Attachment I

Attachment I includes the draft document provided to EFSA, with modifications proposed by the GMO Panel together with an introductory note explaining the nature of the changes.

Introductory note

This draft working document is based on the updated guidance of the GMO Panel which was adopted by EFSA in May 2008 and published in its website (see attachment II), on comments of stakeholders and member states which were provided during the public consultation of EFSA and further discussions.

EFSA was formally consulted on this document by the Commission on 23 February 2009.

EFSA GMO Panel discussed and adopted the present document with the proposed modifications. These have been introduced as tracked changes and comments to the document.

No modifications have been included in Annex I and the main amendments made by EFSA are found in Annex II. The major modifications are explained by section below:

General comments:

It should be noted that although it was explained by the Commission to EFSA, that the word shall should be used for legal reasons, is not always appropriate (e.g Part II section 1.2.2.2(f) or 1.4.4.1)).

When needed an analysis on phenotypic and not only on morphological characteristics should be performed.

Finally a large number of minor clarifications and editorial changes are provided to improve the quality of the document.

Part I:

Section 1: The possibility to need more than one conventional counterpart (e.g in the risk assessment of stacked events) should not be excluded. An amendment of the second paragraph was done to better clarify when a comprehensive safety and nutritional assessment of the GM crop derived food/feed per se should be carried out.

Section 1.1: Clarifications on the concept of substantial equivalence or comparative safety assessment and on the detection of unintended effects are included.

Section 1.2: The text was improved in the subsections and clarifications were added.

Section 2: Clarifications and shortening of the text on Risk Characterisation was suggested as this subject is discussed in Part II section 3 in detail.

Section 2.2 (h): 'for long-term' was deleted since it is not in line with 1.6 'Nutritional assessment'

Section 3.1: The consolidated EFSA opinion on Antibiotic Resistance Marker genes was not adopted when this guidance document was adopted. Therefore, the text should be brought in line with the adopted opinion on ARM.

Section 3.2: The last paragraph has been modified to better clarify when the risk assessment of a stacked event might be applicable for GM stacks with fewer events.

Part II:

Section 1.1: more appropriate text for this paragraph was provided.

Section 1.3: the reference to the novel foods risk assessment was deleted since further guidance needs to be developed.

Section 1.3.1 Description of conventional counterpart was strengthen also with respect to possible additional comparators (e.g. negative segregant). Clarification in case of herbicide tolerant crops was improved

Section 1.3.2: The text was extensively revised by experts of the EFSA GMO Statistics WG. Thus for clarity the whole section is not shown in track changes. Modifications regarded:

- *Better explanation of the simultaneous use of the test of difference and equivalence*
- *Better explanation of the test of equivalence and calculation of equivalence limits*
- *Introduction of reference to the report of the self-task WG on statistics*
- *Introduction of a section describing the experimental set-up in case of simultaneous testing of multiple GMOs in the same trial*
- *Improvement of Figure 1 and its legend*
- *Improvement of interpretation of results*

Section 1.3.4: The last sentence was deleted as its meaning not was clear. Section 1.6 refers to nutritional assessment.

Section 1.3.7: The conclusions were brought in line with the modified text.

Section 1.4: For clarity the first bullet point as an introduction to the following text is strengthened by omitting the bullet formatting.

The last paragraph which refers to testing protocols of single compounds should in EFSAs view be moved to a separate section (currently 1.4.4 in the document) after sections 1.4.1-1.4.3 where the requirements for the testing of single compounds is described.

Section 1.4.1: the widely used terminology 'history of safe use' can be kept if a footnote could provide explanation of this term as proposed. Also part of the text in (e) has been removed as it was explained earlier.

Section 1.4.4.1: In this section important information from the updated guidance document (e.g. selection of doses, stability and equivalence of test diets, the rational behind the study) has been deleted and replaced. Therefore the last sentence of the first paragraph is replaced by the text proposed initially in the updated guidance document

Section 1.4.4.1(b): The 90 day study is not appropriate for testing of allergens as suggested in this paragraph.

The last paragraph of the section 'indications of unintended effects from molecular characterization' has been deleted as it not sufficiently accurate. The results of the bioinformatic analysis of the newly expressed protein are not necessarily unintended effects of the genetic modification. The results of this analysis are part of the requirements in section 1.4.1 and confirmatory studies with the protein are requested in parallel.

Section 1.4.4.1(c): The text of this paragraph is covered by (a) and it is also covered in Part I within section 1 while reference to novel food regulation is not appropriate as guidance needs to be developed as mentioned above. However, the case of GM plants with stacked events where a 90 day feeding study may be required was included to complete this section.

Section 1.4.4.2: The term 'structural alerts' have been deleted as they are inherent characteristics of chemical substances or proteins. If structural alerts are identified the isolated substance/protein should be tested but not the whole food.

Section 3.3: The first bullet point was modified to clarify that it refers to the comparative safety assessment and not to a comprehensive safety assessment.

ANNEX I: PREPARATION AND PRESENTATION OF APPLICATIONS

PART I: GENERAL INFORMATION

- Name and address of the applicant (company or institute);
- Name, qualification and experience of the responsible scientist(s) and contact details of the responsible person for all dealings with EFSA;
- Designation and specification of the GM plant and derived product;
- Scope of the application

  GM food

  ☐ Food containing or consisting of GM plants

  ☐ Food produced from GM plants or containing ingredients produced from GM plants

  GM feed

  ☐ Feed containing or consisting of GM plants

  ☐ Feed produced from GM plants

  GM plants for food or feed uses

  ☐ Products other than food and feed containing of consisting of GM plants with the exception of cultivation

  ☐ Seeds and plant propagating material for cultivation in the EU

  Where an application is limited to either food or feed use, it shall contain a verifiable justification explaining why the authorisation shall not cover both uses in accordance with Article 27 of Regulation (EC) No 1829/2003.

- Unique identifier

  A proposal for a unique identifier for the GM plant and derived products in question, developed in accordance with Commission Regulation (EC) No 65/2004[1] of 14 January 2004 establishing a system for the development and assignment of unique identifiers for genetically modified organisms shall be submitted.

- Where applicable and where relevant to the risk assessment, a detailed description of the method of production and manufacturing. This would include, for example, a description of methods used to process the GM plant materials during the preparation of food/feed, food/feed ingredients, food/feed additives or food flavourings.

[1] OJ L 10, 14.01.2004, p.5 -11

- Where appropriate, the conditions for placing on the market of the food(s) or feed(s) produced from it, including specific conditions for use and handling.

PART II: SCIENTIFIC INFORMATION

Depending on the scope (e.g. limited to derived products), not all the following requirements shall be provided in the application.

1. HAZARD IDENTIFICATION AND CHARACTERISATION

1.1. Information relating to the recipient or (where appropriate) parental plants

- Complete name; (a) family name, (b) genus, (c) species, (d) subspecies, (e) cultivar/breeding line or strain, (f) common name.

- Geographical distribution and cultivation of the plant, including its distribution in Europe.

- Information on the recipient or parental plants relevant to their safety, including any known toxicity or allergenicity.

- Data on the past and present use of the recipient organism, e.g. history of safe use for consumption as food or feed, including information on how the plant is typically cultivated, transported and stored, whether special processing is required to make the plant safe to eat, and the plant normal role in the diet (e.g. which part of the plant is used as a food source, whether its consumption is important in particular subgroups of the population, what important macro- or micro-nutrients it contributes to the diet).

Additional information relating to the recipient or parental plants required for the environmental safety aspects:

- Information concerning reproduction: (i) mode(s) of reproduction, (ii) specific factors affecting reproduction (if any), (iii) generation time;

- Sexual compatibility with other cultivated or wild plant species.

- Survivability: (a) ability to form structures for survival or dormancy, (b) specific factors (if any) affecting survivability.

- Dissemination: (a) ways and extent of dissemination (to include, for example, an estimation of how viable pollen and/or seed declines with distance), (b) special factors affecting dissemination, if any.

- Geographical distribution in Europe of the sexually compatible species.

- In the case of a plant species not grown in the Member State(s), description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts.

- Other potential interactions of the GM plant with organisms in the ecosystem where it is usually grown, or used elsewhere, including information on toxic effects on humans, animals and other organisms.

1.2. Molecular Characterisation

1.2.1. Information relating to the genetic modification

1.2.1.1. Description of the methods used for the genetic modification

1.2.1.2. Nature and source of vector used

1.2.1.3. Source of DNA used for transformation, size and intended function of each constituent fragment of the region intended for insertion

1.2.2. Information relating to the GM plant

1.2.2.1. Description of the trait(s) and characteristics which have been introduced or modified

1.2.2.2. Information on the sequences actually inserted/deleted

1.2.2.3. Information on the expression of the insert

1.2.2.4. Genetic stability of the insert and phenotypic stability of the GM plant

1.2.3. Additional information relating to the GM plant required for the environmental safety aspects

Comment [divekzo1]: This is from the old version - does not correspond anymore to the structure of the present document

1.2.3.1. Information on how the GM plant differs from the recipient plant in: reproduction, dissemination, survivability

1.2.3.2. Any change to the ability of the GM plant to transfer genetic material to other organisms

- Plant to bacteria gene transfer
- Plant to plant gene transfer

1.2.4 Conclusions of the Molecular characterisation

1.3. Comparative analysis

1.3.1. Choice of the conventional counterpart and additional comparators

1.3.2. Experimental design and statistical analysis of data from field trials for comparative analysis

1.3.2.1. Description of the protocol and the experimental design

1.3.2.2. Statistical analysis

1.3.3. Selection of material and compounds for analysis

1.3.4. Comparative analysis of composition

1.3.5. Comparative analysis of agronomic and phenotypic characteristics

1.3.6. Effects of processing

1.3.7. Conclusion

1.4. Toxicology

1.4.1. Toxicological testing of newly expressed proteins

1.4.2. Testing of new constituents other than proteins

1.4.3. Information on natural food and feed constituents

1.4.4. Testing of the whole GM food/feed

1.4.4.1. 90-day feeding study in rodents

1.4.4.2. Additional animal studies [with respect to reproductive, developmental or chronic toxicity]

1.4.4.3. Other animal studies to examine the safety and the characteristics of GM food/feed

1.4.5. Conclusion of the toxicological assessment

1.5. Allergenicity

1.5.1. Assessment of allergenicity of the newly expressed protein

1.5.2. Assessment of allergenicity of the whole GM plant or crop

1.5.3. Conclusion of the allergenicity assessment

1.6. Nutritional assessment

1.6.1. Nutritional assessment of GM food

1.6.2. Nutritional assessment of GM feed

1.6.3. Conclusion of the nutritional assessment

2. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

3. RISK CHARACTERISATION

4. POST-MARKET MONITORING ON GM FOOD/FEED

5. ENVIRONMENTAL ASSESSMENT

5.1. Mechanism of interaction between the GM plant and target organisms

5.2. Potential changes in the interactions of the GM plant with the biotic environment resulting from the genetic modification

5.2.1. Persistence and invasiveness

5.2.2. Selective advantage or disadvantage

5.2.3. Potential for gene transfer

5.2.4. Interactions between the GM plant and target organisms

5.2.5. Interactions of the GM plant with non-target organisms

5.2.6. Effects on human health

5.2.7. Effects on animal health

5.2.8. Effects on biogeochemical processes

5.2.9. Impacts of the specific cultivation, management and harvesting techniques

5.3. Potential interactions with the abiotic environment

6. ENVIRONMENTAL MONITORING PLAN

6.1. General

6.2. Interplay between environmental risk assessment and monitoring

6.3. Case-specific GM plant monitoring

6.4. General surveillance of the impact of the GM plant

6.5. Reporting the results of monitoring

PART III: CARTAGENA PROTOCOL

The application shall provide information required under Article 5(3)(c) and Article 17(3)(c) of Regulation (EC) No 1829/2003 for the purpose of complying with Annex II to the Cartagena Protocol on Biosafety to the Convention on Biological Diversity (the Cartagena Protocol). Depending on the scope of the application, the provided information shall contain as a minimum the information specified in Annexes II or III of Regulation (EC) No 1946/2003 of 15 July 2003 of the European Parliament and of the Council on transboundary movements of genetically modified organisms[2].

The previous paragraph shall not apply to applications concerning only food and feed produced from GMOs or containing ingredients produced from GMOs.

PART IV: LABELLING

The application shall include:

(a) A proposal for labelling in all official Community languages, where a proposal for specific labelling is needed in accordance with Articles 5(3)(f) and 17(3)(f) of Regulation (EC) No 1829/2003;

(b) Either a reasoned statement that the food/feed does not give rise to ethical or religious concerns or a proposal for labelling in all official Community languages in accordance with Articles 5(3)(g) and 17(3)(g) of Regulation (EC) No 1829/2003; and,

(c) When appropriate a proposal for labelling complying with the requirements of Annex IV, A(8) to Directive 2001/18/EC.

PART V: METHODS OF DETECTION AND IDENTIFICATION AND REFERENCE MATERIAL

1. The methods for detection and sampling shall be provided in accordance with Articles 5(3)(i) and 17(3)(i) of Regulation (EC) No 1829/2003. A copy of the completed form for submission of the samples to the Community Reference Laboratory (CRL) and a proof of sending to the CRL (see point 2 below) shall also be provided.

[2] OJ L 287, 5.11.2003, p.1.

2. Samples of the food and feed and their control samples and information as to the place where the reference material can be accessed shall be provided in accordance with Articles 5(3)(j) and 17(3)(j) of Regulation (EC) No 1829/2003. They shall be directly sent to the CRL accompanied by the submission form and the pre-filled acknowledge of receipt below as well as by a copy of the documents describing the methods for detection and sampling.

The following instructions shall be followed in the preparation and the sending of the samples.

