COMMISSION OF THE EUROPEAN COMMUNITIES

Brussels,
C(2009)

Draft

**COMMISSION REGULATION**

**of**

**on implementing rules concerning applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Regulations No (EC) 641/2004 and (EC) No 1981/2006**

**(Text with EEA relevance)**

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**(Text with EEA relevance)**

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed[1], and in particular Articles 5(7) and 17(7) thereof,

After consulting the European Food Safety Authority in accordance with Articles 5(7) and 17(7) of Regulation (EC) No 1829/2003,

Whereas:

(1) Regulation (EC) No 1829/2003 lays down Community procedures for the authorisation and supervision of genetically modified (GM) food and feed and provisions for the labelling of such food and feed, after a scientific evaluation is undertaken on the risks they may present for human and animal health and, as the case may be, for the environment, and after ensuring that they do not mislead the consumer or user and do not differ from the food or feed which is intended to replace to such an extent that its normal consumption would be nutritionally disadvantageous for animals or humans.

(2) It provides in particular that applications for authorisation should adequately and sufficiently demonstrate that the products covered by the Regulation satisfy the requirements laid down in that Regulation, in respect of their proposed uses.

(3) Commission Regulation (EC) No 641/2004 on detailed rules for the implementation of Regulation (EC) No 1829/2003 of the European Parliament and of the Council as regards the application for the authorisation of new genetically modified food and feed, the notification of existing products and

[1] OJ L 268, 18.10.2003, p. 1.

adventitious or technically unavoidable presence of genetically modified material which has benefited from a favourable risk evaluation[2] provides for certain rules concerning applications for authorisation submitted in accordance with Regulation (EC) No 1829/2003. To facilitate the preparation of the applications by the applicant and to ensure that the applications will contain all the information needed for their assessment, it is necessary to provide for more comprehensive and systematic rules concerning the applications for authorisation which should also be specific to each type of GMO (plants, animals and micro-organisms).

(4) These rules should only apply to applications concerning GM plants for food or feed uses, food or feed containing or consisting of GM plants and food or feed produced from GM plants which constitute the vast majority of current applications and for which sufficient experience is available to date. More specific rules than those provided in Regulation (EC) No 641/2004 as regards the other types of GM products may be adopted in the future taking into account the experience gained in those fields.

(5) These rules should specify for the attention of the applicant the general requirements for the presentation and preparation of applications, namely requirements to provide for general and scientific information, including methods of detection, sampling and identification as well as reference material so as to ensure that the application meets the conditions referred to in Article 5 and 17 of Regulation (EC) No 1829/2003. The applicant should also take into consideration the scientific information to be provided in the application as concerns the environmental assessment of genetically modified organisms (GMOs) or food and feed containing or consisting of GMOs set out in Annex II to Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC[3] as well as the applicable guidance published by the European Food Safety Authority in this regard.

(6) It is appropriate to provide for specific rules to ensure that the scientific information required in the application adequately and sufficiently demonstrates that the product does not have adverse effects on human or animal health, does not mislead the consumer or user and does not differ from the food or feed which it is intended to replace to such an extent that its normal consumption would be nutritionally disadvantageous for animals or humans. These rules should take into account relevant international standards, such as the guideline of the Codex Alimentarius for the conduct of food safety assessment derived from recombinant-DNA plant[4].

(7) Depending on the nature of the GM food and feed or their conditions of use, the type and necessity of the studies needed to evaluate their proprieties or their effects may vary. Some degree of flexibility should therefore be granted to applicants regarding the type of studies to be presented in the applications to

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2 OJ L 102, 7.4.2004, p. 14.
3 OJ L 106, 17.4.2001, p. 1.
4 Codex Alimentarius Commission, GL 45-2003.

demonstrate the safety of the genetically modified products concerned. Appropriate verifiable justification should be given in such cases.

(8) It is essential to apply appropriate quality assurance systems to facilities in which toxicological tests are performed in order to ensure that the results are of high quality. Such principles are laid down by Directive 2004/10/EC of the European Parliament and 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.[5] If such tests are carried out outside the Union, they should follow "the OECD Principles of Good Laboratory Practice" (GLP). With regard to studies other than toxicological studies, they should be conducted under ISO or GLP standards or other appropriate quality assurance.

(9) In order to demonstrate that a GM food or feed fulfils the requirements of Regulation (EC) No 1829/2003, experimental testing may be necessary involving laboratory animals. This should be carried out in accordance with Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of the animals used for experimental and other scientific purposes[6], and should be kept to a minimum while ensuring an adequate demonstration of the safety of the GM food and feed.

(10) This Regulation should define the conditions under which a proposal for post-market monitoring of the use of the GM food and feed should be submitted.

(11) The content of this Regulation takes account of the international trade commitments of the Union and of the requirements of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity (the Cartagena Protocol), approved by Council Decision 2002/628/EC[7] and the provisions 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[8].

(12) Studies presented in applications should be carried out in accordance with this Regulation, internationally agreed protocols and the test methods described by the OECD when available.

(13) Applications should include proposals for a unique identifier for each GMO concerned in accordance with Commission Regulation (EC) No 65/2004 of 14 January 2004 establishing a system for the development and assignment of unique identifiers for genetically modified organisms[9].

