European Food Safety Authority

SCIENTIFIC OPINION

Guidance of the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) on the Submission of a Dossier on Food Enzymes for Safety Evaluation by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids

This guidance was published on 24 May 2013, replacing the earlier version published on 23 July 2009. The present revision was endorsed by the Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) on 9 September 2020[1].

Endorsement date	**9 September 2020**
Implementation date	**27 March 2021**

The present guidance has been revised and it is republished with editorial changes: the former chapters "Submission of an application", "Summary of dossier submission" and "Administrative data" have been deleted. The information related to dossier submission have been included 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 of this guidance is under revision, following the request by the European Commission (see mandate M-2020-0115). Until further notice, the present scientific guidance applies.

[1] This revised version of the guidance contains new text compared to the previous version. The new text has been inserted in boxes to be easily identifiable.

Panel members in 2009: Arturo Anadón, David Bell, Mona-Lise Binderup, Wilfried Bursch, Laurence Castle, Riccardo Crebelli, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Thomas Haertlé, Trine Husøy, Klaus-Dieter Jany, Catherine Leclercq, Jean-Claude Lhuguenot, Wim C. Mennes, Maria Rosaria Milana, Karla Pfaff, Kettil Svensson, Fidel Toldrá, Rosemary Waring, Detlef Wölfle.

Acknowledgements: The Panel wishes to thank the members of the EFSA CEP Panel (Panel on Food Contact Materials, Enzymes and Processing Aids) endorsing the document on 9 September 2020: José Manuel Barat Baviera, Andrew Chesson, Claudia Bolognesi, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Claude Lambré, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Vittorio Silano, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, Holger Zorn.

Suggested citation: Guidance of the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids (CEF Panel) on the Submission of a Dossier on Food Enzymes. The EFSA Journal (2009) 1305, 1-26.

Question number: EFSA-Q-2007-080
Correspondence: fip@efsa.europa.eu

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

On 16 December 2008 the following Regulations of the European Parliament and of the Council were adopted:

Regulation (EC) No 1332/2008 on food enzymes[2],

Regulation (EC) No 1333/2008 on food additives[3],

Regulation (EC) No 1334/2008 on flavourings and certain food ingredients with flavouring properties[4] and

Regulation (EC) No 1331/2008 on a common authorisation procedure for food additives, food enzymes and food flavourings[5].

The Regulations entered into force on 20 January 2009.
The Regulation (EC) No 1332/2008 on food enzymes applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. The scope of this Regulation will therefore not extend to enzymes that are not added to food to perform a technological function but are intended for human consumption, such as enzymes for nutritional purposes. Microbial cultures traditionally used in the production of food, such as cheese and wine, and which may incidentally produce them but are not specifically used to produce them should not be considered food enzymes.

Food enzymes shall be subject to safety evaluation by the European Food Safety Authority (EFSA) and approval via a Community list. The inclusion of a food enzyme in the Community list will be considered by the Commission on the basis of the opinion from EFSA, taking into account also other general criteria such as technological need and consumer aspects. For every food enzyme included in the positive list specifications, including the criteria on purity and the origin of the food enzyme shall be laid down.

Since many food enzymes are already on the market in the Community, the transition to a Community positive list should be smooth and should not lead to unfair conditions for enzyme producers. Therefore, the Regulation provides for an initial period of 24 months, after the date of application of the implementing measures foreseen in the common authorisation procedure in Article 9 of Regulation (EC) No 1331/2008, during which applications can be submitted. The establishment of the Community list will take place in a single step procedure after the Authority has expressed opinions on all food enzymes for which sufficient information has been submitted during the 24-month period.

[2] OJ L 354, 31.12.2008, p. 7
[3] OJ L 354, 31.12.2008, p. 16
[4] OJ L 354, 31.12.2008, p. 34
[5] OJ L 354, 31.12.2008, p. 1

In order to increase consistency in common areas the procedural aspects of approval of food enzymes, as well as for the other two sectoral proposals, such as the handling of applications within well-defined deadlines, their evaluation by EFSA and decision making by the Commission, are provided in Regulation (EC) No 1331/2008 on the common authorisation procedure on food additives, food enzymes and food flavourings. This Regulation also provides that implementing measures (Art. 9) shall be adopted by the Commission, within 24 months from the adoption of the Regulation on enzymes, which shall concern in particular the content, drafting and presentation of the application for the evaluation and authorisation of a food enzyme. With a view to the adoption of these implementing measures the Commission consulted the Authority, which, within six months of the date of entry into force of the Regulation on food enzymes, *i.e.* by 20 July 2009 shall present a proposal concerning the data required for risk assessment of the food enzymes.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION in 2007

In accordance with Article 31 of Regulation (EC) No 178/2002, the European Commission has asked the EFSA to establish guidelines to assist applicants in the preparation and submission of applications for the safety evaluation of food enzymes. In addition, the EFSA has been asked to provide the Commission with a proposal concerning the data required for risk assessment of food enzymes with a view to including it in the implementing measures which will lay down amongst other aspects, the content, drafting and presentation of an application for the evaluation and authorisation of food enzymes.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group for the preparation of this guidance document in 2009: A. Anadón, D. Bell, T. Haertlé, T. Hallas-Møller, K.-D. Jany, P. Joudrier, P. Tobback, F. Toldrá, R. Waring and A. Pöting for assisting in the preparation of the draft guidance document under the AFC Panel mandate.

