

## SCIENTIFIC OPINION

### **Guidance for the preparation of dossiers for sensory additives<sup>1,2+</sup>**

**This guidance replaces the earlier version published on 21 October 2009<sup>3</sup>**

**EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)<sup>4</sup>**

European Food Safety Authority (EFSA), Parma, Italy

This guidance document follows the structure and definitions of [Regulation \(EC\) No 1831/2003](#) and its implementing rules ([Regulation \(EC\) No 429/2008](#)). It is intended to assist the applicant in the preparation and the presentation of its application, as foreseen in Article 7.6 of [Regulation \(EC\) No 1831/2003](#). This document does not substitute for the obligation of an applicant to comply with the requirements of [Regulation \(EC\) No 1831/2003](#) and its implementing rules.

A sensory additive is any substance, the addition of which to feed improves or changes the organoleptic properties of the feed, or the visual characteristics of the food derived from animals. The category 'sensory additives' is further grouped into two functional groups:

- (a) colourants:
  - (i) substances that add or restore colour in feedingstuffs;
  - (ii) substances which, when fed to animals, add colours to food of animal origin;
  - (iii) substances which favourably affect the colour of ornamental fish or birds;
- (b) flavouring compounds: substances the inclusion of which in feedingstuffs increases feed smell or palatability.

---

<sup>1</sup> On request from EFSA, Question No EFSA-Q-2009-00832, adopted on 14 October 2009. Revised on 12 November 2009.

<sup>2</sup> This guidance document replaces the previous guidance document for the preparation of dossiers for sensory additives (EFSA-Q-2008-403e), adopted on 16 September 2008

<sup>+</sup> Parts in italics are coming from Regulation (EC) No 429/2008

<sup>3</sup> Revision 2 (9 December 2009). Section 3.1.1 has been amended.

<sup>4</sup> Gabriele Aquilina, Georges Bories, Paul Brantom, Francesca Caloni, Andrew Chesson, Pier Sandro Coconcelli, Noël Dierick, Mikolaj Antoni Gralak, Jürgen Gropp, Ingrid Halle, Nils-Gunnar Ilbäck, Joop de Knecht, Reinhard Kroker, Lubomir Leng, Sven Lindgren, Anne-Katrine Lundbye Haldorsen, Alberto Mantovani, Miklós Mézes, Derek Renshaw, Maria Saarela. Correspondence: [FEEDAP@efsa.europa.eu](mailto:FEEDAP@efsa.europa.eu)

Suggested citation: Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Guidance for the preparation of dossiers for sensory additives. EFSA Journal 2009;7(10):1352. [26 pp.]. doi:10.2093/j.efsa.2009.1352. Available online: [www.efsa.europa.eu](http://www.efsa.europa.eu)

## THE TECHNICAL DOSSIER – GENERAL ASPECTS

*The dossiers must enable an assessment to be made of additives based on the current state of knowledge and permit verification of the compliance of these additives with the fundamental principles for authorisation, which are laid down in Article 5 of [Regulation \(EC\) No 1831/2003](#).*

The studies to be submitted and the extent of them will depend on the additive nature, the functional group, the substance itself, the target animals and the conditions of use. The applicant should refer to [Regulation \(EC\) No 429/2008](#) in order to evaluate which studies and information should be submitted with the application.

Reasons must be given for the omission from the dossier of any data prescribed there.

*The dossier shall include detailed reports of all the studies performed, presented in accordance with the numbering system proposed in [Regulation \(EC\) No 429/2008](#). The dossier shall include references and copies of all published scientific data mentioned and the copies of any other relevant opinions which have already been produced by any recognised scientific body. Where these studies have already been evaluated by a European scientific body following the legislation in force in the Community, a reference to the result of the evaluation should be sufficient and a copy should be provided. Data from studies that have been conducted and published previously or coming from peer review should clearly refer to the same additive as the one subject to the application for authorisation.*

*Studies, including those that have been conducted and published previously or coming from peer review, shall be performed and documented according to appropriate quality standards (e.g., good laboratory practice (GLP) in accordance with [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 or International Organization for Standardization (ISO).*

*Where in vivo or in vitro studies are carried out outside the Community, the applicant shall demonstrate that the facilities concerned comply with the Organisation for Economic Cooperation and Development (OECD) [Principles of Good Laboratory Practice](#) or ISO standards.*

*The determination of physico-chemical, toxicological and eco-toxicological properties must be performed in accordance with the methods established by [Council Directive 67/548/EEC](#) of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, as last amended by [Commission Directive 2004/73/EC](#), or with updated methods recognised by international scientific bodies. The use of methods other than these must be justified.*

The studies involving animals should respect the rules on animal welfare laid down by European Community legislation, and they should not be repeated if not necessary. *The use of in vitro methods or of methods refining or replacing the usual tests using laboratory animals or reducing the number of animals used in these test should be encouraged. Such methods should be of the same quality and provide the same level of assurance as the method they aim to replace.*

*The description of the methods of analysis in feed or water shall be in conformity with the rules of Good Laboratory Practice (GLP) as laid down in [Directive 2004/10/EC](#) and/or EN ISO/IEC 17025. These methods shall comply with the requirements laid down in Article 11 of [Regulation \(EC\) No 882/2004](#) of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.*

*Each dossier shall contain a public summary and a scientific detailed summary in order to enable the additive concerned to be identified and characterised and a labelling proposal as referred to in Article 7(3)(e) of [Regulation \(EC\) No 1831/2003](#).*

A post-market monitoring proposal should be proposed only for those additives which consist of, contain or are produced from genetically modified organisms as required by Article 7(3)(g) of [Regulation \(EC\) No 1831/2003](#).

## 1. SECTION I: SUMMARY OF THE DOSSIER

### 1.1 Public summary according to Article 7(3)(h) of Regulation (EC) No 1831/2003

*The applicant shall submit a summary indicating the main features of the additive concerned. The summary shall not contain any confidential information and shall be structured as follows:*

#### 1.1.1 Contents

- a) name of the applicant(s);
- b) identification of the additive;
- c) method of production and method of analysis;
- d) studies on safety and efficacy of the additive;
- e) proposed conditions for use; and
- f) proposal for post-market monitoring.

#### 1.1.2 Description

- a) name and address of the applicant(s)

*This information shall be provided in all cases. When a dossier is submitted by a group of applicants, the name of each of them should be indicated.*

- b) identification of the additive

*The identification of the additive shall contain a summary of the information required according to Annex II and III of [Regulation \(EC\) No 429/2008](#), depending on the type of the feed additive authorisation. In particular: name of the additive, proposed classification by category and functional group, target species/animal categories and doses.*

- c) method of production and method of analysis

*The manufacturing process shall be described.*

*The general procedures of the analytical methods to be used for the analysis for the official controls of the additive as such, in premixtures, and in feedingstuffs, as required in Annex II and III of [Regulation \(EC\) No 429/2008](#) shall be described. If appropriate, on the basis of the information submitted, the procedure of the method(s) to be used for the analysis for the official controls of the additives or its metabolites in food of animal origin should be included.*

- d) studies on safety and efficacy of the additive

*The conclusion regarding the safety and efficacy of the additive based on the different studies performed shall be given. The results of the studies may be included in a tabular form to support the conclusion of the applicant(s). Only studies required according to Annex III of [Regulation \(EC\) No 429/2008](#) shall be indicated in the summary.*

- e) proposed conditions for use

*The proposal for conditions of use shall be provided by the applicant(s). In particular the applicant shall describe the level of use in water or feed, together with the detailed conditions of use in complementary feedingstuffs. Information is also required where other methods of administration or incorporation in feed or water are used. Any specific conditions for use (e.g. incompatibilities), specific labelling requirements and animal species for which the additive is intended shall be described.*

- f) proposal for post-market monitoring

This part is only required for additives falling within the scope of Community legislation relating to the marketing of products consisting of, containing or produced from GMOs.

