19-20 June 2019

Brussels, Belgium

Technical meeting with stakeholders on applicantions for food enzymes

Toxicological studies and allergenicity

WG members: Alicja Mortensen, Lieve Herman, Claudia Bolognesi, Henk Van Loveren

EFSA: Sandra Rainieri, Natália Kovalkovičová

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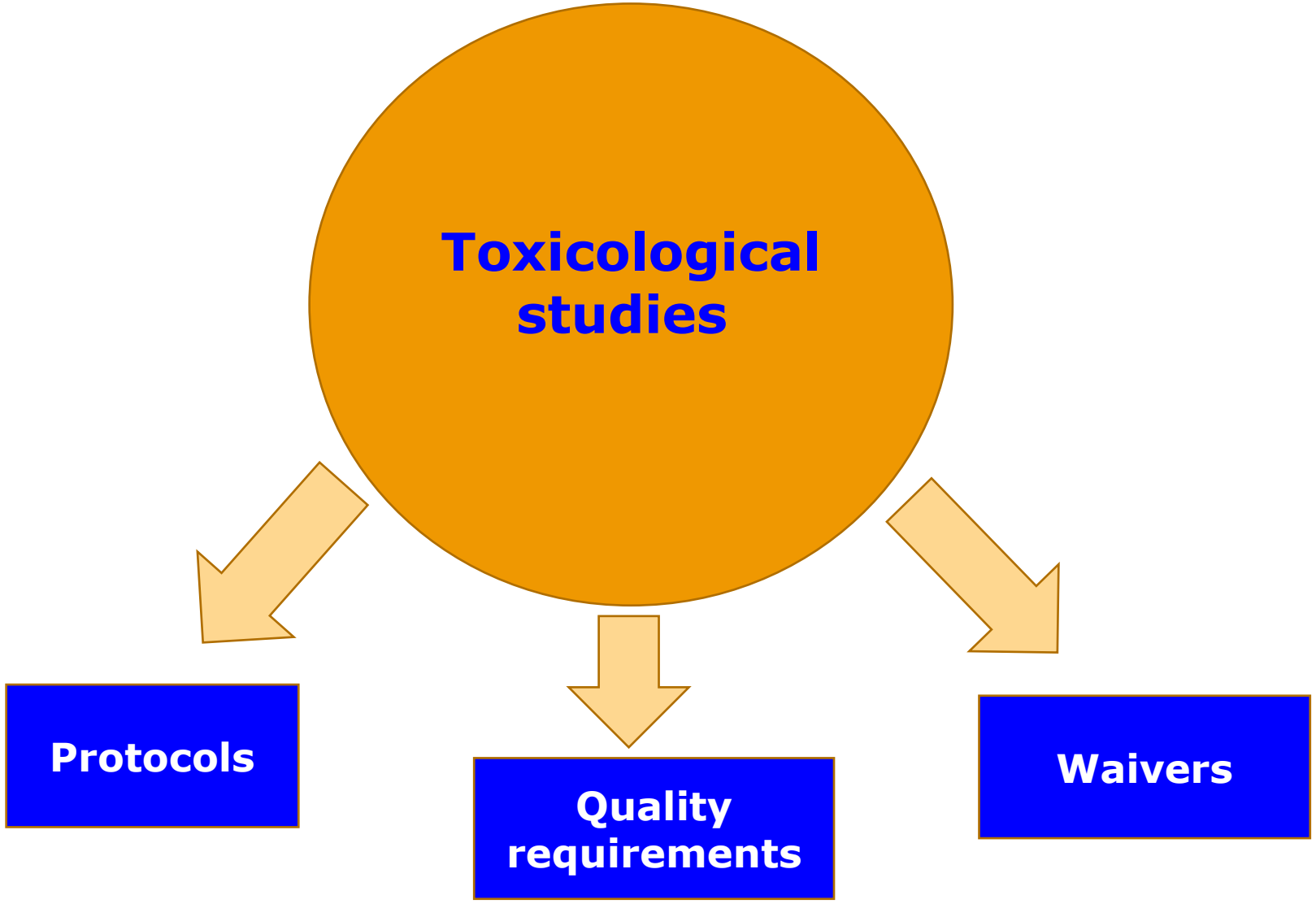

Toxicological studies
Protocols
Quality requirements
Waivers

CONTENT:

PART 1: Toxicological studies and allergenicity

Presenter: Alicja Mortensen, DVM, PhD.