INTRODUCTION

The purpose of this document is to provide guidance to applicants and other interested parties in providing a dossier for safety evaluation accordance with Regulation (EC) No 1332/2008 on food enzymes. It gives guidance on the format (hereafter referred to as a "dossier") of a formal application for the safety assessment of a food enzyme and technical data required, and on the range of toxicological tests generally required. The application is initially made to the European Commission, for further transmission to the European Food Safety Authority, which is responsible for carrying out the safety assessment and providing an opinion on the outcome of the evaluation. All the information necessary to enable EFSA to conduct a safety assessment of a food enzyme must be provided by the applicants in the dossier.

Note: This guidance was published on 24 May 2013, replacing the earlier version published on 23 July 2009. The present revision was endorsed by the Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) on 9 September 2020.

The present version is republished with editorial changes: the former chapters "Submission of an application", "Summary of dossier submission" and "Administrative data" have been deleted. The information related to dossier submission have been included 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 of this guidance is under revision, following the request by the European Commission (see mandate M-2020-0115). Until further notice, the present scientific guidance applies.

General Principles of Risk Assessment of Food Enzymes

In order to enable EFSA to carry out the safety assessment, the following critical issues of risk assessment are:

- **The source[6].** A consideration of safety issues related to the source of the food enzyme (animals, plants, basidiomycetes or micro-organisms). The possibility of infectious agents in the source, measures for their control in the food enzyme and the potential virulence / toxicity of the producer organism/micro-organism have to be considered.
- **The food enzyme**, related to the enzyme protein(s) as well as other constituents, *e.g.* by-products originating from the source organism and residues of any substances and materials used in the production process.
- **Intended and unintended reaction products** resulting either from enzymatic or chemical reactions of the food enzyme with food constituents or from the degradation of the food enzyme during storage and processing of the foodstuff.
- **The dietary exposure of the consumer.** This depends on the residual concentration of the food enzyme(s) and other constituents of the food enzyme in the foods at the time of consumption and the amount and frequency of their consumption.

Each individual food enzyme must be assessed. However, specified food enzymes:

i) with the same catalytic activity (*e.g.* alpha-amylase) and,

ii) produced by the same micro-organism strain and

iii) by the substantially same manufacturing process as described in section 1.2.2, may be grouped in one application.

The guidance document is based on the "Guidelines for the presentation of data on food enzymes of the Scientific Committee for Food (SCF)" (SCF, 1992), taking into account the recommendations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (FAO/WHO, 2006) and national authorities or advisory committees of EU Member States (Agence Française de Sécurité Sanitaire des Aliments (AFSSA) (AFSSA, 2003), Danish Veterinary and Food Administration (DVFA) (DVFA, 2005), UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) (Battershill,1993).

[6] A food enzyme contains an active enzyme (in some instances a blend of two or more enzymes). Furthermore, it may also contain constituents of the source organism (*i.e.* an animal, plant, or microbial material from which an enzyme was isolated) and compounds derived from the manufacturing process, for example, the residues of the fermentation broth.

SCOPE

The scope of this guidance document is confined to the safety evaluation of food enzymes falling within the scope of Regulation (EC) No 1332/2008 on food enzymes (Article 3, Definitions).

"Food enzyme" means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms:

i. containing one or more enzymes capable of catalysing a specific biochemical reaction; and

ii. added to food for a technological purpose at any stage of manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

"Food enzyme preparation" means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

This guidance document is not intended to be exhaustive, since information requirements are likely to vary depending on the food enzyme's function/activity, the properties of the source material, the properties and amounts of any by-products and substances originating from the production process, the history of previous consumption as well as the intended use and the resulting level of human dietary exposure. There may be circumstances where additional data or tests are required for the evaluation of the safety in use. On the other hand, if some of the data stipulated in the guidance document are considered irrelevant, they may be omitted provided that the safety assessment can be adequately addressed. Additionally, progress in science and technology may necessitate periodic updating of this guidance document to reflect new information requirements.

This guidance document does not cover risk assessment for user/worker safety.

TECHNICAL INFORMATION TO BE SUPPLIED WITH AN APPLICATION FOR A FOOD ENZYME

1. Technical Data
2. Toxicological Data
3. Conclusion

1. Technical Data

In this section, the food enzyme should be characterised as completely as possible. The following information should be included and submitted as part of the dossier:

1.1. Identity of the Food Enzyme

The identity and the properties of the food enzyme should be described as completely as possible. The food enzyme sample tested toxicologically should be representative of the food enzyme to be authorised for use in food processing (see Section 2 of the guidance). This should be stated explicitly in the dossier. If the samples are not representative of the commercial product then a justification should be provided. The following paragraphs list the general requirements of dossier submissions to establish the identity of a food enzyme.