## 1.2 Scientific summary of the dossier

*A scientific summary including details of each part of the documents submitted to support the application shall be submitted. This summary shall include the conclusions made by the applicant(s).*

*The summary must follow the order of Annex II of [Regulation \(EC\) No 429/2008](#) and address all the different parts with reference to the relevant pages of the dossier.*

## 1.3 List of documents and other particulars

*The applicant must identify the number and titles of volumes of documentation submitted in support of the application. A detailed index with reference to volumes and pages shall be added.*

## 1.4 List of parts of the dossier requested to be treated as confidential, where necessary

*The list shall make reference to the relevant volumes and pages of the dossier.*

# PART 1 - COLOURANTS

## 2. SECTION II: IDENTITY, CHARACTERISATION AND CONDITIONS OF USE OF THE ADDITIVE; METHODS OF ANALYSIS.

*The additive has to be fully identified and characterised.* For the majority of sensory additives, which are not subject to a specific holder of the authorisation, the paragraphs 2.1.2, 2.1.3, 2.1.4, 2.1.4.2, 2.2, 2.3.1, 2.4.1, 2.4.2, 2.4.4, 2.5, 2.6 apply. For those sensory additives subject to a specific holder of the authorisation (i.e., additives falling within the scope of Community legislation relating to the marketing of products consisting of, containing or produced from GMOs), the whole Section II applies (follow the section II of the guidance for [zootechnical additives](#)).

### 2.1 Identity of the additive

The additive has to be fully identified and characterised. The studies described in this section must be based on the final product(s) for which authorisation is sought. In-house identifiers should be avoided unless embedded in third-party documents. In this case a statement is required to confirm that the identifier(s) refers to the formulation(s) for which the claim is made.

#### 2.1.1 Name of the additive

The name of the additive (characterisation of the active substance(s) as defined in the subsection 2.2.1) should be given.

#### 2.1.2 Proposal for classification

In addition to the classification “sensory additive/colourant” a proposal for the classification of the additive for additional categories<sup>5</sup> and functional groups according to its main functions under Article 6 and Annex I of [Regulation \(EC\) No 1831/2003](#) can be made.

<sup>5</sup> If the applicant applies for one or more categories in addition to sensory additives, reference should be made to the relevant guidance document(s).

Any data from other known uses of the identical active substances (e.g., use in food, human or veterinary medicine, agriculture and industry) must be provided. Any other authorisation as feed or food additive, veterinary drugs or other kind of authorisations of the active substance has to be specified and properly referenced.

### 2.1.3 Qualitative and quantitative composition (active substance, other components, impurities, batch to batch variation)

The active substance(s) and all other components of the additive should be listed, giving the proportion by weight in the final product. Evidence should be provided by the analysis of at least five production batches that the amount and nature of the active substance(s) in the additive specified by the applicant is satisfied in practice.

If the active component of the additive is a mixture of active substances, each of which is clearly definable (qualitatively and quantitatively), the active substance(s) must be described separately and the proportions in the mixture given.

Without prejudice to any request for supplementary information made by the EFSA according to Article 8(2) of [Regulation \(EC\) No 1831/2003](#), the applicant may omit the description of other components with no safety concerns other than active substances or agents for additives not within the scope of [Regulation \(EC\) No 1829/2003](#).

### 2.1.4 Purity

The applicant shall identify and quantify chemical and microbial impurities, substances with toxic or other undesirable properties that are not intentionally added and do not contribute to the activity of additive. Any substances produced via fermentation should be free of antimicrobial activities relevant to the use of antibiotics in humans or animals. In addition the absence of production organisms in the additive should be confirmed.

The protocol used for the routine screening of production batches for contaminants and impurities shall be described.

All the data provided have to support the proposal for a specification of the additive.

Monitoring for contaminants and impurities should be consistent with existing legislation (e.g., [Directive 2002/32/EC](#), or specifications from [European Community food additive authorisations](#)) and recommendations from internationally recognised sources when these are available (e.g., Joint FAO/WHO Expert Committee on Food Additives (JECFA) specifications; [Commission recommendation on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding](#)). Additional measures should be introduced following the HACCP analysis of the specific process, as necessary.

As a guide the following should be considered as minimum requirements:

- for fermentation/cultivation products: microbiological contamination (*Salmonella*, total coliforms, *E. coli*), mycotoxins,<sup>6</sup> heavy metals (Pb, Hg, Cd) and arsenic. *The extent to which spent growth medium is incorporated into the final product shall also be indicated.* For fermentation products produced by genetically modified micro-organisms, identification and quantification of recombinant DNA in the final product should be provided.
- for plant derived substances: microbiological and botanical contamination (e.g., castor oil plant, weed seeds, rye ergot in particular), mycotoxins, dioxins (PCDD/F) and dioxin-like PCBs, pesticides,<sup>7</sup> maximum values for solvents and, where appropriate, substances of toxicological concern known to occur in the original plant;

<sup>6</sup> The selection of mycotoxins for analysis should be made according to the different matrices, where appropriate.

<sup>7</sup> Residues specified under the undesirable substances directive (Directive 2002/32/EC) and any other pesticide residues of potential concern to target animals and/or consumer safety.

- for animal derived substances: microbiological contamination, heavy metals and arsenic and maximum values for solvents, where appropriate;
- for mineral substances: heavy metals and arsenic, dioxins (PCDD/F) and dioxin-like PCBs;
- for products produced by chemical synthesis and processes: all chemicals used in the synthetic processes and any intermediate products remaining in the final product should be identified and their concentrations given.

### 2.1.5 Physical state of each form of the product

EFSA recommends the provision of particle size distribution/dusting potential for solid preparations, specific weight for liquid preparations and solubility or dispersability where the additive is intended to be used in water. Studies on particle size distribution should take into consideration particles of inhalable ( $\leq 100 \mu\text{m}$ ) and respirable ( $\leq 10 \mu\text{m}$ ) size.

## 2.2 Characterisation of the active substance(s)

### 2.2.1 Description

A qualitative description of the active substance(s) should be given. This should include purity and origin of the substance(s), plus any other relevant characteristics.

#### 2.2.1.1 Chemical substances

*Chemically well-defined substances should be described by generic name, chemical name according to the International Union of Pure and Applied Chemistry (IUPAC) nomenclature, other generic international names and abbreviations and/or Chemical Abstract Service (CAS) Number. The structural and molecular formula and molecular weight must be included. Where relevant, data on isomeric forms and accompanying structurally related compounds should be included.*

Additives of plant origin used as a source of colourants should be defined in terms of the plant and part(s) of the plant used and the nature of the extraction method. The Latin name of the plant, including species, and where relevant, recognised variety should also be provided. Trivial name(s) need only to be included when necessary to clarify data included in the dossier.

The microbial origin (bacteria, yeasts, filamentous fungi and micro-algae) of colourants produced by fermentation/cultivation should be described and any history of modification of the production organism should be indicated. *The name and taxonomic classification of each micro-organism shall be provided, according to the latest published information in the International Codes of Nomenclature (ICN). Microbial strains shall be deposited in an internationally recognised culture collection (preferably in the European Union) and maintained by the culture collection for the authorised life of the additive. A certificate of deposition from the collection, which shall specify the accession number under which the strain is held, must be provided.*

### 2.2.2 Relevant properties

#### 2.2.2.1 Chemical substances

*Description of physical and chemical properties should be given. Dissociation constant, pKa, electrostatic properties, melting point, boiling point, density, vapour pressure, solubility in water and in organic solvents,  $K_{ow}$  and  $K_d/K_{oc}$ , mass spectrometry and absorption spectra, NMR data and any other relevant physical properties should be provided where appropriate.*

### 2.2.2.2 Micro-organisms (as source of the additive)

Micro-organisms used as a production strain should not be capable of producing antibiotic substances that are relevant to antibiotics in human and veterinary medicine.

