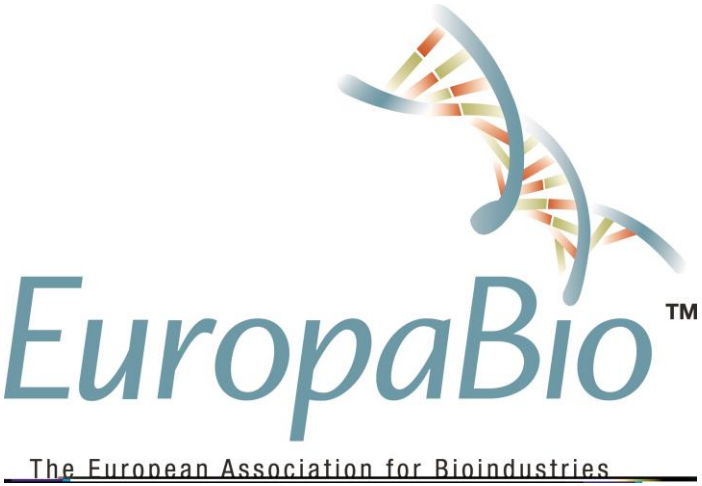

Repeated-dose 90-day Oral Toxicity Studies on Whole Food/Feed

Introduction

- The 2014 EFSA Explanatory Statement is very helpful as:
  - ✓ It clarifies the objectives of the 90-day feeding study; it should detect toxicologically relevant differences.
  - ✓ It addresses major questions on the experimental design and technical details.
  - ✓ It provides precise instructions on how to apply the general principles described in the 2011 EFSA Scientific Committee Guidance.

- The goal of this presentation is to further ask for clarification on a couple of study design elements, which are seen critical for meeting the objectives of the 90-day feeding study.

Outline

- Source of test material
- Incorporation levels
- Toxicological endpoints
- Experimental design
- Statistical design and reporting
- Concluding remarks

Source of test material (1)

- The recommendation is that the production of the test material should be part of the comparative assessment studies.
  - The randomized plot design and objective of the comparative studies do not allow production of material that would be appropriate for the 90-day feeding study. The likelihood of contamination after handling and pooling test material samples is high.

- Another recommendation is to show that the control material and the control diet are not contaminated with GM test material (DNA and protein analyses).
  - This would be more likely to occur if the material is sourced from the comparative assessment study.
  - Quantifying DNA and protein may be not feasible with processed material. The heat processing alteration (*e.g.* heat lability) of the protein may prevent it.

Source of test material (2)

The Explanatory Statement introduces new requirements neither of which are practicable:

- Trials for comparative assessment do not provide a good source of material for feeding studies.

- The analysis of both DNA and protein is likely to be technically challenging if not impossible.
  - Current GLP Guidelines provide for adequate characterisation of the test substances.

Incorporation levels (1)

- Testing at the highest possible level maximizes exposure and thus the potential to detect unanticipated adverse effects.

- Using systematically the highest published incorporation levels is risky for hazard Identification purposes.
  - The highest dose shall be the maximum achievable without causing nutritional imbalance. The incorporation levels may be influenced by the choice of strains (palatability, satiety), the specific crop varieties and the crop growing conditions (nutritional value, toxicants).
  - Historical Control Data were generated with lower percentages of incorporation for soybean, maize and rapeseed in most of the conducted 90-day feeding studies. They are critical for defining a range of acceptable biological variations in order to interpret properly the results.

Incorporation levels (2)

The Explanatory Statement proposes incorporation level reference values:

- The risk of failure is high due to the potential difficulty to interpret the results if the incorporation level is too high or higher to the previous studies conducted in the same laboratory with the same variety of crop.
- The incorporation levels could be alternatively justified by pilot studies or other scientifically sound data.

Toxicological endpoints

- Several endpoints were recommended in addition to the OECD TG 408 recommendations: weight of thyroid, of parathyroid, of sternum with bone marrow, and levels of urine creatinine, of plasma levels for 3 liver enzymes, of red blood cell mean cell volume, of distribution width, of reticulocytes absolute count.
  - Each test animal is monitored on >200 individual endpoints according to the OECD TG408. This currently ensures a thorough examination of the whole animal and that known mechanisms of toxicity will be evaluated. The value of these additional endpoints is unclear without any justifications or triggers.

- Several tests seem also mandatory for assessing the sensory reactivity, grip strength and motor activity
  - These requirements are unclear when there is no evidence of adverse changes in related parameters.

Experimental design (1)

Measuring the water intake and to quantify the feed spillage are new recommendations.

- The relevance of the water intake for the interpretation of data is unclear. Surrogate endpoints allow appropriate evaluation of the effects associated with changes in water consumption (urinalysis, hemoconcentration, renal-associated serum chemistry parameters). The feed consumption is more relevant.
- To quantify the feed spillage is challenging in wire-mesh cages as the spilled feed is mixed with feces. However, measures can be taken to limit the spillage at the beginning of the study. Spillage is uniform across groups when the nutritional balance is done properly.

Experimental design (2)

The water intake and feed spillage measurements are additional requirements which will be difficult to implement:

- How to study them and how to interpret any changes.
- Alternatively, whether feed consumption could be used as a more relevant endpoint than the water intake.
- Alternatively, whether measures should be implemented to limit the spillage rather than trying to quantify the spillage.

Statistical design and reporting

- The recommendation is to present the results on the standardized scale as well as the natural scale
  - The comparison with historical ranges can only be performed on the original scale. The additional standardized scale analysis provides no additional insight over and above the natural scale analyses.

- The type of statistical outputs and analyses are still not fully clear.
  - The standard practice is to analyze and interpret results for each sex separately. Studies are designed so that by sex analyses are suitably powerful. Consequently, there is no necessity to have pooled analysis.
  - The summary statistics as proposed by EuropaBio (mean, min, max, SD, *etc*.) should suffice.
  - The statistical analysis programs, logs and outputs should not be a default requirement.

Conclusions

- Applicants appreciate further clarifications provided through the Explanatory Statement, but are concerned that some new requirements go beyond the Protocol of the Scientific Committee.

- We believe that there is more room for flexibility in the design of future 90-day studies with whole food/feed
  - Scientific justifications are important.
  - Protocol should be simple to ensure its robustness.
  - Practical limitations should be taken into consideration.
  - Design should be accepted in all geographies: 1 global study.

- Europabio would be glad to pursue this exchange at the scientific level.

Thank you!