conclusion that exposure to known levels of the botanical ingredient has occurred in large population groups for many years without reported adverse effects. 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”. However, the Panel considered that the definition of what is considered a significant increase, compared to historical levels, will 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. Methods of extraction of the botanical preparation used as food additive should be considered, since processes differing from the traditional methods of food preparing may lead to compositional differences and concentrate undesirable components. For botanical preparations with a potential to contain toxic, addictive, psychotropic or other substances that may be of concern, presumption of safety 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. Any data on possible drug interaction should be carefully considered. Furthermore, the presumption of safety approach can only be applied when intakes due to the intended levels of use are within the range of intake levels derived from the European Member States’ mean diets or from studies on specific subgroups. It is recognized that the acceptability of presumption of safety approach relies mainly on the objective of not significantly increasing exposures beyond the levels linked to the history of use.

CORE AREAS FOR EVALUATION

4.1. Toxicokinetics (ADME)

4.1.1. General considerations

Toxicokinetics (ADME) is an important tool in human health risk assessment and greater application of toxicokinetics as part of an improved assessment could offer more efficiency, use fewer animals and provide better data for risk assessment purposes. Toxicokinetic data provide valuable information for selection of appropriate species and doses for toxicity testing, and also for risk assessment through the comparison of internal dose in experimental animals and humans. Administration of a chemical does not automatically mean that all of the dose will be systemically available (bioavailable). Therefore, data on systemic exposures to the chemical and its metabolites, as well as an understanding of the major processes involved in its absorption, distribution, metabolism and excretion (ADME), can assist in the interpretation of toxicity studies and the prediction of differences or similarities across animal species or from animal to man (Creton et al., 2009). Toxicokinetic processes and metabolism may become saturated at doses higher than those expected to be relevant to human exposure, which can result in toxicity that would not be relevant to the intended use and usage level (Bus and Reitz, 1992; Counts and Goodman, 1995; Slikker et al., 2004).

- Toxicokinetic data can be derived from a suite of studies covering ADME, including *in vitro*, *in silico* and *in vivo* studies, and single and repeated dose kinetics (Adler et al., 2011). Whole animal studies using single or repeated dosing may be needed to define toxicokinetic parameters. However, the design of toxicokinetic studies should be flexible based on the particular substance being tested.

- Systemic exposure to the parent compound or metabolites is assessed by measuring plasma (or whole blood or serum) concentrations, urinary metabolite patterns, whereas in some cases tissue concentrations may be measured. Commonly measured parameters include the area under the curve (AUC) of plasma concentration of the compound against time after oral administration, maximum concentration (C_{max}), time to reach maximum concentration (T_{max}), elimination half-life (T_{1/2}). Estimates of systemic availability require comparison of results following oral administration with those obtained from intravenous administration. In particular, assessment of systemic exposure greatly aids the interpretation of dose–response relationships, which can be nonlinear due to induction, alteration or saturation of processes involved in the ADME of the compound. Furthermore, toxicokinetic information may be used to determine that a lack of toxicological response is not due to a lack of systemic exposure.
- *In vitro* studies, employing proteins, carrier proteins, enzymes, subcellular fractions, cell cultures, and perfused organs, can also provide useful information for the investigation of absorption, distribution and metabolism, mechanisms of toxicity, effects on enzymes and other specific aspects. Such *in vitro* studies can be especially useful in defining possible species differences.
- Studies in humans should only be performed if there are adequate data from animal and other related studies to demonstrate the likely safety in humans at the proposed level of exposure. Toxicokinetic information in humans can not only provide confirmation of the validity of the animal models used in terms of metabolism, but also whether toxicokinetic parameters estimated from animal data are applicable for humans. This information can be used to define chemical specific adjustment factors.
- For substances with limited systemic availability, studies on the distribution and metabolic fate of the additive may require use of compounds labelled with radioactive or stable isotopes.
- For some food additives such as complex mixtures, conventional metabolism and toxicokinetic studies may not be feasible for all components in the mixture but should be provided for toxicologically relevant constituents. Toxicologically relevant constituents are generally considered to be the major components and those other components with known or demonstrable biological or toxicological activity and should be determined on a case-by-case basis with a scientific justification and the rationale for their selection provided.
- In some cases where a matrix effect is thought to impact on the safety of specific levels of substances by affecting their toxicokinetic parameters, appropriate testing and/or other data should be provided to demonstrate the occurrence of the matrix effect with the preparation and its effect on toxicokinetics. A matrix effect should be judged on a case-by-case basis.

4.1.2. Tiered approach to toxicokinetic testing

Tier 1 Absorption studies and in vitro gastrointestinal metabolism

- The aim of Tier 1 toxicokinetic testing is to establish whether the compound or breakdown products are absorbed from the gastrointestinal tract. There are a number of established models for absorption studies (including *in vitro*, *in vivo* and *ex vivo* models-absorption and bioavailability models). Physicochemical factors which affect absorption are molecular weight, ionisation constant, hydro- and lipophilicity. Demonstration of negligible absorption, either through experimental studies or from theoretical considerations, may provide a scientific justification for not undertaking higher tiered toxicological studies on an additive. The required sensitivity to determine negligible absorption levels will generally necessitate *in vivo* studies using labelled compounds. In general, there is a need for case-by-case evaluation when determining negligible absorption.
- The stability of the compound in the gastrointestinal tract needs to be investigated to ascertain that it neither breaks down nor is metabolised to components that may be absorbed. The use of *in vitro* gastrointestinal metabolism models, including gut flora, may assist in this evaluation. The use of absorption and bioavailability models such as the Ussing chamber (Ussing et al., 1951; Grass et al., 1988; Gotoh et al., 2005) and the inverted sac model (Wilson et al., 1954; Kato et al., 2004) could provide information about the differences in absorption along the

gastrointestinal tract and provide quantitative absorption information (Bohets et al., 2001; Versantvoort et al., 2000).

The Panel considers that assessments of negligible absorption should take into account physicochemical, study design and other parameters. The physicochemical parameters include: chemical structure, molecular weight, octanol water partition coefficient, aqueous solubility, molecular shape, charge and dissociation constants. The study design parameters include percentage of absorption, robustness of study design and performance, sensitivity and specificity of methods of detection, detection limits, amount in faeces and dose accountancy. Other parameters include likelihood of persistence in tissues, predicted metabolic stability, and results of tier 1 studies.

The Panel notes that the TTC might provide a useful comparator in this assessment.

If negligible absorption of the additive, its residuals and its intestinal (e.g. microflora or chemical) breakdown products is demonstrated, a limited number of toxicity studies would be accepted. Further details on toxicity studies required at Tier 1 are given in the respective sections below. In case of absorption of the compound, its metabolites or breakdown products (e.g. microflora or chemical) from the gastrointestinal tract, then Tier 2 toxicokinetic testing should be carried out.

Tier 2 Studies to define distribution, metabolism and excretion and other basic toxicokinetic parameters following a single dose

For some additives (e.g. high molecular weight polymers and mixtures) when there is absorption of low molecular weight components, Tier 2 studies (both in toxicokinetics and in other endpoints) of these components may be more relevant and informative for the risk assessment than such studies on the additive itself.

- *In vivo* assessment of ADME
- Tier 2 toxicokinetic studies (OECD TG 417) should provide data on systemic exposure to the compound and definition of basic single dose toxicokinetic parameters (T_{1/2}, AUC, bioavailability, C_{max} and T_{max}) together with *in vivo* assessment of its absorption, distribution, metabolism and excretion including identification and quantification of metabolites. It is often desirable to have parameters determined at a range of dose levels to examine the linearity of kinetic parameters and possible saturation of these parameters.
- The assessment of the validity of the chosen animal model might require comparative *in vitro* metabolism studies using corresponding animal and human enzymes, subcellular fractions and/or cells.

Tier 3 Studies to define toxicokinetic parameters following repeated administration.

The trigger for requesting Tier 3 studies will be limited or slow excretion or any other mechanism that may underlie possible bioaccumulation. In these cases, the following data should be considered to expand the available database. Further details on human studies are found under the section Additional studies.

- Tier 3 toxicokinetic studies with repeated doses in experimental animals, normally this would involve studies to steady state which would be approximately five terminal half-lives.
- Additional data to help predict the absorption, distribution, metabolism and excretion of a compound in humans.
- Human kinetic data from volunteer studies. It should be done on a case-by-case basis.

