Risk assessment of food enzyme dossiers

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Introduction

Purpose

Clarify toxicological and procedural issues to come to a mutual understanding and so contribute to a smooth and efficient risk assessment process of food enzymes

Background

Published EFSA opinions created concern and uncertainty among AMFEP members on the evaluation of food enzyme dossiers regarding the interpretation of the toxicological data and the derivation of the NOAEL

Interpretation of toxicological data

AMFEP advocates the use of a structured, weight-of-evidence approach for evaluating the biological relevance (adversity) of 90-day toxicity studies and setting a NOAEL, including:

- Historical control data (important to understand the biological variation for a given endpoint)
- Statistical analysis (does not necessarily mean “important” or “meaningful” but is a statistical statement on the property and information content of the data)
- Published critical effect sizes on continuous toxicology data
- Assessment of the presence of a dose-response relationship
- Severity of effect together with effect constellations
- Direct versus indirect effects
- Adaptive responses
- Transient effects and reversibility
- Human relevance

Example (2014 opinions)

- Some EFSA opinions published in 2014 created questions and uncertainty among AMFEP members with regards to EFSA's interpretation of the toxicological data and derivation of the NOAEL
- Two examples are described in the Background document. In summary:

Lipase

- No dose-response (and minor difference, calcium)
- Difference is small (albumin)
- Background findings (thymus)
- Findings not causally related, but combination regarded as adverse by EFSA (albumin, calcium, thymus)
- Lack of scientific rationales for regarding findings as related to treatment/adverse.

Xylanase

- No dose-response and in historical control range (LYMPHO)
- Findings incidental based on historical control range (RBC, MCV, WBC)
- No statistical significance and no dose-response (NEUTRO)
- Lack of scientific rationale for regarding hematopoietic system as disturbed
- Lack of feedback on additional information supplied by applicant

EFSA regarded findings related to treatment and adverse

Example (2017 opinion)

- In a recent EFSA opinion (2017), the interpretation of toxicological data was consistent with that expected based on common practice. In short:

Beta-amylase

- No dose response (several parameters)
- Minor difference (several parameters)

EFSA regarded findings:

- unrelated to treatment
- of no toxicological importance
- attributed to normal biological variation

Scientific transparency

- EFSA's recent guideline on weight of evidence says:
  - *"Transparency and reproducibility are fundamental principles required by EFSA in its scientific assessments. Transparency should apply to all parts of the weight of evidence method, meaning that it should be possible to follow clearly how the input data for the assessment are analysed to produce the conclusions*."
- In the opinions of 2014 the derivation of the NOAELs was not transparent as no sound scientific rationales were provided
- In the more recent opinion from 2017, the assessment process was more descriptive and transparent making it possible to follow how the input data for the assessment were analyzed to reach the conclusion

Risk assessment

Margin of Safety (MoS) or Margin of Exposure (MoE)

- MoS is most relevant to apply in the safety assessment of food enzymes, which are neither genotoxic nor carcinogenic
- A MoS of 200 is sufficient to guarantee the safe use of food enzymes
- The presence of additional information could justify a lower MoS to conclude that the food enzyme is safe

Procedural questions

Anticipation on EFSA's risk evaluation and evaluation outcome

- Role of 3rd parties
- Interaction between the WG and Panel
- Consistency of opinions

Improving the way we work together

- Feedback on provided information
  - Clear guidance in clarification call
  - Insufficient data
  - MoS/MoE insufficient
  - Inconclusive opinion
- Blackening out of part of the opinions
  - How to prevent negative impression on transparency
- How can AMFEP further contribute to a smooth evaluation process?

Supporting slides

Example 1: Lipase

Study: 90-day oral (gavage) toxicity in rats (OECD 408)
Dose groups: 0 – 102 – 340 – 1020 mg TOS/kg/day (10 rats/sex/group)

Endpoint	Results males (Group mean (SD) or incidence) Control - Low - Mid - High	Interpretation	
		Study report NOAEL high-dose	EFSA NOAEL mid-dose
Albumin (g/L)	35 (1.1) - 34 (1.2) - 35 (0.7) - **33** (0.9)**	Very small difference (6%), no toxicological significance	Statistically significant decreases in calcium and albumin in high-dose males in combination with some minor histopathological alterations in thymus were indicative of adverse effects at the highest dose level.
Calcium (µM)	2.85 (0.05) - 2.77 (0.09)* - 2.83 (0.08) - **2.75** (0.06)*	Minor difference (3.5% at high-dose), no dose-response → normal biological variation	
Thymus	Reduced cortical width: 3/10 control males **5/9** high-dose males 5/10 control females	All microscopic findings incidental and of no toxicological importance.	
Thymus	Agonal hemorrhage: 4/10 control males **6/9** high-dose males 2/10 control females		