Strains of micro-organisms belonging to a taxonomic group that includes members known to be capable of producing toxins or other virulence factors shall be subject to appropriate tests to demonstrate at a molecular and, if necessary, cellular level the absence of any cause for concern. As an example on how to assess the potential for toxin production see the technical [guidance on toxin production in \*Bacillus\* spp.](#)

## 2.3 Manufacturing process, including any specific processing procedures

*To define the critical points of the process that may have an influence on the purity of the active substance or additive a detailed description of the manufacturing process shall be given. A material safety data sheet of chemicals used in the production process shall be provided.*

### 2.3.1 Active substance(s)

A description of the production process (e.g., chemical synthesis, fermentation, cultivation, extraction from organic material or distillation and downstream purification steps) used in the preparation of the active substance(s) of the additive should be submitted, if appropriate by means of a flowchart. *The composition of the fermentation/cultivation media should be provided.* For genetically modified micro-organisms used as source of additives and grown under contained conditions, [Directive 90/219/EC](#) applies.

### 2.3.2 Additive

*A detailed description of the manufacturing process of the additive should be submitted. The key stages in the preparation of the additive including the point(s) of introduction of the active substance(s) and other components, and any subsequent process steps affecting the additive preparation should be provided, if appropriate by means of a flowchart.*

## 2.4 Physical-chemical and technological properties of the additive

### 2.4.1 Stability

Stability is assessed through the persistence of the active substance (or rarely by the persistence of colour in feedingstuffs). Data should be provided from at least three batches that include at least one observation at the beginning and one at the end of the storage period.

Where there is a loss of stability, measured by the analytical follow-up of the active substance, potential degradation or decomposition products should be characterised, where appropriate.

Stability studies are not required for metal oxides and carbon black used as colorants.

#### 2.4.1.1 Shelf life of the additive

The stability on exposure to defined environmental conditions (light, temperature, pH, moisture, oxygen and packing material, as appropriate) shall be studied for each formulation of the additive.

The expected shelf-life of the additive as marketed should be proposed, based on at least two model situations covering the likely range of use conditions (e.g., for a solid formulation 25°C, 60% relative air humidity (RH) and 40°C, 75% RH).

If the shelf-life is already established for an additive authorised for use in food, the relevant studies should be summarised. No additional studies would be required.

### 2.4.1.2 Stability of the additive used in premixtures and feedingstuffs

The stability of each formulation of the additive normally should be studied in feedingstuffs manufactured and stored under common conditions, and if relevant, in premixtures. The quantitative and qualitative composition of the premixtures or the feedingstuffs used for the studies should be given.

Stability studies in premixtures and feedingstuffs should be of at least six and three months' duration, respectively.

Stability should be tested preferably in a premixture containing trace elements; otherwise the additive should be labelled as "not to be mixed with trace elements". Stability in feedingstuffs should be assessed in both mash and further processed feed (e.g., pelleted, extruded or canned, including the influence of the respective processing) for the main animal species of the claim.

### 2.4.2 Homogeneity

The capacity for homogeneous distribution of the feed additive in premixtures, feedingstuffs or water must be demonstrated, as appropriate. The same criteria as described under 2.4.1 should be used. As a guide, a minimum of ten sub-samples from a single batch (of the premixture or feedingstuff) should be analysed and the coefficient of variation calculated.

For additives intended to be used in premixtures and feedingstuffs, homogeneity should be studied at different stages of production of premixtures and feedingstuffs (e.g., after mixing, after pelleting), and if appropriate during/after its transportation (or simulated conditions). If homogeneity is demonstrated in the final feedingstuff, there is no need to demonstrate homogeneity of mixing at any preceding stages in feed production (including premixtures).

### 2.4.4 Physico-chemical incompatibilities or interactions

*Physico-chemical incompatibilities or interactions that could be expected with feed, carriers, other approved additives, or medicinal products must be shown.*

## 2.5 Conditions of use of the additive

### 2.5.1 Proposed mode of use in animal nutrition

The proposed use in feed should be defined. *The animal species or categories, age group or production stage of animals shall be indicated, as appropriate, in accordance with the categories listed in Annex IV of [Regulation \(EC\) No 429/2008](#). Possible contra-indications shall be mentioned.*

For additives intended to favourably affect the colour of ornamental fish or birds or that add or restore colour in feedingstuffs, the level of inclusion in complete feedingstuffs should be provided.

For additives which, when fed to animals, add colours to food of animal origin, details of the proposed method of administration and level of inclusion must be provided for premixtures or feedingstuffs. In addition, the proposed dose (minimum and maximum) in the complete feedingstuff and the proposed duration of administration must be provided. If a particular use in complementary feedingstuffs for some animal species or categories is intended, the dose should be proposed and justified.

### 2.5.2 Information related to worker safety

#### 2.5.2.1 Chemical substances

*A material safety data sheet formatted in accordance with the requirements of Commission [Directive 91/155/EEC](#) of 5 March 1991 defining and laying down the detailed arrangements for the system of specific information relating to dangerous preparations in implementation*



of Article 10 of [Directive 88/379/EEC](#) as amended by [Directive 2001/58/EC](#) must be provided. If necessary, measures for the prevention of occupational risks and means of protection during manufacture, handling, use and disposal shall be proposed.

### 2.5.2.3 Labelling requirements

Without prejudice to the labelling and packaging provisions laid down in Article 16 of [Regulation \(EC\) No 1831/2003](#), any specific labelling requirements and, where appropriate, specific conditions for use and handling (including known incompatibilities and contraindications) and instructions for proper use shall be indicated.

## 2.6 Methods of analysis and reference samples

Methods of analysis to determine the active substance in the additive itself and in premixtures and feedingstuffs as appropriate should be submitted. These should be suitable for the official control of the feed additive. If there are residues of concern, a method of analysis of the active substance and/or its metabolites (including the marker residue) in the relevant tissues/products should be provided.

These methods will be evaluated by the Community Reference Laboratory (CRL). Details of the requirements are specified in the [Regulation \(EC\) No 429/2008](#). Applicants should refer to the [guidance provided by the CRL](#).

Methods to determine the identity and the characteristics of the additive (composition of the additive, impurities, physical and chemical properties) should be internationally recognised or otherwise fully described.

## 3. SECTION III: STUDIES CONCERNING THE SAFETY OF THE ADDITIVE

The studies included in this section are intended to permit assessment of:

- *the safety of use of the additive in the target species;*
- *any risk associated with the selection and/or transfer of resistance to antimicrobials and increased persistence and shedding of enteropathogens;*
- *the risks to the consumer of food derived from animals given feedingstuffs containing or treated with the additive or which could result from the consumption of food containing residues of the additive or its metabolites;*
- *the risks from respiratory, other mucosal tissue, eye or cutaneous contact for persons likely to handle the additive as such or as incorporated into premixtures or feedingstuffs; and*
- *the risks of adverse effects on the environment, from the additive itself, or products derived from the additive, either directly and/or excreted by animals.*

Where an additive has multiple active components, each may be separately assessed for safety for consumers and then consideration given to additivity (exclusion of interactions). Alternatively, the complete mixture should be assessed.

### 3.1 Studies concerning the safety of use of the additive for the target species

#### 3.1.1 Tolerance for the target species

*The aim of the tolerance test is to provide a limited evaluation of short-term toxicity of the additive to the target animals. It is also used to establish a margin of safety, if the additive is consumed at higher doses than recommended.*

*All studies reported in this section must be based on the additive described in Section II, except in cases where a concentrated form of the additive is recommended to be tested.*

A tolerance study in the relevant target species/category is required for substances which, when fed to animals, add colour to food of animal origin.

For substances that add or restore colour in feedingstuffs and for substances which favourably affect the colour of ornamental fish or birds, safety for the target animals should be demonstrated with studies performed on animals receiving the additive under the recommended conditions of use. Evidence can also be provided by reference to existing scientific literature.