1.1.1. Name(s), Synonyms, Abbreviations and Classification(s)

i. Common Name(s) and/or Trade Name(s) *(if applicable)*
ii. Enzyme Classification Number of Enzyme Commission of the International Union of Biochemistry and Molecular Biology (IUBMB)[7] *(if applicable)*
iii. Chemical Name(s) *(if applicable)*
iv. Chemical Abstract Service (CAS) Registry Number *(if available)*
v. European Inventory of Existing Chemical Substances Number (EINECS) or European List of Notified Chemical Substances Number (ELINCS) *(if available)*

1.1.2. Chemical Composition and Properties of the Food Enzyme

1.1.2.1. Chemical Composition

The following should be provided:

i. Molecular mass of the food enzyme and subunit structure; and amino acid sequence (if available),
ii. Chemical description of the food enzyme as tested including chemical purity and identity and percentage or concentration of chemical impurities originating from the source and/or the production process (*e.g.* metabolites such as mycotoxins, heavy metals, residues of extraction solvents) and the methods of analysis,
iii. Information on whether the food enzyme is modified by post translational process or by technological procedures,
iv. Information on whether the food enzyme is protein engineered, the nature of the modification and the rational for the modification, *e.g.* enhancing pH or thermal stability,

[7] The IUBMB was formerly the International Union of Biochemistry. The IUBMB assigns each enzyme a recommended name and a 4-part distinguishing number and divides enzymes into six main groups: Oxidoreductases, Transferases, Hydrolases, Lyases, Isomerases and Ligases.

v. Data on the batch-to-batch variability for the relevant parameters,
vi. Data on the reproducibility for relevant parameters,
vii. Any other useful information such as the concentration of the Total Organic Solids (TOS) as defined by JECFA (FAO/WHO, 2006).

1.1.2.2. Proposed Chemical and Microbiological Specification

The proposed specifications should be submitted in a format modelled on recent EU or other internationally accepted specifications. Where the proposed specifications differ from any already existing JECFA or other internationally recognised specification, these specifications should be set out alongside the proposed new specification, and any differences pointed out.

Other data which the applicant considers useful in describing the composition of a food enzyme should also be supplied.

1.1.2.3. Properties of the Food Enzyme

The following should be provided:

i. Information on the principal enzymatic activity, specifying substrates, reaction products and required co-factors. Measurement of the activity should be based on a reference method using a standard substrate. Details of the activity should be given in enzyme activity units (U) per unit weight (specific activity) or by the SI unit (Katal (kat =· mol · s^{-1})[8]). The enzyme assay method and methods for determination of principal and side reactions, along with information on the stability of the food enzyme during food processing/storage should be provided,
ii. The activity of the food enzyme under the conditions of the intended use and the influence of reaction conditions (*e.g.* the optimum pH and temperature, as well as inhibitors, activating compounds and co-factors),
iii. Any subsidiary/side activities should be characterised, if possible and where appropriate. In particular those activities should be specified that might cause adverse effects (*e.g.* protease and phospholipase activities due to their action on the mucous membranes) and/or form toxic metabolites,
iv. Data on the stability of the food enzyme during storage and before use.

[8] The amount of food enzyme present or used in food production can be difficult to determine in absolute terms such as grams. However, parameters such as the activity of the food enzyme or food enzyme preparation used in production are more relevant. The activity is typically measured by an enzyme activity unit (U) which is the amount of enzyme which will catalyse the transformation of one micromole of the substrate per minute under standard conditions (IUPAC, 1974). The SI Unit of activity *(i.e.* enzyme activity*)* is the Katal (kat = mol · s^{-1}·) which was proposed as a replacement for the enzyme activity unit (U) in 1978. It is a derived SI unit for expressing quantity values of activity of enzymes and other catalysts. However, in practice, enzyme activity units are still more commonly used than the Katal.

1.2. Source Materials and Manufacturing Process

1.2.1. Source Materials

Food enzymes are produced from animal, plant, basidiomycete and microbial sources. Note that microbial sources include prokaryotes, protozoa, microalgae, and all fungi (including moulds, yeasts and filamentous fungi), but that fungal basidiomycete fruiting bodies/mycelia are considered together with plant sources. The specific information which should be included and submitted as part of the dossier in the case of animal, plant and basidomycete and microbial sources is outlined below.

The most recent taxonomic classification and identification methods used in determining the classification should be provided including genus, species, sub-species (if appropriate). In the case of micro-organisms and fungi, applicants are recommended to refer to the Organisation for Economic Cooperation and Development (OECD) Guidance Document on the use of Taxonomy in Risk Assessment of Micro-organisms: Bacteria (OECD, 2003).

1.2.1.1. Production from Animal Sources

i. Information should be provided on which animal tissue is used for production as well as history of previous consumption of the tissue in question, in particular on whether there is a documented history of use with absence of human health adverse effects. Information should also be provided as to whether the animal tissue is fit for human consumption or derives from a Cat. 3 Animal By-Product according to Regulation (EC) 1774/2002 as amended.

ii. Information should be provided as to whether animal tissues used for the preparation of food enzymes comply with meat inspection requirements and are handled in accordance with good hygienic practice; if not, justification should be given.

iii. Information should be provided on methods used to ensure the absence of any risk of infectivity (*e.g.* the agent of transmissible spongiform encephalopathies (TSEs), parasites or other zoonotic agents).

iv. Data on non-infectivity should be supplied based on the classification of the tissues in terms of their infectious titre in natural diseases established by the WHO (WHO, 2003).

1.2.1.2. Production from Plant and Basidiomycete Sources

i. The part(s) of the plant or basidiomycete fruiting bodies/mycelia used for the production of the food enzyme should be specified.

ii. Information should be provided on previous consumption, in particular on whether there is a documented history of safe use.

iii. Relevant information should be provided on methods used for ensuring absence of substances that might cause adverse health effects to humans. For any residue of such substances

remaining in the food enzyme, the name and amount should be specified in section 1.1.2.1. and limits should be proposed in section 1.1.2.2.

iv. If a genetically modified plant or fungus is used, information should also be provided on the organism in accordance with the Guidance document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed (EFSA, 2006). If the source is already covered by an authorisation in accordance with Regulation (EC) No 1829/2003[11] on genetically modified food and feed, information concerning the risk assessment and authorisation of the GMO should be provided.