Remarks on results

- Hemorrhage in thymus is a common agonal lesion due to CO_2 euthanasia.
- The above findings are not known as a constellation of related changes (i.e. relationships between changes in plasma levels of albumin/calcium and thymus morphology are not well understood to be causally related).

Example 2: Xylanase

Study: 90-day oral (gavage) toxicity in rats (OECD 408)
Dose groups: 0 – 106 – 390 – 1070 mg TOS/kg/day (10 rats/sex/group)

Endpoint	Results females (Group mean (SD) or incidence) Control - Low - Mid - High	Interpretation	
		Study report NOAEL high-dose	EFSA NOAEL low-dose
RBC (10E12/L)	8.61 (0.21) - 8.53 (0.19) - 8.25 (0.30)**- 8.28 (0.28)* 4% lower at Mid and High	Incidental based on historical data.	Stat.sign. decrease Mid and High
MCV (fl)	52.1 (1.0) - 52.0 (0.7) - 53.0 (1.1) - 53.3 (0.9)* 2% higher at High	Incidental based on historical data.	Stat.sign. increase High
WBC (10E9/L)	9.37 (1.36) - 7.40 (2.74) - 7.42 (1.74)* - 7.19 (1.05)** 21% resp. 23% lower at Mid and High	Incidental based on historical data.	Stat.sign. decrease Mid and High
NEUTRO (10E9/L)	1.17 (0.37) - 0.99 (0.65) - 0.83 (0.25) - 0.87 (0.24) 29% resp. 26% lower at Mid and High	(no difference mentioned)	Considerable decrease Mid and High
LYMPHO (10E9/L)	7.99 (1.12) - 6.25 (2.05)*- 6.41 (1.61) - 6.14 (0.85)* 22%, 20% resp. 23% lower at Low, Mid and High	No dose response, in HCR → No tox significance.	Stat.sign. decrease Low and High
Spleen weight (% of bw)	0.21 (0.02) - 0.23 (0.02) - 0.23 (0.03) - 0.25 (0.02)** 10% resp. 21% higher at Mid and High	Treatment-related increase High. No tox significance.	Small increase Mid Stat.sign. increase High
Spleen (n=10) extramedullary hematopoiesis	minimal 2 - 3 - 3 - 2 slight 0 - 0 - 3 - 4	Treatment-related increase inc. + severity Mid and High. Little tox significance. Exaggeration background.	Small increase incidence + severity Mid and High

Remarks on results:

- Results did not show any changes indicative of disturbance of normal function of the hematopoietic system.
- In response to EFSA's request for clarification of the NOAEL (high-dose) proposed by applicant, an independent expert report (confirming the proposed NOAEL) , micro-photos of spleen sections and historical control data on splenic EMH were provided.

EFSA conclusion:

- Magnitude some effects mid-dose small, but could be considered as likely indicative of the same effects (i.e. decrease RBC and effects spleen) and system disturbance could increase susceptibility, these changes should be considered adverse.

Example 3: beta-amylase (1/2)

Study: 90-day oral (gavage) toxicity in rats (OECD 408)

Dose groups: 0 – 120– 396 – 1199mg TOS/kg/day (10 rats/sex/group)