For details on how to perform and report tolerance studies, see the [technical guidance on tolerance and efficacy studies in target animals](#).

### 3.1.2 Microbial studies

Microbial studies are not required for colourants which are already authorised intended to favourably affect the colour of ornamental fish or birds or intended to add colour to food of animal origin.

Studies are also not required for:

- compounds known or demonstrated not to possess an antimicrobial activity, or whose structure or physical properties preclude antimicrobial activity, at concentrations relevant to feed use.
- additives which consist only of micro-organisms considered by EFSA to qualify for [QPS status](#).

Where required, studies should demonstrate that the additive does not induce cross-resistance to antibiotics used in human or veterinary medicine or encourage the growth and/or shedding of zoonotic agents.

For those additives that in the tolerance test give an indication of an adverse effect possibly related to digestive tract disturbances, studies on the effects on the target animal gastrointestinal microbiota are required.

For the details see the [technical guidance on microbial studies](#).

## 3.2 Studies concerning the safety of use of the additive for consumers

*The aim is to evaluate the safety of the additive for the consumer and to establish potential residues of the additive or its metabolites in food derived from animals given feed or water containing or treated with the additive.* This section consists of metabolic and residue studies (3.2.1.), toxicological (*in vitro* and *in vivo*) studies (3.2.2) and the assessment of consumer safety (3.2.3).

Studies concerning safety for consumers are not required for:

- substances which favourably affect the colour of ornamental fish or birds.
- substances that add or restore colour in feedingstuffs and when fed to animals are essentially not absorbed and excreted unchanged (or if transformed in the digestive tract, its metabolites can be demonstrated to be essentially not absorbed).

For additives already authorised in food, refer to the [technical guidance on additives already authorised in food](#).

For all other colourants, full section applies.

For details on how to assess consumer safety, refer to the [technical guidance on consumer safety](#).

### 3.2.1 Metabolic and residue studies

*The establishment of the metabolic fate of the additive in the target species is a determinant step in the identification and quantification of the residues in the edible tissues or products derived from the animals given the feed or water containing the additive.*

For some additives, depending on their nature or use, it may not always be necessary to carry out metabolic and residues studies.

#### 3.2.1.1 Metabolic studies

The purpose of metabolic studies is to evaluate the absorption, distribution, biotransformation and excretion of the additive in the target species and in a laboratory animal, if necessary.

Metabolic studies are not required if the substance is naturally present in significant amounts in food or feedingstuffs or the substance is a normal constituent of body fluids or tissues.

For all other colourants metabolic studies should be provided.

#### 3.2.1.2 Residue studies

For colourants, the primary objective of residue data is to enable the calculation of consumer exposure.

*Residue studies are required for all substances for which metabolic studies are needed.*

Residue data are required for all colourants which add colour to food of animal origin and for those substances that add or restore colour in feedingstuffs if the additive results in tissue/product retention in the target species. In such cases, the requirement is limited to the measurement of the tissue/product concentration (at steady state) in a group supplemented with the highest recommended dose in comparison to an untreated group.

### 3.2.2 Toxicological studies

*The safety of the additive for the consumer is assessed on the basis of the toxicological studies performed in vitro and in vivo on laboratory animals.*

*Toxicological studies must be carried out with the active substance.* If the active substance is present in a fermentation/cultivation product, this shall be tested. The fermentation/cultivation product tested must be identical to that to be used in the commercial product.

Toxicological studies are required for additives produced by fermentation/cultivation and, on a case by case basis, for additives not already authorised.

For fermentation/cultivation products and for all substances which colour food of animal origin, two *in vitro* and one *in vivo* genotoxicity studies and a subchronic (90 day) oral toxicity study should be provided unless the colourant is produced by a micro-organism considered by EFSA to qualify for [QPS status](#) (or rarely from a commercial strain (lineage) of micro-organism with a substantial history of documented safe use).

Where the production micro-organism belongs to a group in which some strains are known to produce toxins, their presence should be specifically excluded.

For xenobiotic substances (defined as chemicals which are not a natural component of the host organism), the complete set of toxicological studies described in the [guidance for consumer safety](#) is normally required.

Physiological substances (and in the case of colourants, which naturally occur in the diet) whose use results in much higher concentrations than usual in the host organism are considered as xenobiotics. In these cases, the need for toxicological studies should be considered on a case by case basis, taking into account the level and nature of exposure.

### 3.2.3 Assessment of consumer safety

*Consumer safety is assessed by a comparison of the established Acceptable Daily Intake (ADI) and calculated theoretical intake of the additive or its metabolites from food. Where a Tolerable Upper Intake Level (UL) is established, it should be used in place of the ADI.*

### 3.3 Studies concerning the safety of use of the additive for users/workers

*Workers can be exposed mainly by inhalation or topical exposure while manufacturing or handling or using the additive. For example, farm workers are potentially exposed when handling or mixing the additive.*

*An assessment of risk to workers shall be included. Experience in the manufacturing plant is often an important source of information in evaluating the risks to workers from exposure to the additive itself by both airborne and topical routes. Of particular concern are additives/additive-treated feeds and/or animal excreta, which are in, or may give rise to, a dry powdery form, and feed additives which may have allergenic potential.*

*Risks to workers shall be assessed in a series of studies using the additive in the form for which the application has been submitted. Acute inhalation toxicity studies shall be performed unless the product is unlikely to form a respirable dust or mist. Studies on skin irritancy must be performed, and if these give negative results, mucous membrane (e.g. eye) irritancy shall be assessed. Allergenic potential/skin sensitisation potential shall also be assessed. The toxicity data generated to meet consumer safety (see 3.2.2) shall be used to assess the potential systemic toxicity of the additive. All these shall be assessed, if necessary, by direct measurement and specific studies.*

Additives containing microbial biomasses are assumed to be respiratory sensitizers. Studies are not required provided adequate labelling is proposed.

The formulation of the product (e.g., micro-encapsulation) may obviate the need for some or all tests. In such cases, appropriate justification should be provided.

Information on precautionary measures to be taken when handling the additive should be provided (see 2.5.2). *However, use of personal protective devices should only be regarded as a measure of last resort to protect against any residual risk once control measures are in place. It is preferable, for example, to consider reformulation of the product.*

For details on how to assess user/worker safety, refer to the [technical guidance on user safety](#).

### 3.4 Studies concerning the safety of use of the additive for the environment

Administration of additives typically occurs over long periods, often involves large groups of animals and the active substance(s) may be excreted to a considerable extent either as the parent compound or its metabolites.

*To determine the environmental impact of additives, a stepwise approach should be followed. All additives have to be assessed through Phase I to identify those additives which do not need further testing. For the other additives a second phase (Phase II) assessment is needed to provide additional information, based upon which further studies may be considered necessary.*

The impact on the environment as a result of the Phase I assessment will be considered negligible if:

- the substance is a physiological/natural substance whose use will not result in a substantial increase in concentration in the environment; or
- the additive is intended for non food-producing animals only.

For details on how to assess environmental safety, refer to the [technical guidance on environmental risk assessment](#).

#### 4. SECTION IV: STUDIES CONCERNING THE EFFICACY OF THE ADDITIVE

Studies should demonstrate the efficacy for each proposed use under the recommended conditions of use. *Such studies must permit the evaluation of the efficacy of the additive according to common farming practices in the EU.*

- a) For substances which, when fed to animals, add colour to food of animal origin

The change in colour of products obtained from animals receiving the additive should be measured using the appropriate methodology (e.g., colour fan, reflectance spectroscopy). It should be demonstrated that the use of the additive does not adversely affect product stability or organoleptic and nutritional qualities of the food. Evidence of efficacy can be demonstrated in long term studies or, where the relationship between a particular substance and the colour of animal tissues/products is well documented, in short term studies (e.g., bioavailability).

For details on how to perform and report efficacy studies, see the [technical guidance on tolerance and efficacy studies in target animals](#).

