Overview on EFSA data requirements for the safety evaluation of food enzymes applications

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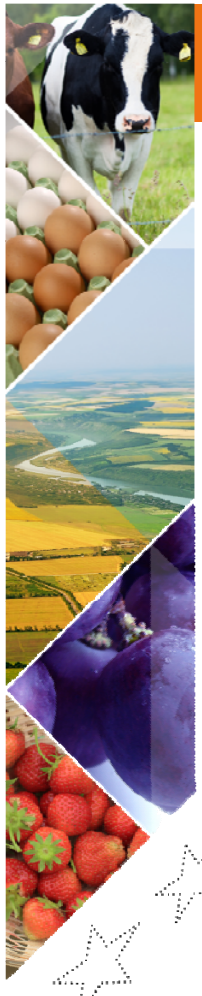
EFSA CEF Panel

Info session on Food Enzymes applications
Parma, 27 May 2014

efsa
European Food Safety Authority

GUIDANCE DOCUMENT BY THE CEF PANEL



European Food Safety Authority

The EFSA Journal (2009) 1305, 1-26

SCIENTIFIC OPINION

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 Material, Enzymes, Flavourings and Processing Aids [1]

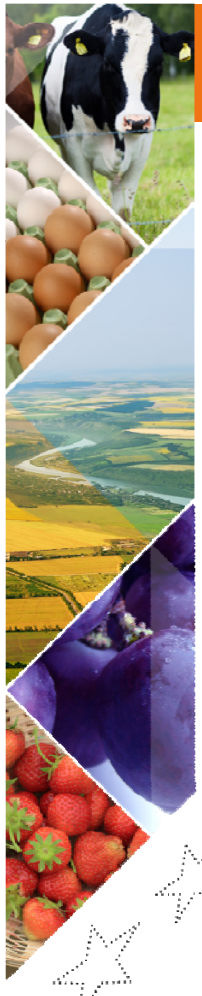
(Question No EFSA-Q-2007-080)

Adopted after public consultation and discussion in the Panel:

23 July 2009

http://www.efsa.europa.eu/en/efsajournal/pub/1305.htm

EXPLANATORY NOTE FOR THE GUIDANCE



European Food Safety Authority

EFSA supporting publication 2014:EN-579

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.

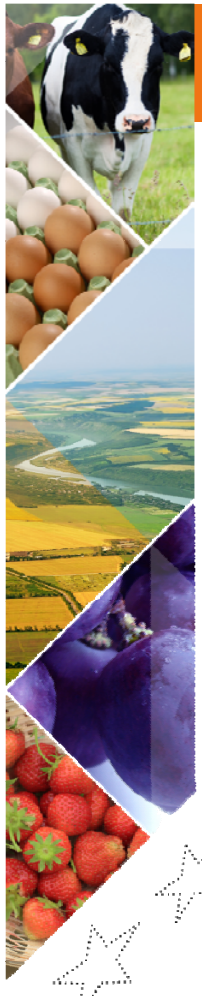
http://www.efsa.europa.eu/en/supporting/doc/579e.htm

Chemical Composition

- Molecular mass and subunit structure and amino acid sequence (if available)
- Chemical description including chemical purity / identity
- Information if modified by post translational or technological procedures
- Information if protein engineered, rational and nature of the modification
- Data on batch-to-batch variability for relevant parameters
- Data on the reproducibility for relevant parameters
- Any other useful information: Total Organic Solids (TOS)

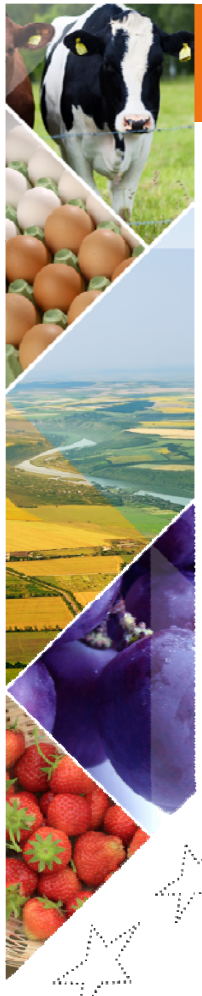
TECHNICAL DATA. EXPLANATORY NOTE

Guidance	Comments/Explanations
Molecular mass and subunit structure; and amino acid sequence (if available)	Feasible with current state of the art
Chemical description including chemical purity and identity	Provide relevant chromatographic or electrophoretic data. Expected impurities, analysis and standardised or validated methods of analysis
Information if modified by post translational or technological procedures	Post translational process means enzymatic or 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.
Data on the batch-to-batch variability for the relevant parameters	Size of batches and frequency of production. Acceptable inter-batch variability on a case-by-case basis



