European Food Safety Authority

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TECHNICAL REPORT OF EFSA

Explanatory Note for the Guidance of the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) on the Submission of a Dossier on Food Enzymes[1]

European Food Safety Authority[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 7 April 2014, replaces the earlier version 2011:EN-177 published on 8 July 2011.

ABSTRACT

EFSA's Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids Panel (CEF) has adopted a 'Scientific Opinion on Guidance on the Submission of a Dossier on Food Enzymes' on 23 July 2009. In this guidance document the data required to conduct a risk assessment for food enzymes are described. It was requested by the Regulations (EC) No 1331/2008 on a common authorisation procedure for food additives, food enzymes and food flavourings of the European Parliament and of the Council. Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 lays down the content of a dossier as well as the procedure to file a dossier for the safety evaluation of a food enzyme.

In order to assist applicants to compose a technical dossier, EFSA issues the updated Explanatory Note for the CEF Panel guidance giving more examples of scientific data need. The Explanatory Note is likely to be updated if needed to incorporate new examples.

KEY WORDS

food enzyme, CEF Panel guidance on the submission of a dossier on food enzymes, application, safety evaluation

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The technical report was further elaborated. To avoid confusion, the original version of the technical report has been removed from the website, but it is available on request.

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BACKGROUND AS PROVIDED BY EFSA

On 16 December 2008 the Regulations (EC) No 1332/2008 on food enzymes[4] and (EC) No 1331/2008 on a common authorisation procedure for food additives, food enzymes and food flavourings[5] of the European Parliament and of the Council were adopted. Both Regulations entered into force on 20 January 2009.

All food enzymes currently on the EU market as well as new food enzymes shall be subject to safety evaluation by the European Food Safety Authority (EFSA) and approval via a Union list.

The legislation stipulated also that EFSA shall present the Commission with a proposal concerning the data required for risk assessment of food enzymes. This task was fulfilled on 23 July 2009, when the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids Panel (CEF) has adopted a scientific opinion concerning the data required for risk assessment of the food enzymes in its 'Scientific Opinion on Guidance of the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids (CEF) on the Submission of a Dossier on Food Enzymes' (EFSA, 2009a).

AMFEP prepared two trial dossiers according to the CEF Panel guidance document. The dossiers were received by EFSA on 3 March 2010 (amylase from *Aspergillum niger*) and on 12 July 2010 (amylase from *Bacillus licheniformis*). They were used as case studies to draft a Technical Report 'Explanatory Note for the Guidance of the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids (CEF) on the Submission of a Dossier on Food Enzymes'. The dossiers do not contain competitive information.

This Explanatory Note is a document giving examples of scientific data needed for risk assessment established in the CEF Panel guidance. It was first published on 8 July 2011. EFSA may consult the Scientific Committee and the CEF Scientific Panel during the process of elaboration of the document. The Explanatory Note is likely to be updated if needed to incorporate new examples.

TERMS OF REFERENCE AS PROVIDED BY EFSA

On the basis of the trial dossiers submitted and taking into account the 'Guidance of the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids (CEF) on the Submission of a Dossier on Food Enzymes', the FIP unit is asked to prepare with the assistance of the Enzyme WG a Technical Report 'Explanatory Note for the CEF Panel Guidance on Food Enzymes'.

[4] Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on food enzymes and amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L354, 31.12.2008, p. 7-15.

[5] Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, p. 1-6.

1. Introduction

This Explanatory Note is addressed to applicants wishing to submit a technical dossier for the safety evaluation of a food enzyme prior to its authorisation and subsequent inclusion in the relevant EU legislation. The purpose is to give examples of scientific data needed for risk assessment established in the CEF Panel Guidance on the submission of a Dossier on Food Enzymes.

It should be stressed that the Explanatory Note is not replacing but complementing the CEF Panel Guidance. The readers are invited to consult the CEF Panel Guidance for the preparation of the technical dossier (EFSA, 2009a) as well as the corresponding EU legislation[6,7,8]. Especially Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 lays down the content of a dossier as well as the application procedure.

