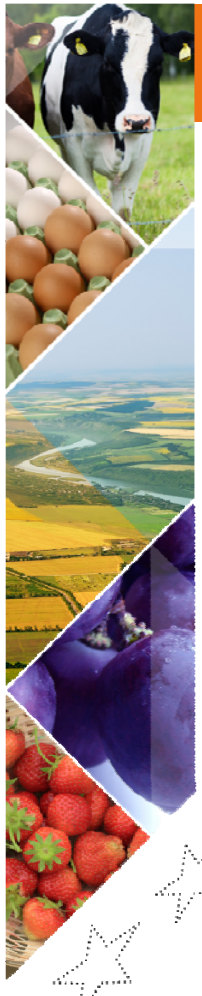
Toxicological assessment: Experiences from the evaluation of food enzymes applications

Kettil Svensson
CEF Panel

Marina Goumenou
EFSA, FIP Unit

Info session on Food Enzymes applications
27 May 2014



PRESENTATION OVERVIEW

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EN-579, 2014

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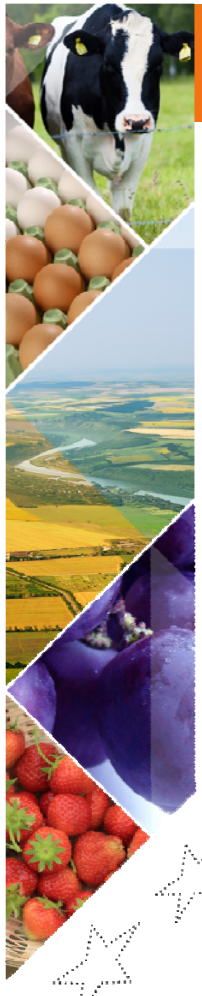
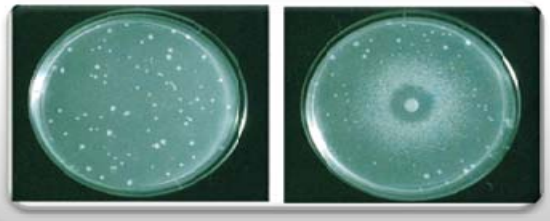
Practical issues



The default assumption

- **The default assumption is that toxicological testing is necessary**.
- However, 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.

Required core set of toxicological data

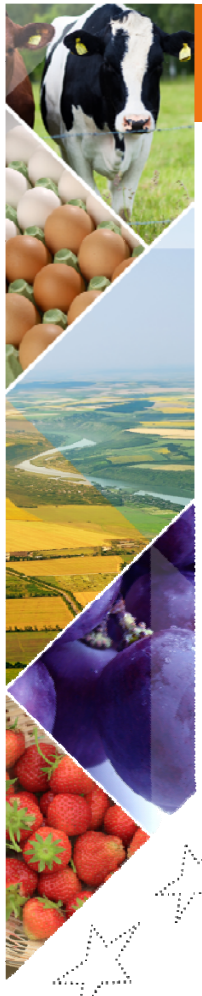


Ames test *

Micronucleus test or
Chromosome aberration test

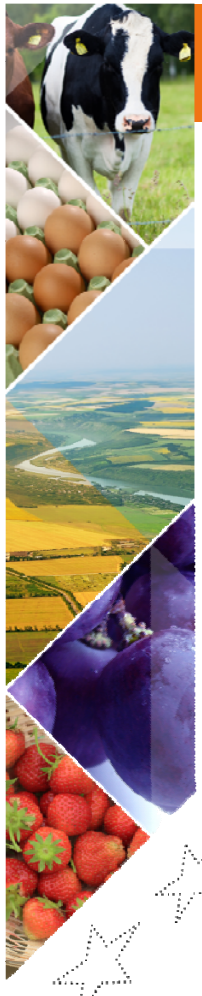
90-day sub-chronic oral
toxicity study

*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.



Tested Batch and toxicological studies

- The tested batch should be characterised. **Evidence (by test results) must be provided that the tested batch is representative of commercial samples.**
- **The parameters used to demonstrate the equivalence of the batch that is toxicologically tested shall be the same as those used to describe the chemical composition**.
- Original study reports must be provided and any effect detected must be reported and commented.