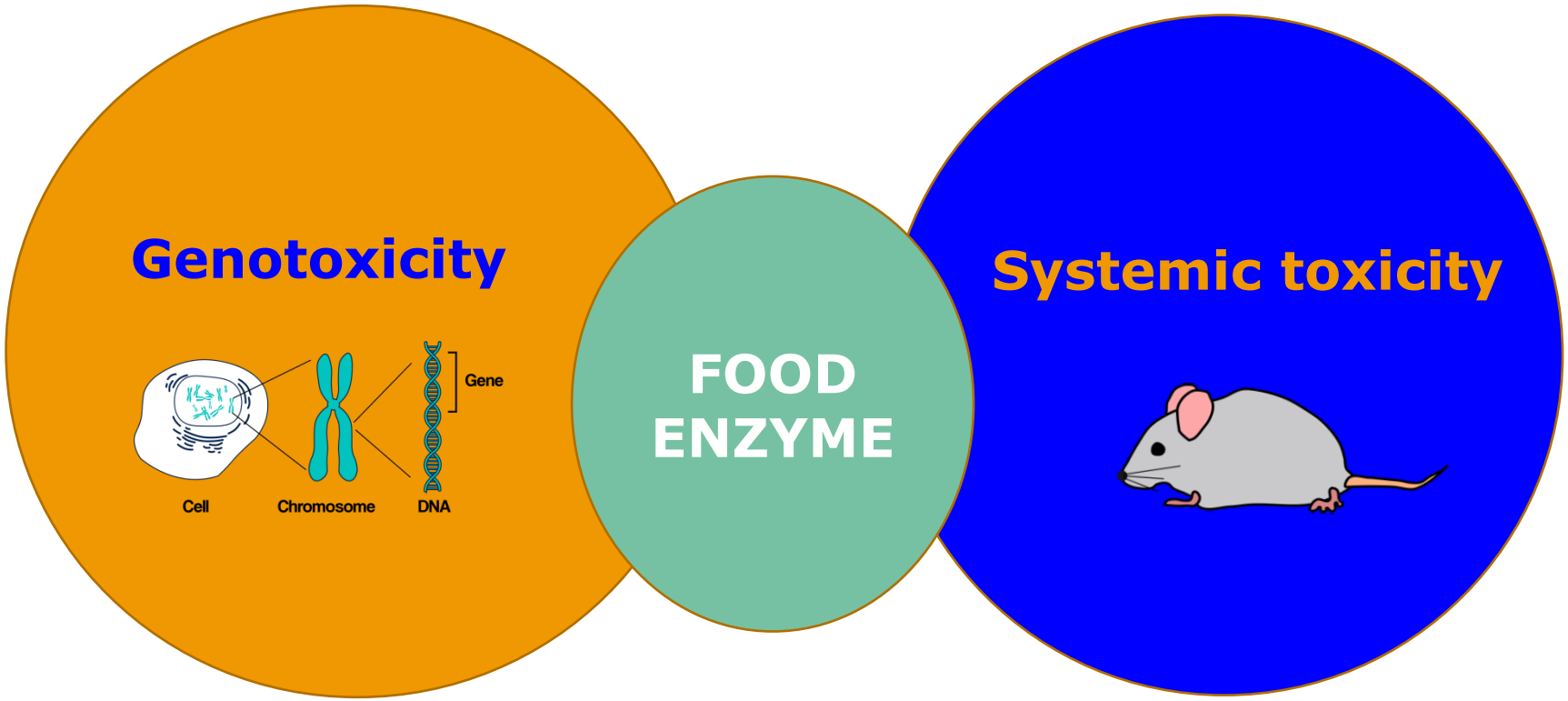
PART 2: Waivers and alternative approaches for toxicological studies

Presenter: Lieve Herman, Dr., PhD.

