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EFSA Guidance on the Submission of a Dossier on Food Enzymes for Safety Evaluation: Toxicological data

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Toxicological data

- Assessment of genotoxicity
- Assessment of systemic toxicity

Default assumption: toxicological testing is necessary

Exceptions: only in specific cases s. also 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

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:168:0021:0023:EN:PDF

Toxicological data

No need for toxicological testing (I)

- Documented history on the safety of the source of the enzyme
- Composition, properties and use in food of the food enzyme
  → no adverse effects on human health
- Provision of detailed rationale
  e.g. edible parts of animals and (non GM) plants

Toxicological data

No need for toxicological testing (II)

- Food enzymes produced by microorganisms 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 tested production process

Toxicological data

No need for toxicological testing (III)

- Food enzyme from a specific strain has been thoroughly tested
- Manufacturing process does not differ significantly for other food enzymes from the same strain
- Decisions on a case – by – case basis

Assessment of genotoxicity

- At least two *in vitro* assays
  - gene mutations
  - chromosomal effects

- Positive results in any of the *in vitro* tests

  → follow-up by *in vivo* testing

Assessment of genotoxicity: *in vitro*

- Test for inducation of gene mutations in bacteria
  (Ames test; OECD 471)

  if not applicable:

  test for inducation of gene mutations in mammalian cells

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

- Chromosomal aberration assay (OECD 473)
  or
  micronucleus assay (OECD 487)
  or
  mouse lymphoma *tk* assay with colony sizing (OECD 476)

Assessment of genotoxicity: *in vivo*

- Choice of the appropriate *in vivo* test is critical
- 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“

Assessment of genotoxicity: *in vivo*

Any of the following tests may be conducted:

- Rodent bone marrow or mouse peripheral blood
- Micronucleus test (OECD 474)
- Rodent bone marrow clastogenicity study (OECD 475)
- Comet assay (single cell gel electrophoresis)
- Test for gene mutations in a transgenic rodent model
- Rat liver Unscheduled DNA synthesis (UDS) test (OECD 486)

Allergenicity

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

- Stepwise approach
  - allergenicity of the source of the food enzyme
  - sequence and / or structural similarities to known allergens
  - concern from this initial screening
    → further analysis as described for the safety evaluation of newly expressed proteins in genetically modified plants (EFSA, 2006)

Review / Conclusions

- For each toxicological study
  - significant findings
  - NOAEL
  - any other relevant information
- Relationship between dose giving rise to effects in animal to likely dietary exposure from use of the food enzyme
- Establishment of margin of safety

Legislation

- 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 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:354:0001:0006:EN:PDF

- Commission Regulation (EU) No 234/2011 implements the common authorisation procedure and establishes the derogation from submitting toxicological data in some specific cases and the possibility of grouping food enzymes under one application under certain conditions http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:064:0015:0024:EN:PDF

- 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 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:354:0007:0015:EN:PDF

- 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 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:168:0021:0023:EN:PDF

- Commission Regulation (EU) No 1056/2012 of 12 November 2012 amending Regulation (EC) No 1332/2008 of the European Parliament and of the Council on food enzymes with regard to transitional measures http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:313:0009:0010:EN:PDF