1.2.1.3. Production from Microbial Sources

Although neither pathogenic nor toxigenic micro-organisms are intentionally used in the production of food enzymes, individual strains of certain microbial fungal species traditionally used as sources of food enzymes may produce toxic secondary metabolites under certain fermentation conditions conducive to the production of these compounds. Some of these micro-organisms are now used as sources of recombinantly expressed enzymes (Olempska-Beer *et al.*, 2006). The key component of evaluating food enzyme safety from microbial sources is the safety assessment of the production strain, in particular, its pathogenic and toxigenic potential (Pariza and Johnson, 2001). In the case of food enzymes produced by fermentation processes using micro-organisms, the following information on the micro-organism is required:

i. Information about the strain used for food enzyme production

- The taxonomic identity of the strain must be provided.
- Details of any documented history of use with absence of human health adverse effects including Qualified Presumption of Safety (QPS) (EFSA, 2005) status should be provided if available.

ii. For genetically modified micro-organisms (GMM), the presence of any factor(s) affecting the genetic stability of the producer strain

Additional information should be provided according to the `Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Micro-organisms and their Derived Products Intended for Food and Feed Use` (EFSA, 2011)[9]

iii. Monitoring of Production Strain

[9] OJ L 268, 18.10.2003, p. 1

The following information shall be provided:

- Details of procedures for the control and monitoring of the microbial source selected for food enzyme production. This may include details on storage conditions of the strain, the industrial pre-culture and culture conditions and their effect on reproducibility between the different batches of food enzymes. Strain monitoring should be sufficient to demonstrate that the strain in use is the same as that described in the dossier.

- Details of procedures for control and monitoring to ensure pure culture and optimum enzyme productivity conditions during fermentation. This may include details of the culture and process conditions designed to ensure the absence of toxins or secondary metabolites harmful to human health.

- Details of procedures for the control of the hygienic conditions throughout recovery and treatments of the food enzyme.

- Details of strain identification methods and results, sufficient to distinguish the production strain from other strains of the same species.

iv. Production Strain Pathogenicity, Toxigenicity and Antimicrobial Resistance

- Information relating to pathogenicity and toxigenicity of the source organism, as well as other properties with potential impact on human health, *e.g.* the production of antibiotics as well as the presence of natural and/or acquired antibiotic/antimicrobial (TH) resistance genes.

- Details of data related to the presence of acquired antimicrobial resistance genes in accordance with the ʾOpinion of the Panel on additives and products or substances used in animal feed (FEEDAP) on the updating of criteria used in the assessment of bacteria for resistance to antibiotics of human or veterinary importance` (EFSA, 2008).

1.2.2. Manufacturing Process

The production process for the food enzyme should be described as completely as possible. A flow chart diagram showing the most important steps in the process should accompany the description.

The following information is required:

i. Description of key steps involved in the production process

If the food enzyme is obtained from a microbial source, information on the fermentation process is required, *e.g.* on process parameters, fermentation media and chemical substances used throughout.

The purification procedure(s) used to obtain the food enzyme should be described including information on the techniques used to remove microbes from the food enzyme and information on extraction solvents, other chemicals, materials and equipment.

Analytical data on a statistically relevant number of manufactured batches representative of the commercial food enzyme demonstrating that the food enzyme complies with the specification set out in 1.1.2.2

ii. Description of operational limits including process controls and quality assurance procedures and how key parameters such as temperature are controlled during production.

iii. In the case of immobilised food enzymes, information on the immobilisation procedure is required, *e.g.* enzyme support materials[10] and immobilisation agents. Information on potential leakage of carriers, immobilisation agents and active enzymes into the food should be provided.

iv. Other relevant information, taking into account recent opinion of EFSA´s Scientific Committee on "The potential risks arising from nanoscience and nanotechnologies on food and feed safety" (EFSA, 2009).

1.3. Reaction and Fate in Food

Information should be provided on the fate of the food enzyme during food processing (see Section 1.1.2) and its behaviour in the food matrix. If relevant any data on intended and unintended reaction products resulting either from enzymatic or chemical reactions of the food enzyme with food constituents or from the degradation of the food enzyme during storage and processing of the foodstuff. If for safety reasons certain food enzymes have to be inactivated experimental studies should be carried out and data from these studies presented to demonstrate the inactivation of both the principal and subsidiary/side enzymatic activities in the final food, if applicable.

In addition, the following is required to allow safety assessment:

- Information on possible adverse effects on nutrients;
- Data related to any possible effects of food enzymes on existing micro-organisms in food (*e.g.* lysozyme can induce germination of microbial spores).

1.4. Case of Need and proposed Conditions of Use

Information should be provided on:

[10] Enzyme support materials should comply with rules for materials intended to come into contact with food under Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food.

i. The technological need/purpose and intended use of the food enzyme,

ii. The mode of action and reactions catalysed by the food enzyme,

iii. The type of foodstuffs in which the food enzyme is intended to be used,

iv. The amount of food enzymes to be added to specific foods (recommended use levels and maximum use levels),

v. The conditions of its use in food processing.

1.5. Dietary Exposure

Potential human exposure to the food enzyme and to any other constituent or by-product of concern should be assessed considering all proposed uses.