THE TOXICOLOGICAL DATA SET

PART 1

EFSA, 2009. Guidance of EFSA Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. The EFSA Journal 2009;1305, 1-26.



GENOTOXICITY

EFSA, 2014. 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. EFSA supporting publication 2014: EN-689. 22 pp

EFSA Scientific Committee, 2011. Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379. 69 pp

GENOTOXICITY *IN VITRO* TESTING

Endpoint	Test	OECD test guideline
Gene mutation	Bacterial reverse mutation assay	471
Chromosomal numerical and structural aberrations	*In vitro* mammalian cell micronucleus test	487
	In vitro mammalian chromosomal aberration test	473

GENOTOXICITY *IN VIVO* TESTING

- **If 1 or 2 *in vitro* tests are positive: *in vivo* follow up needed**

Endpoint	Test	OECD test guideline
Gene mutation	Transgenic rodent cell gene mutation assay	488
Chromosomal numerical and structural aberrations	*In vivo* mammalian erythrocyte micronucleus test	474
DNA damage	*In vivo* Comet assay	489

Examples from published opinions:
Genotoxicity evaluation

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In vitro genotoxicity studies

Nr. of opinions (2014-2019)	Gene mutation		Chromosomal effects numerical and structural
26	Ames test	+	*in vitro* chromosomal aberration test
14	Ames test	+	*in vitro* micronucleus assay

In vivo genotoxicity studies

Nr. of opinions (2014-2019)	
0	Chromosomal aberration test
1	Micronucleus assay
0	Comet assay

Examples from published opinion: Genotoxicity test guidelines

Ames test	**OECD TG 471 version**			**NO OECD-TG**	**Inconclusive**
Year	**1983**	**1994**	**1997**		
Nr. opinions	2	2	35	1	2 Genotoxicity and DNA oxidizing/crosslink

***In vitro* chromosomal aberration test**	**OECD TG 473 version**				**NO OECD-TG**	**Inconclusive**
Year	**1981**	**1983**	**1997**	**2014**		
Nr. opinions	1	2	21	1	1	1 Inconsistent data

***In vitro* micronucleus test**	**OECD TG 487 version**			**NO OECD-TG**	**Inconclusive**
Year	**2007**	**2008**	**2010**		
Nr. opinions	1	1	12		

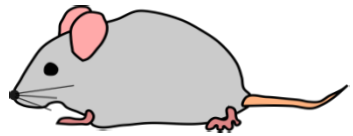
SYSTEMIC TOXICITY

Repeated dose 90-day oral toxicity study in rodents (**OECD TG 408**)

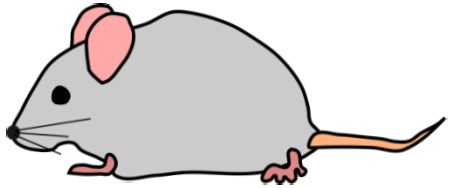
EFSA, 2014. Explanatory Note for the Guidance of the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) on the Submission of a Dossier on Food Enzymes. EFSA supporting publication 2014:EN-689. 22 pp.

Reporting

- Toxicologically and statistically significant findings should be highlighted
- Description of result or discussion:
  - to include an interpretation of the significance of the findings
  - to explain reasons for disregarding any significant finding
- Appropriate historical control values should be provided (e.g. from the last 5 years)
- The no-observed-adverse-effect level (NOAEL) should be identified
- Original study report to be provided



Examples from published opinions:
Test guidelines for repeated dose 90-day oral toxicity study



90-day study	OECD 408 version		NO OECD-TG	Inconclusive
Year	**1981**	**1998**		
Nr. opinions	1	37	1	1 Test item not representative

TOXICOLOGICAL STUDIES

Requirements concerning the test batch:

- Well characterised
- Representative of commercial samples (demonstration by the analytical results)
- Representative of the food enzyme before addition of other components
- Depending on the test the dose units should be: µg TOS/plate, µg TOS/ml or mg TOS/kg bw/day
- Correct calculation of the concentrations/doses in the toxicological studies (TOS must be clear)
- The selection of doses must be justified, if lower than recommended in the respective OECD TGs

EFSA, 2009. Guidance of EFSA Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. The EFSA Journal (2009), 1305, 1-26.
EFSA, 2014. 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. EFSA supporting publication 2014:EN-689. 22 pp.