Evidence of differences in toxicokinetics due to age, physiological state, disease state, etc may require consideration of specific toxicokinetic studies that will refine the risk assessment.

4.2. Genotoxicity

4.2.1. General considerations

As outlined in the EFSA Scientific Committee (SC) opinion on genotoxicity testing strategies (EFSA, 2011d), genetic alterations in somatic and germ cells are associated with serious health effects, which in principle may occur even at low exposure levels. Mutations in somatic cells may cause cancer if

mutations occur in proto-oncogenes, tumour suppressor genes and/or DNA damage response genes and are responsible for a variety of genetic diseases (Erickson, 2010). Accumulation of DNA damage in somatic cells has also been proposed to play a role in degenerative conditions such as accelerated aging, immune dysfunction, cardiovascular and neurodegenerative diseases (Hoeijmakers, 2009; Slatter and Gennery, 2010; De Flora & Izzotti, 2007; Frank, 2010). Mutations in germ cells can lead to spontaneous abortions, infertility or heritable damage to the offspring and possibly to the subsequent generations.

In view of the adverse consequences of genetic damage to human health, the assessment of mutagenic potential is a basic component of chemical risk assessment. To this aim, both the results of studies on mutation induction ("mutagenicity") and tests conducted to investigate other effects on genetic material are taken into consideration. For definitions of the terms "mutagenicity" and "genotoxicity", the EFSA SC opinion on genotoxicity testing strategies (EFSA, 2011d) or the REACH "Guidance on information requirements and chemical safety assessment" (ECHA, 2008) may be consulted.

Genotoxicity testing is performed with the following aims:

- to identify substances which could cause heritable damage in humans,
- to predict potential genotoxic carcinogens in cases where carcinogenicity data are not available, and
- to contribute to understanding of the mechanism of action of chemical carcinogens.

For an adequate evaluation of the genotoxic potential of a chemical substance, different endpoints (i.e. induction of gene mutations, structural and numerical chromosomal alterations) have to be assessed, as each of these events has been implicated in carcinogenesis and heritable diseases.

The genotoxic potential of any new additive has to be assessed as part of the evaluation process. The recommendations concerning genotoxicity testing in this technical guidance are based on the scientific opinion on genotoxicity testing strategies (EFSA, 2011d).

The Scientific Committee recommended a stepwise (tiered) approach for the generation and evaluation of data on genotoxic potential, comprising:

- a basic battery of *in vitro* tests aimed to evaluate the genotoxic potential of the substance assessing induction of gene mutation, structural (clastogenicity) and numerical (aneuploidy) chromosomal alteration,
- consideration of whether specific features of the test substance might require substitution of one or more of the recommended *in vitro* tests by other *in vitro* or *in vivo* tests in the basic battery,
- in the event of positive results from the basic battery, review of all the available genotoxicity data on the test substance, and
- where necessary, conduct of an appropriate *in vivo* study (or studies) to assess whether the genotoxic potential observed *in vitro* is expressed *in vivo*.

Indicator tests, which detect primary DNA damage that may not result in mutations, are not part of the basic battery; however, such tests could be useful in the follow-up of *in vitro* positive results (EFSA, 2011d).

Before embarking on any testing, it is important for the appropriate conduct of the tests to consider other relevant knowledge on the substance. Supporting information may also be available from Structure Activity Relationship (SAR) data, and 'read-across' of data between structurally related substances. This information can also be important for interpretation of genotoxicity testing results and particularly relevant for the choice of any *in vivo* study.

In rare cases there may be scientific grounds (e.g. insufficient metabolic activation *in vitro*, the involvement of specific conditions such as reactions in the gastrointestinal tract or structural similarity with known mutagens/carcinogens) for requiring *in vivo* testing even in case of negative results *in vitro*.

The opinion on genotoxicity testing strategies of the Scientific Committee (EFSA, 2011d) may be consulted for further general aspects such as scope of genotoxicity testing, definition of terms, data interpretation and follow up of e.g. equivocal or inconclusive results.

The Panel noted that the Scientific Committee in its recent statement had clarified guidance on the use of the MOE for low-exposure substances such as impurities, metabolites and degradation products of deliberately added substances which are genotoxic and carcinogenic. The Panel noted that the SC opinion precluded use of the TTC for substances where the EU legislation requires the submission of toxicity data and therefore genotoxicity data would be required for all additives. The Panel further noted that the TTC approach might be helpful when assessing the genotoxicity of low-exposure substances such as impurities, metabolites and degradation products of deliberately added substances for which genotoxicity data may be unavailable. The Panel noted that the Scientific Committee concluded that a TTC of 0.15 µg/day, there was a high probability of protection against carcinogenic genotoxic effects and that this was also likely to cover heritable effects (EFSA, 2011d; 2012c). The Panel therefore considered that genotoxicity data would not always be necessary for impurities, metabolites and degradation products of deliberately added substances in food and feed for which human exposures are below the TTC of 0.15 µg/day.

4.2.2. Tiered approach to genotoxicity testing

The principle of tiered testing to examine genotoxic potential *in vitro* and whether this is expressed *in vivo* is well established in genotoxicity testing strategies. There is a recommended battery of *in vitro* tests that determine possible genotoxicity hazards (EFSA, 2011d). Tier 1 testing is mandatory for all food additives, however as described above the MOE or TTC may be sufficient for impurities, metabolites and degradation products of food additives. A positive result in Tier 1 requires follow-up in Tier 2. This Tier 2 testing determines whether the hazard is expressed *in vivo*. There are a number of reasons why the genotoxic potential may not be observed *in vivo* and in case of negative results it is crucial to demonstrate exposure of the tissue either through direct toxicity or using kinetic data. A valid negative Tier 2 outcome is regarded as showing an absence of genotoxicity *in vivo*. If Tier 2 is positive it is usually assumed that the compound is a somatic cell genotoxin and will be potentially carcinogenic and also mutagenic in germ cells. Such compounds are not considered acceptable as food additives.

Tier 1 Basic test battery

In line with the recommendations of the Scientific Committee (EFSA, 2011d), the following two *in vitro* tests are required as the first step in genotoxicity testing:

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

This combination of tests fulfils the basic requirements to cover the three genetic endpoints with the minimum number of tests; the bacterial reverse mutation assay covers gene mutations and the *in vitro* micronucleus test covers both structural and numerical chromosome aberrations. The addition of any further *in vitro* mammalian cell tests in a basic battery would significantly reduce specificity with no substantial gain in sensitivity (EFSA, 2011d). There may be circumstances under which deviation from the above-mentioned tests may be justified. In such cases a scientific justification should be provided, and additional types of considerations or mechanistic studies may be needed. If there are indications for the substance of interest that specific metabolic pathways would be lacking in the standard *in vitro* systems, or it is known that the *in vitro* test system is inappropriate for that substance or for its mode of action, testing may require either appropriate modification of the *in vitro* tests or use of an *in vivo* test at an early stage of testing. It may be advantageous to include *in vivo* assessment of genotoxicity at an early stage and incorporate such testing within other repeated-dose toxicity studies that will be conducted anyway, especially when the test substance can be dosed up to the limit dose which would be applicable in a separate *in vivo* genotoxicity study. Some practical aspects that need to be considered when combining genotoxicity testing with repeated-dose toxicity testing are described in the SC opinion on genotoxicity testing strategies (EFSA, 2011d).

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

In cases where all *in vitro* endpoints are clearly negative in adequately conducted tests, it can be concluded with reasonable certainty that the substance is not a genotoxic hazard.

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

Tier 2 Follow-up of results from the basic test battery

Before embarking on any necessary follow-up of positive *in vitro* results by *in vivo* testing, not only the results from the *in vitro* testing should be reviewed, but also other relevant data on the substance, such as information about chemical reactivity of the substance (which might predispose to site of contact effects), bioavailability, metabolism, toxicokinetics, and any target organ specificity. Additional useful information may come from structural alerts and 'read-across' from structurally related substances. It may be possible after this to reach a conclusion to treat the substance as an *in vivo* genotoxin. If, after such a review, a decision is taken that *in vivo* testing is necessary, tests should be selected on a case-by-case basis using expert judgement, with flexibility in the choice of test, guided by the full data set available for the substance.