A conservative technique such as the "budget method" (Hansen, 1966; Hansen 1979; Douglass *et al*., 1997; European Commission 1998; FAO/WHO 2008) should be used to assess potential dietary exposure in a standard adult of 60 kg body weight consuming large amounts of the categories of foods and beverages for which use levels have been proposed, assuming that they always contain the food enzyme at its proposed upper use level. If needed, the technique should be adapted to consider the potential higher consumption per kg body weight of these foods and beverages in children. All assumptions and data used for the dietary exposure assessment should be clearly described and justified.
In case the use of the food enzyme is proposed for products specifically designed for infants (12 months) or young children (12-36 months) as defined in the Commission Directive 2006/141/EC, *ad hoc* conservative exposure estimates must be produced taking specifically into account these population groups.

1.6. Information on Existing Authorisations and Evaluations

Information on any existing authorisations and evaluations and/or evaluations by other bodies should be provided. Evaluations performed by the national authorities of the EU Member States may be considered on a case-by-case basis.

2. Toxicological Data

2.1. Toxicological Testing

A decision on the need for toxicological testing on a food enzyme should be made on the basis of already available information, including the source of the enzyme, its composition and properties, any existing toxicological studies and any documented history of use of the enzyme in food as well as foreseen level of exposure.

The default assumption is that toxicological testing is necessary. Exceptions are detailed below (s. section 2.1.2.).

2.1.1. The toxicological Data Set

The core set of toxicological data that is required is set out below.

i. Assessment of genotoxicity

This assessment should start with *in vitro* tests, covering both gene mutations and chromosomal effects (structural and numerical).

Two *in vitro* tests would normally be required:

- a test for induction of gene mutations in bacteria (Ames test; OECD guideline 471). If this assay is not applicable, alternatively a test for induction of gene mutations in mammalian cells, preferably the mouse lymphoma *tk* assay with colony sizing (OECD guideline 476), could be performed;
- an *in vitro* assay for the detection of chromosomal aberration (OECD guideline 473) or the *in vitro* micronucleus assay (Draft OECD guideline 487) or the mouse lymphoma *tk* assay with colony sizing (OECD guideline 476).

In any case at least two *in vitro* assays should be performed.

Positive results in any of the above *in vitro* tests may suggest that food enzyme and/or any residues, degradation products or substances originating from the production process that may be present in the food enzyme are mutagenic. A positive result in genotoxicity testing would then require further assessment to determine whether it is genotoxic *in vivo.* Deliberate addition of a genotoxic carcinogen to food is unacceptable (Barlow *et al*, 2006).
One or more positive *in vitro* tests normally require follow-up by *in vivo* testing, unless it can be adequately demonstrated that the positive *in vitro* findings are not relevant for the *in vivo* situation. This is in line with the general strategy elaborated in the updated WHO/IPCS Harmonised Scheme on mutagenicity testing (Eastmond *et al*., 2009).

The choice of the appropriate *in vivo* test is critical, due to different sensitivities, different endpoints and other variables. It requires expert judgement based on all available information, to be applied case-by-case. For this reason, a flexible approach is preferable to a fixed decision tree.

Guidance for the follow-up of positive results from *in vitro* assays could be taken from a guidance document issued recently by the European Chemicals Agency (ECHA 2008, ECB 2003) which recommends that any of the following tests may be conducted:

1. A rodent bone marrow or mouse peripheral blood micronucleus test (OECD guideline 474) or a rodent bone marrow clastogenicity study (OECD guideline475).
2. A Comet (single cell gel electrophoresis) assay.
3. A test for gene mutations in a transgenic rodent model, *e.g.* using *lacI*, *lacZ* or *cII* as reporter gene present in every tissue.
4. A rat liver Unscheduled DNA synthesis (UDS) test.

According to this ECHA guidance, "the nature of the original *in vitro* response(s) (*i.e.* gene mutation, structural or numerical chromosome aberration) should be considered when selecting the *in vivo* study. For example, if the test substance showed evidence of *in vitro* clastogenicity, then it would be most appropriate to follow this up with either a micronucleus test or chromosomal aberration test or a Comet assay. However, if a positive result were obtained in the *in vitro* micronucleus test, the rodent micronucleus test would be appropriate to best address clastogenic and aneugenic potential.

The rat liver UDS test may be appropriate for substances that appear preferentially to induce gene mutations, although the Comet and transgenic tests are also suitable (Speit, 2008). These latter test systems offer greater flexibility, most notably the possibility of selecting a range of tissues for study on the basis of what is known of the toxicokinetics and toxicodynamics of the substance. It should be realised that the UDS and Comet tests are indicator assays detecting putative DNA lesions. In contrast, the transgenic test measures permanent mutations." (ECHA 2008). A combination of the *in vivo* micronucleus assay and the Comet assay in a single study as suggested by Pfuhler *et al*. (2007) would also be acceptable.

Other studies (*e.g.* DNA adduct studies) could also be relevant in order to clarify the mechanism of genotoxicity.

It should also be taken into account that the sensitivity (ability to detect carcinogens as positive) and specificity (ability to give negative results with non-carcinogens) of such assays have recently been analysed by Kirkland and Speit (2008).

ii. Assessment of systemic toxicity

A subchronic oral toxicity study as described in OECD guideline 408 (OECD, 2000a) should be performed.