- The preparation of the samples and control samples shall follow the specifications laid down in: http://gmo-crl.jrc.ec.europa.eu

- The parcel shall be specified to contain "Free samples", and it shall include the list of all items and their storage instructions. In addition, it is recommended to send an advance notice of the arriving delivery (e.g. at the time of shipment) to: gmo-validation@jrc.ec.europa.eu

FORM FOR THE SUBMISSION OF SAMPLES REFERRED TO IN ARTICLES 5(3)(J) AND 17(3)(J) OF REGULATION (EC) 1829/2003 TO THE COMMUNITY REFERENCE LABORATORY, EUROPEAN COMMISSION - DG JOINT RESEARCH CENTRE

"European Commission - DG Joint Research Centre
Institute for Health and Consumer Protection
Unit "Biotechnology and GMOs"
Community Reference Laboratory
TP 331 Via Fermi 1
I-21020
Ispra (VA), ITALY"

Reference: Date:

The undersigned (name) hereby submits samples of the food/feed and their control samples referred to in Articles 5(3)(j) and 17(3)(j) of Regulation (EC) No 1829/2003 for applications for authorisation in accordance with Articles 5 and 17 of that Regulation:

1. Name of the food and/or feed
2. Trade name (where applicable)
3. Transformation event
4. Unique identifier as defined in Regulation (EC) No 65/2004
5. Place where the reference material can be accessed

An electronic version of this letter has also been sent to:

EFSA: GMO@efsa.eu.int

on: (date of sending dd/mm/yyyy)

Yours faithfully,

Signature:

Enclosures: samples, control samples

ACKNOWLEDGEMENT OF RECEIPT FOLLOWING THE SUBMISSION OF SAMPLES REFERRED TO IN ARTICLES 5(3)(J) AND 17(3)(J) OF REGULATION (EC) 1829/2003 TO THE COMMUNITY REFERENCE LABORATORY, EUROPEAN COMMISSION - DG JOINT RESEARCH CENTRE

Please write your return address below:

Reference:

I confirm that the samples and control samples, concerning the product as specified below have been received by the European Commission, Directorate-General Joint Research Centre, and will be the subject of the verification provided by Article 5 and/or 17 of Regulation (EC) No 1829/2003.

An electronic version of this letter has also been sent to GMO@efsa.eu.int

Name of the food and/or feed:

Trade name (where applicable):

Short description:

Date: (dd/mm/yyyy)

Stamp :

SIGNATURE:

PART VI: ADDITIONAL INFORMATION TO BE PROVIDED FOR GMOS AND/OR FOOD/FEED CONTAINING OR CONSISTING OF GMOS

The information required by Annex III to Directive 2001/18/EC [illegible] nce with Articles 5(5) and 17(5) of Regulation (EC) [illegible] t is not yet covered by the requirements of other parts

Stamp :

PART VII: SUMMARY OF APPLICATIONS FOR [illegible] OR DERIVED FOOD AND FEED

According to Articles 5(3)(l) and 17(3)(l) of Regulation (EC) No 1829/2003, the applications shall include a summary of the dossier in a standardised and easily comprehensible and legible form. This part specifies the format of such summary for genetically modified plants and/or derived food and feed. Depending on the scope of the application, some of the requested information may not be applicable. The summary shall not contain parts which are considered to be confidential.

Comment [divekzo2]: This is from the old document – does not correspond anymore to the structure and content of the present document

1. GENERAL INFORMATION

1.1. Details of application

a) Member State of application
b) Application number
c) Name of the product (commercial and other names)
d) Date of acknowledgement of valid application

1.2. Applicant

a) Name of applicant
b) Address of applicant
c) Name and address of the representative of the applicant established in the Community (if the applicant is not established in the Community)

1.3. Scope of the application

GM food

☐ Food containing or consisting of GM plants

☐ Food produced from GM plants or containing ingredients produced from GM plants

GM feed

☐ Feed containing or consisting of GM plants

☐ Feed produced from GM plants

GM plants for food or feed use

☐ Products other than food and feed containing of consisting of GM plants with the exception of cultivation

☐ Seeds and plant propagating material for cultivation in the EU

1.4. Is the product or the uses of the associated plant protection product(s) already authorised or subject to an other authorisation procedure within the Community?

Yes ☐	No ☐
If yes, specify	

1.5. Has the GM plant been notified under Part B of Directive 2001/18/EC?

Yes ☐	No ☐
If no, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC	

1.6. Has the GM plant or derived products been previously notified for marketing in the Community under Part C of Directive 2001/18/EC?

Yes ☐	No ☐
If yes, specify	

1.7. Has the product been notified/authorised in a third country either previously or simultaneously?

Yes ☐	No ☐
If yes, specify the third country and provide a copy of the risk assessment conclusions, the date of the authorisation and the scope.	

1.8. General description of the product

a) Name of the recipient or parental plant and the intended function of the genetic modification
b) Types of products planned to be placed on the market according to the authorisation applied for and any specific form in which the product must not be placed on the market (seeds, cut-flowers, vegetative parts, etc.) as a proposed condition of the authorisation applied for
c) Intended use of the product and types of users
d) Any specific instructions and/or recommendations for use, storage and handling, including mandatory restrictions proposed as a condition of the authorisation applied for
e) If applicable, geographical areas within the EU to which the product is intended to be confined under the terms of the authorisation applied for.
f) Any type of environment to which the product is unsuited
g) Any proposed packaging requirements
h) Any proposed labelling requirements in addition to those required by law and when necessary a proposal for specific labelling in accordance with Articles 13(2), (3) and 25(2)(c), (d) and 25(3) of Regulation (EC) No 1829/2003. In the case of GMOs, food and/or feed containing or consisting of GMOs, a proposal for labelling has to be included complying with the requirements of Annex IV, A(8) of Directive 2001/18/EC.
i) Estimated potential demand (i) In the Community (ii) In export markets for EC supplies
j) Unique identifier in accordance with Regulation (EC) No 65/2004

1.9. Measures suggested by the applicant to take in case of unintended release or misuse as well as measures for disposal and treatment

2. INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

2.1. Complete name

a) Family name
b) Genus
c) Species
d) Subspecies
e) Cultivar/breeding line or strain
f) Common name

2.2. Geographical distribution and cultivation of the plant, including the distribution in Europe

2.3. Information concerning reproduction (for environmental safety aspects)

(a) Mode(s) of reproduction
(b) Specific factors affecting reproduction
(c) Generation time

2.4. Sexual compatibility with other cultivated or wild plant species (for environmental safety aspects)

2.5. Survivability (for environmental safety aspects)

a) Ability to form structures for survival or dormancy
b) Specific factors affecting survivability

2.6. Dissemination (for environmental safety aspects)

a) Ways and extent of dissemination
b) Specific factors affecting dissemination

2.7. Geographical distribution in Europe of the sexually compatible species (for environmental safety aspects)

2.8. In the case of plant species not normally grown in the Member State(s), description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts (for environmental safety aspects)

2.9. Other potential interactions, relevant to the GM plant, of the plant with organisms in the ecosystem where it is usually grown, or used elsewhere, including information on toxic effects on humans, animals and other organisms (for environmental safety aspects)

3. MOLECULAR CHARACTERISATION

3.1. Information relating to the genetic modification

3.1.1. Description of the methods used for the genetic modification

3.1.2. Nature and source of the vector used

3.1.3 Source of donor DNA, size and intended function of each constituent fragment of the region intended for insertion

3.2. Information relating to the GM plant

3.2.1 Description of the trait(s) and characteristics which have been introduced or modified

3.2.2. Information on the sequences actually inserted or deleted

a) The copy number of all detectable inserts, both complete and partial
b) In case of deletion(s), size and function of the deleted region(s)

c) Subcellular location(s) of insert(s) (nucleus, chloroplasts, mitochondria, or maintained in a non-integrated form), and methods for its determination
d) The organisation of the inserted genetic material at each of the insertion site(s)
e) In case of modifications other than insertion or deletion, describe function of the modified genetic material before and after the modification as well as direct changes in expression of genes as a result of the modification

3.2.3. Information on the expression of the insert

a) Information on developmental expression of the insert during the life cycle of the plant
b) Parts of the plant where the insert is expressed

3.2.4. Genetic stability of the insert and phenotypic stability of the GM plant

3.2.5. Information (for environmental safety aspects) on how the GM plant differs from the recipient plant in

a) Mode(s) and/or rate of reproduction
b) Dissemination
c) Survivability
d) Other differences

3.2.6. Any change to the ability of the GM plant to transfer genetic material to other organisms (for environmental safety aspects)

a) Plant to bacteria gene transfer
b) Plant to plant gene transfer

4. COMPARATIVE ANALYSIS

4.1. Choice of the conventional counterpart and additional comparators

4.2. Experimental design and statistical analysis of data from field trials for comparative analysis

a) Description of the experimental design (Number of locations, growing seasons, geographical spread, replicates and number of commercial varieties in each location)

4.3. Selection of material and compounds for analysis

4.4. Comparative analysis of agronomic and phenotypic characteristics

4.5. Effect of processing

5. TOXICOLOGY

5.1. Toxicological testing of newly expressed proteins
5.2. Testing of new constituents other than proteins
5.3. Information on natural food and feed constituents
5.4. Testing of the whole GM food/feed

6. ALLERGENICITY

6.1. Assessment of allergenicity of the newly expressed protein
6.2. Assessment of allergenicity of the whole GM plant or crop

7. NUTRITIONAL ASSESSMENT

7.1. Nutritional assessment of GM food
7.2. Nutritional assessment of GM feed

8. EXPOSURE ASSESSMENT – ANTICIPATED INTAKE/EXTENT OF USE

9. RISK CHARACTERISATION

10. POST-MARKET MONITORING ON GM FROOD/FEED

11. ENVIRONMENTAL ASSESSMENT

11.1. Mechanism of interaction between the GM plant and target organisms

11.2. Potential changes in the interactions of the GM plant with the biotic environment resulting from the genetic modification

11.2.1. Persistence and invasiveness
11.2.2. Selective advantage or disadvantage
11.2.3. Potential for gene transfer
11.2.4. Interactions between the GM plant and target organisms

11.2.5. Interactions of the GM plant with non-target organisms
11.2.6. Effects on human health
11.2.7. Effects on animal health
11.2.8. Effects on biogeochemical processes
11.2.9. Impacts of the specific cultivation, management and harvesting techniques

11.3. Potential interactions with the abiotic environment

12. ENVIRONMENTAL MONITORING PLAN

12.1. General (risk assessment, background information)
12.2. Interplay between environmental risk assessment and monitoring
12.3. Case-specific GM plant monitoring (approach, strategy, method and analysis)
12.4. General surveillance of the impact of the GM plant (approach, strategy, method and analysis)
12.5. Reporting the results of monitoring

13. DETECTION AND EVENT-SPECIFIC IDENTIFICATION TECHNIQUES FOR THE GM PLANT

14. INFORMATION RELATING TO PREVIOUS RELEASES OF THE GM PLANT (FOR ENVIRONMENTAL SAFETY ASPECTS)

14.1. History of previous releases of the GM plant notified under Part B of the Directive 2001/18/EC and under Part B of Directive 90/220/EEC by the same notifier

a) Notification number
b) Conclusions of post-release monitoring
c) Results of the release in respect to any risk to human health and the environment (submitted to the Competent Authority according to Article 10 of Directive 2001/18/EC)

14.2. History of previous releases of the GM plant carried out outside the Community by the same notifier

a) Release country
b) Authority overseeing the release
c) Release site

d) Aim of the release
e) Duration of the release
f) Aim of post-releases monitoring
g) Duration of post-releases monitoring
h) Conclusions of post-release monitoring
i) Results of the release in respect to any risk to human health and the environment

ANNEX II

SCIENTIFIC REQUIREMENTS FOR RISK ASSESSMENT CONCERNING FOOD AND FEED SAFETY ASPECTS

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Comment [divekzo3]: This reflects the structure of the old document – does not correspond anymore to the structure and content of the present document

PART I

PRINCIPLES AND STRATEGIES FOR RISK ASSESSMENT OF GENETICALLY MODIFIED ORGANISMS

1. GENERAL PRINCIPLES GOVERNING THE COMPARATIVE APPROACH FOR THE RISK ASSESSMENT OF GM PLANTS

The risk assessment strategy for GMOs seeks to deploy appropriate methods and approaches to compare the GMO and derived products with their conventional counterpart[3]. The underlying assumption of this comparative assessment approach for GM plants is that traditionally cultivated crops have a history of safe use for the average consumer or animals. These crops can serve as a baseline for the food/feed safety assessment of GMOs. To this end the concept of substantial equivalence was developed by the OECD (OECD, 1993) and further elaborated by WHO/FAO (WHO/FAO, 2000) for the assessment of the food safety of GMOs. The risk assessment starts with the comprehensive molecular characterisation of the organisms in question followed by the comparative analysis of the relevant characteristics of the GMO and its conventional counterpart with the objectives to characterise the intended effect of the genetic modification and to identify potential unintended effects. The risk assessment then focuses on food/feed safety issues and the nutritional impact issues on any intended or unintended identified differences.

Where no conventional counterpart (s) can be identified, a comparative safety assessment cannot be made and a comprehensive safety and nutritional assessment of the GM crop derived food/feed *per se* should be carried out. This would for instance be the case where the GM food/feed is not closely related to a food/feed with a history of safe use or where a specific trait or specific traits are introduced with the intention of changing significantly the composition of the crop.

1.1. Concept of substantial equivalence or comparative safety assessment

The concept of substantial equivalence is based on the idea that an existing organism used as food/feed with a history of safe use, can serve as a comparator when assessing the safety of the GM food/feed (OECD, 1993). Application of this concept, also denoted as comparative safety assessment (Kok and Kuiper, 2003), serves the purpose of identifying similarities and differences between the GM crop-derived food/feed and the non-GM comparator (or conventional counterpart). The outcome of this comparative analysis will further structure the subsequent assessment procedure, which may include further specific safety, and nutritional testing. This approach should provide evidence on whether or not the GM crop-derived food/feed is as safe as the conventional counterpart. The first step of the approach is the comparative analysis of the agronomic, and phenotypic characteristics of the organisms in question, as well as their chemical composition. Such comparisons should be made between the GM plant and its conventional counterpart grown under the same regimes and environmental conditions.

[3] As defined in Article 2(12) of Regulation (EC) No 1829/2003, "conventional counterpart" means a similar food or feed produced without the help of genetic modification (as defined in Directive 2001/18/EC) and for which there is a well-established history of safe use.

Intended and unintended effects

Introduction of gene(s) in an organism or any other type of genetic modification may result in intended and/or unintended effects in the modified organism. The safety assessment is focussed on the identification and characterisation of such effects with respect to a possible impact on human/animal health.

Intended effects are those that are targeted to occur from the introduction of the gene(s) in question and which fulfil the original objectives of the genetic modification process. Alterations in the phenotype may be identified through a comparative analysis of growth performance, yield, disease resistance, etc. Intended alterations in the composition of a GM plant compared to its conventional counterpart, e.g. the parent, may be identified by measurements of single compounds e.g. newly expressed proteins, macro- and micro-nutrients (targeted approach). Analytical methods used shall meet specific quality and validation criteria.