2. General remarks

A complete technical dossier is requested for each food enzyme regardless of the source material. This holds especially true for food enzymes produced by genetically modified micro-organisms (GMM).
The specific requirements for grouping dossiers under one application are laid down in the Commission Implementing Regulation (EU) No 562/2012.[9] Data need to be specific for the food enzyme under evaluation, especially compositional data (including impurities), TOS, specific activity (enzyme activity per amount of TOS), production method (incl. characterisation of the strain and down-stream processes).
Toxicological studies should be carried out according to the OECD guidelines mentioned in the CEF Panel Guidance and under GLP condition. The food enzymes tested in toxicological studies must be demonstrated to be representative of the commercial product. Evidence of the representativeness should be provided in the dossier.

PRACTICAL EXAMPLES AND EXPLANATIONS

Next chapter follows the order of the CEF Panel Guidance. The left column contains the requirements from the CEF Panel Guidance while the right column contains explanations, examples and comments.
In case no information is given in the column ‘comments’, the knowledge of examples is currently insufficient and the content might be updated in the future.

[6] Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on food enzymes and amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, p. 7-15.
[7] Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, p. 1-6.
[8] Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L64, 11.03.2011, p. 15-24.
[9] Commission implementing Regulation (EU) No 562/2012 of 27 June 2012 amending Commission Regulation (EU) No 234/2011 with regard to specific data required for risk assessment of food enzymes. OJ L 168/21, 28.06.2012, p. 21 -23.

3. Technical Data[10]

3.1. Identity of the Food Enzyme

3.1.2. Chemical Composition and Properties of the Food Enzyme

3.1.2.1. Chemical Composition

CEF Panel Guidance	Comments/Explanations
The following should be provided: **i.** Molecular mass of the food enzyme and subunit structure; and amino acid sequence (if available)	Considering the current state of the art, it should be feasible to provide the amino acids sequence for food enzymes.
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,	Degree of purity and identity: provide relevant chromatographic or electrophoretic data. Expected impurities (e.g. as identified by the JECFA specifications for food enzymes, and those possibly coming from raw materials used in the manufacturing process) should be identified. The rational for their analysis should be provided in the light of the sources, the production and the downstream processes. The methods should be standardised and/or validated, and provided in annexes.
iii. Information on whether the food enzyme is modified by post translational process or by technological procedures,	Updated on version 2014:EN-579; Post translational process means enzymatic/chemical modifications performed in the enzyme protein after its translation by the organism itself (i.e. glycosylation). In eukaryotic expression hosts the applicant should consider that glycosylation could influence the properties of the enzyme.
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,	
v. Data on the batch-to-batch variability for the relevant parameters,	Relevant parameters are mentioned in (ii). Indicate the size of batches and frequency of production. These data may not be available for newly produced food enzymes. Updated on version 2014:EN-579; Acceptable inter-batch variability is decided on a case by case basis and depending on 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	

[10] The numbering of the Chapter & Sections starting with number '3 Technical data' is reflecting the one in the document "Guidance of the Scientific Panel of Food Contact Material, 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.

defined by JECFA (FAO/WHO, 2006)	

3.1.2.2. Proposed Chemical and Microbiological Specification

CEF Panel Guidance	**Comments/Explanations**
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.	

3.1.2.3. Properties of the Food Enzyme

CEF Panel Guidance	**Comments/Explanations**
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)). 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.	The information to be provided must refer to the specific characteristics of the food enzyme. Generally, data should be based on own experimental measurements. Literature data can be used as support if these are of relevance for the food enzyme. Determination methods for the food enzyme activities should be provided in annexes. If possible, correlation to international units should be made.
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),	Temperature and pH optima might be determined in model experiments under laboratory conditions. Provide T- and pH ranges in food items for which the food enzyme should be used.
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,	Indicate also the presence of other significant enzyme activities of the food enzyme. Updated on version 2014:EN-579; Side/subsidiary activities are referring to other activities of the enzymes present in the food enzyme, including activities that may be expressed under different conditions than those intended in the application.
iv. Data on the stability of the food enzyme during storage and before use.	Give practical examples with data for the intended preparations on the market and types and conditions of their storage. Updated on version 2014:EN-579;

	The data on the stability of the food enzyme as such would have to cover at least the recommended time of use under the specified conditions of use.

