Recurrent issues in GMOs applications
A. Dose selection in 28-day toxicological studies on newly expressed proteins (NEPs)
B. Statistical analysis of field trials

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Parma, November 2015

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A. Dose selection in 28-day toxicological studies on newly expressed proteins (NEPs)

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THE FRAME

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European Food Safety Authority

EFSA Journal 2011; 9(5):2150

SCIENTIFIC OPINION

Guidance for risk assessment of food and feed from genetically modified plants[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

3.1. Hazard identification and characterisation

3.1.4. Toxicological assessment

3.1.4.1. Standardised Guidelines for Toxicity Tests

3.1.4.2. Assessment of newly expressed proteins

OECD TG 407

A RECURRENT ISSUE

Dose level selection is a recurrent issue in 28-day toxicity studies in rodents on NEPs in GMO applications

- Justification NOT adherent to OECD TG407
  - based on high fold margin of safety over a "worst case" scenario for human exposure.
- OECD TG 407
  - the high dose should induce adverse changes
  - if no toxicity is expected, a limit test should be conducted (1000mg/kg/day).
- Information on NEPs is available to drive dose selection→limit test

CONSEQUENCES

- LOW DOSES TESTED
- **WEAK HAZARD IDENTIFICATION**

OTHER CONSIDERATIONS

High margins of safety vs. human exposure

- Not supported as the sole justification for dose selection in 28-day toxicological studies in GMO RA by OECD TGs or EFSA Guidances.
- Not addressing animal exposure.

RECOMMENDATIONS

28-day studies in rodents on NEPs should adhere to standardised reference protocols (OECD TG 407) in accordance to EFSA, 2011 and Implementing Regulation (EU) 503/2013.

Dose level selection of 28-day studies in rodents on NEPs should be justified accordingly, in particular as regards the selection of the high dose.

Thank you for your attention

Questions?

Background slides

407 OECD/OCDE

Dosage

18 Generally, at least three test groups and a control group should be used, but if from assessment of other data, no effects would be expected at a dose of 1000mg/kg bw/d, a limit test may be performed. If there are no suitable data available, a range finding study (animals of the same strain and source) may be performed to aid the determination of the doses to be used. Except for treatment with the test substance, animals in the control group should be handled in an identical manner to the test group subjects. If a vehicle is used in administering the test substance, the control group should receive the vehicle in the highest volume used.

19 Dose levels should be selected taking into account any existing toxicity and (toxico-) kinetic data available for the test compound or related materials. The highest dose level should be chosen with the aim of inducing toxic effects but not death or severe suffering. Thereafter, a descending sequence of dose levels should be selected with a view to demonstrating any dosage related response and no-observed-adverse effects at the lowest dose level (NOAEL). Two to four fold intervals are frequently optimal for setting the descending dose levels and addition of a fourth test group is often preferable to using very large intervals (e.g. more than a factor of 10) between dosages.

Limit test

21 If a test at one dose level of at least 1000 mg/kg body weight/day or, for dietary or drinking water administration, an equivalent percentage in the diet, or drinking water (based upon body weight determinations), using the procedures described for this study, produces no observable toxic effects and if toxicity would not be expected based upon data from structurally related compounds, then a full study using three dose levels may not be considered necessary. The limit test applies except when human exposure indicates the need for a higher dose level to be used.

Recurrent issues in GMOs applications
B. Statistical analysis of field trials

Franco M. Neri, Scientific Officer, GMO
Parma, November 2015

STATISTICAL ANALYSIS: TESTING MODEL ASSUMPTIONS

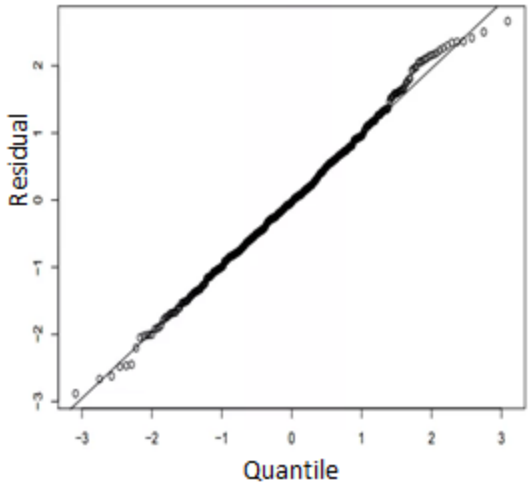
Linear mixed model recommended by EFSA (agronomic-phenotypic and compositional endpoints)