Unintended effects are considered to be consistent differences between the GM plant and its conventional counterpart, which go beyond the primary intended effect(s) of a genetic modification. Unintended effect(s) could potentially be linked to genetic rearrangements or metabolic perturbations and may be predicted or explained in terms of our current knowledge of plant biology and metabolic pathway integration and interconnectivities. Unintended effects may be detected through the comparison of the agronomic and phenotypic or compositional characteristics of the GM plant with its conventional counterpart cultivated under the same conditions. A starting point in the identification of potential unintended effects is analysis of the transgene flanking regions to establish whether the insertion is likely to impact the function of any endogenous gene of known or predictable function. Furthermore, a comparative and targeted analysis should be carried out on single compounds in the GM organism and its conventional counterpart, which represent components of important metabolic pathways in the organism. The components will include macronutrients, micronutrients and secondary metabolites as well as known anti-nutrients and toxins. Statistically significant differences between GM lines and their conventional counterpart, which are not due to the intended modification, may indicate the occurrence of unintended effects, and should be assessed specifically with respect to their safety, allergenic and nutritional impact.

2. THE OBJECTIVES OF THE DIFFERENT STEPS OF THE RISK ASSESSMENT PROCEDURE FOR GM PLANTS AND DERIVED FOOD/FEED AND ISSUES TO BE CONSIDERED

2.1. Objectives of the different steps of the risk assessment

2.1.1. Hazard identification

Hazard identification may be defined as the identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods[4]. Hazard identification is the first step in risk assessment and in case of GM plants is focussed on the identification of differences between the GM plant and its conventional counterpart by using comparative analysis of compositional, agronomic and phenotypic characteristics. Identification of differences will determine the additional

[4] Codex Alimentarius Commission, Procedural Manual, 17th edition.

studies required to characterise these differences with respect to possible impact on human/animal health.

2.1.2. Hazard characterisation

Hazard characterisation is defined as "the qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents which may be present in food. For chemical agents, a dose response assessment should be performed. For biological or physical agents, a dose-response assessment should be performed if the data are obtainable"4.
This step is focussed on a possible quantification of the toxicological/nutritional potential of identified differences between the GM plant and derived food/feed and the conventional counterpart.
The hazard characterisation may be provided useful information from studies on laboratory animals and/or target animals. An appropriate test model (animal species) and suitable test material should be used in order to generate data identifying the onset of adverse effects, and a possible dose-response relationships.

2.1.3. Exposure assessment

The aim of the exposure assessment is the quantitative estimation of the likely exposure of humans and animals to GM plant and derived products (e.g. food/feed, pollen, new constituents)4. With regard to humans and animals, an exposure assessment characterises the nature and size of the populations exposed to a source and the magnitude, frequency and duration of that exposure. For exposure assessment, it is necessary that every significant source of exposure is identified. In particular it is of interest to establish whether the intake of the GM plant derived products and new constituents are expected to differ from that of the conventional product which it may replace. In this respect specific attention will be paid to that GM food/feed which is aimed at modifying nutritional quality. This category of GM food/feed may require post-market monitoring to confirm the conclusion of the exposure assessment (see annex III of this Regulation).

2.1.4. Risk characterisation

Risk characterisation is defined as the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment.

2.2. Elements to be considered for the risk assessment of GM plants

The following elements shall be considered for the risk assessment of GM plants and products:

(a) the characteristics of the donor and recipient organisms;

(b) the genetic modification and its functional consequences, intended as well as unintended;

(c) the compositional characteristics;

(d) the agronomic and phenotypic characteristics;

(e) the influence of processing on the characteristics of the food or feed;

(f) a potential for changes in dietary intake;

(g) the potential toxicity and allergenicity of gene products, plant metabolites and the whole GM plant;

(h) the potential for nutritional impact.

3. SPECIFIC CONSIDERATIONS

3.1. Insertion of marker genes and other DNA not essential to achieved the desired trait

During the process of genetic modification of plants and other organisms, marker genes are normally used to facilitate the selection and identification of genetically modified cells, containing the gene of interest inserted into the genome of the host organism, among the vast majority of untransformed cells. These marker genes shall be carefully selected as they will be subject to a safety assessment.(see also EFSA opinion on ARM genes to be published).

3.2. Risk assessment of genetically modified plants containing stacked transformation events combined by conventional crossing

The risk assessment of stacked events combined by conventional crossing shall follow the general principles provided in this annex although, on a case-by-case basis, not all components of part II of this annex may be relevant. Conversely, additional information may be required.

Where all single events have been assessed by the EFSA GMO Panel, the risk assessment of stacked events should mainly focus on issues related to a) stability of the insert(s), b) expression of the events and c) potential synergistic or antagonistic effects resulting from the combination of the events.

If each event in the highest number of stacked events has been risk assessed, the risk assessment of the stacked events might also be applicable to GM stacks containing fewer of these events. Thus a single risk assessment of such a stack could cover all combinations with fewer of these events. However, applicants need to take into account the potential impact of any reduction in the number of events involved and should provide scientific arguments to support the use of higher level stacks under these circumstances with respect to a, b and c of the paragraph above .

PART II

GUIDELINES FOR THE SUBMISSION OF INFORMATION AND STUDIES CONCERNING FOOD AND FEED SAFETY ASPECTS

1. HAZARD IDENTIFICATION AND CHARACTERISATION

1.1. Information relating to the recipient or (where appropriate) parental plants

Comprehensive information relating to the recipient or (where appropriate) the parental plants shall be provided

- to evaluate all issues of potential concern, such as the presence of natural toxins or allergens;
- to identify the need for specific analyses.

The applicant shall provide the following information:

(a) Complete name; (a) family name, (b) genus, (c) species, (d) subspecies, (e) cultivar/breeding line or strain, (f) common name;

(b) Geographical distribution and cultivation of the plant, including its distribution in Europe;

(c) Information on the recipient or parental plants relevant to their safety, including any known toxicity or allergenicity;

(d) Data on the past and present use of the recipient organism. This information shall include the history of safe use for consumption as food or feed, information on how the plant is typically cultivated, transported and stored, whether special processing is required to make the plant safe to eat, and describe the normal role of the plant in the diet (e.g. which part of the plant is used as a food/feed source, whether its consumption is important in particular subgroups of the population, what important macro- or micro-nutrients it contributes to the diet).

1.2. Molecular Characterisation

1.2.1. Information relating to the genetic modification

Sufficient information shall be provided on the genetic modification:

- to identify the DNA intended for transformation and related vector sequences potentially delivered to the recipient plant;
- to provide the necessary information for the characterisation of the DNA actually inserted in the plant.

1.2.1.1. Description of the methods used for the genetic modification

The applicant shall provide information on the following:

(a) the method of genetic transformation including relevant references;

(b) the recipient plant material;

(c) the strain of *Agrobacterium* if used during the genetic transformation process;

(d) the helper plasmids, if used during the genetic transformation process;

(e) the source of carrier DNA, if used during the genetic transformation process.

1.2.1.2. Nature and source of vector used

The applicant shall provide the following information:

(a) a physical map of the functional elements and other plasmid/vector components together with the relevant information needed for the interpretation of the molecular analyses (e.g. restriction sites, the position of primers used in PCR, location of probes used in Southern analysis). The region intended for insertion should be clearly indicated;

(b) a table identifying each component of the plasmid/vector (including the region intended for insertion), its size, its origin and its intended function.

1.2.1.3. Source of DNA used for transformation, size and intended function of each constituent fragment of the region intended for insertion

Information on the donor organism(s) and on the DNA sequence(s) intended to be inserted shall be provided in order to determine whether the nature of the donor organism(s) or the DNA sequence(s) may trigger any safety issue.

Information regarding the function of the DNA region(s) intended for insertion shall comprise the following elements:

(a) the complete sequence of the DNA intended to be inserted, including information on any deliberate alteration(s) to the corresponding sequence(s) in the donor organism(s);

(b) history of safe use of the gene product(s) arising from the regions intended for insertion;

(c) data on the possible relationship of the gene products with known toxins, anti-nutrients and allergens.

Information regarding each donor organism shall comprise:

– taxonomic classification;

– history of use regarding food and feed safety.

1.2.2. Information relating to the GM plant

1.2.2.1. General description of the trait(s) and characteristics which have been introduced or modified

Information provided under this point may be limited to a general description of the introduced trait(s) and the resulting changes to the phenotype and metabolism of the plant.

1.2.2.2. Information on the sequences actually inserted/deleted

The applicant shall provide the following information:

(a) the size and copy number of all detectable inserts, both complete and partial; this is typically determined by Southern analysis. Probe/restriction enzyme combinations used for this purpose should provide complete coverage of sequences that could be inserted into the host plant, such as any parts of the plasmid/vector or any carrier or foreign DNA remaining in the GM plant. The Southern analysis should span the entire transgenic locus(i) as well as flanking sequences and include all appropriate controls.

(b) the organisation and sequence of the inserted genetic material at each insertion site;

(c) in the case of deletion(s), size and function of the deleted region(s), whenever possible;

(d) sub-cellular location(s) of insert(s) (integrated in nuclear-, plastid-, or mitochondrial chromosomes, or maintained in a non-integrated form) and methods for its determination;

(e) sequence information for both 5' and 3' flanking regions at each insertion site, with the aim of identifying interruptions of known ORFs[5] or regulatory regions. Bioinformatic analysis should be conducted using up-to-date databases with the aim of performing both intraspecies and interspecies homology searches;

(f) ORFs created as a result of the genetic modification either at the junction sites with genomic DNA or due to internal rearrangements of the inserts. The ORFs shall be analysed between stop codons, not limiting their lengths. Bioinformatic analyses shall be conducted to investigate possible similarities with known toxins or allergens using up-to-date databases. The characteristics and versions of the databases shall be provided. Depending on the information gathered, further analyses may be needed to complete the risk assessment.

[5] **O**pen **R**eading **F**rames shall be defined as any nucleotide sequence that contains a string of codons that is uninterrupted by the presence of a stop codon in the same reading frame.

1.2.2.3. Information on the expression of the insert

Information shall be provided:

– to demonstrate whether the intended changes in expression have been achieved;

– to characterise the potential unintended expression of new ORFs identified under 1.2.2.2 as raising a safety concern.

The applicant shall provide the following information:

(a) Methods used for expression analyses together with the raw datasets;

(b) Information on developmental expression of the insert during the life cycle of the plant. The requirement for information on developmental expression shall be considered on a case-by-case basis taking into account the promoter used, the intended effect of the modification and scope of the application;

(c) Parts of the plant where the insert is expressed. Data on expression levels from those parts of the plant that are used for food/feed purposes are considered necessary in all cases. Where tissue-specific promoters have been used, information may be requested on expression of target genes in other plant parts relevant for risk assessment.

(d) Potential unintended expression of new ORFs identified under 1.2.2.2 as raising a safety concern;

(e) The range of concentrations of newly produced proteins or existing plant proteins deliberately modified in the GM food(s) and feed(s) to be placed on the market;

(f) Protein expression data should be obtained from field trials and be related to the conditions in which the crop is grown. Expression analysis could be carried out in parallel with compositional analysis as specified in Section 1.3.2.;

(g) Depending on the nature of the insert, information on the RNA levels could also be necessary;

(h) With regard to the stacking of events by conventional crossing, data shall be provided to establish that the combination of events does not raise any additional safety concerns over protein and trait expression compared with the single events. On a case-by-case basis, and where concerns arise, additional information may be necessary.

1.2.2.4. Genetic stability of the insert and phenotypic stability of the GM plant

Information shall be provided:

– to demonstrate the genetic stability of the transgenic locus(i) and the phenotypic stability and inheritance pattern(s) of the introduced trait(s);

– in case of stacked events, to establish that each of the events stacked in the plant has the same molecular properties and characteristics as in the individual events separately.

Applicants shall provide data from multiple generations or vegetative cycles for single events. The source of the material used for the analysis shall be specified. Data shall be analysed using appropriate statistical methods.

For stacked events comparisons between the insert structures in the original events and the GM stacks could be carried out using plant materials representative of those designed for commercial production. The applicant should justify the plant material used.

To assess genetic stability of the event(s), applicants shall use appropriate molecular approaches detailed in section 1.2.2.2

Conclusions of the Molecular characterisation

- The molecular characterisation shall provide data on the structure of the insert (s), expression and stability of the intended trait(s). This shall also apply to situations where events have been stacked by conventional breeding.

- It shall be specifically indicated whether the molecular characterisation of the genetic modification(s), including stacked events, raises safety concerns with regard to the potential production of proteins/products other than those intended.

- The molecular characterisation shall specifically aim to identify whether the genetic modification(s) raise(s) any issues regarding the potential for producing new toxins or allergens.

- The potential unintended changes identified in this section shall be addressed in the relevant complementary part(s) of the safety assessment.

Comment [divekzo4]: MC conclusions should be either taken out or it should be complete with all bullet points listed before

1.3. Comparative analysis

The comparative analysis of composition and agronomic and phenotypic characteristics represents, together with the molecular characterisation, the starting point to structure and conduct the risk assessment of a new GM plant and its derived products. It aims at:

– identifying similarities and differences in composition, agronomic performance and phenotypic characteristics (intended and unintended alterations) between the GM plant and its conventional counterpart;

– identifying similarities and differences in composition between the GM food/feed and its conventional counterpart.

Where no appropriate conventional counterpart can be identified, a comparative safety assessment cannot be made and thus a safety and nutritional assessment of the products produced from the GM crop shall be carried out that do not have conventional counterparts]. This would be the case where the GM food/feed is not closely related to a food/feed with a history of safe use or where a specific trait or specific traits are introduced with the intention of bringing significant changes in the composition.

1.3.1. Choice of the conventional counterpart[6] and additional comparators

In the case of vegetatively propagated crops, the conventional counterpart shall, in principle, be the non-GM isogenic variety used to generate the transgenic lines and with a history of safe use.

In the case of crops that reproduce sexually, the conventional counterpart shall have a genetic background that is as close as possible to the GM plant and with a history of safe use (since many crops used to produce food and feed are developed using back-crossing, it is important that in such cases, tests for phenotypic, agronomic and compositional similarity use a conventional counterpart with a genetic background that is as close as possible to the GM plant).