RECOMMENDATION FOR PROTOCOLS

- Use **internationally agreed protocols** if available (OECD TG).
- Follow the **most up-to-date edition** of any test guideline.
- Carry out studies according to the principles of **Good Laboratory Practice (GLP)** - Council Directives 2004/10/EC and 2004/09/EC - and accompanied by a statement of GLP compliance of the laboratory conducting the studies.

EFSA, 2009. Guidance of EFSA Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. The EFSA Journal 2009;1305, 1-26.

Examples from published opinions: challenging dossiers

Shortcomings	Category
➢ Doses derived from food enzyme not from TOS **too low** to allow a conclusion on toxicity	All toxicity studies
➢ **Batch** for 90-day study **not representative**; study not considered	Systemic toxicity
➢ Test item not **fully representative** for 90-day study	Systemic toxicity
➢ Confirmatory data not provided. **No conclusion on genotoxicity**	Genotoxicity Ames test
➢ Only 4 strains tested in Ames test. **No conclusion** could be made if enzyme induce gene mutation by **DNA oxidizing or cross-linking**	Genotoxicity Ames test
➢ No data on bone marrow exposure. **Limited validity**	Genotoxicity *In vivo* micronucleus test
➢ **Inconsistencies** in data reporting	Genotoxicity *In vitro* chromosomal aberration test

PROTOCOL DEVIATIONS

- Deviations from protocols include:
  - Exemption from certain tests
  - Use of alternative protocols / assays or tests

  Scientific justification must be provided

- Additional studies might be required on a case-by-case basis with respect to:
  - food enzyme's molecular and functional characteristics
  - fate in food and the gastrointestinal tract
  - extent of potential exposure

EFSA, 2009. Guidance of EFSA prepared by the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. The EFSA Journal 2009;1305, 1-26.

Examples from published opinions: Overview

Period (2014-2019)	Nr. of published opinions: 45
Nr. of applications with toxicological data required	38
Toxicity testing on the food enzyme from the application	*35*
Substitute approach: Different food enzyme / different microbial strain	*3*
Nr. of opinions without toxicity data (enzyme source, QPS, No concern – see slide 34)	7

- **Allergenicity**

ALLERGENICITY

EFSA, 2009. Guidance of EFSA Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. The EFSA Journal 2009;1305, 1-26.

EFSA, 2014. Explanatory Note for the Guidance of the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. EFSA supporting publication 2014:EN-689. 22 pp.

EFSA GMO Panel, 2017. Guidance on allergenicity assessment of genetically modified plants. EFSA Journal 2017;15(5):4862, 49 pp.

FAO/WHO, 2001. FAO/WHO expert consultation on foods derived from biotechnology. Evaluation of allergenicity of genetically modified foods. http://www.who.int/foodsafety/publications/biotech/en/ec_jan2001.pdf, last visited on 31/07/2009.

ALLERGENICITY

EFSA CEF Panel guidance, 2009:

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 GM plants (EFSA, 2006; FAO/WHO, 2001).

The approach used must be detailed: **searches in data bases** must be demonstrated (**for amino acid sequences of food enzymes from both GMM and non GMM**).

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.

Search reports and programs used should be provided in annex.

ALLERGENICITY

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 GMO for the risk assessment of GM plants and derived food and feed (EFSA GMO Panel, 2017).

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.