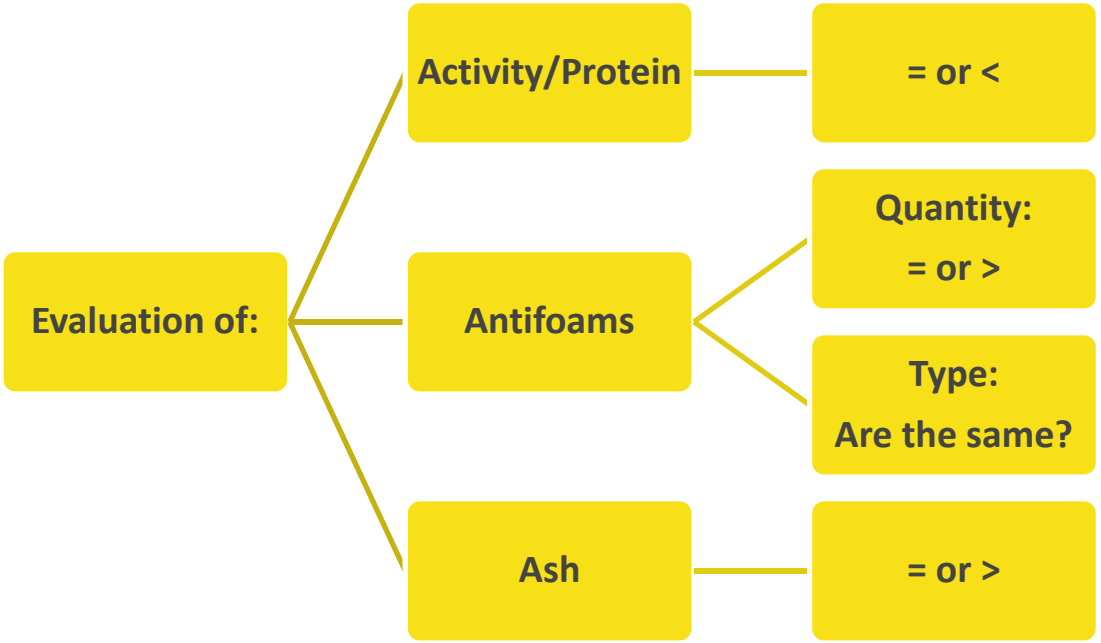
3.1.2.1 Chemical composition (point v.)

- **Updated on version 2014:EN-579;**

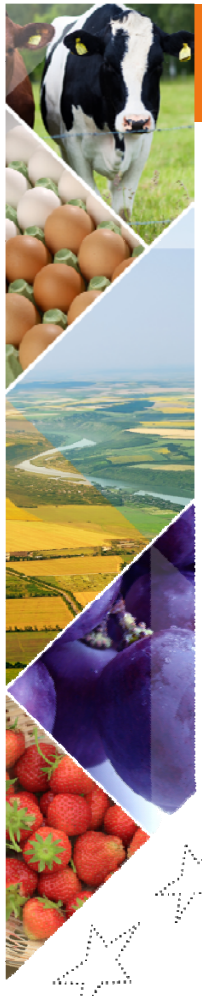
"*Acceptable inter-batch variability is decided on a **case by case basis** and depending on parameters*".

Example of factors affecting the representativeness of tested batch

(NfG 3.1.2.1.v)



Use of cruder test batch could lead to a lower NOAEL and consequently to a lower MOE