Endpoint	Results (Group mean (SD) or incidence) Control - Low - Mid - High	Interpretation Study report(#) NOAEL high dose
Overall weight gain (g)	♂ 276 (28) - 332 (41)** - 328 (38)** - 316 (38)** 20%, 19% resp. 14% higher at Low, Mid and High	No dose response, no similar trend females → no tox importance.
Overall mean food intake (g/wk)	♂ 198 (6) - 211 (8) - 211 (2) - 203 (13) 7%, 3% resp. 3% higher at Low, Mid and High	
Hematocrit (L/L)	♂ 0.48 (0.02) - 0.46 (0.02)* - 0.45 (0.01)* - 0.46 (0.01)** 4%, 6% resp. 3% lower at Low, Mid and High	No dose response, no similar finding females, most values in background range. No tox significance.
Hemoglobin (g/dL)	♂ 15.8 (0.6) - 15.2 (0.7)* - 15.0 (0.4)* - 15.3 (0.5)* 4%, 5% resp. 3% lower at Low, Mid and High	
RBC (10E12/L)	♂ 8.9 (0.25) - 8.6 (0.48) - 8.6 (0.28) - 8.5 (0.38)* 4% lower at High	In background range. No tox significance.
Reticulocytes (10E12/L)	♀ 0.167 (0.05) - 0.173 (0.04) - 0.135 (0.04) - 0.131 (0.02)* 22% lower at High	In background range, due to high control values. No tox significance.
Eosinophils (10E9/L)	♀ 0.14 (0.04) - 0.11 (0.03)* - 0.10 (0.02)* - 0.12 (0.04)* 21%, 29% resp. 14% lower at Low, Mid and High	No dose response, in background range → biological variation.
Platelets (10E9/L)	♀ 857 (182) - 721 (136)* - 725 (56)* - 685 (73)** 16%, 16% resp. 20% lower at Low, Mid and High	No change clotting time → no tox significance.
Potassium (mM)	♂ 4.9 (0.41) - 5.5 (0.53)* - 5.3 (0.53)* - 5.6 (0.41)8** 12%, 8% resp. 14% higher at Low, Mid and High	No dose response, minor difference, in background range, and/or no similar finding females → biological variation.
Protein (g/L)	♂ 67(2.3) - 65 (3.0) - 65 (2.2) - 64 (2.1)* 4% lower at High	
Adrenal weight (adjusted for bw)	♀ 0.062 - 0.072* - 0.068* - 0.072* 16%, 10% resp. 16% higher at Low, Mid and High	No dose response → biological variation
Forelimb grip strength (kg)	♀ 0.84 (0.10) - 0.84 (0.11) - 1.00* (0.11) - 0.91* (0.11) 19% resp. 8% higher at Mid and High	In historical range, no similar trend in males. Direction change does not indicate peripheral neuropathy. No tox significance.
Motor activity	♂ high dose: lower total scores (not stat.sign.) and lower/higher scores some time intervals (stat.sign.)	Scores not consistent, total scores not stat.sign. → biological variation.

(#) EFSA's interpretation on next page

Example 3: beta-amylase (2/2)

EFSA conclusions:

- Increase overall weight gain and feed intake in males : not dose related and therefore considered of no toxicological importance.
- All differences in hematology and clinical chemistry parameters: minor, confined to one sex or lacked dose-relationship and can be attributed to normal biological variation.
- Variation in neurobehavior parameters: showed no consistent association with consumption of the food enzyme.
- NOAEL high dose.

MoS / MoE and UFs across EFSA Panels

Panel	MoE/MoS	Toxicity study length	UFs	Reference
Scientific Committee	MoE for genotoxic and carcinogenic substances Health based limit value, e.g. ADI		Various 100	Opinion on harmonised approach for risk assessment of genotoxic and carcinogenic substances (EFSA, 2005)
CEF Panel	MoS	90-day	300 (limited duration and limited statistical power of the study)	Statement on Safety Evaluation of Smoke Flavourings (EFSA, 2010)
Scientific Committee	MoE		200 (extrapolation from sub-chronic to chronic) No extrapolation factor from subacute to sub-chronic was established	Guidance on default factors (EFSA, 2012b)
CEF Panel	MoE		300 (extrapolation from short term to chronic)	Outcome public consultation draft Exposure Assessment of Food Enzymes (EFSA, 2016a)
ANS Panel	MoS	Carcinogenicity (2-year)	100	Opinion on Titanium dioxide as Food additive (EFSA, 2016b)
ANS Panel	MoS	90-day	200 (extrapolation from 90-day to chronic exposure)	Opinion on potassium polyaspartate as Food additive (EFSA, 2016c)
Scientific Committee	ADI		100	Guidance on Weight of Evidence (EFSA, 2017a)
NDA Panel	MoE	90-day	100 (children) and 200 (adolescents and adults)	Opinion on hydroxytyrosol as Novel food (EFSA, 2017d)
NDA Panel	Margin between daily consumption and dose in rats causing treatment-related effects	90-day	45	Opinion on proline-specific oligopeptidase as Novel food (EFSA, 2017e)