- Testing model assumptions (normality, homogeneity of variance): crucial.
- How to do it? Standard tests or "graphical techniques"? Both are possible. However, the choice should be done with care. Conclusions based on graphical techniques can be subjective. Formal tests are preferable.
- If there is uncertainty on the fulfilment of model assumptions, the outcome of the analysis cannot be used for RA.

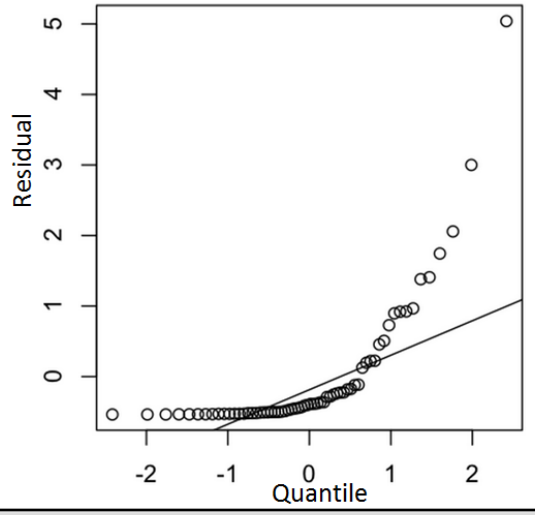
TESTING MODEL ASSUMPTIONS GRAPHICALLY

Normality

Example 1

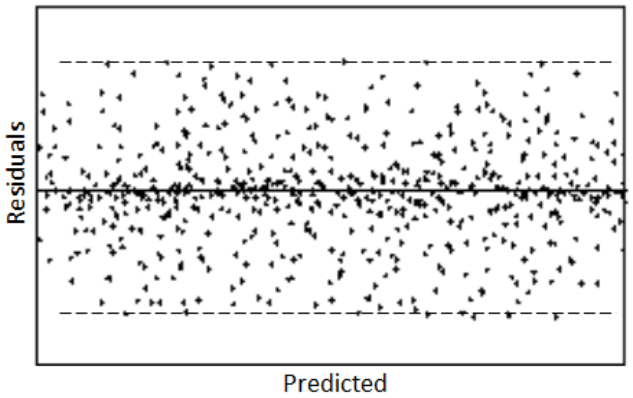


Example 2

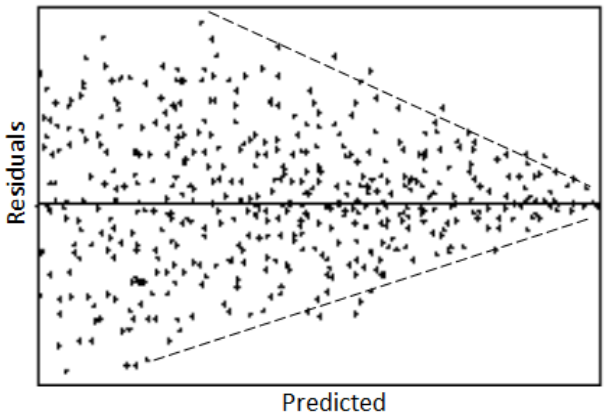


Homogeneity of variance

Example 1



Example 2



MODEL ASSUMPTIONS AND MODEL SELECTION

- If normality and homogeneity of variance are not fulfilled, the recommended strategy is data transformation.

- Other strategies (e.g. use of non-parametric statistics) should be only considered if no adequate scale for the data was found, and should be adequately justified.

SUBMISSION OF DATA

- "The raw data and the programming code used for the statistical analysis shall be given in an editable form." (Implementing Regulation (EU) 503/2013).

- The data files submitted to EFSA should be the same as those used by the applicant for the statistical analysis.

- In some cases, this did not happen because the data files sent to EFSA had been further processed. In a few other cases, they were just the wrong files. In all those cases, several months in the RA process have been lost trying to clarify the issue.

Thank you for your attention

Questions?