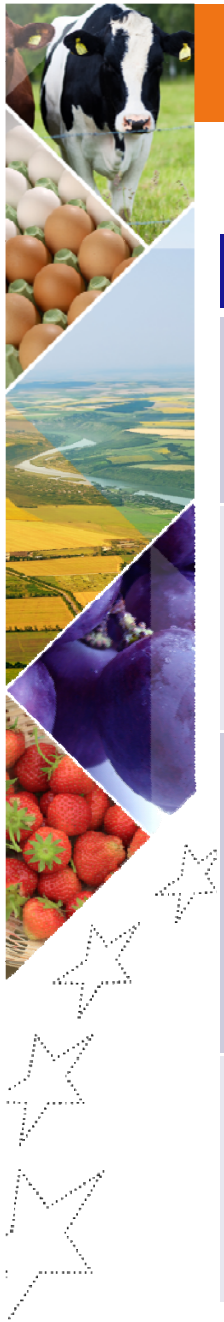
Proposed Chemical and Microbiological Specifications

The proposed specifications should be submitted in a format modelled on recent EU or other internationally accepted specifications.



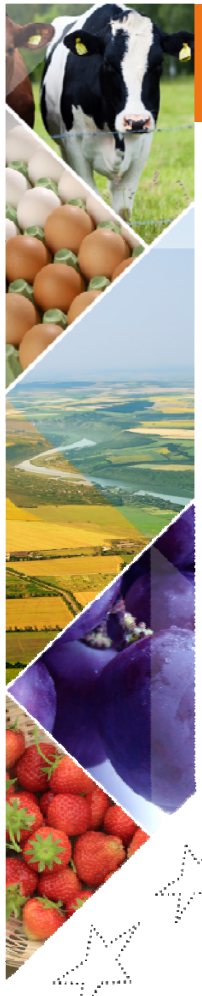
Properties of the Food Enzyme

- Information on the principal enzymatic activity
- The activity of the food enzyme under the conditions of the intended use and the influence of reaction conditions
- Any subsidiary/side activities
- Data on food enzyme stability



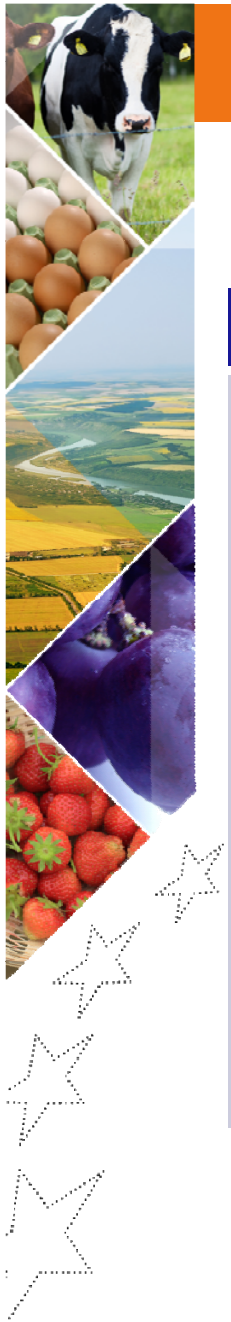
TECHNICAL DATA. EXPLANATORY NOTE

Guidance	Comments/Explanations
Information on the principal enzymatic activity	Specific characteristics of the food enzyme Methods for the food enzyme activities If possible, correlation to international units
The activity of the food enzyme	T and pH optima at laboratory conditions Provide T and pH ranges in food items for which the food enzyme should be used
Any subsidiary/side activities	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
Data on food enzyme stability	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.