In vivo tests should relate to the genotoxic endpoint(s) identified as positive *in vitro* and to appropriate target organs or tissues. Evidence, either from the test itself or from other toxicokinetic or repeated dose toxicity studies, that the target tissue(s) have been exposed to the test substance and/or its metabolites is essential for interpretation of negative results.

The approach to *in vivo* testing should be stepwise. If the first test is positive, no further test is needed, and the substance would be considered as an *in vivo* genotoxin. If the test is negative, it may be possible to conclude that the substance is not an *in vivo* genotoxin. However, in some cases, a second *in vivo* test may be necessary as there are situations where more than one endpoint in the *in vitro* tests is positive and an *in vivo* test on a second endpoint may then be necessary if the first test is negative. It may also be necessary to conduct a further *in vivo* test on an alternative tissue if, for example, it becomes apparent that the substance did not reach the target tissue in the first test. The combination of assessing different endpoints in different tissues in the same animal *in vivo* should be considered.

In line with the recommendation of the EFSA Scientific Committee, the Panel considers the following tests as suitable *in vivo* tests:

- an *in vivo* micronucleus test (OECD TG 474),
- an *in vivo* Comet assay (no OECD TG at present; internationally agreed protocols available, e.g. see <http://www.cometassay.com/>), and
- a transgenic rodent assay (OECD TG 488).

The *in vivo* micronucleus test covers the endpoints of structural and numerical chromosomal aberrations and is an appropriate follow up for *in vitro* clastogens and aneugens. The current OECD TG only considers peripheral blood and bone marrow as target tissues. There may be circumstances in which an *in vivo* mammalian bone marrow chromosome aberration test (OECD TG 475) may be an alternative follow up test. The Panel noted that local genotoxic effects (e.g. in the upper gastrointestinal tract) cannot be ruled out solely on the basis of inactivity in bone marrow, especially for directly acting, electrophilic molecules. The *in vivo* Comet assay is considered a useful indicator test in terms of its sensitivity to substances which cause gene mutations and/or structural chromosomal aberrations *in vitro* and can be performed with many tissues. Transgenic rodent assays can detect point mutations and small deletions and are without tissue restrictions.

When the *in vivo* and *in vitro* results are not consistent, then the differences should be clarified on a case-by-case basis. For example, in the *in vivo* micronucleus test, certain substances may not reach the bone marrow due to low bioavailability or specific tissue/organ distribution. In certain cases, for example when it is known that the test substance is metabolised in the liver and the reactive metabolites formed are too short-lived to reach the bone marrow, even demonstration of the bioavailability of the parent substance in the bone marrow does not indicate that bone marrow is an appropriate target. A negative result of the *in vivo* micronucleus assay can be considered as meaningful only if there is definitive evidence from effects observed in bone marrow or toxicokinetic data that the tested substance as well as the relevant reactive metabolite(s) can reach the bone marrow.

More detailed advice on strategies for *in vivo* follow up is given in the opinion on genotoxicity testing strategies (EFSA, 2011d).

Normally, if the results of appropriate and adequately conducted *in vivo* tests are negative, then it can be concluded that the substance is not an *in vivo* genotoxin. If the results of the *in vivo* test(s) are positive, then it can be concluded that the substance is an *in vivo* genotoxin.

Follow-up of results from Tier 2 by carcinogenicity studies and germ cell assays

The Panel considered that an adequately conducted and powered carcinogenicity study may demonstrate that an *in vivo* genotoxin does not give rise to carcinogenicity. However, mutations in somatic cells are also known to be responsible for a variety of genetic diseases (Erickson, 2010). Furthermore, such an *in vivo* genotoxin may be a germ cell mutagen and it is recognised that standard reproductive toxicity studies do not cover all germ cell effects. The Panel noted that the Scientific Committee concluded that 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, and therefore potentially hazardous to future generations. In the contrary situation, a substance that is negative in tests in somatic tissues *in vivo* would be assumed to be negative in germ cells, also because no germ cell specific mutagen is known. Accordingly, the Scientific Committee concluded that routine testing for genotoxicity in germ cells is not necessary. The Scientific Committee further concluded that clear evidence of genotoxicity in somatic cells *in vivo* has to be considered an adverse effect per se, even if the results of cancer bioassays are negative, since genotoxicity is also implicated in diseases other than cancer (EFSA, 2011d). Hence, careful consideration should be given to animal welfare issues such as suffering and numbers before conducting any further *in vivo* studies.

There is no Tier 3 for genotoxicity testing.

4.3. Toxicity testing (subchronic, chronic and carcinogenicity)

4.3.1. General considerations

The major objective of a toxicity study on a food additive is to provide information on treatment-related changes in blood, urine and clinical biochemistry parameters, gross and histopathological changes in organs and tissues following prolonged exposure to the additive via an appropriate oral route. The clinical observations may also provide information on neurofunctional and neurobehavioral effects of the additive under investigation.

Data from a subchronic toxicity study should normally be submitted. Such studies often establish the main toxicological profile of the substance, providing information on the target organs and tissues affected (hazard identification), on the nature and severity of any effects, and on the dose-response relationships (hazard characterisation). They should allow determination of the relevant BMDL using a BMD analysis or of the dose at which adverse effects found at higher dose levels are no longer observed, i.e. the NOAEL (EFSA, 2009a). The subchronic toxicity study is used for estimating the appropriate dose levels for chronic toxicity studies and it can provide indications for the need for additional studies on particular effects, such as neurotoxic or immunological effects.

Subchronic toxicity will usually be investigated in one species only, normally the rat, although other species may have to be used, either alternatively or additionally. A scientific justification, e.g. metabolic differences, needs to be provided for the choice of species. However, if there is evidence that there are toxicokinetic differences which would question the adequacy of the chosen animal model for the human situation, then testing should be performed in a different, adequate species.

For subchronic and chronic toxicity studies and for carcinogenicity studies, the highest dose level should normally be chosen to identify the principal target organs and toxic effects while minimising suffering, severe toxicity, morbidity, or death. For food additives, which may be relatively non-toxic, it may be impossible for animal welfare reasons to identify such a dose level in a meaningful way.

The highest dose level in chronic or carcinogenicity studies should normally be chosen to elicit some evidence of toxicity, as evidenced by, for example, depression of body weight gain (approximately 10%), and has previously been referred to as the Maximum Tolerated Dose (MTD). In the case of food additives given via the diet, the highest dose should normally not exceed 5% of the diet, in order to avoid nutritional imbalances. This upper dose is acceptable even if no toxicity is produced. The OECD Guidance Document 116 provides additional guidance on dose selection for chronic toxicity and carcinogenicity studies.

Subchronic toxicity

Within Tier 1, a subchronic toxicity study should normally be conducted for a period of at least 90 days (OECD TG 408) in rodents, modified to include assessment of some additional parameters described in the more recent guideline on repeated-dose 28-day oral toxicity study in rodents (OECD TG 407). The additional parameters place more emphasis on endocrine-related endpoints, (e.g. determination of thyroid hormones, gross necropsy and histopathology of tissues that are indicators of endocrine-related effects), and (as an option) assessment of oestrous cycles. The modified 90-day study should allow for the identification of chemicals with the potential to cause neurotoxic, immunological, reproductive organ effects or endocrine-mediated effects, which may warrant further in-depth investigation. Preceding range-finding studies conducted for shorter periods can provide an indication of target organs and help in selection of appropriate doses for 90-day studies. When range-finding studies have been conducted, the results should be submitted. Studies of shorter duration than 90-days are generally not sufficient, by themselves, for evaluation of potential subchronic toxicity.

Chronic toxicity and carcinogenicity

In Tier 2, a chronic toxicity study may reveal effects not evident in subchronic studies, or it may confirm effects observed in subchronic studies at the same or perhaps lower doses. The chronic toxicity of a food additive may be evaluated in a stand-alone study, using the relevant OECD TG 452. Alternatively, the use of a combined protocol to study chronic toxicity and carcinogenicity in the same experiment will often be appropriate in the testing of food additives, in accordance with OECD TG 453. 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 phase. 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. An OECD Guidance Document (No.116) on the design and conduct of chronic toxicity and carcinogenicity studies, supporting OECD TGs 451, 452 and 453 is currently under development, providing useful additional information on dose selection and the conduct of such studies (OECD GD 116).