In all cases, information on the breeding scheme (pedigree) in relation to both the GM plant and the conventional counterpart and justification for the use of the selected conventional counterpart shall be provided. In addition, the applicant may consider the inclusion of a comparator having a closer genetic background to the GM plant than the conventional counterpart (such as a negative segregant).

In the case of herbicide tolerant GM plants, three test materials shall be compared: the GM plant exposed to the intended herbicide, the conventional counterpart treated with conventional herbicide management regimes and the GM plant treated with the same conventional herbicide(s). Such comparison allows the assessment of whether the expected agricultural practices influence the expression of the studied endpoints.

The appropriate conventional counterpart for stacked events shall be selected in accordance with the principles defined previously in the present section. In addition, single parental GM lines or GM lines containing previously stacked events that have been fully risk assessed may also be included as additional comparators. The applicant shall provide detailed information justifying the choice of additional comparators.

1.3.2. Experimental design and statistical analysis of data from field trials for comparative analysis

1.3.2.1. Principles of experimental design

Field trials used for production of material for the comparative analysis shall be performed in order to assess similarities and differences between three test materials: the GM crop, its conventional counterpart and commercial varieties: the objective is to determine whether the GM plant and/or derived food feed is different from its conventional counterpart and/or equivalent to commercial varieties with a history of safe use.

For each endpoint, the comparative analysis shall involve two approaches: (i) a proof of difference, to verify whether the GM plant is different from its conventional counterpart and might therefore be considered a hazard (potential risk) depending on the type of the identified

[6] As defined in Article 2(12) of Regulation (EC) No 1829/2003, "conventional counterpart" means a similar food or feed produced without the help of genetic modification and for which there is a well-established history of safe use.

difference, extent and pattern on exposure; and (ii) a proof of equivalence to verify whether the GM plant is equivalent or not to commercial varieties with a history of safe use, apart from the introduced trait(s). In testing for difference the null hypothesis is that there is no difference between the GMO and its conventional counterpart against the alternative hypothesis that a difference exists. In testing for equivalence the null hypothesis is that the difference between the GMO and the set of commercial varieties is at least as great as a specified minimum size (see section 1.3.2.3.) against the alternative hypothesis that there is no difference or a smaller difference than the specified minimum between the GMO and the commercial varieties. Rejection of the null hypothesis is required in order to conclude that the GMO and the set of commercial varieties are unambiguously equivalent for the endpoint considered. The equivalence limits used for the test of equivalence shall represent appropriately the range of natural variation expected for commercial varieties with a history of safe use. The advantage of using both a test of difference and a test of equivalence is the provision of a richer framework within which the conclusions of both types of assessment are allowed. The two approaches are complementary: statistically significant differences may point to biological changes caused by the genetic modification, but these may or may not be relevant from the viewpoint of food safety. The combination of both tests gives more information for the subsequent toxicological assessment following risk characterization of the statistical results. Further discussion of the principles of equivalence testing, with practical examples, is given in EFSA (2009).

Natural variation may have several sources: variation within a variety arises due to environmental factors and variation between varieties arises due to a combination of both genetic and environmental factors. In order to identify and estimate differences attributable only to genotypes it is essential to control environmental variability. Therefore, commercial varieties shall be included in the experimental design of the field trials and in sufficient numbers to ensure an adequate estimate of the variability required to set the equivalence limits. All test materials (GM crop, conventional counterpart, commercial varieties and any additional test material, where appropriate) shall all be randomized to plots within a single field at each site, usually in a completely randomized or randomized block experimental design. The different sites selected for the trials shall be representative of the range of receiving environments where the crop will be grown, thereby reflecting relevant meteorological, soil and agronomic conditions; the choice shall be justified explicitly. The choice of commercial varieties shall be appropriate for the chosen sites and shall be justified explicitly. Environmental variation is manifest at two scales: site-to-site and year-to-year: many years are required to capture adequately the full range of the year-to-year variation. Since the primary concern is not environmental variation *per se*, but whether potential differences between the test materials vary across environmental conditions, this experimental design defines a minimum number of sites for replication of the field trials, but allows flexibility in the number of years over which those trials are conducted. In the case that sites cover a very restricted geographic range, then replication of trials over more than one year is required.

This experimental design aims at maximizing the efficiency within available resources and providing sufficient statistical power for a wide variety of endpoints with differing variability.

1.3.2.2. Specific protocols for experimental design

Within each site the GM crop, its conventional counterpart and any additional test material, where appropriate, shall be identical for all replicates. In addition, unless there is explicit justification, at each site there shall be at least 3 appropriate commercial varieties of the crop that have a known history of safe use. The number of distinct test materials plus the number of commercial varieties is denoted by *t*. For example, if there are the GM crop, the conventional counterpart plus four commercial varieties, then t=6. The number of results to be obtained for each test material and commercial variety at each site (the replication) is denoted as *r*. The minimum requirements for replication that follow were chosen to give an appropriate number of plots on the basis both of extensive experience with field trials and levels of degrees of freedom for desired precision in simple designed experiments. The minimum level of replication shall be an integer greater or equal to $[15/(t-1)]+1$.

For example, if t=5 (the minimum value) then *r*, the replication, shall be at least 5; if t=6 then *r* shall be at least 4, etc. Notwithstanding these rules, the replication for a field trial shall never be less than r=4 at any site.

Each field trial shall be replicated at a minimum of 8 sites, chosen to be representative of the range of likely receiving environments where the crop will be grown. The trials may be conducted in a single year, or spread over multiple years. The commercial varieties may vary between sites, but unless there is explicit justification, there shall be at least 6 different commercial varieties used over the entire set of trials.

When it is desirable to assess several different GM plants for one crop species (e.g. *Zea mays*) the production of material for the comparative assessment of these different GM crops may be produced simultaneously, at the same site and within the same field trial, by the placing of the different GM plants and their appropriate conventional counterparts in the same randomized block. This is subject to two conditions which shall be strictly met: (i) each of the appropriate counterpart shall always occur together with its particular GM crop in the same block; (ii) all the different GM crops and their counterparts and all the commercial varieties used to test equivalence with those GM crops shall be fully randomized within each block.

As an example, suppose at a particular site, GM1, GM2 and GM3 denote three different GM maize crops; NIC1, NIC2 and NIC3 denote their respective conventional counterparts; and that CV1, CV2, CV3 and CV4 denote four commercial varieties to be used for the estimation of equivalence limits and equivalence testing of the three GM crops. Then, assuming that a minimum number of four randomized blocks are used, one example of the randomized allocation of plants to plots within blocks may be:

Block	Plot									
	1	2	3	4	5	6	7	8	9	10
1	GM2	CV2	CV1	GM3	NIC3	NIC1	CV3	GM1	NIC2	CV4
2	CV2	GM2	CV3	NIC3	NIC2	GM1	NIC1	CV4	CV1	GM3
3	NIC1	NIC3	GM1	CV1	GM3	NIC2	CV2	CV4	CV3	GM2
4	GM3	GM2	CV1	NIC1	CV2	NIC2	NIC3	CV3	CV4	GM1

For the purposes of statistical analysis the GM crops shall all be assessed separately. Hence, for GM1, only plots 2, 3, 6, 7, 8, 10 in block 1 enter the analysis; for GM2, only plots 1, 2, 3, 7, 9, 10 in block 1, enter the analysis, and similarly for GM3.

If the number of plots per block required for such a trial were to exceed 16, then a partially balanced incomplete block design may be used, if desired, to reduce the number of plots per block, by excluding some of the GM crops and their appropriate comparator(s) from each block. This is subject to two conditions which shall be strictly met: (i) each conventional counterpart shall always occur together with its particular GM crop in the same block; (ii) all of the commercial varieties shall appear in each of the incomplete blocks and be fully randomized with the GM crops and their conventional counterparts.

For example, a trial at a site with 5 commercial varieties, each to be tested for equivalence against 6 different GM crops, each with its conventional counterpart, would require a minimum of 4 randomized blocks each with 17 plots per block. These could be replaced, if desired, by 6 incomplete randomized blocks each of 13 plots per block, each comprising the 5 commercial varieties plus 4 of the 6 GM crops, each with its appropriate conventional counterpart. As already stated above for the case of a single GM crop assessment, when several different GM crops are used simultaneously at the same site in this way, all of the crops involved and all of the commercial varieties in the trial shall be appropriate for that site, and the requirement of a minimum of 4 replicates per site and of 8 sites in total is unchanged.

The field trials shall be adequately described, giving information on important parameters such as management of the field before sowing, date of sowing, soil type, herbicide use, climatic and other cultivation/environmental during growth and time of harvest, as well as the conditions during storage of the harvested material.

(c) Statistical analysis

Analysis of data shall be presented in a clear format, using standardised scientific units. The raw data and the programming code used for the statistical analysis shall be given in an editable form.

Data transformation may be necessary to ensure normality and to provide an appropriate scale on which statistical effects are additive. For many endpoint response variables, a logarithmic transformation may be appropriate. In such cases, any difference between the GM and any

other test material is interpreted as a ratio on the natural scale. However, for other endpoints the logarithmic transformation may not be optimal and the natural scale or another scale may be more suitable.

The analysis shall address all field trials simultaneously and shall be based on the full dataset from all sites.

The applicant shall provide for each site a table or graph, giving, for each (transformed) endpoint, the means and standard errors of means of the GM crop, its conventional counterpart, the commercial varieties and any other test material, where applicable.

The total variability in each endpoint observed in the field trials shall be estimated and partitioned using an appropriate statistical model in order to derive a confidence interval and to set equivalence limits (FDA, 2001) based on the variability observed among the commercial varieties. The confidence interval is used in the test of difference and in the test of equivalence, whereas equivalence limits are used only in the latter.

Linear mixed models are recommended for the statistical analysis of differences, the estimation of equivalence limits representing the range of background variation for commercial varieties, and equivalence testing. These mixed models shall include but not be restricted to the following factors, each with a number of levels appropriate to the chosen experimental design: (i) fixed factor(s) describing the appropriate contrasts between GM crop, comparator(s) and the group of all commercial varieties; (ii) a random factor describing the variation within the group of commercial varieties; (iii) a random factor describing variation between sites; (iv) a random factor describing variation between blocks within sites; and (v) a random factor describing the interaction between commercial varieties and sites, commonly termed the genotype x environment interaction.

Full details shall be given, for each endpoint analysed, listing: (a) the assumptions underlying the analysis, (b) full specification of the model chosen, including indication of fixed and random effects, (c) results of any test of interaction between the test materials and sites, (d) degrees of freedom, (e) the estimated variation for each fixed effect, together with the appropriate estimated residual variation with which it is compared, and appropriate variance components for the random factors, (f) any other relevant statistics. The likely impact of other growing conditions not tested in the trial shall be discussed.

The analysis proceeds by testing for difference and for equivalence applying the same mixed model described above to each endpoint. Specifically, for a particular endpoint the mean difference between the GM and its conventional counterpart is computed and a 90% confidence interval constructed around it. In addition, an upper and lower equivalence limit shall be set for each endpoint, according to the variability observed between commercial varieties. Each equivalence limit shall be calculated as the estimated mean of all commercial varieties plus or minus the product of t times the standard error of the difference between the mean GM and the mean of the commercial varieties, estimated from the mixed model above. Here, t represents the two-tailed 95^{th} percentile of the t distribution with appropriate degrees of freedom from the mixed model, calculated if necessary using the Kenward-Roger method. Upper and lower equivalence limits are assumed to be symmetrical, as expected for a normal distribution, around the point estimator of the mean of all commercial varieties.

All these calculated quantities shall be displayed, for all the endpoints simultaneously, on a single graph or a few graphs. The graph shall show the line of zero difference between the

GM and its conventional counterpart and, for each endpoint: the lower and upper equivalence limits, the mean difference between the GM and its conventional counterpart and its confidence interval (see figure 1).

When in addition to the conventional counterpart other test material(s) is used as comparator(s), the mean difference and its confidence interval for all comparators shall be displayed on one graph, referring all of these to the same zero line defined by the conventional counterpart. For example, suppose that for a particular endpoint the mean for the GM was 0.60, the mean for its conventional counterpart was 0.29, the mean of the commercial varieties was 0.50, the mean of the additional comparator was 0.46, the lower equivalence limit was 0.19, and the upper equivalence limit was 0.81. Then on the graph, all values would be referred to the baseline of 0.29, and the mean GM would be displayed as 0.31, the additional comparator as 0.17, the lower equivalence limit as -0.1, and the upper equivalence limit as 0.52. There is no need for the mean of the commercial varieties about which the equivalence limits are symmetric to be displayed, but if it were it would be displayed as 0.21. Note that the line of zero difference on the logarithmic scale corresponds to a multiplicative factor of unity on the natural scale. The horizontal axis shall be labelled with values that specify the change on the natural scale. In the case of logarithmic transformation, changes of 2x and ½x will appear equally spaced on either side of the line of zero difference.