3.2. Source Materials and Manufacturing Process

3.2.1. Source Materials

3.2.1.1. Production from Animal Sources

CEF Panel Guidance	Comments/Explanations
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.	Updated on version 2014:EN-579; An example can be given for rennet (chymosin): There are different types of rennet commercially available which may differ in their origin (e.g. animal, vegetable, microbial or recombinant rennet) or physical state (liquid, powder or paste). Rennet paste is a crude form of rennet and the dossiers for this form of rennet should follow the data requirements as laid down in this chapter.
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).	

3.2.1.2. Production from Plant and Basidiomycete Sources

CEF Panel Guidance	Comments/Explanations
i. The part(s) of the plant or basidiomycete fruiting bodies/mycelia used for the production of the food enzyme should be specified.	Provide data on strain identification (e.g. strain numbers) and cultivar identification. For mycelia (fungal sources), refer to section 3.2.1.3. Updated on version 2014:EN-579; The application must provide evidence that enzymes extracted from the different parts of the plant correspond to the claimed food enzyme(s). As an example a dossier of a plant–derived food enzyme e.g. bromelain containing two enzymes (stem bromelain and fruit bromelain) may be covered under one dossier provided that the parts

	of the plant (e.g. fruit, stem) used for the production of the food enzyme and the most recent taxonomic classification including genus, species and sub-species (if appropriate) are specified (e.g. Ananas comosus).
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 3.1.2.1 and limits should be proposed in section 3.1.2.2.	
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 1829/200310 on genetically modified food and feed11, information concerning the risk assessment and authorisation of the GMO should be provided.	

3.2.1.3. Production from Microbial Sources

CEF Panel Guidance	**Comments/Explanations**
Although neither pathogenic or 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 microorganisms 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.	Provide the evidence that the strain / strain lineage used is covered by the QPS status (e.g. data for 16S rRNA (for bacteria). If the strain / strain lineage is not QPS, demonstrate that the strain does not produce toxins (see section 3.1.2.1, ii). Updated on version 2014:EN-579; Mutants from a specific strain that has been thoroughly tested for safety, have to be re-tested if additional mutations are performed.

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 Products Intended for Food and Feed Use` (EFSA Panel on Genetically Modified Organisms (GMO), 2011).	Updated on version 2014:EN-579; Recipient strain is the one receiving the genetic modification which is subject of the application. Parental strain is the closest non-GM microorganism with direct genealogical link to the GMM subject of the application. On page 26 of the GMM GD, "expression host" refers to the recipient. The applicant may use information on the safety of the parental strain. In these cases the parental strain if already safety assessed, can be used as the starting point. It is important that the relationship between the parental strain and the recipient strain as well as the modification steps applied on this parental strain to result in the recipient strain are described. When genetic modification steps are used, these steps should be described in detail, following the GMM GD. Information should be provided on the insert copy number and on whether the insert remains in the vector or is transferred to the genome. However, the GMM GD does not request that the integration site is defined and characterized in any case. This can be requested on a case-by-case basis if deemed necessary for the risk assessment. The documentation requested for the GMMs containing antibiotic resistance marker genes (ARMs) is described in the GMM GD. For strains containing ARMs the risk assessment on the presence/absence of recombinant DNA (2.2.3), the absence of the GMM in the product (2.2.1) and the inactivation of the GMM cells and evaluation of the presence of remaining physically intact cells (2.2.2) will be scrutinized. Sufficient information has to be provided on the results and the methodology (including sampling methodology) used.
iii. Monitoring of Production Strain 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	If these aspects are covered by an implemented HACCP program, relevant parts of such a program (e.g. table with critical control points and measures) could be provided to meet the requirements.

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 'Opinion 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).	Provide information e.g. which toxins are screened for, how often and provide the results of measurements. Updated on version 2014:EN-579; Lipopeptides may also exert antibacterial or antifungal properties. Their absence would be dependent on the purification procedure so that when using production strains able to generate lipopeptides, their presence in the final product should be checked. If information is already provided in previous sections, please refer to those.