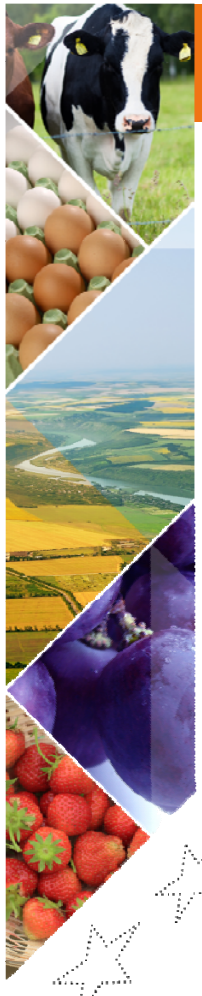
Production from animal sources

- Which animal tissue and information on previous consumption and if documented history of safe use
- Compliance with meat inspection requirements and handling in accordance with good hygienic practice
- Methods used to ensure absence of any risk of infectivity
- Data on non-infectivity based on the classification of the tissues in terms of their infectious titre in natural diseases



TECHNICAL DATA. EXPLANATORY NOTE

Guidance	Comments/Explanations
Which animal tissue and information on previous consumption and if documented history of safe use	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



Production from plant and basidiomycete sources

- The part(s) of the plant or basidiomycete fruiting bodies/mycelia used
- Information on previous consumption and if documented history of safe use
- Methods used to ensure absence of any risk
- If a genetically modified plant or fungus is used, information on the organism in accordance with the GMO guidance document

TECHNICAL DATA. EXPLANATORY NOTE

Guidance	Comments/Explanations
The part(s) of the plant or basidiomycete fruiting bodies/mycelia used	Provide data on strain identification (e.g. strain numbers) and cultivar identification Evidence that enzymes extracted from the different parts of the plant correspond to the claimed food enzyme(s) Example: 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) and the most recent taxonomic classification including genus, species and sub-species (if appropriate) are specified (e.g. Ananas comosus).

Production from microbial sources

- Information about the strain used for food enzyme production
- For GMM, the presence of any factor(s) affecting the genetic stability of the producer strain
- Monitoring of production strain
- Production strain pathogenicity, toxigenicity and antimicrobial resistance

GMM: information given on GMM presentation

TECHNICAL DATA. EXPLANATORY NOTE

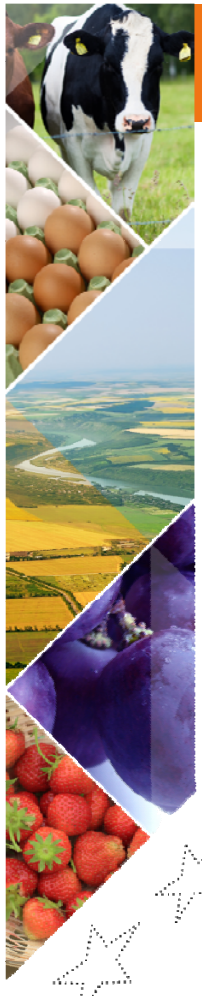
Guidance	Comments/Explanations
Information about the strain used for food enzyme production	Provide evidence for QPS status, e.g. data for 16S rRNA (for bacteria) If not QPS, demonstrate it does not produce toxins Mutants from a specific strain that has been thoroughly tested for safety, have to be re-tested if additional mutations are performed.
For GMM	***Information given on GMM presentation***
Monitoring of production strain	If covered by an implemented HACCP program, relevant parts (e.g. table with critical control points and measures) could be provided
Production strain pathogenicity, toxigenicity and antimicrobial resistance	Which toxins are screened, how often and measurements performed Lipopeptides may also exert antibacterial or antifungal properties. Their presence in the final product should be checked.

Manufacturing process

- Description of key steps involved in the production process
- Description of operational limits including process controls and quality assurance procedures
- If used, information on the immobilisation procedure
- Other relevant information (i.e. nanotechnology?)