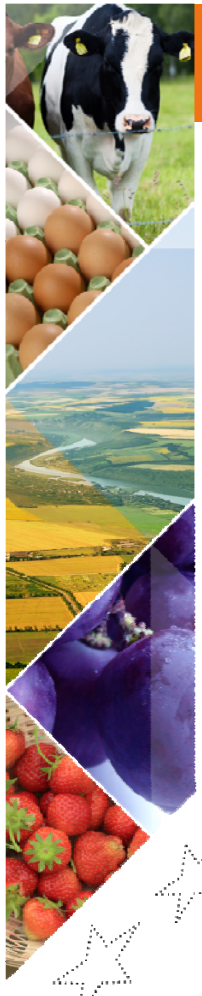


Toxicological data set

- **Updated on version 2014:EN-579;**

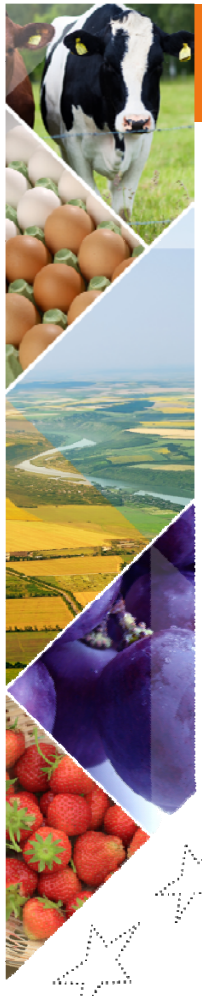
"***The test substance in the OECD guidance should refer to TOS.*** *It is recommended that the maximum dose chosen for all toxicological tests should be based on the amount TOS (e.g. for Ames test 5 mg TOS/plate provided that the enzyme is soluble and none cytotoxic in the test as discussed in the OECD guidance). Depending the test the dose units should be expressed as µg TOS/plate, µg TOS/ml or mg TOS/kg b.w./day.*

The selection of the lower doses must be justified and discussed in detail".



Default maximum doses based on the OECD GDs

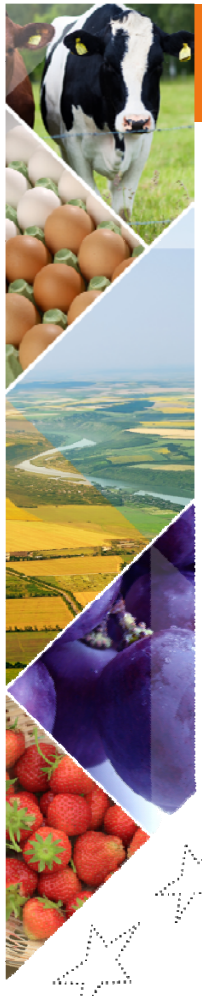
Test		
	Ames test	5000 µg TOS / plate
	Chr. Ab. or MN	5000 µg TOS / ml
	90 days oral toxicity study	Higher dose should produce toxicity or Limit test (1000 mg TOS/ kg b.w. / day)



Assessment of genotoxicity

- 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 micronucleus assay (OECD guideline 487). An in vitro chromosomal aberration assay (OECD guideline 473) and a mouse lymphoma tk assay with colony sizing (OECD guideline 476), would still be acceptable if such studies have already been performed following the Note for Guidance from 2011.



Update assessment of genotoxicity – Chromosome Aberrations

- **Updated on version 2014:EN-579;**

*"For an adequate evaluation of the genotoxic potential of a chemical substance, different endpoints (i.e. induction of gene mutations, structural **and numerical** chromosomal alterations) have to be assessed, as each of these events has been implicated in carcino-genesis and heritable diseases."*

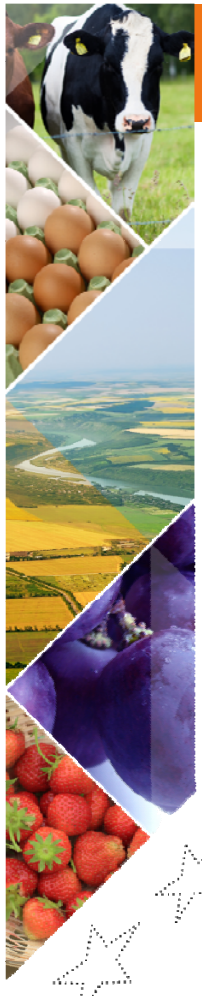
NOTE FOR GUIDANCE EN-579, 2014

Update assessment of genotoxicity – Ames test

- **Updated on version 2014:EN-579;**

"In the case of the Ames test, in order to overcome potential problems with the histidine in the food enzyme batch, it is recommended to expose the Salmonella strains to the tested food enzyme in the liquid culture ***("treat and plate assay",*** *instead of the traditionally "plate incorporation assay")".*

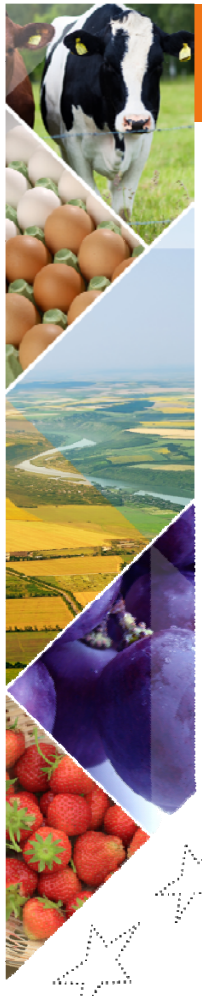
"In the case of the Ames test and when ***the food enzyme may affect the performance of S9*** *(e.g.* ***inactivation*** *by phospholipase), it would be advisable to test the positive control also in the presence of the food enzyme so as to show that the S9 performance is not affected".*