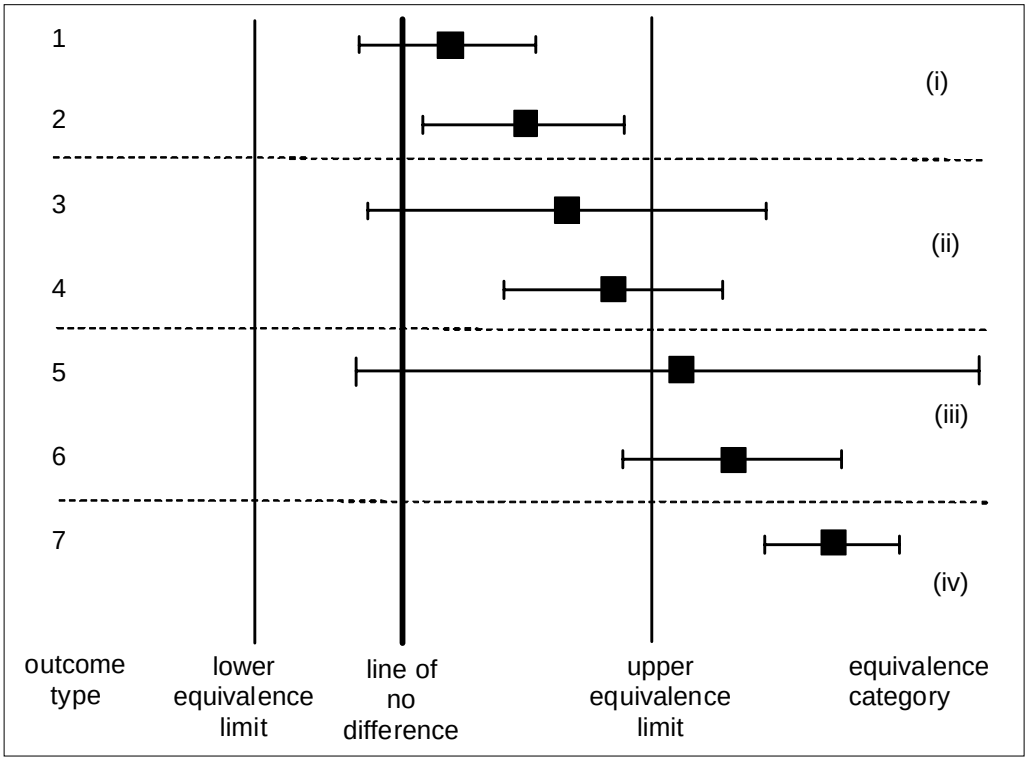

Figure 1. Simplified version of a graph for comparative assessment. The 7 outcome types possible for one single endpoint are shown. Only the upper equivalence limit is considered. Shown are: the mean of the GM crop on an appropriate scale (square), its confidence interval (bar), a vertical line indicating zero difference (for proof of difference), and vertical lines indicating equivalence limits on the same scale (for proof of equivalence). For outcome types 1, 3 and 5 the null hypothesis of no difference cannot be rejected: for outcomes 2, 4, 6 and 7 the

GM crop is different from its comparator. Regarding interpretation of equivalence, four categories (i) - (iv) are identified: in categories (i) and (iv) there is a significant equivalence and non-equivalence, respectively, in categories (ii) and (iii) equivalence and non-equivalence, respectively, are more likely than not.

Both the difference test and the equivalence test may be implemented using the well-known correspondence between hypothesis testing and the construction of confidence intervals. In the case of equivalence testing the approach used shall follow the two one-sided tests (TOST) methodology (e.g. Schuirmann, 1987) by rejecting the null hypothesis when the entire confidence interval falls between the equivalence limits. The choice of the 90% confidence interval corresponds to the customary 95% level for statistical testing of equivalence.

Since the confidence interval graph is used also for the test of difference, each difference test will have a 90% confidence level. Although 1 in 10 of these tests is expected to yield a significant result by chance alone, the applicant shall report and discuss all significant differences observed between the GMO, its conventional counterpart and, where applicable, any other test material, focussing on their biological relevance (see section 3. on Risk Characterisation).

Regarding proof of difference, each outcome from the graph shall be categorised as follows and the respective appropriate conclusion shall be drawn:

- Outcome types 1, 3 and 5: the confidence interval bar overlaps with the line of no-difference. The null hypothesis of no difference cannot be rejected and the appropriate conclusion is that there is no evidence that the GM crop and its conventional counterpart differ.

- Outcome types 2, 4, 6 and 7: the confidence interval bar does not overlap with the line of no-difference. The null hypothesis of no difference must be rejected and the appropriate conclusion is that the GM crop is different from its conventional counterpart.

Regarding proof of equivalence, each outcome from the graph shall be categorised as follows, and the respective appropriate conclusion shall be drawn:

- Outcome types 1 and 2: the confidence interval bar lies entirely between the equivalence limits. The appropriate conclusion is that the GM is equivalent to the set of commercial varieties.

- Outcome types 3 and 4: the confidence interval bar lies between the equivalence limits, but at least one of the ends of the confidence interval falls outside the equivalence limits on the graph. The appropriate conclusion is that equivalence between the GM and the set of commercial varieties is more likely than not. Further evaluation may be required.

- Outcome types 5 and 6: the confidence interval bar lies outside the equivalence limits, but the confidence interval overlaps with at least one of the equivalence limits. The appropriate conclusion is that equivalence between the GM and the set of commercial varieties is less likely than not. Further evaluation is required.

- Outcome type 7: the confidence interval bar lies entirely outside the equivalence limits. The appropriate conclusion is that there is non-equivalence between the GM and the set of commercial varieties. Further evaluation is required.

In case of significant difference and/or lack of equivalence, further analysis shall be done to assess whether there are interactions between any of the test material and site, possibly using a standard ANOVA approach. Whatever approach is adopted, details shall be given, for each endpoint analysed, listing: (a) the assumptions underlying the analysis, and, when appropriate: (b) degrees of freedom, (c) the estimated residual variation for each source of variation, and variance components, (d) any other relevant statistics. These additional analyses are intended to aid the interpretation of any significant differences found and to study potential interactions between test materials and other factors.

1.3.3. Selection of material and compounds for analysis

Analysis of the composition is crucial when comparing the GM plant and/or derived food/feed product with its conventional counterpart. The material to be used for the comparative assessment shall be selected while taking into account the uses of the GM plant and the nature of the genetic modification. Analysis shall normally be carried out on the raw agricultural commodity, as this usually represents the main point of entry of the material into the food/feed production and processing chain. Additional analysis of processed products (food/feed, food ingredients, feed materials, food/feed additives or food flavourings), may be necessary on a case-by-case basis (see also section 1.3.6.). The preparation of the tested material and the analyses shall be carried out according to appropriate quality standards.

1.3.4. Comparative analysis of composition

Besides the analysis on the level of the newly expressed proteins (see section 1.2.2.), the compositional analysis shall be carried out on an appropriate range of compounds. In each case, proximates(Proximate analysis), fibre fractions, non structural carbohydrates, key macro- and micro-nutrients, anti-nutritional compounds, natural toxins, and allergens shall be determined. Information on the key nutrients, anti-nutrients, and toxins as well as other secondary plant metabolites characteristic for specific crop plant species are provided in OECD consensus documents on compositional considerations for new plant varieties being published in the Series [?]on the Safety of Novel Foods and Feeds.

Key nutrients are those components that have a major impact on the diet, i.e. proteins, carbohydrates, lipids/fats, fibre, vitamins and minerals. The vitamins and minerals selected for analysis shall be those which are present at levels which are nutritionally significant and/or which make nutritionally significant contributions to the diet at the levels at which the plant is consumed. The specific analyses required will depend on the plant species examined, but shall include a detailed assessment appropriate to the intended effect of the genetic modification, the considered nutritional value and use of the plant. For example, a fatty acid profile shall be included for oil-rich plants (main individual saturated, mono-unsaturated and poly-unsaturated fatty acids) and an amino acid profile (individual protein amino acids and main

non-protein amino acids) for plants used as an important protein source. Measures of plant cell wall components are also required for the vegetative parts of plants used for feed purposes.

Key toxins are those compounds, inherently present in the recipient plant, whose toxic potency and levels may adversely affect human/animal health. The concentrations of such compounds shall be assessed according to plant species and the proposed use of the food/feed product (NETTOX, 1998).

Similarly, anti-nutritional compounds, such as digestive enzyme inhibitors, and already identified allergens shall be studied. Compounds other than the key nutrients, key toxins, and anti-nutrients and allergens identified by the OECD consensus documents may be included in the analyses on a case-by-case basis. The OECD consensus documents, therefore, provide a minimum list of compounds for analysis. The characteristics of the introduced trait may trigger further analysis of specific compounds including metabolites of potentially modified metabolic pathways.

For events stacked by conventional crossing the selection of the nutrients, anti-nutrients, allergens and natural toxins to be analysed and considered in the comparative assessment shall be carried out as well according to OECD consensus documents on the key components. Where appropriate, on a case-by-case basis additional compounds could be selected for analysis depending upon the introduced traits.

1.3.5. Comparative analysis of agronomic and phenotypic characteristics

Compositional analysis represents a key component of the comparative approach for identifying unintended effects during the risk assessment process. However, unintended effects may also manifest themselves through, for example, changes in susceptibility to biotic and abiotic stresses, through morphological and developmental changes or through modified responses to agronomic and crop management regimes. Therefore, the comparison between the GM plant and its conventional counterpart shall address also plant biology and agronomic traits, including common breeding parameters (e.g. yield, plant morphology, flowering time, day degrees to maturity, duration of pollen viability, response to plant pathogens and insect pests, sensitivity to abiotic stress). The protocols of these field trials shall follow the specifications made under section 1.3.2.

Where events are stacked by conventional crossing there may also be changes to agronomic and phenotypic characteristics. Possible differences in phenotypic characteristics and agronomic properties of stacks shall be assessed in field trials over at least one season. On a case-by-case basis, additional information on agronomic traits of the stacked events may be necessary from additional field trials.

1.3.6. Effects of processing

Food or feed produced from GM plants may include food ingredients, feed materials, food additives, feed additives, flavourings, and certain products used in animal nutrition. These compounds can range from single compounds to complex mixtures. Genetic modification can target metabolic pathways resulting in changes in the concentration of non-protein substances or in new metabolites (e.g. nutritionally enhanced foods, functional foods).

Processing includes, for example, making silage, oilseed extraction, refining or fermentation. Processed products may be assessed together with the assessment of the GM plant for the safety of the genetic modification, or a processed product may be assessed separately. The applicant shall provide the scientific rationale for the risk assessment of these products. On a case-by-case basis, experimental data may be required.

The applicant shall assess whether or not the processing and/or preserving technologies applied are likely to modify the characteristics of GM end products compared with their respective conventional counterpart. This would require the description of the different processing technologies in sufficient detail, paying special attention to the steps which may lead to significant changes in the product content, quality or purity. If the GM plant (or relevant parts of it) is considered safe for consumption, and there is no reason to suspect that the products would be any different from their respective conventional counterpart, further toxicological tests with the processed products are normally not requested. This is also the case when the product is assessed separately and there is no reason to suspect that it would be any different from its conventional counterpart. Depending on the product, information may be necessary on the composition, level of undesirable substances, nutritional value and metabolism, as well as on the intended use.

The applicant shall assess any potential risk associated with horizontal gene transfer from the processed product to humans, animals and micro-organisms, shall intact and functional DNA remain after the processing events. Depending on the nature of the newly expressed protein(s), it may be necessary to assess the extent to which the processing steps lead to the concentration or to the elimination, denaturation and/or degradation of these protein(s) in the final product.

1.3.7. Conclusion

The conclusion of the comparative analysis shall clearly state:

(a) whether agronomic and phenotypic characteristics of the GM plant are, except for the introduced trait(s), different to the characteristics of its conventional counterpart and/or equivalent or not to the reference varieties, taking into account natural variation;

(b) whether compositional characteristics of the GM food/feed are, except for the introduced trait(s), different to the characteristics of its conventional counterpart and/or equivalent or not to the reference varieties, taking into account natural variation;

(c) whether there are characteristics for which the GM plant or the GM food/feed are, except for the introduced trait(s), different to the characteristics of its conventional counterpart and/or not equivalent to the reference varieties, taking into account natural variation, which need further investigation.

(d) whether, in the case of events stacked by traditional crossing, there are indications of interactions between the combined events.

1.4. Toxicology

The toxicological impact of any changes resulting from the expression of introduced genes or any other type of genetic modification, e.g. gene silencing or over-expression of an endogenous gene, shall be assessed.

Toxicological assessment shall identify, adverse effects of single compounds and determine the highest dose level(s) that do not result in adverse effects. From data obtained from an appropriate animal study an acceptable daily intake (ADI) for humans may be derived by using uncertainty or safety factors that take into account differences between test animal species and humans, and inter-individual variations among humans. This internationally accepted approach is similar to that applied for testing chemicals in foods and is described in detail by FOSIE, the European project "Food Safety in Europe: Risk Assessment of Chemicals in Food and Diet" (FOSIE, 2002, EFSA opinion on benchmark approach in preparation).

Toxicological assessment shall be performed:

(a) to demonstrate that the intended effect(s) of the genetic modification has no adverse effects on human and animal health. The potential deviations from the conventional counterpart may require different toxicological approaches and varying degrees of testing.

(b) to demonstrate that unintended effect(s) of the genetic modification(s) that have been identified or assumed to have occurred based on the preceding comparative molecular, compositional or phenotypic analyses, have no adverse effects on human and animal health.

The requirements of toxicological testing shall be considered on a case-by-case basis and will be determined by the outcome of the comprehensive comparative analysis, i.e. the differences identified between the GM product and its conventional counterpart, including intended as well as unintended changes. In principle, the assessment shall consider the presence of (a) newly expressed proteins, (b) the potential presence of other new constituents and/or (c) possible changes in the level of natural constituents beyond normal variation. The specific information requirements and testing strategies are outlined in the following sections.

There may be circumstances, when the applicant considers that a decision on safety can be taken without conducting some of the tests recommended in this chapter (see below) and/or that other tests are more appropriate. In such cases the applicant shall state the reasons for not submitting the required or recommended studies or for carrying out studies other than those mentioned below.

Toxicology studies designed to evaluate risks to human and/or animal health complement each other. Most studies recommended for the assessment of the safety of the GM food are relevant for the assessment of GM feed. Testing methodologies are basically the same and the same level of data quality is required.

Besides the exposure of consumers and animals through intake of food and feed, any adverse effect(s) on individuals that could be due to their exposure to GM food/feed material as part of their professional activities e.g. farming, seed processing shall be reported by the applicant.

Appropriate studies shall be performed to further characterise these indications of potential adverse effects.

1.4.1. Testing of newly expressed proteins

All newly expressed proteins shall be evaluated. The studies required to investigate the potential toxicity of a newly expressed protein shall be selected on a case-by-case basis, depending on the knowledge available with respect to the protein's source, function/activity and history of human/animal consumption. In the case of proteins expressed in the GM plant where both the plant and the newly expressed proteins have a history of safe use[7], specific toxicity testing may not be required.

If specific testing is required, it is essential that the tested protein is equivalent to the newly expressed protein as it is expressed in the GM plant. If, due to the lack of sufficient amount of test materials (e.g. plant proteins), a protein produced by micro-organisms is used, the structural, biochemical and functional equivalence of this microbial substitute to the newly expressed plant protein shall be demonstrated. In particular, comparisons of the molecular weight, amino acid sequence, post-translational modification, immunological reactivity and, in the case of enzymes, the enzymatic activity, are needed to provide evidence for the equivalence. In case of differences between the plant expressed protein and its microbial substitute the significance of these differences for the safety studies shall be evaluated.