TECHNICAL DATA. EXPLANATORY NOTE

Guidance	Comments/Explanations
Description of key steps involved in the production process	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
Description of operational limits including process controls and quality assurance procedures	Identify the critical steps for fermentation, recovery and purification and explain how are under control If covered by an implemented HACCP program, relevant parts (e.g. table with critical control points and measures) could be provided

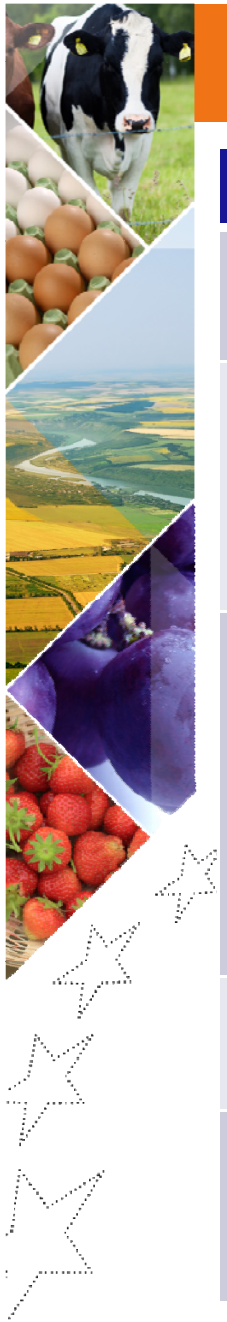


TECHNICAL DATA. REACTION AND FATE IN FOOD

- Fate of the food enzyme during food processing
- Behaviour of the food enzyme in the food matrix
- If needed, demonstrate the inactivation of both the principal and subsidiary/side enzymatic activities in the final food
- Information on possible adverse effects on nutrients
- Data on any possible effects on existing microorganisms in food

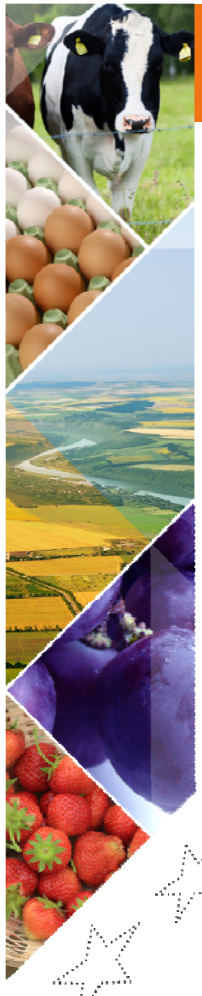
CASE OF NEED AND PROPOSED CONDITIONS OF USE

- The technological need/purpose and intended use of the food enzyme
- The mode of action and reactions catalysed
- The type of foodstuffs in which the food enzyme is intended to be used
- The amount of food enzymes to be added to specific foods
- The conditions of its use in food processing



TECHNICAL DATA. EXPLANATORY NOTE

Guidance	Comments/Explanations
The technological need/purpose and intended use	Proposed use in food and recommendations made to customers
Mode of action and reactions catalysed	Refer to the foods covered by the proposed conditions of use - matrix effects on activity in intended uses - side reactions depending on food
The type of foodstuffs to be added to specific foods	The food categorization system described in Annex III of Regulation 1565/2000 is not especially developed for food enzymes. Necessary to identify the types of foods / food processes (e.g. baking, brewing) in which the enzyme is intended to be used.
Amount of food enzymes to be added	Recommended and maximum use levels for all intended foods
The conditions of its use in food processing	Typical pH and temperature ranges and any cofactors needed according to specified food processing

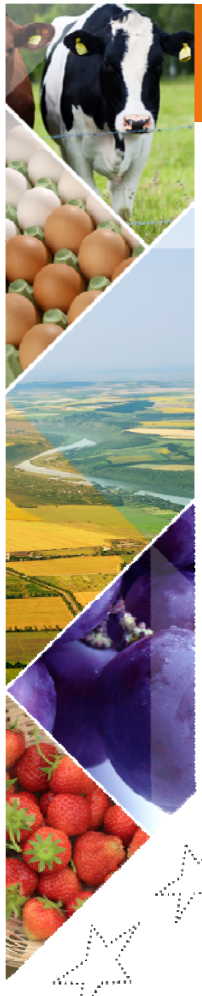


TECHNICAL DATA. DIETARY EXPOSURE

- Potential human exposure to the food enzyme and to any other constituent or by-product of concern

- A conservative technique such as the “budget method” should be used to assess potential dietary exposure in a standard adult of 60 kg bw. If needed, it should be adapted to consider the potential higher consumption in children.