Update assessment of genotoxicity – Chromosome Aberrations (2)

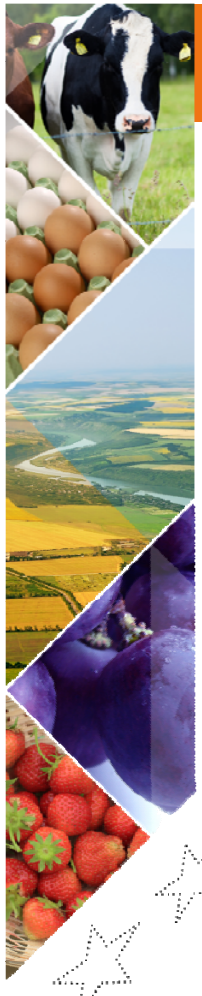
- In its opinion the Scientific Committee (EFSA Panel on Genetically Modified Organisms (GMO), 2011), suggested the following two *in vitro* tests as the first step in genotoxicity testing
  - **a bacterial reverse mutation assay (OECD TG 471), and**
  - **an *in vitro* mammalian cell micronucleus test (OECD TG 487).**

The opinion states that "this combination of tests fulfils the basic requirements to cover the three genetic endpoints with the minimum number of tests; the bacterial reverse mutation assay covers gene mutations and the *in vitro* micronucleus test covers both structural and numerical chromosome aberrations."



Assessment of genotoxicity in vivo

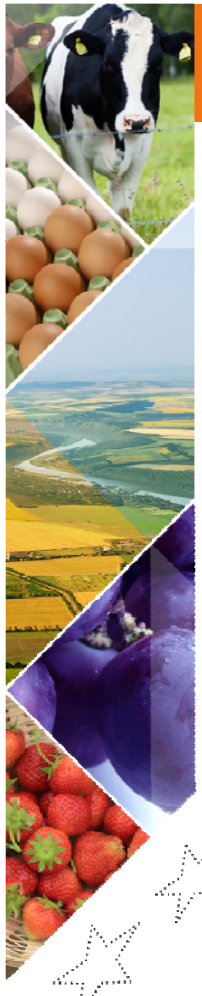
- In the case that any genotoxicity was identified in vitro genotoxicity in vivo should be tested in accordance with the Scientific Opinion on Genotoxicity testing strategies applicable to food and feed safety assessment (EFSA Scientific Committee, EFSA Journal 2011; 9(9):2379)



Assessment of systemic toxicity

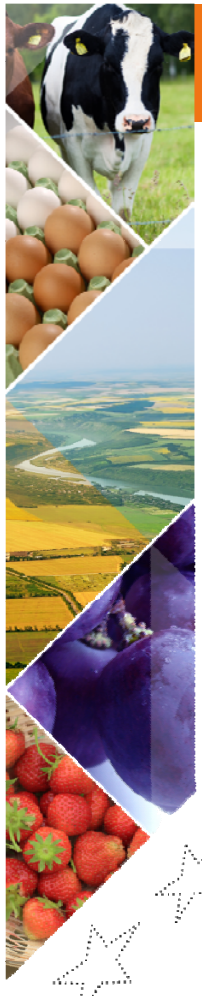
- A subchronic oral toxicity study (OECD 408) should be performed.

FOR FURTHER DETAILS SEE CEF PANEL GUIDANCE



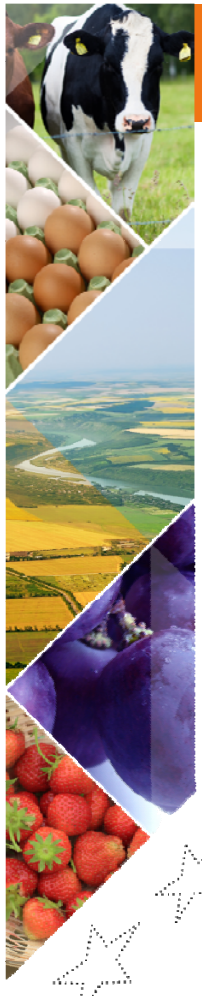
When toxicological testing may not be needed

- 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 food enzyme, the composition and the properties of the food 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.



