TOX 90-DAY DATA SUBMISSION

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Meeting with Industry

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EUROPEAN FOOD SAFETY AUTHORITY

TOX 90-day study with GM FF: Risk Assessment

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CLARIFICATION CLARIFICATION CLARIFICATION

Pre-Validation | Post-Validation

FDP Unit Completeness Checks

Preparatory Work to Risk Assessment

Phase 1. NIF Unit & ISA contractor(s)
Check for compliance to EU regulatory frameworks and for the adequateness of the 90-day tox study and its statistical design

Check for adequateness of 90-day tox study design
Check for adequateness of 90-day statistical design

Risk assessment

Phase 2. GMO Panel
Assessment of the study results
- ❑ In-life toxicology & clinical observations
- ❑ Ophthalmology & neurobehavioral evaluation
- ❑ Clinical pathology evaluation
- ❑ Anatomic pathology evaluation

Food & Feed Working Group

TOX 90-day studies: assessment of adequateness

Aim of the "TOX 90-day data submission" template: to list those requirements which:

1. guarantee the full compliance to EU regulatory frameworks
   - ❑ IR 503/2013
   - ❑ GLP standard
   - ❑ EFSA SC GD 2011
   - ❑ EFSA 2014
   - ❑ OECD TG 408
2. ensure the adequateness of materials and diets used in TOX 90-day studies

and related overall improvement of the risk assessment

A. GENERAL INFORMATION

B. COMPLIANCE WITH REGULATORY REQUIREMENTS
- ❑ GLP standard of reference
- ❑ OECD standard of reference
- ❑ EFSA standard of reference

C. STUDY DESIGN
- ❑ Scenario hypothesis-related
- ❑ Experimental design

D. INFORMATION ON MATERIALS AND DIETS
- ❑ source material, item & diet - GM
- ❑ source material, item & diet - non-GM comparator
- ❑ source material, item & diet - non-GM reference

E. TEST SYSTEM
- ❑ Animal model
- ❑ Housing conditions

F. EXPERIMENTAL ENDPOINTS
- ❑ In-life observations
- ❑ Clinical pathology
- ❑ Necropsy findings
- ❑ Terminal body and organ weights
- ❑ Histopathological findings

G. STUDY REPORTING

TOX 90-day study with GM FF: compliance to EU regulatory frameworks

1.4.4.1. 90-day feeding study in rodents with whole genetically modified food/feed

The applicant shall include a 90-day feeding study with whole food and feed in rodents for the assessment of food and feed containing, consisting of or produced from genetically modified plants with a single transformation event or with stacked transformation events which are not obtained by conventional crossing of genetically modified plants containing a single transformation event.

In the case of stacked transformation events obtained by conventional crossing of genetically modified plants containing one or several transformation event(s), a 90-day feeding study with whole food and feed in rodents shall be included for each of the genetically modified plant with a single transformation event which was used. An additional 90-day feeding study with whole food and feed in rodents with the genetically modified plant with the stacked transformation events shall be included where indications of potential adverse effects are identified during the assessment of: (i) the stability of the inserts; (ii) the expression of the inserts; and (iii) the potential synergistic or antagonistic effects resulting from the combination of the transformation events.