- If food enzyme is proposed for products specifically designed for infants (0-12 months) or young children (12-36 months), *ad hoc* conservative exposure estimates must be produced.



TECHNICAL DATA. EXPLANATORY NOTE

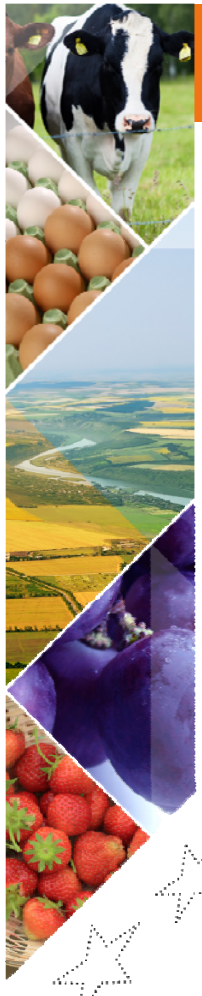
Comments/Explanations

Information is to be provided even if enzyme is produced by QPS microorganism.

The assumption in the FAO/WHO report (2009) for food consumption and proportion of solid food and non milk liquid beverages should be used to calculate the 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 calculated use indicates potential concern with high consumers.

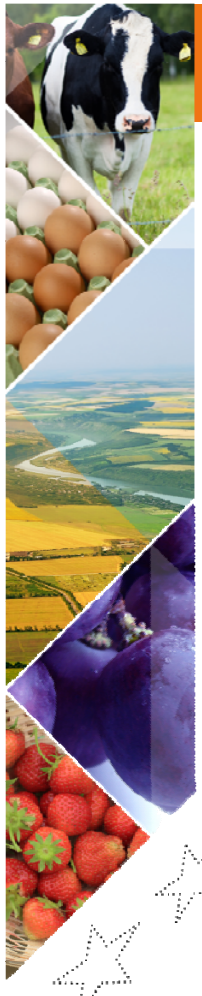


TECHNICAL DATA. EXPLANATORY NOTE

Comments/Explanations

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 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.



TOXICOLOGICAL DATA

- Assessment of genotoxicity. At least two *in vitro* assays:
  - A test for induction of gene mutations in bacteria (Ames test, OECD 471) or, if not applicable, in mammalian cells (preferably mouse limphoma *tk* assay with colony sizing, OECD 476)
  - An *in vitro* assay for the detection of chromosomal aberration (OECD 473) or

    in vitro micronucleus assay (OECD 487) or

    mouse lymphoma *tk* assay with colony sizing (OECD 476)

- Assessment of systemic toxicity:
  - A subchronic oral toxicity study (OECD 408)

TOXICOLOGICAL DATA

Comments/Explanations

Evaluation genotoxic potential: different endpoints (i.e. induction of gene mutations, structural and numerical chromosomal alterations) to assess

The Scientific Committee suggested the following two *in vitro* tests (that cover the three genetic endpoints with the minimum number of tests) as the first step in genotoxicity testing:

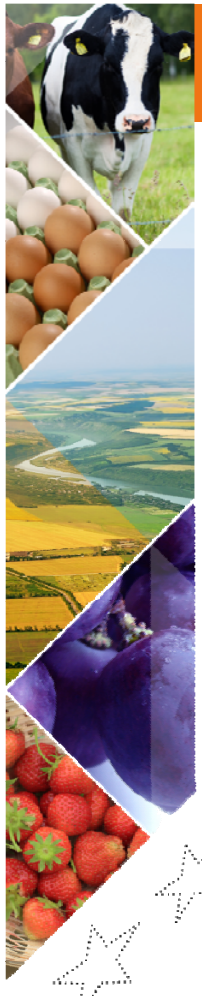
- a bacterial reverse mutation assay (OECD TG 471), and
- an *in vitro mammalian cell micronucleus test* (OECD TG 487).

Ames test: To overcome potential problems with histidine in the food enzyme batch, the Salmonella strains should be exposed to the tested food enzyme in the liquid culture ("treat and plate assay", instead of the traditionally "plate incorporation assay").

