European Food Safety Authority

Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use

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Preparation of the First
Working Party of Governmental Experts on Enzymes
Brussels, 8 July 2011

Guidance Document

- First GD was released in 2006 following EC mandate
- Scope:
  - Requirements for GMM applications under Regulation (EC) No 1829/2003
  - Risk assessment of food and feed produced using GMMs, irrespective of the legislation they might fall into
- Updated GD was released in 2011. It reflects the scientific information considered relevant by the GMM WG and the criteria applied for the safety assessment

Assessment of trial descriptions

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ASSESSMENT

→ In concordance with the updated GMM GD

→ According to current practices of EFSA

→ Considering individual aspects of the trial descriptions

TECHNICAL REPORT

→ Structured according to the outline of an opinion

→ For each point, missing information (required for the RA) is stated

→ Conceived as further guidance for the preparation of applications

Food enzyme product category

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Category 1: Chemically defined purified compounds and their mixtures; both GMMs and newly introduced genes are absent

Category 2: Complex products; both GMMs and newly introduced genes are absent

ERA not needed according to the GMM GD

Most enzymes

Category 3: Products derived from GMMs; GMMs capable of multiplication or of transferring genes are not present; newly introduced genes are still present

ERA: possible transfer of recombinant DNA into other organisms

Category 4: Products consisting of or containing GMMs capable of multiplication or of transferring genes

Food enzymes and products of Categories 1 and 2

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Chapter III, Section B.1. – Information relating to the GMM

Characteristics of the recipient or (when appropriate) parental organism

- Full taxonomic and physiological description

  QPS qualification can be used to justify safety, fulfilment of specific QPS qualifications for the strain used should be documented

- History of previous use should be provided at the strain level

- Detailed description and risk assessment of any previous genetic modifications made to the strain before the final modifications leading to the final production strain

* EFSA Journal 2011;9(6):2284

Recommendations in the EFSA Scientific Report*	
B. licheniformis	***A. niger***
Strain-specific QPS qualification	**Mycotoxin under industrial fermentation**
Relationship between recipient and production strains	
Original data Origin of all sequences – identity of donor at strain level; Vector details	**Southern; ARM genes; Origin of all sequences; sequences of cloning remnants**

Food enzymes and products of Categories 1 and 2

Chapter III, Section B.1. – Information relating to the GMM

Characteristics of the origin of the inserted sequences

Full description of all DNA fragments introduced and their origin; three possible origins considered:

•DNA from defined organisms: Taxonomic identification, safety characterisation (case-by-case)

•Synthetic DNA: strategy for the design, full sequence, sequence and function of the encoded protein, similarities with natural sequences and function in natural organisms

•Nucleic acids directly extracted from environmental samples: type of sample, full sequence, sequence and function of the encoded protein, similarities with natural sequences and function in known organisms

Recommendations in the EFSA Scientific Report	
B. licheniformis	***A. niger***
Origin of promoter and other fragments	
Similarity of new gene to original at sequence and functional level	**Similarity of new gene to original at sequence and functional level**
How protein differs functionally from natural sequence; Bioinformatic analysis-updated database, discussion of hits	

Food enzymes and products of Categories 1 and 2

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Chapter III, Section B.1. – Information relating to the GMM

Description of the genetic modification

- Full description of the vector, including sequence, physical and genetic maps, and description and origin of each element
- Method used for the genetic modification
- Location of the inserted DNA and, where relevant, information on possible further mobilisation
- Description of the sequences actually inserted, replaced or modified. Copy number of the inserts experimentally documented
- Experimental demonstration of the absence of any vector sequences not intended to remain in the GMM, based on Southern analysis (PCR not sufficient)

Recommendations in the EFSA Scientific Report	
B. licheniformis	***A. niger***
	Clarification
Southern analysis: quality and detailed information	**Southern analysis: quality and detailed information**
Absence of ARM genes, vector sequences	

Food enzymes and products of Categories 1 and 2

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Chapter III, Section B.1. – Information relating to the GMM

Information relating to the GMM

- Description of any new traits and characteristics of the GMM
- Structure and amount of any vector and/or donor nucleic acid remaining in the GMM

  Genetic map, experimental documentation on the copy number and on the absence of sequences not intended to be inserted
- Documented stability of the GMM (only Category 2)
- Description of identification and detection techniques of the inserted sequences
- Changes in the GMM, which may potentially affect its safety for humans and animals (only Category 2)

Recommendations in the EFSA Scientific Report	
B. licheniformis	***A. niger***
Southern analysis: quality and detailed information; Absence of ARM genes, vector sequences	**Southern analysis: quality and detailed information**

Food enzymes and products of Categories 1 and 2

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Chapter III, Section B.2. – Information relating to the product

Information on the production and preparation process

- Description of the stages of the production process of the GMM (cultivation, fermentation), and of the method used to remove the cells
- Demonstration of the absence of the GMM in the product:

  For cases where the production strain is removed

  Use of a recognised method for the count of viable cells. Detection of possible stressed cells must be ensured by including a resuscitation step

  Description, specificity, reliability and efficacy of the method used

  Data from several batches with reliable sampling methods

Recommendations in the EFSA Scientific Report	
B. licheniformis	***A. niger***
Process of product purification; Steps of inactivation or elimination; Method used to test the presence of production strain: - Cultivation media - Detection sensitivity -Differentiation of production strain from background - At least 3 independent batches, each in triplicate, proper sampling method	**Process of product purification; Steps of inactivation or elimination; Method used to test presence of production strain: - Cultivation media - Detection sensitivity -Differentiation of production strain from background - At least 3 independent batches, each in triplicate, proper sampling method**

Food enzymes and products of Categories 1 and 2

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Chapter III, Section B.2. – Information relating to the product

Information on the production and preparation process

- Information on the inactivation of the GMM cells and evaluation of the presence of remaining physically intact cells:

  For cases when the production strain is destroyed but not removed from the product

  Description, specificity, reliability and efficacy of the method used to inactivate/destroy the cells

  Verification of the absence of viable GMM by using targeted method

  Verification of the possible presence of spores, stressed cells, VBNC (viable but not cultivable) cells and physically intact dead cells

  Data from several batches with reliable sampling methods

Recommendations in the EFSA Scientific Report	
B. licheniformis	***A. niger***

Food enzymes and products of Categories 1 and 2

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Chapter III, Section B.2. – Information relating to the product

Information on the production and preparation process

- Information on the possible presence of recombinant DNA:
  - PCR-based method with documented reliability, efficacy and sensitivity
  - At least one recombinant genetic element, and all functional genes of possible concern (e.g. antimicrobial resistance genes, virulence genes, genes encoding toxic compounds) should be targeted
  - All DNA in the product should be tested. Lysis steps should be applied to the purification method. Positive and negative controls should be used.
  - Samples spiked with control DNA in different dilutions until DNA extinction to check the limit of detection
  - Data from several batches with reliable sampling methods

Recommendations in the EFSA Scientific Report

B. licheniformis	*A. niger*
Presence of recombinant DNA by PCR - Original data must be provided - At least 3 independent batches, each in triplicate, proper sampling method	**Presence of recombinant DNA by PCR - Original data must be provided - At least 3 independent batches, each in triplicate, proper sampling method**
Transformation/ DNA uptake assay is not substitute for PCR analysis	**Transformation/ DNA uptake assay is not substitute for PCR analysis**