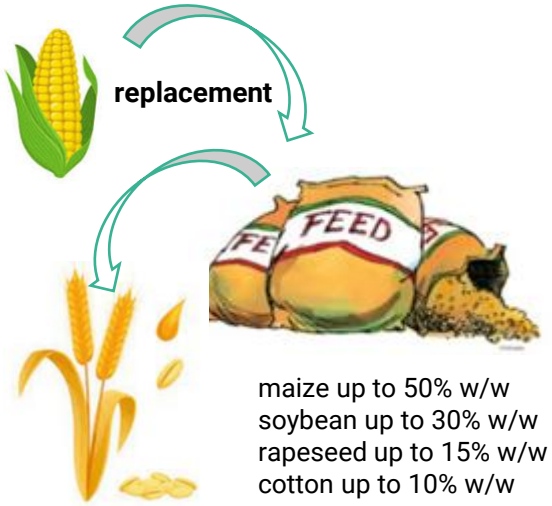
The design of the toxicity study with genetically modified food and feed should be performed according to the 'subchronic oral toxicity test repeated-dose 90-day oral toxicity study in rodents' (see Table 1) following an adapted protocol. In principle a minimum of two test doses and a negative control shall be used. The highest dose shall be the maximum achievable without causing nutritional imbalance; the lowest dose shall contain the tested food and/or feed in an amount always above the anticipated human/target animal intake level. The genetically modified food and feed analysed should be relevant to the product to be consumed. In the case of herbicide tolerant genetically modified plants, the tested material should come from the genetically modified plant exposed to the intended herbicide. Whenever possible, information on natural variation of test parameters shall be derived from historical background data rather than from the inclusion of reference varieties, consisting of commercially available food and feed derived from non-GM plants with a history of safe use, in the experiments. The statistical analysis shall focus on the detection of possible differences between the test material and its control. A power analysis to estimate a sample size capable of detecting a pre-specified biologically relevant effect size with a specified power and significance level should be used. More detailed guidance for performing this study is provided in the EFSA Guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed ([1]).

Provisions for the TOX 90-day study design (IR 503/2013)

- ❑ Reference to OECD TG 408
- ❑ Reference to EFSA SC GD 2011 → EFSA 2014
- ❑ GLP compliance * (EFSA GLP WG position)
- ❑ Test doses
- ❑ Test material
- ❑ Historical control data vs reference groups
- ❑ Statistical design

TOX 90-day study with GM FF: peculiarity of the test item

ppm (mg/kg diet)



maize up to 50% w/w
soybean up to 30% w/w
rapeseed up to 15% w/w
cotton up to 10% w/w

90-day feeding study with a chemical substance (test item)

- ❑ test item added & mixed in a commercial diet in proportion of ppm (mg/kg diet)
- ❑ commercial standard diet manufactured under non-GLP quality standards (e.g. ISO)
- ❑ mixing of test item into the commercial standard diet performed according to GLP

90-day feeding study with GM plant food/feed (test item)

- ❑ test item incorporated as ingredients in a commercial diet in percentage of the diet (w/w), replacing standard ingredients (e.g. cereals/protein sources) upon proper formulation
- ❑ manufacturing of the test diet is commonly outsourced to external companies
- ❑ mixing of test item into the commercial standard diet performed under quality standards (e.g. ISO) different from GLP

TOX 90-day study with GM FF: Adequateness of test materials and diets




Harvesting *Source material*	Processing *item*	Manufacturing *diet*	Administration *diet*
maize grains	→ ground grains	→	→
soybean seeds rape seeds cotton seeds	→ defatted toasted meal	→ pellet or meal	→ 
production plan	processing report	manufacturing report	90-day study report

“Checklist” for adequateness of 90-day tox study design

TOX 90-day studies: EFSA GLP-WG Recommendations

TOX 90-day studies with GM FF are unique:
- required only in the EU
- mainly performed outside the EU

TOX 90-day diet Manufacturing:
- critical phase from a GLP point of view
- commonly outsourced to feed manufacturer operating under quality standards (e.g. ISO) different from GLP
- in most cases excluded from GLP, jeopardizing the GLP status of the whole study

Supplementary information to assess the adequateness of feed materials and diets used in TOX 90-day studies:
- in most cases has been provided separately by the applicant
- should be an integral part of the main Study Report; falls under the responsibility of the Study Director

Required information when GLP is claimed for the whole study except for diet manufacturing:
- the homogeneity and stability of the diet
- the effective role of the Quality Assurance in the quality control of all the phases related to diet manufacturing
- the Study Director must ensure that the diet preparation is performed according to established protocols
- the description of the diet composition.