Ames test: 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

TOXICOLOGICAL DATA. *IN VIVO*

- Choice of the appropriate *in vivo* test is critical
- One or more positive *in vitro* tests normally require follow-up by *in vivo* testing, unless it can be demonstrated that the positive *in vitro* findings are not related for the *in vivo* situation.
- Flexible approach
- ECHA guidance (2008): "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"
- The toxicological studies should be performed on a batch representative of the food enzyme before addition of other components of the food enzyme preparation

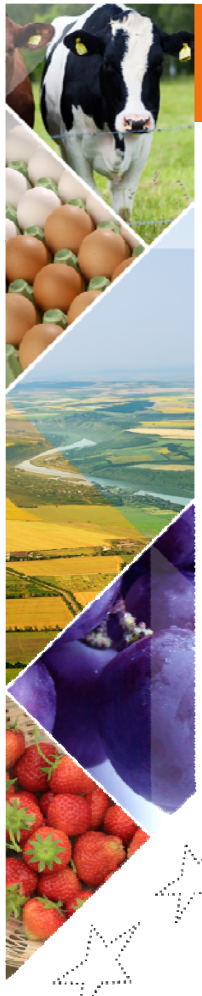


TOXICOLOGICAL DATA. EXPLANATORY NOTE

Comments/Explanations

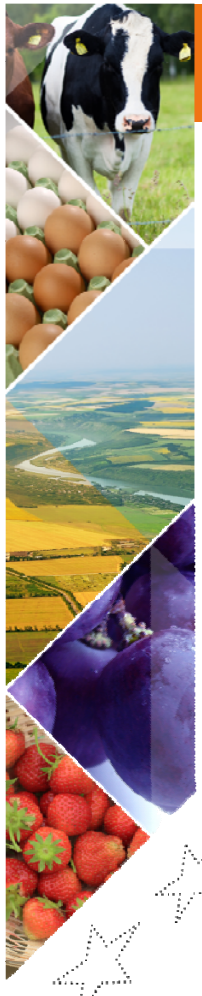
The tested batch should be characterised and evidence provided that is representative of commercial batches. Use same parameters as those used for the chemical composition

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 of 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).



TOXICOLOGICAL DATA. WHEN NO NEEDED

- Documented history on the safety of the source of the enzyme
- Composition, properties and use in food of the food enzyme demonstrate no adverse effects on human health. Provision of detailed rationale (e.g. edible parts of animals and non GM plants)
- Food enzymes produced by microorganisms with status QPS if it can be demonstrated that there are no concerns related to any residues, degradation products or substances from the tested production process
- Food enzyme from a specific strain has been thoroughly tested and manufacturing process does not differ significantly for other food enzymes from the same strain. Decisions on a case-by-case basis

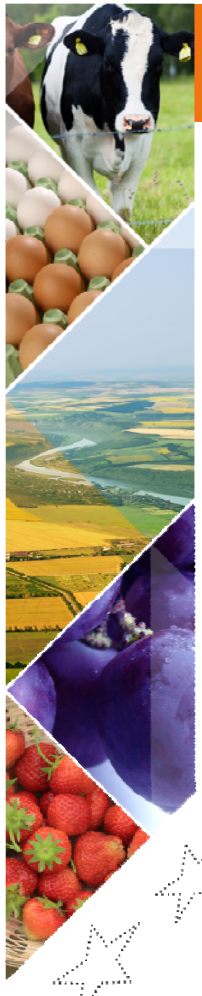


TOXICOLOGICAL DATA. WHEN NO NEEDED

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. Journal OJ L168, 21-23

Enzymes derived from:

- Edible parts of plants or animals intended to be or reasonably expected to be ingested by humans
- Micro-organisms having the QPS-status

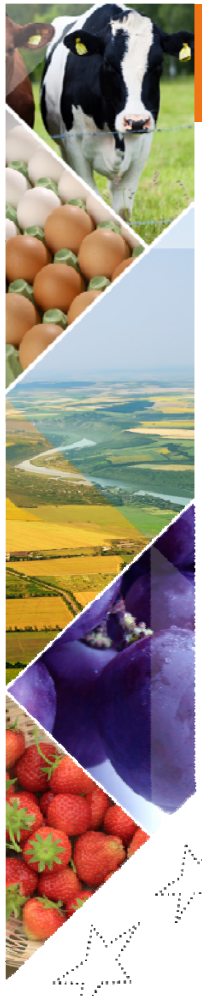


TOXICOLOGICAL DATA. WHEN NO NEEDED.