To demonstrate the safety of newly expressed proteins, the applicant shall provide:

(a) A molecular and biochemical characterisation of the newly expressed protein, including determination of the primary sequence, molecular weight, studies on post-translational modifications and a description of the function. In the case of newly expressed enzymes, information on the enzyme activities including the temperature and pH range for optimum activity, substrate specificity, and possible reaction products shall also be provided. Also the potential interaction with other plant constituents should be evaluated.

(b) An up-to-date search for homology to proteins known to cause adverse effects, e.g. toxic proteins. A search for homology to proteins exerting a normal metabolic or structural function may also contribute valuable information. The database(s) and the methodology used to carry out the search shall be specified.

(c) A description of the stability of the protein under processing and storage conditions and the expected treatment of the food/feed. The influences of temperature and pH changes shall normally be examined and potential modification(s) of the proteins (e.g. denaturation) and/or production of stable protein fragments generated through such treatments shall be characterised.

[7] for consumption as food (Codex Alimentarius, 2003)

(d) Data concerning the resistance of the newly expressed protein to proteolytic enzymes (e.g. pepsin), e.g. by *in vitro* investigations using appropriate and standardised tests. Stable breakdown products shall be characterised and evaluated with regard to the potential risks linked to their biological activity.

(e) Repeated dose toxicity studies using laboratory animals. Such studies are of particular importance in case the newly expressed protein is structurally and functionally related to proteins which have the potential to adversely affect human or animal health.

(f) If a repeated dose toxicity study is required, a repeated dose 28-day oral toxicity study with the newly expressed protein in rodents shall be performed (OECD, 1995). Depending on the outcome of the 28-day toxicity study, further targeted investigations may be required an analysis of immunotoxicity.

Acute toxicity testing of the newly expressed proteins of GM plants is of little additional value for the risk assessment of the repeated human and animal consumption of GM food/feed and is therefore discouraged.

When the genetic modification results in the expression of two or more proteins in the GM plant and, based on scientific knowledge, a possibility of synergistic or antagonistic interactions of safety concerns is identified, studies with combined administration of proteins shall be performed.

1.4.2. Testing of new constituents other than proteins

Identified new constituents other than proteins shall be evaluated. This may include toxicological testing on a case-by-case basis, which includes an assessment of their toxic potency and occurrence in the GM food/feed. To establish the safety of new constituents having no history of safe use[8], information analogous to that described in the "Guidance on submissions for food additive evaluations by the Scientific Committee on Foods" (SCF, 2001) and Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives[9] shall be provided. This implies the submission of information on a core set of studies and the consideration of whether or not any other type of study might also be appropriate. Normally, the core set includes information on metabolism/toxicokinetics, sub-chronic toxicity, genotoxicity, chronic toxicity, carcinogenicity and reproduction and developmental toxicity (for specific OECD guidelines for animal tests, see Table 1 of section 1.7). Genotoxicity test protocols are given in Table 2 of section 1.7.

[8] for consumption as food (Codex Alimentarius, 2003)
[9] OJ L 133, 22.5.2008, p.1.

1.4.3. Information on natural food and feed constituents

The present section shall only apply when the intended or unintended effect of the modification is that the content of such natural food and feed constituents is altered beyond the natural variation.

Natural food and feed constituents comprise a large variety of substances: macro- and micronutrients, anti-nutrients, and natural toxins as well as other secondary plant metabolites. To demonstrate the safety of the altered content of natural food and feed constituents a detailed risk assessment based on the knowledge of the physiological function and/or toxic properties of these constituents shall be submitted. The result of this assessment will determine if, and to what extent, toxicological tests shall be provided.

1.4.4 Test methods for single substances

Comment [b5]: Indicative to be brought in line with the final numbering

Internationally agreed protocols and test methods described by the OECD (OECD, 1995) or in accordance with the requirements of Article 13 of Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and establishing a European Chemicals Agency[10] shall be used for toxicity testing. Adaptations of these protocols or use of any methods that differ from such protocols shall be justified. Studies shall be carried out according to the principles of Good laboratory Practice (GLP) described in Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances[11] and be accompanied by a statement of GLP-compliance. A non-exhaustive list of validated test protocols is provided in section 1.7.

1.4.4. Testing of the whole GM food/feed

The risk assessment of the GM plant and derived food/feed is primarily based on molecular characterisation, comparative agronomic, phenotypic and comprehensive compositional analysis, and the toxicological evaluation of the identified intended and unintended effects. Under the circumstances presented hereunder, specific toxicological studies with the whole GM food/feed shall be carried out.

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1.4.4.1. 90-day feeding study in rodents

The design of the 90-day rodent feeding study for assessment of the safety and nutritional properties of the GM food and feed shall be adapted from the OECD 90-day oral rodent toxicity study, Guideline 408 (OECD, 1995). The aim of the study is to establish whether the GM food and feed is as safe and nutritious as its traditional comparator, and to demonstrate the absence of unintended changes in the GM food of toxicological concern (EFSA, , 2008).

[10] OJ L 396, 30.12.2006, p.1.
[11] OJ L 50, 20.02.2004, p.44.

Special attention must be paid to the selection of doses and the avoidance of problems of nutritional imbalance. The highest dose level should be the maximum achievable without causing nutritional imbalance. Stability of test diets and nutritional equivalence between control and test diets are other important aspects to consider. If designed and carried out properly such a study is of sufficient specificity, sensitivity and predictivity to act as a sentinel study in order to detect in a comparative manner toxicologically relevant differences as well as nutritional deficiencies/improvements that may be due to the expression of new substances, intended alterations in levels of natural compounds or unintented effects (König, A. 2004, Report of the EFSA GMO Panel working group on Animal Feeding Trials, 2008).

When such studies are conducted, the control diet(s) shall include the conventional counterpart and where appropriate additional comparator(s).

Ninety-day studies with rodents are normally of sufficient duration for the identification of general toxicological effects of compounds that would also be seen after chronic exposure. In general, long term, chronic toxicity testing of whole GM food and feed is not expected to generate information additional to what is already known from subchronic testing and from *in silico/in vitro* testing. However, the subchronic, 90-day rodent feeding study is not designed to detect effects on reproduction or development, other than effects on adult reproductive organ weights and histopathology.

The applicant shall include a 90-day feeding study in rodents in the following cases:

(a) GM plants with extensive intended genetic modifications

In case the composition of the GM plant is modified substantially, the testing program shall include at least a 90-day feeding study in rodents.

Examples are GM plants which have been extensively modified in order to cope with environmental stress conditions like drought or high salt conditions, and GM plants with quality or output traits with the purpose to improve human or animal nutrition and/or health. Through insertion of multiple genes or gene cassettes the internal metabolism in these GM plants may have changed significantly, leading to profound compositional alterations which may have an impact on the health or nutritional status of the consumer. Moreover besides intended alterations in the composition, unintended and unpredicted changes may take place, which may not always be detected by the usual compositional analyses of major macro and micro nutrients, or naturally occurring toxins, and which may impact on human/animal health or nutritional status.

(b) Indications for unintended effects and remaining uncertainties in risk assessment

If there are indications or remaining uncertainties regarding the potential occurrence of unintended effects, based on the preceding molecular, agronomical, phenotypical and/or compositional analysis, the testing program shall include at least a 90-day toxicity study in rodents.

Indications for unintended effects from molecular characterization

The molecular characterisation of the GM event shall specifically identify whether the event(s) raise(s) any issues regarding the potential for alterations in metabolic pathways

which may have a negative impact on the safety and nutritional value of the GM plant and derived food/feed like for instance the production of new toxins.

To this end information on the sequences actually inserted/deleted in the GM plant, on the organisation of the inserted genetic material at the insertion site, and sequence information on flanking regions may indicate possible interruptions of known open reading frames (ORFs) or regulatory regions and/or on the potential for producing novel chimeric proteins.

Indications for unintended effects from the comparative analysis

Each of the outcomes of the comparative analysis, as described in Section 1.3, shall be evaluated with respect to possible impact on the safety and/or nutritional properties of the GM crop, in particular those situations where differences in composition between the GM plant and its conventional counterpart have been observed and where equivalence cannot unambiguously be established. In this respect, the applicant shall assess the information on the type and function of the constituent(s), which showed a difference, its relevance for human/animal health (essential nutrient), and its toxicological profile. The outcome of this assessment shall determine whether animal feeding trials with the whole food/feed shall be performed.

(c) Stacked events

In the case of GM plants obtained through conventional breeding of parental GM lines (stacked events), possible interactions between the expressed proteins, new metabolites and original plant constituents should be assessed. If the potential for adverse interactions is identified, feeding trials with the GM food/feed are required. Indications for possible interactions may be provided by (i) the outcome of the molecular analysis, (ii) the knowledge of the mode of action of the newly expressed proteins, (iii) information on the response to combined administration of proteins to target organisms and (iv) information on the effects on the activity of target enzymes.

1.4.4.2. Animal studies with respect to reproductive and developmental toxicity testing

The subchronic 90-day rodent feeding study is not designed to detect effects on reproduction or development, other than effects on adult reproductive organ weights and histopathology. Thus, in some cases, testing of the whole food and feed beyond a 90-day rodent feeding study may be needed.

In cases of indications from the subchronic study (e.g. functional, and/or histological modifications of nervous, endocrine, reproductive or immunological tissues/organs) or other information on whole GM plant derived food and feed suggest the potential for reproductive, developmental or chronic toxicity, the performance of such testing shall be considered. OECD protocols for reproductive, developmental and chronic toxicity testing (see Table 1 of section 1.7) can be adapted for the testing of whole GM plant derived food and feed.

1.4.4.3. Other animal studies to examine the safety and the characteristics of GM food/feed (see also sections 1.6.1. and 1.6.2.)

Supplemental information to 90-day feeding studies in rodents on the possible influence of intended and unintended effects may be obtained from comparative growth studies conducted with young rapidly growing animal species (broiler chicks as animal model for non-ruminants; lambs for ruminants; or other rapidly growing species). Because of their rapid weight gain such animals are sensitive to the presence of certain undesirable substances in their feed (ILSI, 2003). Studies of this type are, however, limited to those materials suitable for inclusion in their diets and which can be nutritionally matched to a suitable control diet.

Livestock feeding studies with target animal species shall be considered, on a case-by-case basis and be hypothesis driven. The focus shall be on the safety of newly expressed constituents, on the identification and characterisation of unintended effects, and on the nutritional impact of any intentional, substantial, compositional modifications of the GM plant (see also section 1.6 and EFSA, 2008)

1.4.4.4. Interpretation of relevance of animal studies

Any effects observed in the animal trials shall be evaluated by experts in order to identify relevant effects. The experts' experience will facilitate the interpretation of the observed effects with respect to potential consequences for the health of humans and animals and thus assess their relevance for the safety of food and feed derived from the GM product. This interpretation may be supported by additional information and considerations, including the examples discussed below.

Information on the background variability in a given parameter may be obtained from data from other animals of the same species/strain tested in the same or other experiments, or from internationally harmonised databases. Even if the change observed in a certain parameter falls within this background range of variability, further considerations are required with respect to a dose-response relationship, gender specificity, and linkage with other changes, to identify any plausible cause.

Dose-response relationships in parameters that have been changed (i.e. commensurate increases in changes at increased doses) provide a strong indication for an effect of the tested compound. Conversely, the absence of such a dose-response relationship may indicate that the effect is accidental or spurious.

In tests where animals of both genders are used, changes occurring in animals of one gender only may still be relevant indicators of an effect, depending on the parameter being changed and the mechanism by which the change may have been caused. For example, animals of one gender may be more or even specifically prone to changes caused by a certain compound than animals of the other gender, such as in the case of endocrine effects.

Possible inter-relationships between observed changes in single parameters may strengthen the notion that an effect has occurred. For example, liver damage, which may be observed in the liver itself as a change in histopathology, gross pathology, and organ weights, may also be evident from the changed levels of certain liver-derived compounds, such as enzymes, bilirubin, etc., in serum.

With regard to the potential cause for an observed effect, the likelihood of causality shall be taken into account, not only for the test compound, but also for other factors that may have also influenced the outcomes (e.g. body weight decrease due to reduced intake of less palatable diet). Supportive data for a hypothesis of causality between the test compound and effects in test animals may include, for example, predictive data for plausible effects from *in vitro* and *in silico* experiments and dose-response relationships observed in the animal test.

1.4.5. Conclusion of the toxicological assessment

The conclusion of the toxicological assessment shall indicate whether:

(a) the information provided and the testing strategy used to assess the intended and/or unintended changes of the GM food/feed are considered adequate.

(b) Potential adverse effects identified in other parts of the safety assessment have been confirmed or discarded;

(c) the available information on the newly expressed protein(s) and other new constituents resulting from the genetic modification gives indications of potential adverse effects in particular, whether and at which dose levels adverse effects were identified in specific studies;

(d) the information on natural constituents of which the levels are different from those in its conventional counterpart provides indications of potential adverse effects, in particular, whether and at which dose levels adverse effects were identified in specific studies;

(e) adverse effects have been identified in the studies made on the whole GM food/feed and at which dose levels;

The results of the toxicological characterisation shall be evaluated in the light of anticipated intake of the GM food/feed.

1.5. Allergenicity

Allergy is an adverse reaction which, by definition, is immune-mediated and particularly involves IgE antibodies. It affects individuals who have a genetic predisposition (i.e. atopic individuals). This section mainly deals with the risks to those individuals when exposed to foods (and pollen) derived from GMOs with regard to sensitisation or to elicitation of an allergic reaction.

The majority of the constituents that are responsible for allergenicity of foods as well as of pollens are proteins. Some protein breakdown products, i.e. peptide fragments, may conserve part of the allergenicity of the native protein and thus can also be considered as allergens. The specific allergy risk of GMOs is associated i) with exposure to newly expressed protein(s) that can be present in edible parts of the plants or in the pollen. This point is related to the biological source of the transgene and ii) with alterations to the allergenicity of the whole plant and derived products e.g. due to over-expression of natural endogenous allergens as an unintended effect of the genetic modification. This point is related to the biology of the host itself.

1.5.1. Assessment of allergenicity of the newly expressed protein

Allergenicity is not an intrinsic, fully predictable property of a given protein but is a biological activity requiring an interaction with individuals with a pre-disposed genetic background. Allergenicity therefore depends upon the genetic diversity and variability in atopic humans. Given this lack of complete predictability it is necessary to obtain, from several steps in the risk assessment process, a cumulative body of evidence which minimises any uncertainty with regard to the protein(s) in question.