In rats, chronic toxicity studies will normally be carried out for a 12-month period. Carcinogenicity studies should cover the majority of the lifespan of the animals, generally 24 months in the rat and 18 or 24 months in the mouse, in accordance with OECD TG 453. In utero exposure is not required in carcinogenicity studies unless specific considerations suggest otherwise.

Information to be derived from these studies should include histopathological investigations and clinical observations including ophthalmology, measurements of body weight, food/water consumption and food efficiency, made at appropriate intervals as specified in the OECD Test Guidelines. For additives where previous subacute or subchronic toxicity tests indicated the potential to cause neurofunctional or neurobehavioral effects, further investigations of such effects should be carried out using appropriate methodology (referred to under Additional Studies). Microscopic examination should cover all organs and tissues in the body. It is however acceptable to examine control and top dose animals only for microscopic changes, provided no significant treatment-related pathological changes are observed in the top dose group. Tissues from lower dose groups should always be retained in case further examination is required.

Carcinogenicity and chronic toxicity studies will usually be investigated only in one species, the rat. Traditionally, carcinogenicity testing for food additives has been conducted in two species, the rat and the mouse, as recommended in the 2001 SCF Guidelines. In recent years there has been considerable debate about the value of the two rodent species approach to carcinogenicity and about the continued use of the mouse as a second species, particularly within the ICH (ICH, Proceedings of the Third International Conference, 1995). A number of studies have assessed the relative individual contribution of rat and mouse carcinogenicity studies and whether the use of rats or mice alone would result in a significant loss of information on carcinogenicity relevant to human risk assessment. This debate has led to the suggestion that there may be no need for routine conduct of two long-term rodent carcinogenicity studies, with the rat being the preferred species for testing. Overall, the Panel supports this position and considers that it is appropriate to perform the carcinogenicity studies in the rat only.

Strategies for carcinogenicity testing

OECD TG 451 indicates that before commissioning carcinogenicity studies, all available data should be evaluated. These data include the identity, chemical structure, and physico-chemical properties of the additive; results of any *in vitro* or *in vivo* toxicity tests including genotoxicity tests; anticipated use(s) and potential for human exposure; available (Q)SAR data, mutagenicity/genotoxicity, carcinogenicity and other toxicological data on structurally-related substances; available toxicokinetic data (single dose and also repeated dose kinetics where available) and data derived from other repeated exposure studies. Assessment of carcinogenicity should only be carried out after initial information on toxicity has been obtained from 90-day toxicity and/or longer-term toxicity tests. In the event of a carcinogenic response being demonstrated in the study, additional mechanistic information together with good data on toxicokinetics are usually essential for risk assessment, both with respect to extrapolation to humans and possible determination of a threshold for non-genotoxic carcinogens.

4.3.2. Tiered approach to toxicity testing

The **Tier 1** for toxicity testing consists of a modified 90-day toxicity test (OECD TG 408 with extended parameters from the OECD 407) that should allow for the identification of chemicals with the potential to cause neurotoxic, immunological or reproductive organ effects or endocrine-mediated effects, which may warrant further in-depth investigation at higher tiers. The results from the repeated dose 90-day oral toxicity can be used to identify a BMDL or a NOAEL.

In the case of food additives for which Tier 1 toxicokinetics testing indicates a lack of systemic availability, the Tier 1 studies should look for both pathological and physiological effects in the gastrointestinal tract. The effects of unabsorbed materials on gastrointestinal function and tolerance also need to be investigated.

Tier 2 Studies on chronic toxicity (12 months) and carcinogenicity in a single species, generally the rat. Either separate studies (OECD TGs 452 and 451, respectively) or the combined study (OECD TG 453). Carcinogenicity study in a second species would only be triggered by the results in the preferred species (equivocal results or species specific findings) or by observations from specialised studies to investigate the mode of action or mechanism of toxicity or carcinogenicity observed.

Tier 3 In the last decades, several alternative models including short-term tests with transgenic mouse models (p53+/-, rasH2, Tg.AC, Xpa-/- and Xpa-/-p53+/-) have been developed to add to or refine the classical carcinogenicity bioassay, and may provide appropriate information at Tier 3. Although not a complete replacement to the rodent 2-year cancer bioassay, transgenic mouse models are a refinement and may result in a significant reduction in the use of experimental animals.

Tier 3 may also include specialised testing for neurotoxicity, immunotoxicity or endocrine-mediated effects. The purpose of investigations into mechanisms and modes of action is to determine the relevance for man of effects observed in the test species as part of their mode of action framework.

4.4. Reproductive and developmental toxicity

4.4.1. General considerations

Food additives showing systemic availability should be tested in reproductive toxicity and developmental toxicity studies. The objective of a reproductive toxicity study is to provide information about effects and potency of food additives on male and female libido, fertility, on the female's ability to carry pregnancy to term, on maternal lactation and care of the young, on the prenatal and postnatal survival, growth, functional and behavioural development of the offspring, on the reproductive capacity of the offspring and to identify histologically any major target organs for toxicity (including reproductive organs) in the parents and offspring. The major objective of a prenatal developmental toxicity study is to identify the potential of a substance to cause lethal, teratogenic or other toxic effects on the embryo and foetus, by examination for embryonic and foetal resorptions or deaths, foetal weight, sex ratio, and external, visceral and skeletal morphology. Exposure to an additive, prenatally via the mother and postnatally via maternal milk, may also impair postnatal development and function, including neurological function and behaviour, immunological function and endocrine activity.

Decisions on whether tests are necessary for reproductive and developmental toxicity will need to be considered in the light of the toxicity data and toxicokinetics information available. For a decision on whether a developmental toxicity study will be necessary, consideration also needs to be given as to whether the substance may cross the placenta. Such information may not be readily available, since ADME studies do not routinely include pregnant animals.

4.4.2. Tiered approach to reproductive and developmental toxicity testing

Tier 1

The data from Tier 1 subchronic toxicity testing are relevant when considering the need for reproductive and developmental testing in Tier 2.

- The repeated dose 90-day oral toxicity study (OECD TG 408) offers only limited information on reproductive toxicity and no information on developmental toxicity; it can inform about effects on the reproductive organs and, if assessed, the oestrous cycle, but it does not assess fertility and the whole reproductive cycle from in utero exposure onwards, through sexual maturity to conception, gestation, prenatal and postnatal development.

Decisions on whether tests are necessary for reproductive and developmental toxicity need to be considered in the light of the toxicity data and toxicokinetics information available. If the Tier 1 toxicokinetic study shows that the test substance is systemically available in the test species (normally rodents) or suspected to be systemically available in humans, Tier 2 testing for reproductive and developmental toxicity is required. Indications of effects on reproductive organs or parameters in the 90-day oral toxicity will also trigger Tier 2 testing for reproductive and developmental toxicity.

- Where absorption is negligible, Tier 2 testing for reproductive and developmental toxicity studies need not be performed.

Tier 2

- Tier 2 testing for reproductive and developmental toxicity comprises a prenatal developmental toxicity study (OECD TG 414) in the rabbit and an Extended One-Generation Reproduction Toxicity Study (EOGRTS) (OECD TG 443). Cohorts for the preliminary assessment of additional more specific endpoints should be routinely incorporated in the EOGRTS for studies on food additives (see details below). When evaluating existing additives, the Panel could consider a multi-generation study, instead of an EOGRTS, acceptable, provided that sufficient information on possible neurotoxicity and immunotoxicity is available (for example from an extended 90-day study, OECD TG 408).
- In the EOGRTS, administration of the test substance should normally be via the diet or by oral gavage to both sexually mature male and female animals covering a defined pre-mating period (minimum of 2 weeks) and a 2-week mating period, with parental males being treated until at least the weaning of the F1, for a minimum of 10 weeks, and parental females during pregnancy and lactation until weaning of the F1. Dosing of the F1 offspring should begin at weaning and continue until scheduled necropsy in adulthood. The testing will be conducted in one laboratory species only, primarily rodents, with the rat being the preferred species of choice provided that careful consideration has been taken in relation to all the other available information. However, based on other information available, alternative species can be used provided that a rationale is outlined by the applicant.
- The EOGRTS (OECD TG 443) in the rat will provide information evaluating specific life stages not covered by the other toxicity studies; fertility and reproductive function, and short-to long-term developmental effects from exposure during pregnancy, lactation and prepubertal phases as well as effects on juveniles and adult offspring will be assessed, by efficiently integrating several endpoints that cover the whole reproductive cycle (from gametogenesis through to maturation of the following generation) as well as preliminary assessment of additional more specific endpoints (i.e. developmental neurotoxicity and developmental immunotoxicity). According to the OECD guideline (TG 443), the selected parameters to be measured fall into the following categories:

- reproductive endpoints
- developmental (pre- and postnatal) endpoints
- specific endpoints (developmental neurotoxicity, immunotoxicity and endocrine disruption)

and focus on physical, functional and behavioural development in animals exposed from the beginning of embryogenesis through to adulthood. Relevant observations generally include pup body weight, pre-weaning physical and functional developmental landmarks including reflex development, the onset of sexual maturity as measured by vaginal opening in females and cleavage of the balanopreputial gland in males, sensory and locomotor function, and some indication of cognitive ability (learning and memory).