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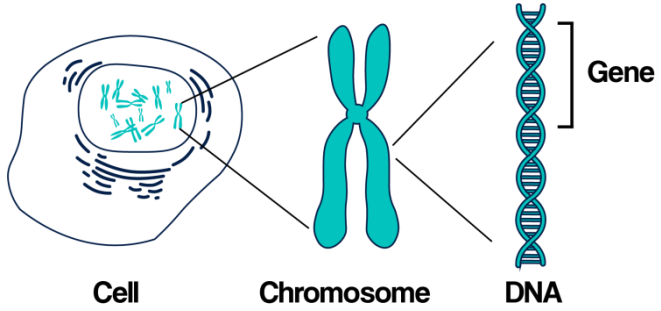
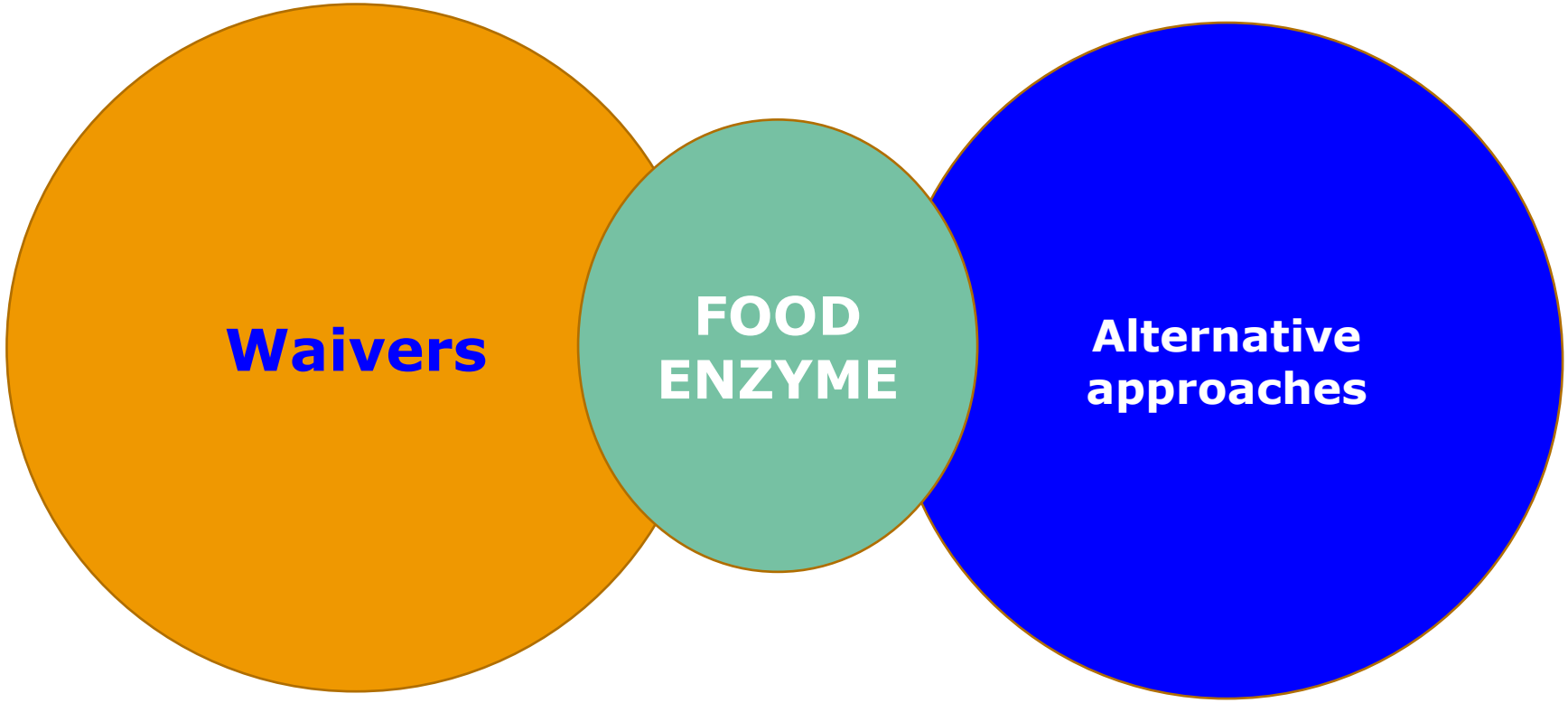
Thank you very much for your attention



Part 2 is comming now

Part 2



WAIVERS

Toxicological studies might be waived for:

- Food enzymes derived from edible parts of non-GMO plants and animals not posing health problems

- Food enzymes derived from microorganisms considered qualified for the presumption of safety

EFSA, 2009. Guidance of EFSA prepared by the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. The EFSA Journal 2009; 1305, 1-26.