Toxicological studies should be conducted using internationally agreed protocols if available. Test methods described by OECD and other provisions adopted under European legislation are recommended. The most up-to-date edition of any test guideline should be followed. Studies should be carried out according to the principles of Good Laboratory Practice (GLP) described in Council Directives 2004/10/EC[11] and 2004/09/EC[12] and accompanied by a statement of GLP compliance of the laboratory conducting the studies.

The toxicological studies should be performed on a batch representative of the food enzyme before addition of other components of the food enzyme preparation.

There may be circumstances under which it may be appropriate to deviate from the above-mentioned core set. Such deviations include exemption from certain tests or use of alternative protocols or use of alternative assays or tests. In such cases a scientific justification should be provided and additional types of considerations or mechanistical studies may be needed.

[11] OJ L 50, 20.2.2004, p. 44
[12] OJ L 50, 20.2.2004, p. 28

In the event that the toxicological studies listed above are not sufficient for a safety assessment additional studies might be required on a case-by-case basis depending on the knowledge available with respect to the food enzyme's molecular and functional characteristics as well as its fate in food and the gastrointestinal tract and the extent of potential exposure.
For example, studies addressing possible health effects resulting from long-term exposure, including possible effects in the gastrointestinal tract, may be necessary, as may additional testing on the possible allergenicity of the food enzyme (see section 2.2). Decisions on whether additional studies are needed will be taken by EFSA on a case-by-case basis.

2.1.2. When toxicological testing may not be needed

While administrative and technical data shall be provided for all notified food enzymes, the requirement for toxicological data may in some cases be reduced or completely waived; the justification for not supplying toxicological data may include:

- A documented history on the safety of the source of the food enzyme, the composition and the properties of the food enzymes as well as its use in food, demonstrating no adverse effects on human health when consumed in a comparable way, supported by any existing toxicological studies. In such cases, a detailed rationale must be provided to EFSA for evaluation, *e.g*. edible parts of animals and (non-GM) plants.

- Food enzymes produced by micro-organisms that have been given a status of Qualified Presumption of Safety (QPS), if it can be demonstrated that there are no concerns related to any residues, degradation products or substances originating from the total production process (EFSA, 2005).
- If a food enzyme from a specific strain has been thoroughly tested and the manufacturing process does not differ significantly for other food enzymes from the same strain, the full testing battery may be waived for these food enzymes. This will be decided on a case-by-case basis.

The detailed justification shall be provided in the dossier. However, EFSA may request further clarification.

2.1.3. Data reporting

The data reported for standard toxicological tests should follow the recommendations for data reporting given in the relevant OECD guidelines. For each study performed it should be stated and supported by analytical data for the specification as defined in section 1.1.2.2, that the test material is representative of the food enzyme as described in the dossier.

2.1.4. Review of the toxicological and exposure data and conclusions

For each toxicological study, the significant findings should be highlighted, together with the no-observed-effect level (NOEL) and/or the no-observed-adverse-effect level (NOAEL) if one has been determined, and any other relevant information. Where effects in animals are seen, the relationship between the dose giving rise to effects and likely dietary exposure from use of the food enzyme should be discussed to establish an appropriate margin of safety. The reasons for disregarding any findings should be carefully explained. Where relevant, the conclusions should include an interpretation of the significance of the findings.

2.2. Allergenicity

At present, validated testing methods to predict the allergenicity of the enzyme protein or its breakdown products after oral intake are not available. However, some information on the potential allergenicity of food enzymes can be obtained by applying the integrated, stepwise case-by-case approach used in the safety evaluation of the newly expressed proteins in genetically modified plants (EFSA, 2006; FAO/WHO, 2001). The allergenicity of the source of the food enzyme should be considered and a search for amino acid sequence and/or structural similarities between the expressed protein and known allergens should be undertaken where possible. If there is cause for concern from this initial screening, further analysis may be undertaken, *e.g.* as described in Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006).

If other studies are available, which may have been conducted for other purposes, such as the assessment of safety at the workplace (*e.g.* sensitisation studies), they should be submitted.

3. Conclusion

An overall assessment of the safety data and toxicological tests including rationales for the inclusion or exclusion of specific tests, discussion of their adequacy and any uncertainties, *e.g.* differences in specification between the tested and commercialised product or structural similarities to known allergens should be provided. The overall evaluation of potential human risk should be made in the context of known or anticipated human exposure.

REFERENCES

AFSSA (2003). Guide to the constitution of dossiers for the use of enzyme preparations in food, 26 September 2003.

Barlow S, Renwick AG, Kleiner J, Bridges JW, Busk L, Dybing E, Edler L, Eisenbrand G, Fink-Gremmels J, Knaap A, Kroes R, Liem L, Müller DJG, Page S, Rolland V, Schlatter J, Tritscher A, Tueting W and Würtzen G (2006). Risk assessment of substances that are both genotoxic and carcinogenic. Report of an International Conference organized by EFSA and WHO with support of ILSI Europe. Food and Chemical Toxicology, 44, 1636-1650.

Battershill JM (1993). Guidelines for the safety assessment of microbial enzymes used in Food. Food Addives and Contaminants, 10 (5), 479-488.

Douglass JS, Barraj LM, Tennant DR, Long WR and Chaisson CF (1997). Evaluation of the Budget Method for screening food additive intakes. Food Additives and Contaminants, 14,791-802.

DVFA (2005). Bekendtgørelse nr 22 af 11/01/2005 om tilsætningsstoffer til fødevarer med senere ændringer. Ministeriet for Familie- og Forbrugeranliggender.

