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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 Evaluation by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids (CEF) [1]

(Question No EFSA-Q-2007-080)

Adopted after public consultation and discussion in the Panel:

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16 **TABLE OF CONTENTS**

49

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[3],

Regulation (EC) No 1333/2008 on food additives[4],

Regulation (EC) No 1334/2008 on flavourings and certain food ingredients with flavouring properties[5] and

Regulation (EC) No 1331/2008 on a common authorisation procedure for food additives, food enzymes and food flavourings[6].

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 measure 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.

[3] OJ L 354, 31.12.2008, p.7

[4] OJ L 354, 31.12.2008, p.16

[5] OJ L 354, 31.12.2008, p.34

[6] 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 an implementing measure (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 this implementing measure 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 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 measure which will lay down amongst other aspects, the content, drafting and presentation of an application for the evaluation and authorisation of food enzymes.

INTRODUCTION

The purpose of this document is to provide guidance to petitioners and other interested parties wishing to introduce food enzymes (as defined in below) into the European Union (EU) market in 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 on the administrative 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 (EFSA), 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 petitioners in the dossier.

General Principles of Risk Assessment of Food Enzymes

In order to enable the EFSA to carry out the safety assessment, the following aspects should be addressed in the dossier, as outlined in more detail in this guidance document:

- **Safety of the source.** A consideration of safety issues related to the source of the food enzyme (animals, plants, fungi 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.
- **Safety assessment of 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.

- **Safety assessment of 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 anticipated dietary exposure of the consumer.** This depends on the residual concentration of the 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.

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, 1990) 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).

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 catalyzing 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.

The 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 occupational health and safety aspects.

SUBMISSION OF AN APPLICATION

Applications shall be submitted in accordance with Regulation (EC) 1331/2008 and its implementing measure referred to in Article 9 of this Regulation. The applicant shall provide all available data relevant for the evaluation by the EFSA. For the purposes of the current guidelines, the definitions laid down in the Regulation (EC) 1332/2008 on food enzymes shall apply.

Petitioners should note that the Commission will make available to competent authorities in Member States in full form any dossier submitted to the Authority for a safety assessment (Art. 4 of the Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings). Confidentiality of information provided by the applicant will be treated in accordance with Article 12 of the Regulation (EC) No 1331/2008. Therefore applicants should mark clearly which of the information provided they wish to be treated as confidential and provide verifiable justification in such case.

The applicant should submit a dossier with the full information, both on paper and in electronic format on standard physical media (*e.g.* CD ROM). It has to be declared by letter that the electronic and the paper versions are identical.

In addition to the complete version with the full information, applicants are requested to provide a second version of the CD ROM without the confidential information. This version will be made available to anyone who might submit a request for access to documents to the EFSA.

Any specific literature reference (such as scientific papers) mentioned and used to support the petition must be supplied in the dossier as full length paper. When reference is made to a book or to extensive publications, only the relevant parts need be supplied.

Applicants may deviate from the guidelines, provided valid and documented scientific reasons are given in the dossier. In all cases the EFSA may request additional data.

INFORMATION TO BE SUPPLIED WITH AN APPLICATION FOR A FOOD ENZYME

In order for the EFSA to carry out a safety evaluation of a food enzyme, petitioners and other interested parties wishing to introduce food enzymes into the EU market according to Regulation (EC) No 1332/2008 on food enzymes should include the following information when submitting a dossier[7]:

Title of the dossier (including enzyme name and enzyme source)

i. Summary of Dossier Submission
ii. Administrative Data
iii. Technical Data
iv. Toxicological Data
v. Conclusion

[7] Dossiers are initially submitted to the European Commission for onward transmission to the EFSA, and may be made available to outside parties. Petitioners should therefore indicate if any parts of the dossier are confidential business information in accordance to the legislation provisions. Petitioners are requested to retain copies of their petition.