In line with the recommendations of the Codex ad hoc Intergovernmental Task Force on Foods Derived from Biotechnology (Codex Alimentarius, 2003), an integrated, stepwise, case-by-case approach shall be used in the assessment of possible allergenicity of newly expressed proteins.

The source of the transgene shall be considered carefully to make clear whether or not it encodes an allergen. Information shall specify at what stage of the development of the plant and in what organs of the plant the allergenic protein may be expressed. When the introduced genetic material is obtained from wheat, rye, barley, oats or related cereal grains, the applicant shall assess the newly expressed proteins for a possible role in the elicitation of gluten-sensitive enteropathy or other enteropathies which are not IgE mediated.

Where events have been stacked by conventional crossing, the applicant shall provide an assessment of any potential for increased allergenicity to humans and animals on a case-by-case approach. These potential effects may arise from additive, synergistic or antagonistic effects of the gene products.

In every case the first step in the assessment shall be a search for sequence homologies and/or structural similarities between the expressed protein and known allergens using various algorithms to identify overall structural similarities. Strategies for identification of sequences that may correspond to potential linear IgE binding epitopes shall be conducted by a search for identical peptidic fragments in the amino acid sequence of the test protein to peptidic fragments of known allergens. The number of contiguous identical amino acid residues used in the search setting shall be based on a scientifically justified rationale in order to minimise the potential for false negative or false positive results[12]. The use of different homology searching strategies based on the sequences available in relevant databases may identify several scenarios. These include a high degree of homology, with or without conservation of the allergenicity, or a low degree of homology with conservation of allergenicity (Mills et al., 2003).

The second step for assessing the potential that exposure to the newly expressed proteins might elicit an allergic reaction in individuals already sensitised to cross reactive proteins, is based on in vitro tests that measure the capacity of specific IgE from serum of allergic patients to bind the test protein(s).

[12] It is recognised that the 2001 WHO/FAO consultation suggested moving from 8 to 6 identical amino acid segment searches. The smaller the peptide sequence used in the stepwise comparison, the greater the likelihood of identifying false positives. Conversely, the larger the peptide sequence used the greater the likelihood of false negatives, thereby reducing the utility of the comparison.

If the source of the introduced gene is considered allergenic, but no sequence homology of the newly expressed protein to a known allergen is demonstrated, specific serum screening of the expressed protein shall then be undertaken with appropriate sera from patients allergic to the source material using relevant validated immunochemical tests. If a positive IgE response occur, the newly expressed protein may then be considered very likely to be allergenic. If no IgE binding is observed, the newly expressed protein shall undergo pepsin resistance tests and additional testing (see third step below).

If the source is not known to be allergenic but if there are consistent indications of sequence homology to a known allergen, the specific serum screening shall be conducted with sera from patients sensitised to this allergen in order to confirm or exclude an IgE cross-reactivity between the newly expressed protein and this allergen. The results of the screening are interpreted as in the previous paragraph.

As a third step, the applicant shall consider the following additional tests:

(a) Pepsin resistance test. Stability to digestion by proteolytic enzymes has long been considered a characteristic of allergenic proteins. Although it has now been established that no absolute correlation exists (Fu et al., 2002), resistance of proteins to pepsin digestion is still proposed as an additional criterion to be considered in an overall risk assessment. In the case that a rapid and extensive degradation of a protein in the presence of pepsin is not confirmed under appropriate conditions, further analysis shall be conducted to determine the likelihood of the newly expressed protein being allergenic. It could also be useful to compare intact, pepsin digested and heat denatured proteins for IgE binding.

(b) Targeted serum screening. As proposed in the FAO/WHO expert consultation (WHO/FAO, 2001) targeted serum screening aims to assess the capacity of the newly expressed protein to bind to IgE in sera of individuals with clinically-validated allergic responses to categories of foods broadly related to the gene source.

Specific (as well as targeted) serum screening requires a sufficient number and sufficient volumes of relevant sera from allergic humans. These might not always be available either because the allergy is not frequent or for other reasons. The use of existing models and the development and validation of new alternative models that may substitute for and/or complement the use of human biological material for evidence of cross reactivity and elicitation potency shall be considered. These approaches would include the search for T-cell epitopes, structural motifs, in vitro cell based assays using animal or humanised-animal immune cells, etc. They also include appropriate in vivo animal models.

(c) Animal models are certainly also useful tools for the assessment of the sensitising potential of newly expressed proteins, i.e. their capacity to induce an allergic immune response with the synthesis of specific IgE in individuals that have never been exposed to those proteins nor to proteins that cross react with them.

1.5.2. Assessment of allergenicity of the whole GM plant or crop

When the host of the introduced gene is known to be allergenic, the applicant shall test any potential change in the allergenicity of the whole GM food by comparison of the allergen repertoire with that of the conventional counterpart.

These approaches shall be applied on a case-by-case basis depending on the available information on the allergenic potential of the source and/or the host.

To this purpose, the applicant may use modern analytical tools including profiling techniques. These tools although still in development, may provide, in association with human and animal serum or cell-based assays, valuable additional information.

The integrated process applies to the assessment of the allergenicity of the edible components and the pollen of GM crops (i.e. covers both food and respiratory allergy risk).

In addition, the applicant shall provide, where available, information on the prevalence of occupational allergy in workers or in farmers who have significant exposure to GM plant and crops, or to the airborne allergens they may contain.

Regarding animal health, allergenicity is not an issue that needs to be specifically addressed.

1.5.3. Conclusion of the allergenicity assessment

The conclusion of the allergenicity assessment shall clearly indicate:

– whether the novel protein(s) is likely to be allergenic;

– whether the GM food is likely to be more allergenic than the conventional counterpart.

When there is a likelihood of allergenicity in one of the above mentioned cases, the GM food shall be further characterised in the light of anticipated intake of the GM food and appropriate conditions for placing on the market, including labelling, shall be proposed.

1.6. Nutritional assessment

Nutritional evaluation shall be provided:

(a) to demonstrate that introduction of the GM food/feed into the market is not nutritionally disadvantageous to humans and animals, respectively. This evaluation shall include the relevance for the nutrition of newly expressed proteins, other new constituents, and changes in the levels of natural constituents in the GM food/feed, as well as potential alterations in the total diet of the consumer;

(b) to demonstrate that unintended effects of the genetic modification that were identified or that may be assumed to have occurred based on the preceding molecular, compositional or phenotypic analyses (see sections 1.2. and 1.3.), have not adversely affected the nutritional value of the GM food/feed;

For stacked events combined by conventional breeding, an assessment of the potential changes in nutritional value that might arise from synergistic or antagonistic effects of the gene products including compositional changes shall be provided. This may be particularly relevant where the combined expression of the newly introduced genes has unexpected effects on biochemical pathways.

Compositional analysis is the starting point and cornerstone for the nutritional assessment of food and feed material. The applicant shall provide analyses of all the key components outlined in the consensus documents prepared by OECD (OECD a) for the respective food/feed plants. Analyses of additional components shall be determined on a case-by-case basis and depend on the introduced trait(s).

The nutritional assessment of GM food/feed shall consider:

(a) the composition of the GM food/feed with regard to the levels of nutrients and anti-nutrients (see compositional studies as described in section 1.3.4);

(b) the bioavailability and biological efficacy of nutrients in the food/feed taking into account the potential influences of transport, storage and expected treatment of the foods;

(c) the anticipated dietary intake of the food/feed (see section 2) and resulting nutritional impact.

When the comparative analysis has identified compositional characteristics of the GM food/feed that are different and/or not equivalent to the characteristics of its conventional counterpart, their nutritional relevance shall be assessed on the basis of current scientific knowledge. If this assessment does conclude on the nutritional equivalence between the GM food/feed and its conventional counterpart, no further studies are recommended. By contrast if, on the basis of the assessment of the information obtained from the comparative analysis, it is not possible to conclude to nutritional equivalence, further studies shall be carried out.

Information on nutritional aspects is available in case a subchronic (90-day) feeding study in rodents using the whole GM food/feed is provided. This study, in addition to toxicological aspects, also provides valuable information on nutritional aspects since it starts with juvenile animals in rapid growth phase that are sensitive to effects on weight gain.

1.6.1. Specific considerations for the nutritional assessment of GM food

The applicant shall determine the necessity and design of nutritional studies on the basis of the introduced trait(s), the outcome of the comparative analysis, and of the subchronic (90-day) feeding study, where available. Supplemental information regarding the nutritional value may be obtained from comparative growth performance studies conducted with other animal species, e.g. broiler chickens (see sections 1.4.4 and 1.6.2), addressing the nutritional assessment of GM feed (ILSI 2003, ILSI 2007). When nutritional studies are conducted, the control diet(s) shall include the conventional counterpart and where appropriate additional comparator(s).

GM foods modified to provide additional health benefits to the consumer as compared to conventional foods, may benefit specific populations or sub-populations while others may be

at risk from the same food. In cases where an altered bioavailability needs to be established and may raise concern for sub-population(s), the level of the nutrient in the food shall be determined, taking into account all the different forms of the compound. The methods to test for bioavailability shall be selected on a case-by-case basis depending on the nutrient or other constituent, the food containing these constituents, as well as the health, nutritional status and dietary practices of the specific population(s) anticipated to consume the food.

1.6.2. Specific considerations for the nutritional assessment of GM feed

The applicant shall determine the necessity and design of further nutritional studies on the basis of the introduced trait(s), the outcome of the comparative analysis, and the subchronic (90-day) feeding study, where available.

In the case of GM feed with improved nutritional characteristics, livestock feeding studies with target animal species shall be conducted on a case-by-case basis to assess the impact on the feed. In the case of GM crops modified for improved content and bioavailability of nutrients, livestock studies with target species shall be conducted to determine the bioavailability of individual nutrients in the GM crop compared to its conventional counterpart and a range of conventional varieties. In the case of GM crops specifically modified with traits to enhance animal performance through increased nutrient density (e.g. increased oil content) or an enhanced level of a specific nutrient (e.g. an essential amino acid or a vitamin), an appropriate control diet using its conventional counterpart shall be formulated by supplementing it with the specific nutrient to the extent of the change effected in the GM crop. Regarding co- products (e.g. oilseeds meals), from which the ingredient targeted by the genetic modification has been extracted, these may be compared with co-products derived from the conventional counterpart and other conventional varieties as additional comparators (on the basis that all these products are low in the component targeted by the genetic modification).

Target animal feeding studies shall span either the growing and/or finishing period to slaughter for chickens, pigs, and cattle for fattening or a major part of a lactation cycle for dairy cows, or laying cycle for laying hens or quails. For feedstuffs intended only for aquaculture, growth studies with aquatic species such as carp or other typical herbivores are preferable.

Various experimental designs might be necessary to demonstrate that the nutritionally improved GM plant fulfils the expected nutritional value as discussed in the Report of the EFSA GMO Panel Working Group on Animal Feeding Trials (EFSA, 2008). The exact experimental design and statistical approaches of feeding experiments in food producing animals to test the nutritional value of GM plants modified for enhanced nutritional characteristics will depend on a number of factors and include choice of animal species, type of plant trait(s) studied and the size of the expected effect. The experimental diets need to be formulated in such a way that the key measured endpoints are responsive to a difference in the quantity and/or availability of the nutrient in question. Endpoint measurements will vary with the target species used in the study, but will include feed intake, body weight, animal performance and bioavailability of nutrients (see Flachowsky and Böhme 2005, EFSA, 2008, ILSI, 2007).

1.6.3. Conclusion of the nutritional assessment

The conclusion of the nutritional assessment of GM food/feed shall indicate:

– whether the GM food/feed is nutritionally equivalent to its conventional counterpart, taking natural variations into account.

The results of the nutritional assessment shall be evaluated in the light of anticipated intake of the GM food/feed.

1.7. Standardised guidelines for toxicity tests

The applicant shall use for toxicity testing internationally agreed protocols and test methods described by the OECD (OECD, 1995) or in accordance with the requirements of Article 13 of Regulation (EC) No 1907/2006. Use of any methods that differ from such protocols shall be justified. Studies shall be carried out according to the principles of Good laboratory Practice (GLP) described in Council Directive 2004/10/EC and be accompanied by a statement of GLP-compliance. A non-exhaustive list of validated test protocols which, where necessary, shall be used in a possibly adapted form for GMO toxicological testing is provided in tables 1 and 2 below.

It is emphasized that not all of these protocols have to be applied for toxicological testing of GM plant derived food/feed. Application of test protocols depends on the type of GM plant derived food/feed, type of the genetic modification and resulting intended and unintended alterations, intended use and exposure/intake, and the available knowledge. Some of the tests are relevant for the assessment of risks at the worplace.

Table 1: Non-exhaustive list of validated test protocols for chemicals which may be used in a possibly adapted form for GMO toxicological testing (OECD, 1995) (modified from FOSIE, 2002).

No.	Subject	Note
407	Repeated Dose 28-day Oral Toxicity Study in Rodents	Updated guideline, adopted 3 October 2008
408	Repeated Dose 90-Day Oral Toxicity Study in Rodents	Updated guideline, adopted 21 September 1998
410	Repeated Dose Dermal Toxicity:21/28-Day	Original guideline, adopted 12 May 1981
415	One-Generation Reproduction Toxicity	Original guideline, adopted 26 May 1983
416	Two-Generation Reproduction Toxicity Study	Updated guideline, adopted 22 January 2001
417	Toxicokinetics	Original guideline, adopted 4 April 1984
421	Reproduction/Developmental Toxicity Screening Test	Original guideline, adopted 27 July 1995

424	Neurotoxicity Study in Rodents	Original guideline, adopted 21 July 1997
451	Carcinogenicity Studies	Original guideline, adopted 12 May 1981
452	Chronic Toxicity Studies	Original guideline, adopted 12 May 1981
453	Combined Chronic Toxicity/Carcinogenicity Studies	Original guideline, adopted 12 May 1981
402	Acute Dermal Toxicity	Updated Guideline, adopted 24 February 1987
406	Skin Sensitisation	Updated guideline, adopted 17 July 1992

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1329 Table 2: Genotoxicity tests as described by OECD guidelines (OECD, 1995) (Modified from
1330 the Report of the EFSA GMO Panel working group on Animal Feeding Trials, EFSA, 2008):

No.	Title
OECD 471	Bacterial reverse mutation test
OECD 473	In vitro mammalian chromosome aberration test
OECD 474	Mammalian erythrocyte micronucleus test
OECD 475	Mammalian bone marrow chromosome aberration test
OECD 476	In vitro mammalian cell gene mutation test
OECD 479	In vitro sister chromatid exchange (SCE) assay in mammalian cells
OECD 480	Saccharomyces cerevisiae, gene mutation assay
OECD 481	Saccharomyces cerevisiae, mitotic recombination assay
OECD 482	DNA damage and repair, unscheduled DNA synthesis in mammalian cells in vitro
OECD 487	Draft guideline on: In vitro mammalian cell micronucleus test

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2. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

An estimate of the expected intake is an essential element in the risk assessment of GM food/feed and also required for the nutritional evaluation. Information shall be provided on the intended function, the dietary role, and the expected level of use of the GM plant-derived food/feed product(s).