- b) For substances that add or restore colour in feedingstuffs:

Evidence of the efficacy of the additive should be demonstrated using laboratory-based studies by means of appropriate criteria as reflected in recognised acceptable methods in comparison with an appropriate control feed.

The studies should be designed to cover a representative range of feedingstuffs to which the additive will be applied. Results should be statistically evaluated and differences between groups accepted at  $P \leq 0.05$ . Non-parametric tests may be necessary when a low number of observations is available.

- c) For substances which favourably affect the colour of ornamental fish and birds:

Colour changes should be measured using the appropriate methodology. Evidence of efficacy may also be provided by other experimental studies (e.g. bioavailability) or by reference to scientific literature.

#### 4.6 Studies on the quality of animal products where this is not the effect claimed (substances that add or restore colour in feedingstuffs).

Evidence should be given that the additive does not have a negative effect or other unintended effect on the organoleptic and nutritional (and if appropriate, hygienic and technological) characteristics of food deriving from animals fed the treated feed.

Evidence can be based on physiological/metabolic considerations or given by reference to scientific literature. Specific studies may be necessary in case of substances for which residue studies are required. An unsupplemented group should be compared with a group receiving the highest dosage proposed for the additive. *The data should allow statistical evaluation.*

#### 5. SECTION V: POST-MARKET MONITORING PLAN

A post-market monitoring plan is required only for sensory additives that are products consisting of, containing or produced from GMOs, in order to trace and identify any direct or indirect, immediate, delayed or unforeseen effects resulting from the use of the additive on human or animal health or the environment, in accordance with the characteristics of the products concerned.

*The design of the monitoring plan shall be detailed on a case-by-case basis and identify who (e.g., applicant, users) will carry out the various tasks that the monitoring plan requires, who is responsible for ensuring that the monitoring plan is set into place and carried out appropriately.* The post-market monitoring plan should in all cases ensure that there is a route

by which the competent control authorities, the Commission and the EFSA are informed of any observed adverse effects.

## PART 2 – FLAVOURING COMPOUNDS

For the purpose of the evaluation of applications of these products, flavourings are classified in the following subgroups:

1. Natural products:
  - 1.1. Natural products - botanically defined.
  - 1.2. Natural products - non-plant origin.
2. Natural or corresponding synthetic chemically defined flavourings.
3. Artificial substances.

*In general, in the case of the group 'natural products', whole plants, animals and other organisms and parts of these or products thereof resulting from very limited processing such as crushing, grinding or drying (e.g., many herbs and spices), shall not be considered as falling under the functional group flavourings of the category sensory additives.*

If sufficient structural/metabolic similarity exists between flavouring compounds (e.g., there is already an established food flavouring group evaluation, FGE), a group-based application and evaluation can be performed.

Natural products (extracts) whose composition is well defined may be assessed on the basis of their major and characteristic components, taking into account any components of known toxicological concern. As a guide, all components representing more than 20% of the natural product solids should be considered as major components. However, in some cases, a lower concentration may apply.

## 2. SECTION II: IDENTITY, CHARACTERISATION AND CONDITIONS OF USE OF THE ADDITIVE; METHODS OF ANALYSIS.

*The additive has to be fully identified and characterised.* For the majority of sensory additives, which are not subject to a specific holder of the authorisation, the paragraphs 2.1.2, 2.1.3, 2.1.4, 2.1.4.2, 2.2, 2.3.1, 2.4.1, 2.4.2, 2.4.4, 2.5 and 2.6 apply. For those sensory additives subject to a specific holder of the authorisation (i.e., additives falling within the scope of Community legislation relating to the marketing of products consisting of, containing or produced from GMOs), the whole Section II applies.

Because of the difficulty of identifying all of the active components in a natural product, the major/characteristic component(s) should be considered, for the purpose of this document, as the active substance(s).

### 2.1 Identity of the additive

The additive has to be characterised and fully identified. The studies described in this section must be based on the final product(s) for which authorisation is sought. In-house identifiers should be avoided unless embedded in third-party documents. In this case a statement is required to confirm that the identifier(s) refers to the formulation(s) for which the claim is made.

#### 2.1.1 Name of the additive

A name of the additive should be proposed. This should reflect the production process and source or the active substance, as appropriate (see subsection 2.2.1).

### 2.1.2 Proposal for classification

In addition to the classification “sensory additive/flavouring compounds” a proposal for the classification of the additive for additional categories<sup>8</sup> and functional groups according to its main functions under Article 6 and Annex I of [Regulation \(EC\) No 1831/2003](#) can be made.

The relevant subgroup (see above) to which the flavouring compound belongs must be indicated. In case the product does not fit into any of the above subgroups, this shall be mentioned and justified.

Any data from other known uses of the product or its characteristic components (e.g., use in food, human or veterinary medicine, agriculture and industry) must be provided. Any other authorisation for use in feed or food, as a veterinary drug or other kind of authorisation should be specified and properly referenced.

### 2.1.3 Qualitative and quantitative composition (active substance(s), other components, impurities, batch to batch variation)

The active substance(s) and the other components of the additive should be listed, giving the proportion by weight in the final product. Evidence should be provided by the analysis of at least five production batches that the nature and amount of the active substance(s) in the additive specified by the applicant is satisfied in practice.

Natural products and artificial substances in which the constituents cannot be described by precise chemical formula (e.g., plant polymers, smoke flavourings<sup>9</sup>) and/or where not all can be identified should be characterised by the constituent(s) contributing to its activity and/or typical major constituent(s).

Without prejudice to any request for supplementary information made by the EFSA according to Article 8(2) of [Regulation \(EC\) No 1831/2003](#), the applicant may omit the description of other components with no safety concerns other than active substances for additives not within the scope of [Regulation \(EC\) No 1829/2003](#).

### 2.1.4 Purity

*The applicant shall identify and quantify chemical and microbial impurities and substances with toxic or other undesirable properties that are not intentionally added and do normally not contribute to the activity of additive. Any substances produced via fermentation should be free of antimicrobial activities relevant to the use of antibiotics in humans or animals and the absence of viable production organisms in the additive should be confirmed.*

*The protocol used for the routine screening of production batches for contaminants and impurities should be described.*

The data provided should support a proposal for a specification of the additive.

Monitoring for contaminants and impurities should be consistent with existing legislation (e.g., [Directive 2002/32/EC](#), or specifications from [European Community food additive authorisations](#)) and recommendations from internationally recognised sources when these are available (e.g., Joint FAO/WHO Expert Committee on Food Additives (JECFA) specifications; [Commission recommendation on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding](#)). Additional measures should be introduced following the HACCP analysis of the specific process, as necessary.

Emphasis will be placed on the minimum assay value for the active substance(s) and the identification of any other components to 99% (w/w) of the additive.

<sup>8</sup> If the applicant applies for one or more categories in addition to sensory additives, reference should be made to the relevant guidance document(s).

<sup>9</sup> As defined in Regulation (EC) No 1334/2008. OJ L 354, 31.12.2008, p. 34



As a guide the following should be considered:

- For all flavouring additives: process-related impurities (e.g., residual solvents, degradation products).
- For natural products and natural chemically defined compounds: carry-over of any compound of toxicological concern from the starting material, microbiological contamination, mycotoxins and pesticides,<sup>10</sup> heavy metals (Pb, Hg, Cd) and arsenic, dioxins (PCDD/F) and dioxin-like PCBs.
- For fermentation/cultivation products: in addition to the above, the extent to which spent growth medium is incorporated into the final product should also be indicated. For fermentation products produced by genetically modified micro-organisms, identification and quantification of recombinant DNA in the final product should be provided.
- For products produced by chemical synthesis: all chemicals used in the process and any intermediate products remaining in the final product should be identified and quantified.
- For smoke flavourings: polycyclic aromatic hydrocarbons should be identified and where appropriate, quantified.