When toxicological testing may not be needed

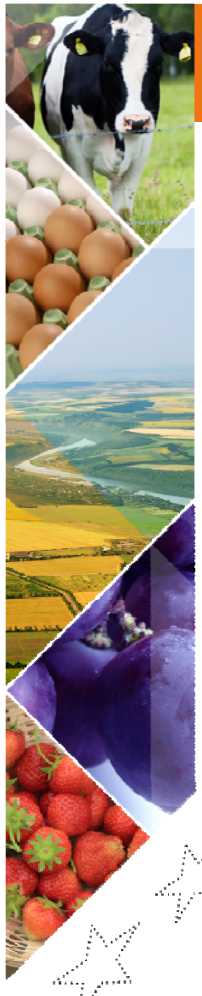
- - Food enzymes produced by micro-organisms that have been given a status of Qualified Presumption of Safety (QPS), if it can be demonstrated that there are no concerns related to any residues, degradation products or substances originating from the total production process (EFSA, 2005).
- - If a food enzyme from a specific strain has been thoroughly tested and the manufacturing process does not differ significantly for other food enzymes from the same strain, the full testing battery may be waived for these food enzymes. This will be decided on a case-by case basis. The detailed justification shall be provided in the dossier. However, EFSA may request further clarification.



When toxicological testing may not be needed, comment

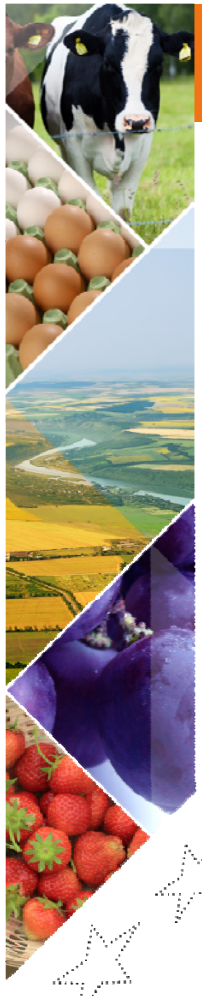
- The QPS status of the production microorganism shall exempt from toxicological studies, provided that absence of concern from residues, impurities, degradation products linked to the total production process (production, recovery and purification) is demonstrated and supported by experimental data.
- **Updated on version 2014:EN-579;**

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.



Review of toxicological data

The reasons for disregarding any findings should be carefully explained. Where relevant, the conclusions should include an interpretation of the significance of the findings. Conclusions drawn should be product specific (Production strain or strain lineage, and enzyme specific).



Update assessment of genotoxicity

Ames test

- Ideally S9 functionality should be tested also in the presence of enzyme (appropriate positive control + S9 + food enzyme).

- Cells viability should be sufficient in order to allow for adequate test sensitivity especially when the “treat and plate” assay is used. For example TA97 could be used in case TA1537 cell viability is considerable affected by the enzyme (with or without S9).

Update assessment of genotoxicity

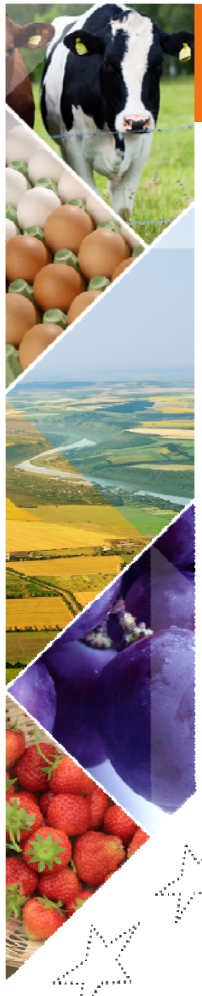
Chromosomal Aberration Test

- OECD 473 updated draft (Dec 2013) for the *in vitro* chromosomal aberration test ;

"The purpose of the *in vitro chromosomal aberration test is to identify agents that cause* ***structural*** *chromosomal aberrations in cultured mammalian cells"*

"*This test is not designed to measure aneuploidy. An in vitro micronucleus test (35) would be recommended for the detection of aneuploidy."*

- In order to measure numerical aberrations specific counts should be made in the slides . Ideally an aneugenic positive control (e.g. Vinblastine) should be used in addition to the clastogenic one.



Repeated Dose 90-day Oral Toxicity Study

- The selection of the highest dose should be based on the criteria mention on the OECD GD 408 and referring on TOS
- The evaluation of all finding should be exhaustively discussed and statement for non relevance, incidental observations etc should be fully justified.
- During the evaluation specific consideration should be made regarding the chemical nature of enzymes and the fact that what is tested is mixture and not a pure substance.