TOX 90-day "Checklist" is currently in use by EFSA to assess the adequateness and full compliance of the 90-day study design:
- endorsed by EFSA GLP-WG Experts
- applicants might provide the filled-in "checklist" alongside the submission of a 90-day study in GMO applications

The GLP WG of EFSA expressed the recommendation that in future, applicants should endeavor to perform the whole study under GLP, including diet manufacturing

TOX 90-day study “checklist” - GMO Stakeholder’s meeting 23-24 October 2019

3) Technical aspects of 90-day feeding studies with rodents

At the June ad hoc meeting and in our letter dated 20 September 2019 (with reference EW/SM/yog (2019) – OC-2019-21831841), we sought for feedback on the following scientific aspects of 90-day studies with rodents:

1) the feasibility to use test material that is representative from the field trials material supporting the comparative assessment,
2) the need for a proper characterisation of the test (and control) material,
3) the feasibility and possible implementation for future 90-day studies to test diet incorporating 50% maize as reported by Steinberg et al. (2019).

During the meeting, Europabio and EFSA addressed these three questions in their respective PowerPoint presentations and in the following discussion. You will find below EFSA conclusions:

1) EFSA welcomes the informative presentation by Dr Duska Stojsin and acknowledges the significant limitations (e.g. insufficient quantity of grains produced, heterogeneity of harvested grains from different plots) in using field trials for comparative analysis to generate the material for feeding studies. Subsequently EFSA agrees with EuropaBio that the test material for feeding studies can only be generated through dedicated *ad hoc* studies. Therefore, applicants are requested to provide these *ad hoc* studies as well as detailed information on the conditions of material production (e.g. herbicides treatments). Over the last years EFSA systematically requested such information to applicants when not provided in the application.

2) As indicated at the June meeting, the material used for the feeding studies needs to be adequately described for its integrity, identity and purity. EFSA expects to receive systematically comprehensive information on test material characterisation by applicants. The checklist (see attachment) used by EFSA to assess the completeness and appropriateness of the information on feeding studies might guide applicants in preparing their applications and providing the necessary information.

from the follow up EFSA Letter to GMO Stakeholder’s Meeting October 23-24th 2019]

A previous, similar version of the “checklist” used by EFSA to assess the adequateness of TOX 90-day study design to EU Regulatory frameworks was made available to GMO applicants on 19-12-2019

The aim was to support applicants in preparing their applications and providing the necessary information to ensure full compliance

However, 2020-to date - still recurrent and specific Additional Data Request re. the full compliance of TOX 90-day study design to EU regulatory frameworks.

“TOX 90-DAY DATA SUBMISSION” TEMPLATE 2024

EXPECTED ADDED VALUE OF THE USE OF “TOX 90-day information for data submission” Template

- ❑ ensure completeness/compliance of the submitted TOX 90-day studies to EU regulatory frameworks.
- ❑ reduce the number of ADRs [i.e. diet formulation, statistical analyses]
- ❑ higher speed to complete the Risk Assessment of TOX 90-day studies by the FF-WG Experts

"TOX 90-DAY DATA SUBMISSION" TEMPLATE 2024 PROPOSED WAY FORWARD

PROPOSED WAY FORWARD

- ❑ in line with BI, invitation to provide suggestions how to optimize the use of the "TOX 90-day data submission" Template [proposed timeline mid May]
- ❑ "TOX 90-day data submission" Template [consolidated version] available to GMO applicants
- ❑ Autumn 2024 GMO Stakeholder's meeting [TBC]: joint evaluation of "TOX 90-day data submission" Template in terms of:
  - added value in terms of reduction of ADRs and timing to complete the RA of TOX 90-day studies by the FF-WG Experts
  - GMO applicant's effort [i.e. time to complete "TOX 90-day information for data submission" Template]
  - define the most appropriate format of the "TOX 90-day information for data submission" Template