On the basis of representative consumption data for products derived from the respective conventional plants, the anticipated average and maximum intake of the GM food/feed shall be estimated. Probabilistic methods may be useful to determine ranges of plausible values rather than single values or point estimates. If possible, particular sections of the population with an expected high exposure shall be identified and shall be considered within the risk assessment. Any assumptions made in the exposure assessment shall be described. Recent developments in methodologies and appropriate consumption data shall be used. Data on import and production quantities may provide additional information for the intake assessment.

The concentrations of the newly expressed proteins, other new constituents and natural constituents, of which the levels have been altered as a result of the genetic modification (e.g. due to changes in metabolic pathways) in those parts of the GM plant intended for food or feed use shall be determined by appropriate methods. Expected intake of these constituents shall be estimated taking into account the influences of processing, storage and expected treatment of the food/feed in question, e.g. potential accumulation or reduction. In cases where the genetic modification has resulted in an altered level of a natural constituent, or if a new constituent occurs naturally in other food/feed products, the anticipated change in total intake of this constituent shall be assessed considering realistic as well as worst case intake scenarios.

Information on known or anticipated human/animal intake of analogous GM food/feed and on other routes of exposure to the respective new and natural constituents, including amount, frequency and other factors influencing exposure, shall be provided.

3. RISK CHARACTERISATION

3.1. Introduction

Risk characterisation of GM plants and derived foods/feed is based on data from hazard identification, hazard characterisation, and on exposure/intake data. A comprehensive risk characterisation shall be carried out considering all the available evidence from several analysis including molecular analysis, phenotypic, agronomical and compositional analysis, toxicity and allergenicity testing. The risk characterisation may give indications for the requirement of specific activities for post-market monitoring of GM food/feed.

The risk characterisation shall demonstrate whether the hazard identification and subsequent characterisation is complete or not. It is essentially an iterative process. Integration and evaluation of data from hazard characterisation and exposure assessment allow to determine whether an appropriate risk characterisation may be finalised, or whether further data shall be generated in order to complete the risk characterisation. For instance if an increased intake of a GM derived food/feed by humans or animals may be expected, further data on toxicity at extended dose ranges may have to be generated. The quality of existing data and information

shall be discussed. It shall be clear from the discussion how this body of information has been taken into account when the final risk characterisation is determined.

Any uncertainties inherent in the different stages of the risk assessment shall be highlighted and quantified as much as possible. Distinction shall be made between uncertainties that reflect natural variations in ecological and biological parameters (including variations in susceptibility in populations), and possible differences in responses between species.

An estimation of uncertainties in experimental data may be derived from proper statistical analysis. While it may be more difficult to quantify uncertainties in assumptions (e.g. extrapolation of data from animals to humans), but those should be highlighted.

Depending on the issue to be addressed and the available data, risk characterisation may be qualitative and, if possible, quantitative. The conditions for the estimated risk, and associated uncertainties, should be as precise as possible. For instance, expressions like 'no/negligible/acceptable/significant risk' must, in principle, be accompanied by further numerical quantification in terms of probability of exposure and/or occurrence of adverse effects.

3.2. Issues to be considered for risk characterisation

Risk assessment of GM plants shall be carried out in an integrative manner and on a case-by-case basis depending on the type of genetic modification, taking into consideration cultivation practice of the GM plant and use of the derived foods/feed for human/animal consumption. To this aim, the applicant shall take into account the different issues considered in hazard identification and characterisation and exposure steps. The outcomes of these issues have to be considered together in the risk characterisation step. The list of issues provided in this section is by no means exhaustive.

3.2.1. Molecular characterisation

Evaluation of the characteristics and previous use of the donor and the recipient organism is a key element to identify the need for specific analyses e.g. occurrence of specific toxins, or allergens in the unmodified recipient plant which may be unintentionally increased as result of the genetic modification.

Transformation protocols, molecular characterisation strategies and the specificity and sensitivity of the methods used shall be discussed in relation to the intentional and possibly unintentional insertion and expression of gene sequences.

Where flanking sequence analysis has identified chimeric ORFs, it should be demonstrated how approaches like bioinformatic analysis, compositional/agronomical analysis and possibly animal feeding trials with the whole GM food/feed contribute to the safety impact. The value of the results obtained should be evaluated in the light of the available knowledge on the structure and function of genomic databases of the crop species in question.

In cases where traits are stacked through the interbreeding of existing approved GM lines, additional risks which may arise from the combined effects of the stacked genes *e.g.* on biochemical pathways should be evaluated.

3.2.2. Comparative analysis

An important issue to be evaluated is whether the comparative analysis between the GM plant and its conventional counterpart with respect to agronomic, morphological and compositional characteristics has been carried out appropriately according to current guidelines. and what evidence is available that the conventional crop can be taken as a reference for safe environmental cultivation and human/animal use. Protocols for and performance of field trials should be evaluated, and the data generated assessed to confirm they are representative for the proposed cultivation conditions of the GM plant.

The goal of the comparative safety assessment is to identify possible differences between the GM plant and its conventional counterpart. The choice of the conventional counterpart is key and its selection shall be justified in particular with respect to its history of safe use. The risk characterisation shall concentrate on statistically significant differences in the composition of the GM plant compared to its conventional counterpart and whether these differences are likely to have an impact on food and feed safety or nutrition. Moreover, an analysis shall be made of the uncertainties associated with the comparative analysis.

The goal of the comparative safety assessment is to identify possible differences between the GM plant and its conventional comparator. The choice of the comparator is key and its use should be justified. The risk characterisation should concentrate on statistically significant differences in the composition of the GM plant compared to its non-GM comparator and whether these differences are likely to have an impact on environment, and/or food and feed safety or nutrition. Moreover, an analysis should be made of the uncertainties associated with the comparative analysis.

The unintended effects of the genetic modification are expected to result in differences or lack of equivalence between the GM plant and its conventional counterpart that may be observed in field trials representative of the range of receiving environmental conditions. A difference or lack of equivalence that is consistently observed under all or most conditions can be an indicator of such unintended effects. Whilst sporadic differences or lack of may reflect the inherent variability known to occur in the GM plant and the conventional counterpart or, for specific endpoints be due to chance alone, they may also highlight a strong influence of special environmental conditions on the expression of a difference.

If statistically significant differences and/or non-equivalences are observed, using the methodology as described under section 1.3.2, the following background data may be considered to put them into context with respect to their potential relevance for the human/animal health.

3.2.2.1. Data on variability inherent to the plant, the plant variety and the environment.

Commonly considered is the range of levels observed for the compounds known to occur in the conventional counterpart and in commercial varieties. This variability may be caused by differences that are genotype-dependent, environmentally dependent, or caused by genotype x environment interactions. In addition, the range of levels observed in a broad spectrum of food and feed representative for the human and animal diet may be taken into account. The rationale for considering this variability in the safety assessment is that it reflects the levels of the specific compound to which consumers may be exposed.

3.2.2.2. Information of variation of constituents from databases.

The databases used for comparison shall be specified and adequately assessed for their quality (e.g. type of material analyzed, analytical method used, sampling methods and strategies). No formal statistical analysis shall be carried out, but ranges as well as mean values shall be reported and considered. These data would indicate whether the GM lines fall within the natural range in component concentrations found in non-GM comparators. The influence of environmental factors on phenotypical and compositional characteristics of plants shall be taken into account when comparing analytical data from field studies with literature data.

Based upon one or more of the considerations above, the applicant shall establish whether the differences and/or lack of equivalence observed are to be considered relevant for further consideration in the risk assessment process or if the difference and/or lack of equivalence does not raise safety concerns.

3.2.3. Food/feed safety in relation to intake

The data generated to estimate possible risks to human/animal health associated with the consumption of GM plant derived foods/feed shall be evaluated with respect to the expression of new proteins/metabolites as well as significantly altered levels of original plant proteins/metabolites in GM foods/feed. If single constituents and/or whole GM food/feed were found to induce adverse effects in specific studies, dose response relationships, threshold levels, delayed onset of adverse effects, risks for certain groups in the population, use of uncertainly factors in extrapolation of animal data to humans shall be presented.

The relevance of short-term toxicity data in order to predict possible long-term adverse effects of newly expressed proteins/new metabolites in the GM food/feed shall be discussed as well as the absence of specific data (e.g. on reproductive and developmental toxicity) if applicable. Moreover when feeding trials with whole GM food/feed have been carried out, the relevance of their outcome shall be evaluated with respect to experimental limitations (e.g. dose range, dietary composition, confounding factors).

Data on the characteristics of the new compounds present in the GM plants including potential biological effects in humans and animals shall be considered. If the compounds have known adverse health effects and maximum levels for the presence of these compounds in the plant or derived products were laid down in specific legislation, these maximum levels shall be taken into account. Otherwise, reference values for acceptable or tolerable levels of intake, such as the Acceptable Daily Intake (ADI) or Tolerable Upper Intake Level (UL), shall be considered in relation to the anticipated intake. In cases where the compound has been safely consumed in food, the intake levels of consumers from a conventional diet can implicitly be considered as safe.

Information on the effects of processing on the new compounds shall be evaluated. Potential accumulation / depletion in food / feed products entering the human / animal diet shall be considered. The relevance of differences resulting from chemical reactions known to occur under processing conditions shall be evaluated.

In cases where more complex genetic modifications are produced, e.g. via transfer of multiple genes in a single construct, re-transformation of pre-existing GM lines, and trait stacking through conventional breeding of GM parents, strategies for the assessment of any risk(s) associated with possible interactions between the newly expressed proteins, new metabolites

and original plant constituents shall be discussed. A holistic approach for the assessment shall be demonstrated considering all available information on e.g. the mode of action of the newly expressed proteins, the molecular and compositional/agronomical characteristics of the GM plant, and where applicable on the outcome of animal toxicity studies and feeding trials. Where animal feeding trials are not performed an explanation shall be provided as to why these were not considered necessary.

Data provided to assess the allergenic potential of newly expressed proteins in GM plants shall be evaluated with respect to introduction of new allergenic proteins into the food/feed plants a possible provocation of allergic reactions of susceptible individuals, as well as information to demonstrate that the genetic modification process does not cause unwanted changes in the characteristics and/or levels of expression of endogenous allergenic proteins in the GM crop derived food. In particular the test models used shall be discussed with respect to specificity, predictability and validation status.

With respect to intake estimations of GM plant derived foods for humans, the applied methodologies shall be evaluated with respect to uncertainties associated with the prediction of long-term intake. Specific attention shall be paid to those GM foods which are aimed at modifying nutritional quality. For the GM products in questions the requirement for post-market monitoring shall be discussed as a necessary mechanism for determining changes to overall dietary intake patterns of the GM food, to what extent this has occurred and whether or not the product induces known (side) effects or unexpected side effects. If the performance of post-market monitoring is deemed necessary, the reliability, sensitivity and specificity of the proposed methods shall be discussed.

3.3. The result of risk characterisation

In accordance with Articles 4 and 16 of Regulation (EC) No 1829/2003, the applicant shall ensure that the final risk characterisation clearly demonstrates that:

- Consumption of foods/feed derived from GM plants is as safe for humans/animals as the conventional counterpartsthe GM food does not differ from the food which it is intended to replace to such an extent that its normal consumption would be nutritionally disadvantageous for the consumer;

- the GM feed does not harm or mislead the consumer by impairing the distinctive features of the animal products compared to conventionally produced feed,

- the GM feed does not differ from the feed which it is intended to replace to such an extent that its normal consumption would be nutritionally disadvantageous for animals and humans.

The applicant shall clearly indicate what assumptions have been made during the risk assessment in order to predict the probability of occurrence and severity of adverse effect(s) in a given population, and the nature and magnitude of uncertainties associated with establishing these risks.

The applicant shall also include detailed information justifying the inclusion or the non inclusion in the application of a proposal for labelling in accordance with Articles 5(3)(f) and 17(3)(f).

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ANNEX III: POST-MARKET MONITORING OF GM FOOD/FEED

Where appropriate a Post Market Monitoring (PMM) programme shall be performed for GM food/feed. The appropriateness of performing a PMM is indicated by findings in the pre-market safety assessment. Furthermore, as pre-market risk assessment studies cannot fully reproduce the diversity of the populations who will consume the marketed product, the possibility therefore remains that unpredicted side effects may occur in some individuals of the population, such as those with certain disease states (i.e. allergic consumers), those with particular genetic/physiological characteristics or those who consume the products at high levels. Indeed, risk assessment also relies on an estimate of exposure to the food/feed, which is variable and subject to uncertainty before the food/feed is marketed. A PMM shall therefore address the following questions: i) is the product use as predicted/recommended? ii) are known effects and side-effects as detected during the pre-market risk assessment as predicted? and iii) does the product induce unexpected side effects?

However a PMM does not substitute for a thorough pre-marketing toxicological and nutritional testing programme but complements it in order to confirm the pre-market risk assessment. It may increase the probability of detecting rare unintended effects. Therefore the PMM for GM food/feed shall be designed to generate a reliable and validated flow of information between the different stakeholders in order to potentially relate GM food/feed consumption to any (adverse) effect on health. However it shall be realized that a PMM may not always have the sensitivity to estimate individual intakes of a specific food item or intakes of particular age groups.

Given the practical difficulties in performing a PMM, it shall be required only in specific cases .Those cases could include GM (functional) food/feed with altered nutritional composition and modified nutritional value and/or food/feed genetically modified to achieve specific health benefits. This could be the case for a GM food/feed proposed as an alternative or as a replacement for a traditional food/feed. Because of its specific properties, the intake of this GM food/feed might be increased compared to the intake of the conventional counterpart, which could result in a significant impact on the long-term nutritional and health status of some individuals of the population.

A similar approach could be developed for feed with improved nutritional characteristics.