- The EOGRTS protocol includes endpoints, termed 'triggers'(e.g. P fertility, F1 oestrous cycle evaluation, F1 litter parameters and developmental landmarks, F1 pup survival postnatally and malformations, and F1 live birth index and body weight) which can be used for determining whether assessment of a second generation (F2) is required. Where these triggers are positive, the EOGRTS may be extended to include the F2 generation which may help clarify any equivocal findings or provide further characterisation on fertility in the F1 mating. It is expected that with the additional parameters evaluated in the F1 generation in the EOGRTS, the F2 with their limited parameter assessments would seldom affect the hazard characterisation for risk assessment (Piersma et al., 2011). However, when predicted human exposures are considered adequately characterised, MOE considerations may be factored into the decision to require the assessment of a F2 generation. Consideration should also be taken on all the other information available.

Tier 3

In devising appropriate Tier 3 testing, a case-by-case approach should be adopted with careful consideration given to animal welfare issues and on all available data. Tier 3 testing is triggered by results in Tier 2 studies and might comprise of additional studies for e.g. endocrine, developmental neurotoxicity (OECD TG 426), and mode of action studies which could include both guideline studies and experimental studies designed on a case-by-case basis.

4.5. Additional studies

In addition to the core areas for evaluation, the Panel noted that other tests may be required to allow an adequate risk assessment. These studies generally examine specific biological processes which may not be fully considered in the core areas for evaluation. Other studies that may be relevant and useful for assessing the risk and establishing the safety of an additive include immunotoxicity, hypersensitivity and food intolerance, studies on neurotoxicity, endocrine activity and mechanisms and modes of action.

4.5.1. Human studies

Introduction

Useful information could be gained from human studies conducted before or after the marketing of a food additive. Similarly, experience gained from the investigation of the safety of human therapeutic agents may be applicable in some circumstances to human studies with food additives. A complete package of tier 1 testing (kinetics, 90-day study and genotoxicity) would probably be sufficient data for safety assessment for single or short-term repeated administration human studies under clinically controlled conditions.

Indications for human volunteer studies

Studies of food additives in humans should only be performed if there are adequate data from animal and other related studies to demonstrate the likely safety in humans at the proposed level of exposure. Any proposed studies should have clear scientific objectives and adequate protocols, include provisions for review in the event of occurrence of unexpected results, and comply with the relevant ethical and legal standards. These include approval by an appropriately constituted review or ethical body, adherence to the principles of informed consent by volunteers, and the maintenance of records that are open to inspection.

Types of human volunteer studies

Human volunteer studies are generally of two types: absorption, metabolism, distribution and elimination studies, and tolerance studies. Other special studies e.g. on allergy, behaviour or cognitive function may sometimes be appropriate. Human volunteer studies may also be indicated when knowledge is required about special subgroups of the general population who may be genetically predisposed to low tolerance or particularly exposed to certain additives. Studies of the absorption, metabolism, distribution and elimination of additives in humans would greatly enhance the predictive value of the traditional chemical, biochemical and toxicological investigations in laboratory animals used to demonstrate safety. Comparison of the results of such human studies with those obtained in laboratory animals enables validation of the database acquired in animal experiments and the detection of any significant differences between animals and humans, which can be of importance for the interpretation of unusual or adverse findings.

Gastrointestinal absorption may be followed by determination of blood levels at intervals after administration, giving some indication of bioavailability. Information on kinetics and metabolism following absorption can be obtained from blood and urine measurements. Human studies are particularly appropriate for investigating tolerance of a substance or a food. They may be appropriate, for example, for investigating symptoms which cannot be studied in animals (e.g. headaches, gastrointestinal discomfort). They include physical examination, blood chemistry, haematology, urine analysis and in some cases organ function tests. At the same time monitoring for any adverse reactions, and recording their nature, frequency, intensity and dose relationship should be carried out. A number of publications contain useful information on the conduct of clinical studies (EMEA, 2002).

4.5.2. Immunotoxicity, Hypersensitivity/allergy and Food Intolerance

In exposed individuals, food additives may interact with the immune system in several ways and induce changes in the immune response resulting in either immunosuppression or immunostimulation. Immunostimulation may lead to hypersensitivity reactions, including autoimmunity and allergy. An allergic response to an additive can be induced by the presence of allergenic components or residues, in particular proteins, or alternatively because the additive itself is an allergen (e.g. a protein or a peptide) or capable of acting as a hapten.

Preliminary experimental data indicative of an effect on the immune system may be obtained from the Tier 1 and Tier 2 testing strategies for (sub)chronic toxicity testing, and these may trigger further Tier 3 studies investigating immunotoxicity.

Immunotoxicity

The tiered approach to testing outlined in this guidance includes, at **Tier 1**, a 90-day study in rats (OECD TG 408). This study involves investigation of the effect of the food additive on a number of parameters that may be indicative of an immunotoxic or immunomodulatory effect. These include: changes in spleen and thymus weights relative to body weight in the absence of overt toxicity, histopathological changes in these and other organs of the immune system (e.g. bone marrow, lymph nodes, Peyer's patches), as well as changes in total serum protein, albumin:globulin ratio and in the haematological profile of the animals, notably in lymphocyte numbers and in the total and differential blood cell counts.

The effects may be confirmed or, alternatively, seen for the first time in **Tier 2** studies, notably the EOGRTS (OECD TG 443), but also in chronic toxicity/carcinogenicity studies conducted according to OECD TGs 452, 451 or 453. In the EOGRTS, a cohort of animals is specifically dedicated to assess the potential impact of exposure on the developing immune system. In subchronic and chronic studies, haematological and clinical chemistry data are generally provided, together with phenotypic analysis of spleen cells (T-, B-, NK-cells) and bone marrow cellularity. The EOGRTS provides additional information on the primary IgM antibody response to a T cell dependent antigen, such as sheep red blood cells (SRBC), or keyhole limpet hemocyanin (KLH).

The evaluation of the potential of a food additive to adversely affect the immune system may be based on an integrated assessment of the results obtained from these toxicity studies (Tiers 1 and 2). If, these results indicate that the food additive has such a potential, additional Tier 3 studies should be

considered, on a case-by-case basis. These will normally be designed to investigate the underlying mechanisms of the effects seen, and/or their biological significance.

Tier 3 studies may include specialised functional, mechanistic, and disease model studies (Draft Guidance for Immunotoxicity risk assessment for chemicals – WHO/IPCS, 2012). The Panel noted that there are no OECD guidelines for these extended specialised studies, but based on IPCS, such studies may include the following:

- mitogen stimulation assays for B and T cells
- natural killer cell functional analysis, macrophage quantification and functional analysis, interleukin-2 functional analysis, cytokines production by lymphocytes
- complement assays: total serum haemolytic activity or individual components (C3a, C5a, ...)
- kinetic evaluation of humoral response to a T-cell-dependent antigen (primary and secondary responses to SRBC, tetanus toxoid or other), or to a T-independent antigen such as pneumococcal polysaccharides, trinitrophenyl-lipopolysaccharide, or other
- delayed-type hypersensitivity response to a known sensitizer of T effector cells, or reversibility evaluation
- infectivity challenge (*Trichinella*, *Candida* or other in rat, *Listeria* or other in mouse), or tumour challenge (MADB106 or other in rat, or PYB6 sarcoma in mouse).
- Alternative methods using human cells from umbilical cord such as hematopoietic progenitor clonogenic assays.