Comments/Explanations

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.

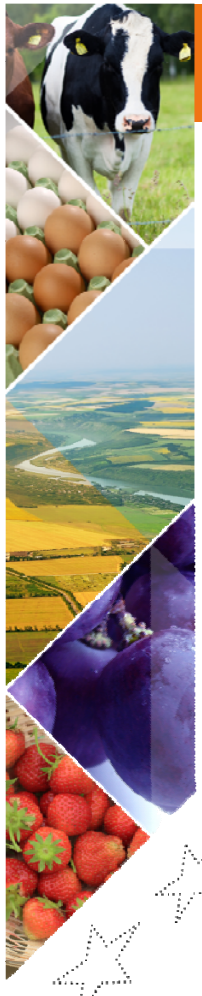
Regarding the genetic modification concerns, microbial strains obtained using self cloning have to be risk assessed following the GMM guidance document. They are exempt from toxicological testing provided they are complying with the requirements of Reg. 562/2012



GROUPING OF APPLICATIONS (ONE DOSSIER)

Regulation (EU) No 562/2012

- Edible parts of plants or animals intended or reasonably expected to be ingested by humans
- Microorganisms having the QPS-status
- Microorganisms for production of enzymes authorised by competent authorities either in France or Denmark following SCF guidelines (1992)
- If the enzymes have the same catalytic activity
- If derived from the same source (species level)
- If obtained by a substantially same production process
- If source materials are not genetically modified

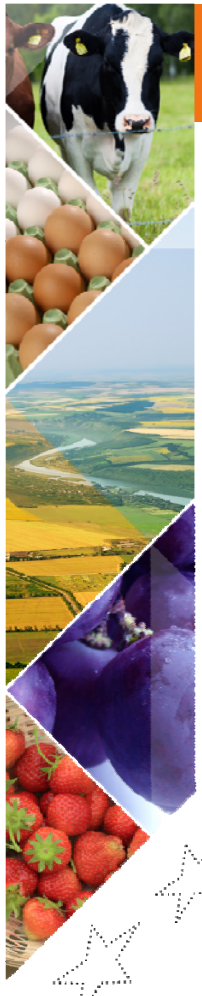


ALLERGENICITY

- No validated testing methods to predict allergenicity of the enzyme protein or its breakdown products after oral intake

- Potential allergenicity through stepwise case-by-case approach:
  - allergenicity of the source of food enzyme
  - sequence and / or structural similarities to known allergens

- In case of concern from the initial screening, proceed to further analysis as described for the safety evaluation of newly expressed proteins in GM plants (EFSA, 2006)

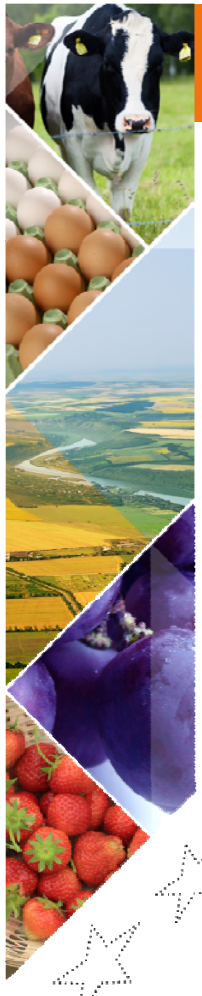


ALLERGENICITY. EXPLANATORY NOTE

Comments/Explanations

The approach used must be detailed: searches in data bases must be demonstrated

Search reports and programs used should be provided in annex



REVIEW / CONCLUSIONS

- For each toxicological study:
  - significant findings
  - NOAEL
  - any other relevant information
- Relationship between dose giving rise to effects and likely dietary exposure from use of the food enzyme
- Establishment of the margin of exposure
- Conclusions drawn should be product specific