## 2.2 Characterisation of the active substance(s)

### 2.2.1 Description

A qualitative description of the active substance(s) should be given. This should include purity and origin of the substance(s), plus any other relevant characteristics. Data to establish the identity of the active substance(s) should be provided (e.g., by mass spectrometry, nuclear magnetic resonance).

For natural products, identification tests should be specific for the preparation, and optimally should be discriminatory with regard to substitutes/adulterants that are likely to occur. Identification solely by chromatographic retention time, for example, is not regarded as being sufficiently specific. A combination of techniques for the separation and structural analysis is considered suitable (e.g., HPLC/MS<sup>n</sup>, GC/MS<sup>n11</sup>).

An overview of the natural occurrence of the active substance(s) in materials used as feed/food should be provided.

#### 2.2.1.1 Chemical substances

Natural or corresponding synthetic chemically defined flavourings should be described by generic name, chemical name according to the International Union of Pure and Applied Chemistry ([IUPAC](#)) nomenclature, other commonly used generic names and abbreviations, the FLAVIS number in connection with relevant chemical group and/or Chemical Abstract Service ([CAS](#)) Number. The structural and molecular formula and molecular weight must be included. Where relevant, data on isomeric forms (e.g., geometrical or optical isomers) and accompanying structurally related compounds should be included.

For artificial substances, the same requirements apply, where appropriate.

For natural products - botanically defined, the characterisation should include the scientific name of the plant of origin, its botanical classification (family, genus, species, if appropriate subspecies and variety) and the common names and synonyms in official European languages. Synonyms in other language(s) should be given only if relevant to the place of origin. The parts of the plant used (leaves, flowers, seeds, fruits, tubers, etc) should be indicated. The

<sup>10</sup> The selection of mycotoxins and pesticides for analysis should be made according to the different matrices, where appropriate. Residues specified under the undesirable substances directive (Directive 2002/32/EC) and any other pesticide residues of potential concern to target animals and/or consumer safety.

<sup>11</sup> <sup>n</sup>: indicates multiple mass spectrometry systems

place of cultivation of the plant, the identification criteria and other relevant aspects of the plants should be indicated. Specifications of the applicant for any plant material supplied by a third party should also be provided. For complex mixtures of many compounds obtained by an extraction process, it is recommended to follow the relevant terminology such as essential oil, absolute, tincture, extract and related terms<sup>12</sup> widely used for botanically defined flavouring products to describe the extraction process. *The major components shall be identified and quantified and their range or variability provided.* The phytochemical marker(s) characteristic of the plant of origin must be included. *Special attention shall be given to impurities as mentioned in subsection 2.1.4. The concentrations of substances of toxicological concern for humans or animals which may occur in the plant from which the extract is produced shall also be reported.*

For natural products of non-plant origin, an equivalent approach to the above may be used.

The microbial origin (bacteria, yeasts, filamentous fungi and micro-algae) of flavourings produced by fermentation/cultivation should be described and any history of modification of the production organism should be indicated. The name and taxonomic classification of each micro-organism should be provided, according to the latest published information in the International Codes of Nomenclature. Microbial strains should be deposited in an internationally recognised culture collection (preferably in the European Union) and maintained by the culture collection for the authorised life of the additive. A certificate of deposition from the collection, which should specify the accession number under which the strain is held, must be provided.

### 2.2.2 Relevant properties

*Description of physical and chemical properties shall be given.* These include, where appropriate, dissociation constant, pKa, electrostatic properties, melting point, boiling point, density, vapour pressure, inflammability, autoignition temperature, explosivity, solubility in water and in organic solvents,  $K_{ow}$  and  $K_d/K_{oc}$  and any other relevant physical properties. These may be in the form of the material safety data sheet.

Micro-organisms used as a production strain should not be capable of producing antibiotic substances that are relevant to antibiotics in human and veterinary medicine. Strains of micro-organisms belonging to a taxonomic group that includes members known to be capable of producing toxins or other virulence factors should be subject to appropriate tests to demonstrate at a molecular and, if necessary, cellular level the absence of any cause for concern. As an example on how to assess the potential for toxin production see the technical [guidance on toxin production in \*Bacillus\* spp.](#)

### 2.3 Manufacturing process, including any specific processing procedures

To define the critical points of the process that may have an influence on the purity of the active substance or additive a detailed description of the manufacturing process (e.g., chemical synthesis, fermentation, cultivation, hydrolysis, extraction from organic material or distillation and downstream purification steps, including specifications/material safety data sheets for the chemicals used) should be given. The composition of the fermentation/cultivation media should be provided. For genetically modified micro-organisms used as source of additives and grown under contained conditions, [Directive 90/219/EC](#) applies.

A material safety data sheet for the active substance/additive (see 2.5.2.1) must be provided. Any additional specifications applied to the active substance/additive must also be submitted. The solvents used must be specified. The current maximum levels set for residual solvents used in veterinary drugs ([VICH guidance GL18](#)) should not be exceeded.

<sup>12</sup> Defined in Appendix 4 of the Council of Europe's Report no. 1 on "Natural sources of flavourings", Volume I, Strasbourg, 2000.

## **2.4 Physical-chemical and technological properties of the additive**

### **2.4.1 Stability**

Stability is generally measured by the analytical follow-up of the active substance(s) or by persistence of sensory property. For natural products (and some artificial substances) stability may be assessed by monitoring the concentration of one or more appropriate marker substances. Data should be provided from at least three batches that include at least one observation at the beginning and one at the end of the storage period.

Where there is a loss of stability, potential degradation or decomposition products should be characterised, where appropriate.

#### **2.4.1.1 Shelf-life of the additive**

The expected shelf-life of the additive as marketed should be proposed, normally based on the results of at least two model situations covering the likely range of use conditions (e.g., for a solid formulation 25°C, 60% relative air humidity (RH) and 40°C, 75% RH).

If the shelf-life is already established for an additive authorised for use in food, the relevant studies should be summarised. No additional studies would be required.

#### **2.4.1.2 Stability of the additive used in premixtures and feedingstuffs**

The proposal of the applicant for the stability of the additive in premixtures should be supported by data. In this case, the premixtures could consist only of a blend of different flavouring compounds (flavouring premixture). The quantitative and qualitative composition of the premixtures used for the studies should be given.

Stability studies in feedingstuffs are not required for flavourings intended to impart smell to feedingstuffs.

For those substances intended to improve palatability of feedingstuffs, the stability of each formulation of the additive normally should be studied in feedingstuffs manufactured and stored under common conditions, and if relevant, in premixtures. The quantitative and qualitative composition of the feedingstuffs used for the studies should be given.

Stability studies in premixtures and feedingstuffs should be of at least six and three months' duration, respectively.

#### **2.4.1.3 Stability of the additive used in water or aqueous media**

The stability of the additive intended to be distributed via the water supply or using aqueous media should be studied under conditions simulating practical use (e.g., environment and water temperature, time).

### **2.4.2 Homogeneity**

Homogeneity studies are not required for flavouring compounds.

### **2.4.4 Physico-chemical incompatibilities or interactions**

Physico-chemical incompatibilities or interactions that could be expected with feed, carriers, other approved additives or medicinal products must be shown.

## **2.5 Conditions of use of the additive**

### **2.5.1 Proposed mode of use in animal nutrition**

The proposed use in feed or water should be defined. The animal species or categories, age group or production stage of animals should be indicated, as appropriate, in accordance with

the categories listed in Annex IV of [Regulation \(EC\) No 429/2008](#). Possible contraindications should be mentioned.

Details of the proposed method of administration, the proposed dose (minimum and maximum) in the complete feedingstuff, and the proposed duration of administration must be provided. If a particular use in complementary feedingstuffs for some animal species or categories is intended, the dose should be proposed and justified.