Allergy

At present there are no validated studies in laboratory animals which would allow assessment of the potential of a substance to cause allergic reactions in susceptible individuals following oral exposure. Studies on dermal or inhalation sensitisation may provide relevant information for possible hazards from occupational exposure to additives and could be helpful in assessing consumer safety even if their relevance to oral allergenicity remains unclear. Any available data on double-blind placebo-controlled oral food challenges, or prick testing in humans should be used. These data may be already available e.g. in the case where the food additive has already been studied for other studies such as in drugs.

Where the additive is a potential allergen (e.g. a protein or a peptide) or contains residues of proteins or other known potential allergenic molecules, the principles discussed in the EFSA Guidance on the Allergenicity of GMOs should be followed in evaluating allergenic components. These principles for the determination of allergenicity include the investigation of structural aspects of the protein or peptide, *in silico* (or bioinformatics) approaches, IgE binding and cell-based methods, analytical profiling techniques and animal models (EFSA, 2010).

Since no single experimental method yields decisive evidence for allergenicity and allergic responses, a weight of evidence approach taking into account all the information obtained from various test methods is recommended.

Where allergenicity of a food additive has been identified, it has generally been accepted to date that defining a threshold/NOAEL for such effect is difficult since different thresholds exist for induction and elicitation of the allergenic response together with idiosyncratic reactions. Therefore, the Panel will take such an adverse effect into account on a case-by-case basis.

Intolerance reactions

Intolerance reactions to food additives are not of immunological origin. They can be due to genetically defined metabolic specificities or to still other undefined causes (NIAID-Sponsored Expert Panel et al., 2010; Guandalini and Newland, 2011; Hayder and Bartholomaeus, 2011). These reactions are mediated by active substances such as bioactive amines, histamine, or tyramine. Such reactions are difficult to predict and mostly rely on human studies reporting observations of adverse effects.

At present, no validated experimental *in vitro* and *in vivo* methods are available which would allow assessment of a substance's potential to cause intolerance reactions in susceptible individuals following oral exposure. Moreover, it is not feasible to undertake clinical studies of sufficient power prior to marketing. Any data from post-marketing surveillance may identify possible sensitive individuals.

4.5.3. Neurotoxicity

Initial indications of potential neurotoxic effects of a test substance will be obtained through the 90-day toxicity study (Tier 1). Other information, such as screening results, SARs or physicochemical properties indicative of any neurotoxic potential should also be considered.

Where initial indication of potential neurotoxicity is seen at Tier 1, further neurotoxicity testing (OECD TG 424) should be considered. Such testing is aiming to confirm or further characterise (and quantify) the potential neurotoxic response induced by the test substance and should be carried out on a case-by-case basis. Information from the other studies should also be considered to improve the design with respect to dose selection in order to address confounding effects by general toxicity. Further specialised studies can also be performed to elucidate mechanisms in order to extrapolate from animals to humans and to further characterise and complete the risk assessment.

The tiered approach to testing outlined in this guidance includes, at Tier 1, a 90-day study in rats (OECD TG 408). This study involves investigation of the effect of the food additive on a number of parameters that may be indicative of a neurotoxic effect. These include: changes in clinical signs, functional observational battery, motor activity and brain weight relative to body weight in the absence of overt toxicity, histopathological changes in this organ.

The effects may be confirmed or, alternatively, seen for the first time in Tier 2 studies, notably the EOGRTS (OECD TG 443), but also in chronic toxicity/carcinogenicity studies conducted according to OECD TGs 452, 451 or 453. In the EOGRTS, a cohort of animals is specifically dedicated to assess the potential impact of exposure on the developing nervous system. In the studies, data will be derived from detailed clinical observations, auditory startle, a functional battery, motor activity and neuropathology assessments of the F1-pups and adult animals.

The evaluation of the potential of a food additive to adversely affect the nervous system may be based on an integrated assessment of the results obtained from these toxicity studies (Tiers 1 and 2). If these results indicate that the food additive has such a potential, additional Tier 3 studies should be considered on a case-by-case basis. These will normally be designed to investigate the underlying mechanisms of the effects seen, and/or their biological significance.

Tier 3 studies may include more extensive behavioural and morphological tests in a developmental neurotoxicity study. Guidance for these tests can be found in OECD TG 426.

5. Supplementary information

5.1. Integrated testing strategies

The Panel noted the continuing development of integrated testing strategies (ITS) and welcomed the use to complement the data required in this guidance. Alternative methods may be used aiming to fulfil the goals as determined by the concept of the 3Rs. ITS are anticipated to refine, reduce or (partly) replace (3Rs) current traditional toxicological approaches (EC, 2008; National Research Council, 2007; van Leeuwen et al., 2007). ITS approaches comprise methods that can efficiently generate toxicological data for both hazard identification and risk assessment, hereby aiming to reduce costs and minimize the need for experimental animals.

The most recent review on ITS, the alternative methods available and the time frame to further develop the methods for a full replacement of *in vivo* testing was published by a group of authors with respect to the requirements of the 7th amendment to the European Union's Cosmetics Directive (76/768/EEC¹⁵) (Adler et al., 2011). This review is also applicable to the present guidance.

5.2. Mechanisms and Modes of action

Studies on the mode of action may be used to investigate the relevance of findings in animals for humans. These studies can examine the mode of action for carcinogenic effects or other endpoints such

¹⁵ Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products. <http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1976L0768:20100301:en:PDF>

as endocrine disruption and should use the appropriate MOA (mode of action) frameworks when assessing the data (IPCS, 2006; Boobis, 2006; Boobis, 2008).

5.3. Review of published literature

Applicants should review the published literature for relevant references. This should be based on the principles underpinning systematic reviews. The methods used to identify relevant data and other information, including the scope and criteria of literature searches, should be described.

5.4. Reporting of studies

Overview and evaluation of toxicological data

In compiling the data in the submission, applicants should also seek to interpret the data and draw conclusions. The significant findings of each study (both commissioned and published) should be highlighted, together with identification of the POD, the BMDL5 value for continuous data, the BMDL10 value for quantal data or the NOAEL, if one has been determined, and any other relevant information. There should also be an evaluation of the whole dossier clearly describing the POD from individual studies and identifying the critical one. The reasons for disregarding any findings should be carefully explained. Where necessary, the conclusions should include an interpretation of the importance of the findings in terms of possible mechanisms underlying any effects observed, a discussion of whether these are relevant to humans and, if so, the possible importance of the extrapolation of such findings to humans.

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Glossary

Absorption (oral): The uptake of a chemical after oral administration from the gut into the cells lining the gut wall by transcellular processes and/or into the blood or lymph by either transcellular processes through the gut epithelium and/or by a paracellular pathway(s) (e.g. persorption). Because of potential pre-systemic elimination, absorption does not necessarily lead to systemic availability, but absorption is necessary for a chemical to become systemically available.

Systemic availability is the proportion of the parent compound that after absorption reaches the (post-hepatic) systemic blood circulation. Thus, a chemical that is absorbed and completely eliminated (including any hepatic-derived metabolites) via the hepato-biliary circulation, is not considered to have been systemically available. In Toxicology, the term "**bioavailability**" is often used interchangeably with the term "systemic availability". In most cases, systemic availability is related to the toxicity (if not the metabolites are the active species). However, if the target organ for a chemical's toxicity is the liver, then systemic availability is not a pre-requisite for toxicity.

Absorption can be determined by measuring the disappearance of the chemical from the gut, by measuring the cumulative excretion of the chemical and its metabolite(s) in urine and bile, and by comparison of the area under the concentration-time curve after oral versus intravenous administration (provided that first pass metabolism in the gut wall and biliary excretion are excluded).

Systemic availability can be determined by comparing the area under the concentration-time curve after oral versus intravenous administration.

ADI: The ADI is the estimated maximum amount of an agent, expressed on a body mass basis, to which individuals in a (sub)population may be exposed daily over their lifetimes without appreciable health risk (standard human 70 kg). The ADI is listed in units of mg/kg of 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 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.

Batch: The quantity of material produced in one operation BMD: The benchmark dose is a dose level, derived from the estimated dose-response curve, associated with a specified change in response (i.e. low incidence of risk generally in the range 1-10%), the Benchmark Response (BMR). The BMD approach makes extended use of the dose-response data from studies in experimental animals or from observational epidemiological studies to better characterise and quantify potential risks. The BMD approach is of particular value for i) situations where the identification of a NOAEL is uncertain, ii) providing a Reference Point for the Margin of Exposure in case of substances that are both genotoxic and carcinogenic, and iii) dose-response assessment of observational epidemiological data. The BMD approach is used as an alternative to the traditionally used NOAEL approach.