3.2.2. Manufacturing Process

CEF Panel Guidance	**Comments/Explanations**
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 3.1.2.2	Describe in detail the key steps performed to produce the food enzyme. Provide specifications of the agents and reagents used in the process demonstrating their suitability for the production of food enzymes.
ii. Description of operational limits including process controls and quality assurance procedures and how key parameters such as temperature are controlled during production.	Identify the critical steps for fermentation, recovery and purification; explain how these critical steps are under control. If the above steps are covered by an implemented HACCP program, relevant parts of such a program (e.g. table with critical control points and

	measures) could be provided to meet the requirements.
iii. In the case of immobilised food enzymes, information on the immobilisation procedure is required, *e.g*. enzyme support materials 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, 2009b).	

3.3. Reaction and Fate in Food

CEF Panel Guidance	**Comments/Explanations**
Information should be provided on the fate of the food enzyme during food processing (see Section 3.1.2) 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).	Provide specific data for the food enzyme.

3.4. Case of Need and proposed Conditions of Use

The purpose of the use will not be evaluated by the Panel, but this information may include related safety aspects. The information below is also important to assess exposure, by specifying conditions of use. Even if some of this information has been detailed elsewhere in the dossier, it should be summarised here. Information should be provided on:

CEF Panel Guidance	**Comments/Explanations**
Information should be provided on: **i.** The technological need/purpose and intended use of the food enzyme,	Provide here proposed use in food and recommendations made to customers.
ii. The mode of action and reactions catalysed by the food enzyme,	Updated on version 2014:EN-579; Reactions should refer to the foods covered by the proposed conditions of use. specific issues to be addressed:

	- matrix effects on activity in intended uses - side reactions depending on food
iii. The type of foodstuffs in which the food enzyme is intended to be used,	The food categorization system described in Annex III of Regulation 1565/2000[11] is not especially developed for food enzymes. However in order to assess consumer exposure and safety margin it is necessary to identify the types of foods / food processes (e.g. baking, brewing) in which the enzyme is intended to be used. (see also section 3.5 below). All intended uses must be described.
iv. The amount of food enzymes to be added to specific foods (recommended use levels and maximum use levels),	Recommended use levels must be reported for all intended foods as identified in (iii).
v. The conditions of its use in food processing.	Updated on version 2014:EN-579; Typical pH and temperature ranges and any cofactors needed according to the specified food processing.

3.5. Dietary Exposure

CEF Panel Guidance	**Comments/Explanations**
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, 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 (0- 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.	This information is to be provided even if enzyme is produced by QPS microorganism. The assumption in the FAO/WHO report (FAO/WHO, 2009) for food consumption and proportion of solid food and non milk liquid beverages should be used to calculate dietary exposure. In case of very limited or specific applications (one food category), alternative techniques that allow to assess exposure in high consumers may be used. A more refined exposure assessment should be performed if the use calculated according to the method described in the FAO/WHO report (s. also "Budget Method") indicates potential concern with high consumers. Updated on version 2014:EN-579; The exposure assessment covers not only the food enzymes but also any constituent or by-product of concern that may be present in the food enzyme. The exposure must be assessed considering all proposed uses and this would be especially relevant for exposure of high consumers. There is no specific food category system for food enzymes that can be used when submitting an

[11] Commission Regulation (EC) No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council. OJ L 180, 19/07/2000, p. 8-16.

	application. The food category system established in Commission Regulation (EU) No 1129/2011 amending Annex II of Regulation (EC) No 1333/2008 may not be useful for this purpose, because the functionality of food enzymes is substrate dependent and thus not directly linked to food categories. Food enzymes may be used in the manufacturing of food ingredients or during food processing or may be immobilised on an insoluble matrix. Information on the use of the enzyme in food processing (e.g. starch processing) can be mentioned instead.

3.6. Information on Existing Authorisations and Evaluations

CEF Panel Guidance	Comments/Explanations
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.	The information requested here is for the specific food enzyme which is the topic of the application. Evaluations performed for the identical food enzyme in Denmark and France according to the SCF Guidelines shall not exempt the food enzyme from a safety assessment by EFSA. However, it could be construed as a support to the history of safe use of the food enzyme.

4. Toxicological Data

4.1. Toxicological Testing

CEF Panel Guidance	Comments/Explanations
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 4.1.2).	