Commission Implementing Regulation (EU) No 562/2012 of 27 June 2012 amending Commission Regulation (EU) No 234/2011.

WAIVERS: Enzymes from edible parts of plants and animals

Application must include:

- Documented history of safety of the enzyme source
- Composition and property of food enzyme
- Use of the food enzyme in food demonstrating no adverse effects on human health supported by any existing toxicological studies
- Consumption data

EFSA, 2009. Guidance of EFSA prepared by the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. The EFSA Journal 2009; 1305, 1-26.

Commission Implementing Regulation (EU) No 562/2012 of 27 June 2012 amending Commission Regulation (EU) No 234/2011.

WAIVERS:
Enzymes from QPS microorganisms

- QPS provides a safety status for microorganisms at the level of a taxonomic unit (TU) (species, genus) intentionally used in the food and feed chain, triggered by an EFSA dossier.

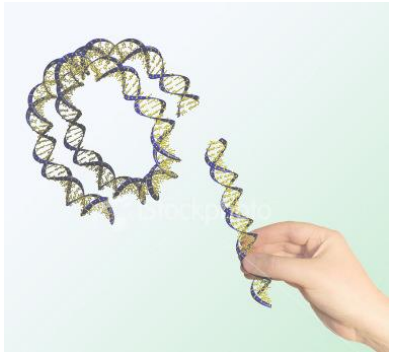
- Microorganism used in the production of a food enzyme with a QPS status - application may not need to provide specific toxicological test data.

- Safety concerns are, where possible and reasonable in number, reflected as 'qualifications' which should be assessed at the strain level.

EFSA BIOHAZ Panel, 2018. Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 7: suitability of taxonomic units notified to EFSA until September 2017. EFSA Journal 2018;16(1):5131, 43 pp.

WAIVERS:
Enzymes from QPS microorganisms

- QPS list is updated continuously and summarizes the QPS TUs with a QPS status and indications of the qualifications to be checked at strain level (link: https://zenodo.org/record/2565996#.XOzrjXduKUk).



- Genetically modified strains can be covered by the QPS concept: Statement published in 2018 (EFSA Journal 16(1): 5131).
  - 'In the case of GMMs being used as production organisms for which the recipient strain qualifies for the QPS status, and for which the genetic modification does not give rise to safety concerns, the QPS approach can be extended to the genetically modified production strain.'

EFSA BIOHAZ Panel, 2018. Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 7: suitability of taxonomic units notified to EFSA until September 2017. EFSA Journal 2018;16(1):5131, 43 pp.

REQUIREMENTS FOR QPS STATUS STRAINS

- The QPS status of the TUs to which the strain in the application belongs, does not imply automatically that the strain itself has the QPS status.
- There are requirements for defining the QPS status at strain level.
- Guidance for applicants is available. Statement on the characterisation of microorganisms used for the production of food enzymes.

EFSA CEP Panel, 2019. Statement on the characterisation of microorganisms used for the production of food enzymes. EFSA Journal 2019;17(6):5741, 13 pp.

REQUIREMENTS FOR QPS STATUS STRAINS

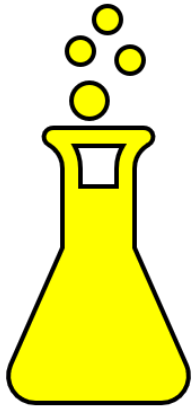
Requirements in application dossier for defining the QPS status at strain level:

- Taxonomic identification of the microorganism
- Confirmation that the existing qualifications are met
  - A generic qualification for all QPS bacterial TUs applies in relation to the absence of acquired genes conferring resistance to clinically-relevant antimicrobials
  - *Bacillus* spp. absence of toxigenic potential
  - End use 'only QPS for production purposes'; not for use as living organisms (e.g. some yeast species as *Candida* and *Komagatella*) (absence of viable organisms in products produced by these organisms)
- Safety of the genetic modification

EFSA CEP Panel, 2019. Statement on the characterisation of microorganisms used for the production of food enzymes. EFSA Journal 2019;17(6):5741, 13 pp.