BMDL:	The lower boundary of the confidence interval on the benchmark dose. The BMDL accounts for the uncertainty in the estimate of the dose-response that is due to characteristics of the experimental design, such as sample size. The BMDL can be used as the Reference Point (Point of Departure) for derivation of a health-based guidance value or margin of exposure.
BMDL5:	The benchmark dose lower confidence limit 5% is the dose where the response is likely to be smaller than 5% (where the term likely is defined by the statistical confidence level, usually 95%-confidence). BMDL5 is used for continuous data.
BMDL10:	The benchmark dose lower confidence limit 10% is an estimate of the lowest dose which is 95% certain to cause no more than a 10% cancer incidence in rodents (EFSA, 2009a). BMDL10 is used for quantal data.
CSAFs:	(Chemical-specific adjustment factor) A modified default 10-fold uncertainty factor that incorporates appropriate data on species differences or human variability in either toxicokinetics or toxicodynamics.
ENM:	A nanomaterial produced either intentionally or unintentionally (due to the production process) to be used in the food and feed area. It refers to a material with at least one size measurement between approximately 1 and 100nm. Within the context of the EFSA ENM Guidance (EFSA, 2011a), the term "engineered" is equivalent to the term "manufactured" and/or "proposed" as used in other reports.
Food additive:	Food additives are substances that are not normally consumed as food itself but are added to food intentionally for a technological purpose.
Food category:	A number of foods where food additives are already authorised or are to be authorised at the same maximum intended use level or the maximum permitted level, as listed in Annex II of Commission Regulation 1129/2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. For example, confectionary non-alcoholic beverages, fine bakery wares, etc.
Food intolerance:	Any illness or biochemical or metabolic abnormality caused by the ingestion of any food or dietary component without implying any specific mechanism. Food intolerances occur through non-immunological reactions to food (or do not include any immune mechanisms). Occasionally food intolerances cause symptoms similar to those of food allergies (pseudo-allergic reactions). Pseudo-allergic reactions can be triggered in various ways such as interaction with the central or peripheral nervous system, non-specific release of mediators, enzyme inhibition due to hereditary or pharmacologically induced enzyme deficiencies and pharmacological properties of some natural food constituents (e.g. biogenic amines). Other synonyms include pseudo-hypersensitivity reactions or non-allergic hypersensitivity.
LOAEL:	(Lowest-Observed-Adverse-Effect-Level) Lowest concentration or amount of a substance, found by experiment or observation, that causes an adverse alteration of morphology, functional capacity, growth, development or lifespan of the target organism distinguishable from normal (control) organisms of the same species and strain under the same defined conditions of exposure.
MOE:	Ratio from a Point of Departure (Reference point) for the critical effect to the theoretical, predicted, or estimated exposure dose or concentration. For the purposes of risk assessment by the ANS Panel the term Margin of Exposure (MOE) is used when compounds are genotoxic and carcinogenic for comparison of the exposure with a benchmark dose.
MOS:	Ratio from a Point of Departure for the critical effect to the theoretical, predicted, or estimated exposure dose or concentration. For the purposes of risk assessment by the ANS panel the term Margin of Safety (MOS) is used for

compounds which are not genotoxic for which a threshold approach can be applied.

NOAEL: (No-Observed-Adverse-Effect-Level) 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.

Non-ENMS: (or Natural Nanomaterials) Materials from mineral or animal origin that have inherent nanostructure properties.

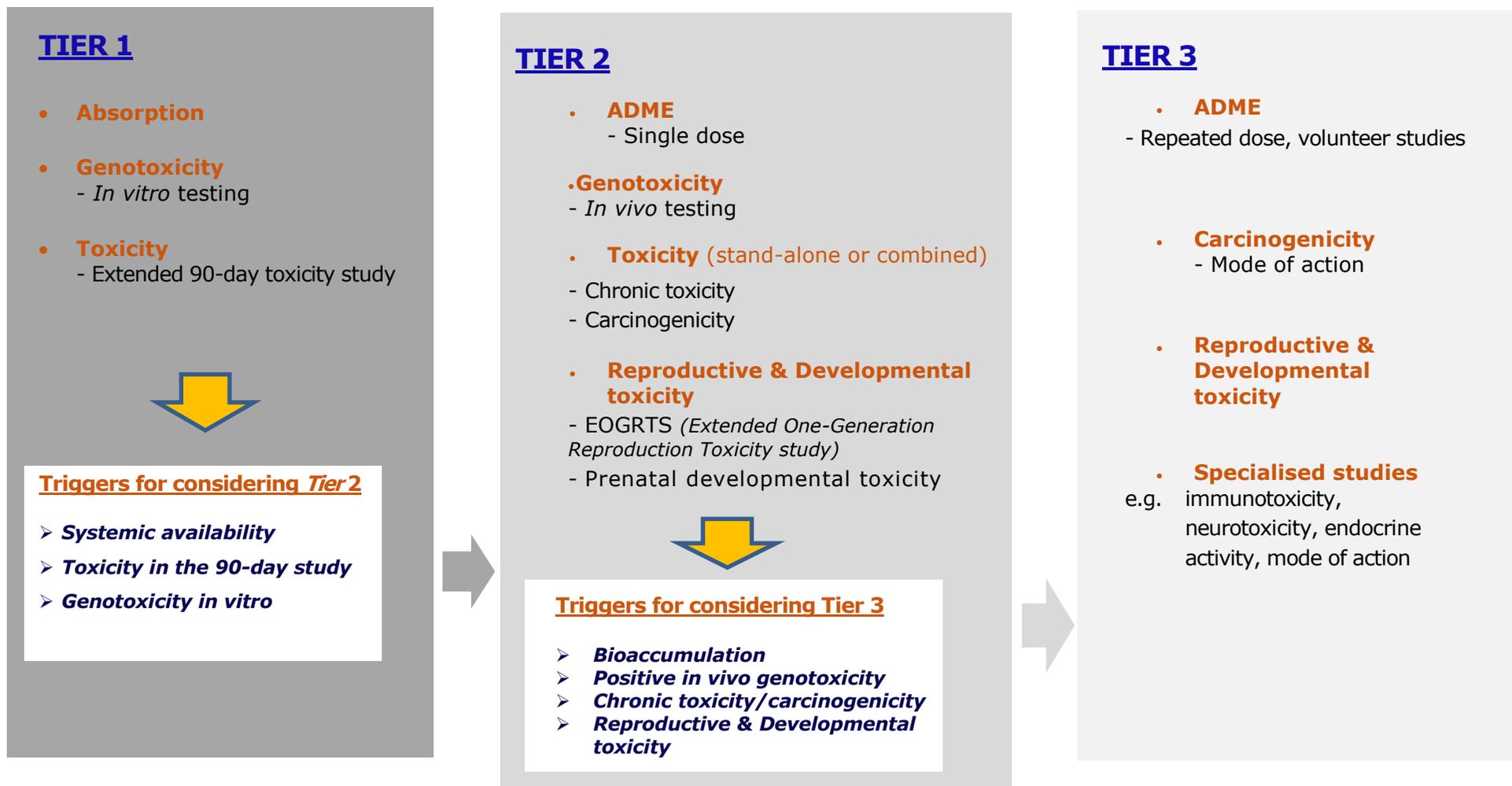
Abbreviations

ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism and Excretion
ANS	Scientific Panel on Food Additives and Nutrient Sources added to food
AUC	Area under the curve
B cell	B lymphocyte
BMD	Benchmark dose
BMDL	Benchmark dose Lower
CAS	Chemical Abstracts Service
CSAF	Chemical-Specific Adjustment Factor
DCM	Dietary and Chemical Monitoring unit
EC	European Commission
EFSA	European Food Safety Authority
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of Notified Chemical Substances
EMA	European Medicines Agency
ENM	Engineered Nanomaterials
EOGRTS	Extended One-Generation Reproduction Toxicity study
EU	European Union
FAIM	Food additive intake(s) model
GD	Guidance document (OECD)
GLP	Good Laboratory Practice
GMM	Genetically Modified Microorganism
GMO	Genetically Modified Organism
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IgE	Immunoglobulin E
IgM	Immunoglobulin G
IPCS	International Programme on Chemical Safety
ITS	Integrated Testing Strategies
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KLH	Keyhole Limpet Hemocyanin
LOAEL	Low Observed Adverse Effect Level
LOD	Limit of detection
LOQ	Limit of quantification
MOA	Mode of action
MADB106	Rat mammary adenocarcinoma derived cell line
MOE	Margin of Exposure