4.1.1. The toxicological Data Set

CEF Panel Guidance	Comments/Explanations
The core set of toxicological data that is required is set out below.	The tested batch should be characterised. Evidence (by test results) must be provided that the tested batch is representative of commercial samples. The parameters used to demonstrate the equivalence of the batch that is toxicologically tested shall be the same as those used to describe the chemical composition.

	Original study reports must be provided and any effect detected must be reported and commented. Updated on version 2014:EN-579; The test substance in the OECD guidance should refer to TOS. It is recommended that the maximum dose chosen for all toxicological tests should be based on the amount TOS (e.g. for Ames test 5 mg TOS/plate provided that the enzyme is soluble and none cytotoxic in the test as discussed in the OECD guidance). Depending the test the dose units should be expressed as µg TOS / plate, µg TOS/ml or mg TOS/kg b.w./day. The selection of the lower doses must be justified and discussed in detail.
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. FOR FURTHER DETAILS SEE CEF PANEL GUIDANCE	Updated on version 2014:EN-579; For an adequate evaluation of the genotoxic potential of a chemical substance, different end-points (i.e. induction of gene mutations, structural and numerical chromosomal alterations) have to be assessed, as each of these events has been implicated in carcinogenesis and heritable diseases. In its opinion the Scientific Committee (EFSA Panel on Genetically Modified Organisms (GMO), 2011), suggested the following two *in vitro* tests as the first step in genotoxicity testing • a bacterial reverse mutation assay (OECD TG 471), and • an *in vitro* mammalian cell micronucleus test (OECD TG 487). The opinion states that "this combination of tests fulfils the basic requirements to cover the three genetic endpoints with the minimum number of tests; the bacterial reverse mutation assay covers gene mutations and the *in vitro* micronucleus test covers both structural and numerical chromosome aberrations." In the case of the Ames test in order to overcome potential problems with histidine in the food enzyme batch, the Salmonella strains it is recommended to exposed to the tested food enzyme in the liquid culture ("treat and plate assay", instead of the traditionally "plate incorporation assay"). In the case of the Ames test and when the food enzyme may affect the performance of S9 (e.g. inactivation by phospholipase), food enzyme should be added to the positive control so as to show that the S9 performance is not affected. Recommendations on the performance of bacterial mutagenicity tests for enzymes are given in the Appendix.

ii. Assessment of systemic toxicity A subchronic oral toxicity study (OECD 408) should be performed. FOR FURTHER DETAILS SEE CEF PANEL GUIDANCE	

4.1.2. When toxicological Testing may not be needed

CEF Panel Guidance	Comments/Explanations
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.	The QPS status of the production microorganism shall exempt from toxicological studies, provided that absence of concern from residues, impurities, degradation products linked to the total production process (production, recovery and purification) is demonstrated and supported by experimental data. Updated on version 2014:EN-579; Regarding the genetic modification concerns, microbial strains obtained using self cloning have to be risk assessed following the GMM guidance document. They are exempt from toxicological testing provided they are complying with the requirements of Reg. 562/2012.

4.1.3. Data reporting

CEF Panel Guidance	Comments/Explanations
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 3.1.2.2, that the test material is representative of the food enzyme as described in the dossier.	

4.1.4. Review of the toxicological and exposure data and conclusions

CEF Panel Guidance	Comments/Explanations
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.	Conclusions drawn should be product specific (Production strain or strain lineage, and enzyme specific).

4.2. Allergenicity

CEF Panel Guidance	Comments/Explanations
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.	The approach used must be detailed: searches in data bases must be demonstrated. Search reports and programs used should be provided in annex.

5. Conclusion

CEF Panel Guidance	Comments/Explanations
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.	A product-related conclusion based on the data in previous sections should be given. Toxicity data from a safe strain lineage (See Section 4.1.2) may be used.

6. Dossier Bibliography

CEF Panel Guidance	Comments/Explanations
In submitting a dossier, a full bibliography should be included and full copies of all references quoted should be provided. References should be quoted as follows: **i.** Published Data - **Journals:** Author(s) (full list including all names and initials), date, title of article, journal, volume number, page numbers. - **Books:** Author(s), title of chapter/book, editor(s) (if relevant), publisher, location, date, page numbers (if relevant). - **Internet:** Organisation, title of report, website and access date	If existing, patents literature should also be provided (copies should be in annexes). Bibliography can be provided in electronic form only.