## 2.5.2 Information related to users/workers safety

### 2.5.2.1 Chemical substances

*A material safety data sheet formatted in accordance with the requirements of Commission Directive 91/155/EEC of 5 March 1991 defining and laying down the detailed arrangements for the system of specific information relating to dangerous preparations in implementation of Article 10 of Directive 88/379/EEC as amended by Directive 2001/58/EC must be provided. If necessary, measures for the prevention of occupational risks and means of protection during manufacture, handling, use and disposal shall be proposed.*

### 2.5.2.3 Labelling requirements

*Without prejudice to the labelling and packaging provisions laid down in Article 16 of Regulation (EC) No 1831/2003, any specific labelling requirements and, where appropriate, specific conditions for use and handling (including known incompatibilities and contraindications) and instructions for proper use shall be indicated.*

## 2.6 Methods of analysis and reference samples

Details of the requirements are specified in [Regulation \(EC\) No 429/2008](#). These methods will be evaluated by the Community Reference Laboratory (CRL). Applicants should refer to Regulation (EC) No 378/2005<sup>13</sup> and the [guidance provided by the CRL](#).

If residues of concern are identified/recognised, methods for their analysis in the relevant tissues/products will be required (e.g., the active substance, its metabolites, the proposed marker substance or any other substance of toxicological concern contained in the additive).

Methods to determine the identity and the characteristics of the additive (composition of the additive, impurities, physical and chemical properties) should be internationally recognised or otherwise fully described.

## 3. SECTION III: STUDIES CONCERNING THE SAFETY OF THE ADDITIVE

The studies included in this section are intended to permit assessment of:

- *the safety of use of the additive in the target species;*
- *any risk associated with the selection and/or transfer of resistance to antimicrobials and increased persistence and shedding of enteropathogens;*
- *the risks to the consumer of food derived from animals given feedingstuffs containing or treated with the additive or which could result from the consumption of food containing residues of the additive or its metabolites;*
- *the risks from respiratory, other mucosal tissue, eye or cutaneous contact for persons likely to handle the additive as such or as incorporated into premixtures or feedingstuffs; and*
- *the risks of adverse effects on the environment, from the additive itself, or products derived from the additive, either directly and/or excreted by animals.*

<sup>13</sup> Last amended by [Regulation \(EC\) No 885/2009](#). OJ L 254, 26.9.2009, p. 58

Where the additive has already been assessed for safety for food use by a European scientific body, a copy of the most recent safety assessment should be provided. This should be supplemented with any relevant data subsequently produced. Reference to any assessments made by other bodies (e.g., JECFA) should be included in the dossier.

All assessments of safety must be based on the highest proposed use level in animal nutrition.

The safety of natural products may be assessed on the basis of major and characteristic components and also considering minor substances of toxicological concern. Applicants are advised to refer to the “[EFSA Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern](#)”. Alternatively, the natural product as such may be assessed. If the major or characteristic components are not already authorised as chemically defined flavourings or as feed additives, then it has to be verified whether they are substances of toxicological concern for humans or animals, and their toxicological properties have to be provided. Use of the source material as a recognised food or feedstuff would be taken into consideration.

### 3.1 Studies concerning the safety of use of the additive for the target species

Any known pharmacological or related properties should be reported for natural or corresponding chemically defined flavourings and reference made to relevant scientific literature (preferably from peer-reviewed journals).

#### 3.1.1 Tolerance for the target species

*The aim of the tolerance test is to provide a limited evaluation of short-term toxicity of the additive to the target animals. It is also used to establish a margin of safety, if the additive is consumed at higher doses than recommended.*

*All studies reported in this section must be based on the additive described in Section II.*

For flavourings already authorised for use in food, the safety for target species may be assessed by a comparison between the level of intake by the target species from feed and that by humans from food.<sup>14</sup> Any metabolism and toxicological data on which the assessment for human use was made shall be submitted.

If the use level in the target animals [expressed as quantity per metabolic body weight (usually mg/kg<sup>0.75</sup>)] is similar to that in humans (or less), a tolerance study is normally not required. The figures for body weight and feed intake to be used for the different categories of major species are given in Table 1.

**Table 1.** Body weight and feed intake of major species

Animal category	Body weight (kg)	Metabolic body weight (kg <sup>0.75</sup> )	Mean feed intake (g/day)
Chickens for fattening	2	1.7	120
Turkeys for fattening	12	6.4	400
Laying hens	2	1.7	120
Piglets	20	9.5	1000
Pigs for fattening	100	31.6	3000
Sows	200	53.2	6000
Veal calves (milk replacer)	100	31.6	2000
Cattle for fattening	400	89.4	8000
Dairy Cows	650	128.7	20000
Salmonids	2	1.7	40

<sup>14</sup> Until guidance on the calculation of human exposure to food flavourings is made available by EFSA, applicants are advised to make use of the Single Portion Exposure Technique (SPET) used by JECFA (Sixty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives. Summary and conclusions. Geneva, 17-26 June 2008).

Mean values for dogs are not tabulated due to the high variation in adult body weight. For cats, a body weight of 3 kg should be considered. For the purpose of intake comparison, feed consumption of 2% of the respective body weight could be taken.

Where the proposed level of intake of the target animal is higher than that of humans, or when the compound is not authorised for use in food, the safety for target species may be assessed taking into account:

- the thresholds of toxicological concern (TTC),<sup>15</sup> according to the Cramer structural class to which the compound was assigned. If the intended maximum feed concentration of a flavouring compound in feed for target species is below the concentration indicated in Table 2 according to its Cramer class, no tolerance studies are required. The “maximum acceptable feed concentrations” in Table 2 are derived from the thresholds of the TTC approach, including a safety factor of 100, and based on approximate body weight and feed intake of animal categories.

**Table 2.** Maximum acceptable feed concentrations of flavouring compounds according to their Cramer classes

	Maximum acceptable feed concentration by Cramer structural class (mg/kg)		
	Class III	Class II	Class I
Major species (all categories)			
Poultry	0.05	0.30	1.00
Pigs	0.05	0.30	1.00
Cattle	0.08	0.50	1.50
Salmonids	0.08	0.50	1.50
Non food-producing animals	0.08	0.50	1.50

or

- the feed concentration derived from the lowest NOAEL (or by benchmark dose procedure) of appropriate substance-specific toxicological studies,<sup>16</sup> applying a safety factor of 100. The conclusions obtained for an individual flavouring may be extended to other flavourings belonging to the same structural group (e.g., an FGE).

Tolerance studies are needed when the safety of the proposed dose for the target species cannot be established from the procedures described above.

For natural products (extracts), for which no substances of recognised toxicological concern are identified, the same procedure as described above should be followed for each major component. Alternatively, the safety of a whole extract could be assessed based on specific toxicological studies (see above) or directly investigated in a tolerance study.

When needed, tolerance studies should be performed in the relevant target species/categories of animals. If the application is for all animal species, tolerance studies are required in only three major target species (a monogastric, a ruminant, poultry or a salmonid) provided that they show a comparable and wide margin of safety (at least ten). The conclusions obtained for an individual flavouring may be extended to other flavourings belonging to the same structural group (e.g., an FGE).

For details on how to perform and report tolerance studies, see the [technical guidance on tolerance and efficacy studies in target animals](#).

<sup>15</sup> JECFA (FAO/WHO, 1996, Food additive series 35, IPCS, WHO Geneva); Barlow, S. 2005. Threshold of toxicological Concern (TTC). A tool for assessing substances of unknown toxicity present at low levels in the diet. ILSI Europe Concise Monograph Series.

<sup>16</sup> These should include at least a 90 day oral toxicity study

### 3.1.2 Microbial studies

Microbiological studies may be required when:

- the additive is or contains a substance known or demonstrated to have a significant antimicrobial effect at feed concentration.
- a tolerance test gives an indication of adverse effect possibly related to intestinal disorders.