MOS	Margin of Safety
MS	Mass Spectroscopy
MTD	Maximum Tolerated Dose
NK	Natural killer cell
NMR	Nuclear Magnetic Resonance
NOAEL	No Observed Adverse Effect Level
OECD	The Organisation for Economic Co-operation Development
PAH	Polycyclic Aromatic Hydrocarbon
POD	Point of Departure
PYB6	Mouse polyomavirus-induced tumour cell line
QPS	Qualified Presumption of Safety
(Q)SARs	(Quantitative) structure-activity relationships
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
3-Rs	Replacement, refinement, reduction
SAR	Structure activity relationship
SC	EFSA Scientific Committee
SCF	Scientific Committee on Food
SCOOP	Scientific Cooperation
SRBC	Sheep Red Blood Cells
tADI	Temporary ADI
T cell	T lymphocyte
TG	Testing guideline (OECD)
TTC	Threshold of Toxicological Concern

Appendix A – Tiered toxicity testing for food

Although higher tier testing may be required based on results in one of the core areas, such testing would only be required in relevant core areas e.g. where results from absorption or the 90-day study require further tier 2 studies but tier 1 is negative, there would be no need for tier 2 genotoxicity.



Appendix B – Data Requirements For The Evaluation Of Food Additive Applications

USE A STATEMENT FROM THE PANEL MADE AT THE TIME

Scientific Statement of the Panel on Food Additives and Nutrient Sources added to Food (Question No EFSA-Q-2007-188)

B.1. Introduction

The present statement defines the general data requirements, while specific scientific approaches are suggested in the guidance for food additives applicable at the time of the application. During its second plenary meeting in September 2008, the Panel endorsed provisionally the guidance document for food additive evaluations adopted by the Scientific Committee on Food (SCF) in 2001. In order to reflect current thinking in risk assessment, the Panel will commence a detailed reappraisal of the guidance in September 2009. It is anticipated that, following a period of public consultation, this new guidance will be finalised in July 2011. Applicants should also take into consideration the opinions adopted in 2009 by the Scientific Committee of EFSA on Nanoscience and Nanotechnologies, on the use of the benchmark dose approach in risk assessment and on the replacement and reduction of animal testing, as well as the guidance on transparency in the scientific aspects of risk assessments adopted in 2009 and the guidance on the safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements adopted in 2008.

B.2. Data requirements

A dossier submitted in support of an application for the evaluation of a food additive should enable an assessment to be made of the additive based on the current state of knowledge and permit verification that the additive does not, on the basis of the scientific evidence available, pose a safety concern to the health of the consumer at the level of use proposed, as laid down in Article 6 of Regulation (EC) No 1333/2008.

The application dossier should include all the available data relevant for the purpose of the risk assessment.

The documentation on the gathering of the data used in the dossier should also be provided. This documentation should specify the data gathering conducted and especially the literature search strategies (assumptions made, key words used, databases used, limitation criteria, etc.).

The comprehensive outcome of the literature search should also be provided. The individual raw data of the unpublished studies should be available. The individual results of examinations and raw data, including microscopic slides, should also be available. The safety evaluation strategy and the corresponding testing strategy should be described and justified with rationales for inclusion and exclusion of specific studies. Information should be provided on:

- the applicant and the application dossier (administrative data)
- the identity and characterisation of the additive (including the proposed specifications and analytical method)
- the manufacturing process
- the stability, reaction and fate in foods to which the additive is added
- the case of need and proposed uses
- the existing authorisations and evaluations
- the exposure assessment
- the biological and toxicological data.

Regarding the biological and toxicological data, the following core areas should normally be covered:

- Toxicokinetics
- Subchronic toxicity
- Genotoxicity
- Chronic toxicity/carcinogenicity
- Reproductive and developmental toxicity

Applicants are reminded that for each study performed it should be stated whether the test material conforms to the proposed or existing specification. Where the test material differs from this specification, the applicant should demonstrate the relevance of these data to the food additive under consideration.

Overall conclusions should be proposed by the applicant on the safety of the proposed uses of the additive. The overall evaluation of potential human risk should be made in the context of known or likely human exposure, including that from other sources. Details of any applications made to other evaluation bodies or regulatory agencies together with their status and outcome should be disclosed. During the evaluation process, EFSA may request any additional data that is considered necessary for the safety assessment.

B.3. Additional Technical Information

Petitioners are also advised to provide reviews of the scientific literature for their additive and to report these with their criteria search strategies and search terms. These reviews should also summarise any existing authorisations for the additive including pending or unsuccessful submissions for any uses together with the basis used by the relevant authorities in making these decisions. The Panel will make its own evaluation on the specifics of the application and is not bound by these evaluations. Whilst other evaluations might inform the decision, petitioners are reminded that there can (and will) be differences in the scientific interpretation of the significance of findings and that the acceptability of individual findings is judged within the basis of the risk management context which determines the acceptability of both risk and uncertainty. Systematic reviews provide a tool for undertaking these literature reviews. The Panel encourages the application of the key elements of the systematic review process. The minimum requirements for a literature review are the search strategies applied, the definition of inclusion and exclusion criteria, documentation of how these were applied to the searches and a review of those papers meeting the inclusion criteria. After the initial premarketing evaluation these searches and reviews need to be kept up to date to facilitate future re-evaluations.

Appendix C – Specifications as required by the Commission

E number	
Synonyms	
Definition	
EINECS	XX-XX-X
Colour Index No	
Chemical names	
Chemical formula	
Molecular/ Atomic weight /Weight average molecular weight	
Particle size of powder	
Assay ¹⁶	
Description	
Appearance of a solution	
Identification	
Spectrophotometry, spectrometry, chromatography, Infra Red, X-ray diffraction	
Density/specific gravity	XX (20°C) (25/25°C)
Refractive Index	
Specific rotation	
pH	XX-XX (XX% aqueous solution)
Degree of hydrolysis/ decomposition/ properties during burning	
Precipitation reaction	
Colour reaction	
Melting range or point	XX to XX °C
Viscosity	
Solubility	
Boiling point	
Specific identification tests and parameters	
Congealing range	
Distillation range	
Drop point	
Isoelectric point	
Solidification point	
Sublimation point	
Vapour pressure	
Microscopic observation/ examination	
Purity	
Loss on drying	
Loss on ignition	
Water or HCl insoluble matter	
Water content	
Conductivity	
Acid/Hydroxyl value	
Acidity/ alkalinity	
Oil content	
Fat	
Protein	
Total sugars	

¹⁶ In accordance with Directive 2008/84/EC on specifications of food additives others than colours and sweeteners, the following definition of assay taken from the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006) (<ftp://ftp.fao.org/docrep/fao/009/a0691e/a0691e00a.pdf>) 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.

Starch	
Sodium chloride	
Ash	Not more than XX% (XXX°C)
Viscosity	Not less/more than XXX mPa.s
Wax	
Residual Solvents	Not more than XXmg/Kg
Residue on ignition	
Non-volatile residue	
Organic Volatile impurities	
Aldehydes	
Unsaponifiable matter	
Saponification value	
Ester value	
Iodine value	
Peroxide value / peroxides	
Oxidising/reducing substances	
Readily carbonisable substances	
Specific parameters for impurities	
Other specific parameters indicating the degree of purity	
Chlorinated compounds	
3-Monochloropropane-1,2-diol (3 MCPD)	
Polycyclic Aromatic Hydrocarbons	
Organic compounds other than colouring matters	
Pentachlorophenol	
Epoxides	
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
Aluminium/ aluminium oxides	Not more than XX mg/Kg (expressed as Al)
Copper	
Nickel	
Antimony	
Chromium	
Selenium	
Fluorides	
Microbiological criteria	
<i>Salmonella spp</i>	
<i>Escherichia Coli</i> (coliforms)	
<i>Staphylococcus aureus</i>	
Yeasts and moulds	
Total bacterial count	
Total plate count	
Other safety or purity related microbiological criteria	