Eastmond DA, Hartwig A, Anderson D, Anwar WA, Cimino MC, Dobrev I, Douglas GR, Nohmi T, Phillips DH and Vicers C (2009). Mutagenicity testing for chemical risk assessment: update of the WHO/IPCS harmonized scheme. Mutagenesis. 24 (4), 341-349.

EFSA (2005). Opinion of the Scientific Committee on a request from EFSA related to a generic approach to the safety assessment by EFSA of micro-organisms used in food/feed and the production of food/feed additives. The EFSA Journal, 226, 1-12.

EFSA (2006). Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. The EFSA Journal, 99, 1-100.

EFSA (2008). Technical guidance: Update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance. Prepared by the Panel on Additives and Products or Substances used in Animal Feed, Adopted on 18 June 2008. The EFSA Journal, 732, 1-15.

EFSA (2009). Opinion of the Scientific Committee on the Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety. The EFSA Journal, 958, 1-39.

EFSA (2011). Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified microorganisms and their products intended for food and feed use. The EFSA Journal, 9, 1-54.

EFSA (2021) Administrative guidance on the preparation of applications on food improvement agents (food enzymes, food additives and food flavourings).

ECHA (2008) Guidance on information requirements and chemical safety assessment Chapter R.7a: Endpoint specific guidance.

European Chemicals Bureau (2003). Technical Guidance Document (TGD) on Risk Assessment of New and existing Chemical Substances and biocides following European Regulations and Directives (abbreviated title) (second edition), EUR 20418 EN/1

European Commission (1998). Report on methodologies for the monitoring of food additive intake across the European Union (Final report submitted by the Task Co-ordinator 16 January 1998), Reports of a working Group on Scientific Co-operation on questions relating to food. Task 4.2. SCOOP/INT/REPORT/2. European Commission Directorate General III Industry, Brussels.

European Union (2002a). Regulation (EC) No 178/2002 of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal L31/1, p1, 01/02/2002.

European Union (2002b). Regulation (EC) No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption. Official Journal L 273, p.1, 10.10.2002.

European Union (2003). Regulation (EC) No 1829/2003 of the European Parliament and Council of 22 September 2003 on genetically modified food and feed. Official Journal L168/1, p1, 18/10/2003European Union (2004a). Directive 2004/09/EC of the European Parliament and of the Council of 11 February 2004 on the inspection and verification of good laboratory practice (GLP). Official Journal L50/28, p28, 20/02/2004.

European Union (2004a). Directive 2004/09/EC of the European Parliament and of the Council of 11 February 2004 on the inspection and verification of good laboratory practice (GLP). Official Journal L50/28, p28, 20/02/2004.

European Union (2004b). 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. Official Journal L50/44, p44, 20/02/2004.

European Union (2004c). Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. Official Journal L338/4, p4, 13/11/2004.

European Union (2004d). Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. Official Journal L139/1, p1, 30/04/2004, Corrected version in Official Journal L 226, p3, 25/06/2004.

European Union (2006). Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC. Official Journal L401/1, p1, 30/12/2006.

European Union (2008a). Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. Official Journal L354/1, p1, 31/12/2008.

European Union (2008b). Regulation (EC) No 1332/2008 of the European Parliament and of the Council on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/199, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. Official Journal L354/7, p7, 31/12/2008.

FAO/WHO (2001). FAO/WHO expert consultation on foods derived from biotechnology. Evaluation of allergenicity of genetically modified foods. FAO/WHO, January 2001, Rome

http://www.who.int/foodsafety/publications/biotech/en/ec_jan2001.pdf

FAO/WHO (2006). General Specifications and Considerations for Enzyme Preparations Used in Food Processing. Compendium of food additive specifications, sixty-seventh meeting. FAO JECFA Monographs 3, 2006 (ISBN 92-5-105559-9). Last visited on 19 June 2006 ftp://ftp.fao.org/docrep/fao/009/a0675e/a0675e00.pdf.

FAO/WHO (2008). Dietary exposure assessment of chemicals in food. Report of a Joint FAO/WHO Consultation, 2-6 May 2005, Annapolis, Maryland, USA. WHO: Geneva. http://whqlibdoc.who.int/publications/2008/9789241597470_eng.pdf .

Hansen SC (1966). Acceptable daily intake of food additives and ceiling on levels of use. Food Cosmet Toxicol,4,427–432.

Hansen SC (1979). Conditions for use of food additives based on a budget for an acceptable daily intake. Food Protect, 42,429–432.

Kirkland D and Speit G (2008). Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens III. Appropriate follow-up testing in vivo. Mutat. Res. 654, 114-132.

OECD (1997). Mammalian Erythrocyte Micronucleus Test. Guideline 474, adopted 26.05.1983. In ninth Addendum to the OECD Guidelines for the Testing of Chemicals. Organisation for Economic Co-operation and Development, Paris, June 1997.

OECD (1997). Mammalian Bone Marrow Chromosome Aberration Test. Guideline 475, adopted 04.04.1984. In ninth Addendum to the OECD Guidelines for the Testing of Chemicals. Organisation for Economic Co-operation and Development, Paris, June 1997.

OECD (1997). Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *In Vivo*. Guideline 486, adopted 21.07.1997. In ninth Addendum to the OECD Guidelines for the Testing of Chemicals. Organisation for Economic Co-operation and Development, Paris, June 1997.