For the details on how to perform microbial studies, see the [technical guidance on microbial studies](#)

### 3.2 Studies concerning the safety of use of the additive for the consumer

*The aim is to evaluate the safety of the additive for the consumer and to establish potential residues of the additive or its metabolites in food derived from animals given feed or water containing or treated with the additive.* This section consists of metabolic and residue studies (3.2.1.), toxicological (*in vitro* and *in vivo*) studies (3.2.2) and the assessment of consumer safety (3.2.3).

Studies concerning safety for consumers are not required for additives intended to be used in non-food producing animals only.

**If the use of the flavouring compound as feed additive is not expected to lead to the exposure of the consumer to a different qualitative pattern of metabolites than when used in food (evidence can be provided by literature, database search, etc.), the following applies:**

Assessment of consumer safety is not necessary for:

- flavouring compounds already authorised for use in food for which an acceptable daily intake (ADI) is not specified.<sup>17</sup>
- extracts in which the major components fall in the category above, and no substances of toxicological concern are identified.
- flavouring compounds or extracts already authorised as additives for a food-producing species at a similar or higher use level.

Residue data in food of animal origin and an estimation of total consumer exposure (direct intake<sup>18</sup> plus that resulting from use of the flavouring compound in animal feed<sup>19</sup>) are required for:

- flavourings already authorised for the use in food with an established ADI (or some other indicator of maximum intake).
- natural products (extracts) in which one or more of the major components have an established ADI (or some other indicator of maximum intake), and no substances of toxicological concern are identified.

An estimation of total consumer exposure (direct intake plus that resulting from use of the flavouring compound in animal feed) should be provided for:

- flavouring compounds authorised for use in food following the TTC approach.

<sup>17</sup> Without an explicit indication of the upper limit of intake, assigned to substances of very low toxicity, following an evaluation by a European or international body.

<sup>18</sup> Until guidance on the calculation of human exposure to food flavourings is made available by EFSA, applicants are advised to make use of the Single Portion Exposure Technique (SPET) used by JECFA (Sixty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives. Summary and conclusions. Geneva, 17-26 June 2008).

<sup>19</sup> A refined exposure model for residues in food of animal origin is under development and will be made available as part of the technical guidance on consumer safety.

- natural products (extracts) in which the major components have been assessed following the TTC approach, and no substances of toxicological concern are identified.

If the use of the additive in feed is likely to result in a consumer exposure exceeding the threshold applied, further toxicological studies would be required.

**If the pattern of metabolites is (expected to be) qualitatively different when used in feed than when used in food, further toxicological/residue data may be required.**

Full section 3.2 applies (i.e., a complete set of metabolism, residue and toxicology data) for:

- flavouring compounds not authorised for use in food
- natural products (extracts) in which one or more major components have not been assessed for safety

unless they can be assigned to a FGE previously assessed, in which case the TTC approach can be applied.

In the case of natural products (extracts), tests may be done for the individual components or alternatively for the whole product.

### 3.2.1 Metabolic and residue studies

*The establishment of the metabolic fate of the additive in the target species is a determinant step in the identification and quantification of the residues in the edible tissues or products derived from the animals given the feed or water containing the additive.*

The purpose of metabolic studies is to evaluate the absorption, distribution, biotransformation and excretion of the additive in the target species and in a laboratory animal, if necessary.

The requirement for residue studies is limited to a comparison of residue levels in tissues and products from an untreated group to the group administered the highest dose of the additive proposed without a withdrawal time.

### 3.2.2 Toxicological studies

*The safety of the additive for the consumer is assessed on the basis of the toxicological studies performed in vitro and in vivo on laboratory animals.*

If toxicological studies are required, two *in vitro* and one *in vivo* genotoxicity studies<sup>20</sup> and a subchronic (90 day) oral toxicity study should be provided. For artificial flavourings, which are considered as xenobiotics, a complete set of toxicological studies is normally required.

For details on how to assess consumer safety, refer to the [technical guidance on consumer safety](#).

### 3.3 Studies concerning the safety of use of the additive for users/workers

*Workers can be exposed mainly by inhalation or topical exposure while manufacturing or handling or using the additive. For example, farm workers are potentially exposed when handling or mixing the additive.*

*An assessment of risk to workers shall be included. Experience in the manufacturing plant is often an important source of information in evaluating the risks to workers from exposure to the additive itself by both airborne and topical routes. Of particular concern are additives/additive-treated feeds and/or animal excreta, which are in, or may give rise to, a dry powdery form, and feed additives which may have allergenic potential.*

<sup>20</sup> Genotoxicity testing is under review by the Scientific Committee of EFSA, and these requirements may be adapted depending on the outcome of this discussion.



Studies concerning the safety for the users/workers are generally not required for those additives which are authorised as food additives or approved as components for foodstuffs in the European Union. Precautionary measures set for the additive when used in food and experience in handling the product should be taken into account when considering the advice provided to users of the feed additive.

For the other flavourings, risks to users/workers should be assessed in a series of studies using the additive in the form for which the application has been submitted. *Acute inhalation toxicity studies shall be performed unless the product is unlikely to form a respirable dust or mist. Studies on skin irritancy must be performed, and if these give negative results, mucous membrane (e.g., eye) irritancy shall be assessed. Allergenic potential/skin sensitisation potential shall also be assessed. The toxicity data generated to meet consumer safety should be used to assess the potential systemic toxicity of the additive. All these should be assessed, if necessary, by direct measurement and specific studies.*

The formulation of the product (e.g., micro-encapsulation) may obviate the need for some or all tests. In such cases, appropriate justification should be provided.

Information on precautionary measures to be taken when handling the additive should be provided (see 2.5.2). *However, use of personal protective devices should only be regarded as a measure of last resort to protect against any residual risk once control measures are in place. It is preferable, for example, to consider reformulation of the product.*

For details on how to assess user/worker safety, refer to the [technical guidance on user safety](#).

### 3.4 Studies concerning the safety of use of the additive for the environment

Administration of additives typically occurs over long periods, often involves large groups of animals and the active substance(s) may be excreted to a considerable extent either as the parent compound or its metabolites.

*To determine the environmental impact of additives, a stepwise approach should be followed. All additives have to be assessed through Phase I to identify those additives which do not need further testing. For the other additives a second phase (Phase II) assessment is needed to provide additional information, based upon which further studies may be considered necessary.*

The impact on the environment as a result of the Phase I assessment will be considered negligible if:

- the substance is a physiological/natural substance whose use will not result in a substantial increase in concentration in the environment; or
- the additive is intended for non food-producing animals only.

For details on how to assess environmental safety, refer to the [technical guidance on environmental risk assessment](#).

## 4. SECTION IV: STUDIES CONCERNING THE EFFICACY OF THE ADDITIVE

In general, studies should demonstrate the efficacy for each proposed use under the recommended conditions of use. Such studies must permit the evaluation of the efficacy of the additive according to common farming practices in the EU.

### Flavourings intended to influence feed smell

For flavourings already authorised for use in food, where the functions of the additive applied for feed use and described for food use are similar, no further demonstration of efficacy is generally necessary.

For flavourings not authorised for use in food, efficacy may be demonstrated by submission of studies, peer-reviewed publications (preferably recent) and/or material other than studies.

### **Flavourings intended to increase feed palatability**

Evidence of increased feed palatability should be demonstrated by means of animal studies in the appropriate target species. If the application is for all animal species, then at least studies should be provided for a ruminant and a monogastric mammals and a poultry species.

For details on how to perform and report efficacy studies, see the [technical guidance on tolerance and efficacy studies in target animals](#).

#### **4.6 Studies on the quality of animal products**

Evidence should be given that the additive does not have an effect on the sensory/technological characteristics of food deriving from animals fed the treated feed.

Evidence can be given by reference to scientific literature or experience from practical use. If specific studies are performed, food products from an unsupplemented group should be compared with equivalent products from a group receiving the highest dosage proposed for the additive. The data should allow statistical evaluation.

## **5. SECTION V: POST-MARKET MONITORING PLAN**

See Section V in Part 1 of this guidance.