vi. Bibliography, References (original papers) and Reports

1. Summary of Dossier Submission

A summary of the data which has been submitted by any interested party or parties in support of a food enzyme should be provided at the start of the dossier. The summary should follow the same order as described for the main dossier and include the following information:

vii. Identity of the Food Enzyme
viii. Chemical Composition and Properties of the Food Enzyme
ix. Source materials and Manufacturing Process
x. Reaction and Fate in Foods
xi. Proposed Uses in specific Food Products or Food Categories
xii. Main Findings of Toxicological Tests

Petitioners should also present their own conclusions as to the likely safety-in-use of the food enzyme, drawing attention to any unusual features in the data presented.

The summary should not contain any confidential information as it will be made available to the public on request.

2. Administrative Data

A dossier on a food enzyme for safety evaluation by the EFSA should include information on the following:

i. Name, address and other contact details of the petitioner(s), and/or other interested parties *(e.g.* company, organisation, *etc*.) to include telephone number(s), fax number(s), email(s)
ii. Name, address and other contact details of the manufacturer(s) of the food enzyme (if different from above) to include telephone number(s), fax number(s), email(s)
iii. Name, address and other contact details (if different from above) of the person(s) responsible for the dossier
iv. Date of submission of the dossier
v. Scope of the application (*e.g.* new food enzyme or previously evaluated enzyme produced by new production methods, new starting materials, extension of use)
vi. Reference to any related food enzyme(s) already evaluated/authorised on the market within the EU or internationally
vii. A table of contents for the submitted dossier

3. 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:

3.1 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 (s. Section 4 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.

3.1.1 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) [8] *(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)*

[8] 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

3.1.2 Chemical Composition and Properties of the Food Enzyme

3.1.2.1 Chemical Composition

The following should be provided:

i. Molecular mass of the enzyme and subunit structure; and amino acid sequence,
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 enzyme is modified by post translational process or by technological procedures,
iv. Information on whether the enzyme is genetically 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,
vi. Data on the reproducibility for relevant parameters.

3.1.2.2 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 petitioner considers useful in describing the composition of a food enzyme should also be supplied.

3.1.2.3 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) and/or by the SI unit, Katal (kat =· mol · s^{-1}) [9]. The enzyme assay method and methods for determination of principal and side reactions, along

[9] 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.

with information on the stability of the food enzyme during food processing/storage should be provided,

ii. The activity of the 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.

3.2 Source Materials and Manufacturing Process

3.2.1 Source Materials

Food enzymes are produced from animal, plant, fungal and microbial sources. The specific information which should be included and submitted as part of the dossier in the case of animal, plant and fungal 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, petitioners 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).

3.2.1.1 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).

3.2.1.2 Plant and Fungal Sources

i. The part(s) of the plant or fungus 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 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, 2006a). If the source is already covered by an authorisation in accordance with Regulation (EC) No 1829/2003 on genetically modified food and feed[10], information concerning the risk assessment and authorisation of the GMO should be provided.

3.2.1.3 Microbial Sources

Although neither pathogenic or toxigenic micro-organisms are intentionally used in the production of food enzymes, certain microbial fungi traditionally used as sources of food enzymes can 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 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

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

Modified Micro-organisms and their Derived Products Intended for Food and Feed Use` (EFSA, 2006b).

Accordingly, information should be provided on the recipient or (when appropriate) the parental organism, the donor organism(s), nature of the transferred genetic material, the genetic modification process and information relating to the GMM in comparison with its conventional counterpart.

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 enzyme production. This may include details on storage conditions of the strain, the industrial pre-culture and culture conditions and their effect on strain drift and 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. This should include the reference number/name of the strain, which should be deposited in a strain collection, preferably at an independent and internationally recognised organisation, preferably in the EU. The producer organism must be maintained by the strain collection.

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 acquired antimicrobial/antibiotic (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).

3.2.2 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 extraction solvents, other chemicals, materials and equipment.