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ABBREVIATIONS

EC	European Commission and Enzyme Commission
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agricultural Organization
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
GLP	Good Laboratory Practice
GM	Genetically Modified
GMM	Genetically Modified Micro-organisms
GMO	Genetically Modified Organisms
HACCP	Hazard Analysis and Critical Control Points
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 Organization

APPENDIX

Recommendations on the performance of bacterial mutagenicity tests for enzymes

1. Introduction

Low levels of released amino acids from soluble materials can cause moderate increases in the number of revertant colonies on the plate, whereas higher levels lead to overgrowth of the background lawn, making counting of revertant colonies impossible. For poorly soluble material, the released amino acids can be present at high levels in localized spots on the plate, leading to the growth of 'pseudorevertant' colonies.
To avoid misinterpretations when testing histidine/ tryptophan containing compounds, it has been proposed that a modified pre-incubation method with extensive washing prior to plating could be employed (Aeschbacher et al., 1983; Kirkland et al., 1995, Thompson et al., 2005); various complex washing methods have been demonstrated (Mitchell et al, 1980; O'Connor et al, 1984; Verhagen et al., 1994).
Other amino acids, like arginine, may also interfere with Ames test (Khandoudi et al., 2009). Finally, a high level of bacteriostatic activity may limit the study of high doses (Mitchell et al, 1980) and the use of such variant of the test can be useful.

2. Methodology

2.1. Initial considerations

Except for the treatment of bacteria, the methodology and the reagents (medium, metabolic activation system, preparation of test item, solvents, culture conditions, choice of doses, etc) are the same as those used for the plate incorporation or pre-incubation Ames test (OECD guideline 471).
The test should be performed on the complete set of strains as recommended in the OECD guideline 471: four strains of *S. typhimurium* (TA1535; TA1537 or TA97a or TA97; TA98; and TA100) and one strain of *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102.

2.2. Treatment of the bacteria

A 0.5 mL aliquot of S9 mix or phosphate buffer 0.2 M pH 7.4 is combined with 0.1 mL late log bacterial culture in a sterile container. A 0.1 ml aliquot of the test solution containing the food enzyme is added. Bacteria and treatment are incubated for 90 min with shaking at 37°C. After the 90 min pre-incubation, a large volume (10 to 15 mL) of a wash solution of Oxoid No. 2 nutrient broth in phosphate buffered saline is added and the washed bacteria are collected by centrifugation (e.g. at 2000 g for 30 min). All but about 0.7-1 mL of the supernatant is removed and discarded, and the bacteria are re-suspended in the residual supernatant prior to mixing with the overlay agar and pouring onto the surface of a minimal agar plate (1.5% agar, Vogel–Bonner medium E, 2% glucose). In some cases, it is possible to perform a second washing of the bacteria. The plates are inverted and incubated at 37°C for 48 to 72 h. After the incubation period, the number of revertant colonies per plate is counted.

2.3. Controls

Concurrent strain-specific positive and negative (solvent or vehicle) controls, both with and without metabolic activation, should be included in each assay. Positive control concentrations that demonstrate the effective performance of each assay should be selected. Sterility control is included in each experiment.

2.4. Data and reporting

Treatment of results, evaluation and interpretation of results and test report are the same than for the plate incorporation or pre-incubation Ames test (OECD Guideline 471).

2.5. Historical controls

Negative (solvent) and positive historical control specific of this methodology should be available. The strains should also yield spontaneous revertant colony plate counts within the frequency ranges expected from the laboratory's historical control data and preferably within the range reported in the literature.

3. Recommendations for the test strategy

When negative results are obtained from the classical Ames assay, no further bacterial test is required, but if the results are positive, the relevance of these results might be clarified by a modified pre-incubation version of the Ames assay. If it is demonstrated or suspected that free histidine and/or arginine are present in the test system, a modification of the Ames assay should be applied. Different modified tests are available. The choice of the modified Ames test could be a case-by-case decision on which is the most applicable. A scientifically-based justification should be provided for the choice of any method.

4. Literature

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