OECD (2000a). Repeated dose 90-day oral toxicity study in rodents. Guideline 408, adopted 21.09.1998. In Eleventh Addendum to the OECD Guidelines for the Testing of Chemicals. Organisation for Economic Co-operation and Development, Paris, June 2000.

OECD (2000b). Bacterial Reverse Mutation Test. Guideline 471, adopted 21.07.1997. In Eleventh Addendum to the OECD Guidelines for the Testing of Chemicals. Organisation for Economic Co-operation and Development, Paris, June 2000.

OECD (2000c). *In vitro* Mammalian Cell Gene Mutation Test. Guideline 476, adopted 21.07.1997. In Eleventh Addendum to the OECD Guidelines for the Testing of Chemicals. Organisation for Economic Co-operation and Development, Paris, June 2000.

OECD (2000d). *In vitro* Mammalian Chromosomal Aberration Test. Guideline 473, adopted 21.07.1997. In Eleventh Addendum to the OECD Guidelines for the Testing of Chemicals. Organisation for Economic Co-operation and Development, Paris, June 2000.

OECD (2003). Guidance document on the use of taxonomy in risk assessment of micro-organisms: bacteria. OECD Series on Harmonisation of Regulatory Oversight in Biotechnology. 2003; ENV/JM/MONO, 2003 OECD.

OECD (2004) *In vitro* Micronucleus Test. Guideline 487. Draft New Guideline (June 2004).

Olempska-Beer ZS; Merke, RI; Ditto MD and DiNovi MJ (2006) Food-processing enzymes from recombinant micro-organisms - a review. Regulatory Toxicology and Pharmacology, 45 (2), 144-158.

Pariza MW and Johnson EA (2001). Evaluating the safety of microbial enzyme preparations used in food processing: Update for a new century. Regulatory Toxicology and Pharmacology, 33, 173-186.

Pfuhler S, Albertini S, Fautz R, Herbold B, Madle S, Utesch D and Poth A – Gesellschaft fuer Umwelt-Mutationsforschung (2007). Genetic toxicity assessment: employing the best science for human safety evaluation part IV: Recommendation of a working group of the Gesellschaft fuer Umwelt-Mutationsforschung (GUM) for a simple and straightforward approach to genotoxicity testing. Toxicol. Sci., 97, 237-240.

SCF (1992). Guidelines for the presentation of data on food enzymes. Opinion expressed on 11 April 1991. Reports of the Scientific Committee for Food, twenty seventh series. EUR 14181 EN, Luxembourg 1992.

The International Union of Pure & Applied Chemistry and the International Union of Biochemistry (IUPAC) (1974). Enzyme nomenclature Recommendations. Eur. J. Biochem, 45, 1-3.

WHO (2003). Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products. WHO/BCT/QSD/03.01. Geneva, World Health Organization, pp. 1–26.

ABBREVIATIONS

AFSSA	Agence Française de Sécurité Sanitaire des Aliments
CAS	Chemical Abstract Service
COT	UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
DVFA	Danish Veterinary and Food Administration
EC	European Commission and Enzyme Commission
EC/IUBMB	Enzyme Commission of the International Union of Biochemistry and Molecular Biology
EFSA	European Food Safety Authority
EINECS	European Inventory of Existing Chemical Substances
ELINCS	European List of Notified Chemical Substances
EU	European Union
FAO	Food and Agricultural Organisation
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
GLP	Good Laboratory Practice
GMM	Genetically Modified Micro-organisms
GMO	Genetically Modified Organisms
GMP	Good Manufacturing Practice
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
OECD	Organisation for Economic Cooperation and Development
QPS	Qualified Presumption of Safety
SCF	Scientific Committee on Food
TSE	Transmissible Spongiform Encephalopathies
TOS	Total Organic Solids
WHO	World Health Organisation

ANNEX I Definitions

Enzyme activity unit (U) - The amount of enzyme which will catalyse the transformation of one micromole of the substrate per minute under standard conditions (IUPAC, 1974). **Enzyme activity unit (kat)** - Katal is the SI unit of activity consisting of the amount of enzyme which will catalyse the transformation of one mole of the substrate per second. Katal was proposed as a replacement for the enzyme activity unit (U) in 1978. One kat = 60 x 10^6 U.

Enzyme specific activity - Enzyme activity units (U) or SI units (kat) per unit weight.

Food enzyme[13] - A product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms, containing one or more enzymes capable of catalyzing a specific biochemical reaction and added to food for a technological purpose at any stage of manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

Food enzyme preparation[14] - A formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

Food enzyme preparation - A formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution

Micro-organism - Word used to include prokaryotes, protozoa, microalgae, and all fungi (including moulds, yeasts and filamentous fungi). However, fungal basidiomycete fruiting bodies/ mycelia are considered together with plant sources.

Source materials - Animal, plant, basidiomycete fruiting bodies / mycelia or microbial sources that may be used for the production of the food enzyme.

Total Organic Solids (TOS) To distinguish the proportion of the enzyme preparation derived from the source material and manufacturing process from that contributed by intentionally added formulation ingredients, the content of total organic solids (TOS) is calculated as follows:

% TOS = 100 - (A + W + D)

where:

A = % ash, W = % water and D = % diluents and/or other formulation ingredients.

[13] As defined in Regulation (EC) 1332/2008 on food enzymes
[14] As defined in Regulation (EC) 1332/2008 on food enzymes