Application must also include:

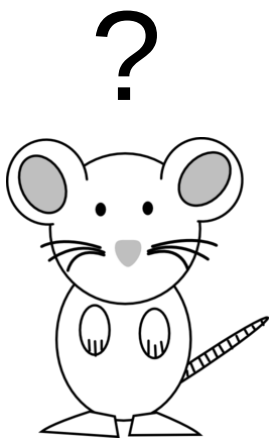
- Experimental data demonstrating absence of concern from:



  - Residues
  - Impurities
  - Degradation products linked to the total production process (production, recovery and purification)

EFSA, 2009. Guidance of EFSA prepared by the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. The EFSA Journal 2009; 1305, 1-26.
EFSA, 2014. 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. EFSA supporting publication 2014:EN-689. 22 pp.

Substitution of toxicological studies by one from other strain (for non-QPS):



- Same enzyme produced by a different microorganism: no substitution of toxicological studies possible.
- Different enzyme produced by the same or very related production microorganism: substitution could be possible, evaluated on case by case basis.

EFSA, 2009. Guidance of EFSA prepared by the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. The EFSA Journal 2009; 1305, 1-26.

Commission Implementing Regulation (EU) No 562/2012 of 27 June 2012 amending Commission Regulation (EU) No 234/2011.

CASE BY CASE EVALUATION

- The **manufacturing process** must be comparable and not differ significantly for alternative enzymes from the same strain.

- No conventional modification steps between the two different production strains have been carried out.

CASE BY CASE EVALUATION

- If different GM microorganisms are employed the **genetic modification** should be compared carefully.

  - *All insertions made in both strains should be documented in detail by WGS data*
  - *Insertions in the production strain may not induce possible expression of genes of concern*
  - *Insertions in the production strain may not alter the possible presence of products of concern in the end product (e.g. deletion of proteinase genes which could alter the break down of unwanted proteins in the end product)*

Examples from published opinions:
Overview

Period (2014-2019)	Toxicological studies				
Nr.	**Required**		**Not required**		
	Tox studies available	Substitute approach	Enzyme source (plant/animal)	QPS	No concern (weight of evidence including QPS TU, no confirmation AMR but absence of cells and DNA of production strain in final product)
45	35	3	4	2*	1

* Toxicological studies provided as supporting evidence

REFERENCES:

- Council Directive 2004/10/EC. OJ L 50, 20.2.2004, p. 44.
- Council Directive 2004/09/EC. OJ L 50, 20.2.2004, p. 28.
- EFSA, 2005. Opinion of the Scientific Committee on a request from EFSA related to a generic approach to the safety assessment by EFSA of micro-organisms used in food/feed and the production of food/feed additives. The EFSA Journal, 226, 1-12.
- EFSA, 2006. Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. The EFSA Journal, 99, 1-100.
- EFSA, 2009. Guidance of EFSA prepared by the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. The EFSA Journal 2009; 1305, 1-26.
- EFSA Scientific Committee, 2011. Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379, 69 pp.
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- EFSA CEP Panel, 2019. Statement on the characterisation of microorganisms used for the production of food enzymes. EFSA Journal 2019;17(6):5741, 13 pp.
- FAO/WHO, 2001. FAO/WHO expert consultation on foods derived from biotechnology. Evaluation of allergenicity of genetically modified foods. FAO/WHO, January 2001, Rome, http://www.who.int/foodsafety/publications/biotech/en/ec_jan2001.pdf, last visited on 31/07/2009.
- https://zenodo.org/record/2565996#.XOzrjXduKUk
- Reg. 562/2012. 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.

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