If relevant, information relating to the techniques used to remove microbial cells from the product, the process used to purify the product from the microbial growth medium and the degree of purity achieved should also be provided.

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

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

iii. Description of the food safety management system which should include details of good manufacturing practice (GMP)[11], Hazard Analysis Critical Control Point (HACCP) (European Union, 2004) and recall and traceability (European Union, 2002) procedures employed by the manufacturer.

iv. In the case of immobilised food enzymes, information on the immobilisation procedure is required, *e.g.* enzyme support materials[12] and immobilisation agents. Information on potential leakage of carriers, immobilisation agents and active enzymes into the food should be provided.

v. 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).

3.3 Reaction and Fate in Food

i. 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, information should be provided on residues, degradation products and interactions with the food matrix. If for

[11] GMP include systems of quality control and quality assurance, employee qualifications, maintenance standards for equipment, control of raw materials and product stability.

[12] 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.

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.

ii. Data should be provided on the nature and stability of the main reaction products and any possible reaction and/or degradation products that are formed during processing, storage or preparation of the foods. In addition the following is required to allow safety assessment:

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

3.4 Case of Need and proposed Conditions of Use

Information should be provided on:

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.

3.5 Dietary Exposure

Petitioners should produce refined or *ad hoc* exposure estimates using other methods based on consumption data for these food categories. Such methodologies should be base on conservative assumptions and should take into account 1) hypothetical high consumers of the food products or of the food categories susceptible to contain the enzyme and 2) the different consumption patterns of the European countries. All assumptions and the data used for calculating refined exposure estimates must be clearly described and discussed.
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), *ad hoc* exposure estimates must be produced taking specifically into account these population groups.

3.6 Information on Existing Authorisations and Evaluations

Information on any existing authorisations and evaluations and/or evaluations by other bodies should be provided.

4. Toxicological Data

4.1 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 4.1.2).

4.1.1 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)

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, and represent a carcinogenic hazard. Deliberate addition of a genotoxic carcinogen to food is unacceptable (Barlow *et al*, 2006). A positive result in genotoxicity testing would then require further assessment to determine whether it is genotoxic *in vivo.*

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[13] and 2004/09/EC[14] 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 necessary to deviate from the above mentioned core set. Such deviations include use of alternative protocols, or use of alternative assays. In such cases a scientific justification should be provided and additional types of considerations or mechanistical studies may be needed.

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 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 enzyme (see section 4.2). Decisions on whether additional studies are needed will be taken by EFSA on a case-by-case basis.

4.1.2 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 enzyme, the composition and the properties of the 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).

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

4.1.3 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,

[13] OJ L 50, 20.2.2004, p. 44

[14] OJ L 50, 20.2.2004, p. 28

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

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 are seen, the relationship between the dose giving rise to effects and likely dietary exposure from use of the food enzyme should be discussed. The reasons for disregarding any findings should be carefully explained. Where relevant, the conclusions should include an interpretation of the significance of the findings.

4.2 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, 2006a; WHO/FAO, 2001). The allergenicity of the source of the enzyme should be considered and a search for amino acid sequence and/or structural similarities between the expressed protein and known allergens (*e.g.* using http://www.allergome.org) should be undertaken. 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, 2006a).

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. Apart from this, petitioners should provide any existing information on indications and/or evidence of allergenicity in workers producing and handling the food enzyme as well as in consumers of enzyme-treated foodstuffs.

5. 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.

6. Dossier Bibliography

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

651 **ii.** Unpublished Data
652 - Name of petitioner, title of report, report reference, name of investigator(s) (if any),
653 name of laboratory, address of laboratory, date**.**
654
655 **iv.** Appended Papers and Study Reports
656 - Full copies from the references cited which are essential to the safety evaluation should
657 be included in the dossier.
658
659 **v.** Copies of all unpublished study reports should be submitted in full. Summaries or abstracts
660 of unpublished studies are not sufficient.
661

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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
WHO	World Health Organisation