(14) This Regulation replaces provisions laid down in Regulation (EC) No 641/2004 as regards GM plants for food or feed uses, food or feed containing or consisting of GM plants and food or feed produced from GM plants. However, Regulation

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5 OJ L 50, 20.2.2004, p. 44.
6 OJ L 358, 18.12.1986, p. 1.
7 OJ L 201, 31.7.2002, p. 48.
8 OJ L 287, 5.11.2003, p. 1.
9 OJ L 10, 14.1.2004, p. 5.

(EC) No 641/2004 should continue to apply as regards other types of GM products (GM animals, GM micro-organisms). Moreover, certain provisions of Regulation (EC) No 641/2004 are obsolete. It follows that Regulation (EC) No 641/2004 should be amended accordingly.

(15) Commission Regulation (EC) No 1981/2006 of 22 December 2006 on detailed rules for the implementation of Article 32 of Regulation (EC) No 1829/2003 of the European Parliament and of the Council as regards the Community reference laboratory for genetically modified organisms[10] should be amended to include references to this Regulation.

(16) According to Articles 5 (7) and 17 (7) of Regulation (EC) No 1829/2003, the Commission shall consult the European Food Safety Authority before establishing implementing rules with regard to the applications for authorisations under that Regulation. In accordance with the indicated Articles, the European Food Safety Authority has been consulted on those rules.

(17) The implementing rules have been drawn up on the basis of current scientific and technical knowledge. Therefore, the Commission should monitor any developments in this field and the publication of new or additional guidance by the European Food Safety Authority.

(18) It is necessary to provide for transitional measures in order to enable the applicants to comply with those rules and for the current applications or the applications close to being submitted to proceed without unnecessary delays.

(19) The measures provided for in this Regulation are in accordance with the opinion of the Standing Committee on the Food Chain and Animal Health,

HAS ADOPTED THIS REGULATION:

# CHAPTER I

# General provisions

*Article 1*
*Scope*

This Regulation shall apply to applications submitted under Regulation (EC) No 1829/2003 for authorisation of genetically modified (GM) plants for food or feed uses, food and feed containing or consisting of GM plants, and food and feed produced from GM plants.

[10] OJ L 368, 22.12.2006, p. 99.

*Article 2*
*Definitions*

For the purposes of this Regulation the definitions set out in Articles 2 and 3 of Regulation (EC) No 178/2002 of the European Parliament and the Council[11] shall apply.

# CHAPTER II

# General requirements

*Article 3*
*Preparation and presentation of the application*

1. The application shall be submitted in accordance with Annex I and shall contain all the information and particulars required therein, in accordance with the requirements set out in Articles 4 to 6.

2. The application, as well as any supplementary information submitted during the authorisation procedure, shall clearly state which parts of the application are claimed to be confidential, providing verifiable justification in accordance with Article 30 of Regulation (EC) No 1829/2003. Parts claimed to be confidential shall be included at the time of submission of the application but separately from the main application.

# CHAPTER III

# Specific Requirements

*Article 4*
*Scientific Requirements for the safety assessment of GM food and feed*

1. Information, including studies, required for the risk assessment as referred to in Article 5(3)(a) to (f) and (h) and in Article 17(3)(a) to (f) and (h) of Regulation (EC) No 1829/2003 to be included in the application shall be provided in accordance with the requirements set out in Annex II.

2. By way of derogation from paragraph 1, an application may be accepted even if it does not satisfy all the requirements set out in that paragraph, provided that the applicant submits verifiable justification for each element not complying with those requirements.

3. Toxicological studies shall be conducted in facilities which comply with the requirements of Directive 2004/10/EC or, if they are carried out outside the Union,

[11] OJ L 31, 1.2.2002, p. 1.

they shall follow “the OECD Principles of Good Laboratory Practice” (GLP). The applicant shall provide evidence to demonstrate that those requirements are complied with.

4. Studies other than toxicological studies shall be conducted under ISO or GLP standards or other appropriate quality assurance. In case where quality assurance standards other than ISO or GLP standards are used, the applicant shall provide a thorough description of the quality assurance system under which they have been conducted.

5. Where trials involve animals as referred to in Council Directive 86/609/EEC, the results of those trials shall not be considered confidential in accordance with Article 30(3)(d) of Regulation (EC) No 1829/2003.

6. Without prejudice to Article 31 of Regulation (EC) No 1829/2003 on data protection, when studies have been already submitted for the purposes of an application to the European Food Safety Authority, a reference to such studies and the results of the evaluation may, with the agreement of the Authority, be made in the framework of another application.

*Article 5*
*Requirements applicable for post-market monitoring of GM food and feed*

When necessary, a proposal for post-market monitoring regarding the use of food for human consumption and/or the use of feed for animal consumption shall be submitted in accordance with Annex III.

*Article 6*
*Requirements concerning the methods of detection, sampling, identification and reference material*

The application shall meet the specific requirements concerning the methods of detection, sampling and identification as well as the reference material referred to in Articles 5.3 (i) and (j) and 17.3 (i) and (j) of Regulation (EC) No 1829/2003, as set out in Annex IV.

# CHAPTER IV

# Final provisions

*Article 7*
*Amendments to Regulation (EC) No 641/2004*

Regulation (EC) No 641/2004 is amended as follows:

1) Article 1 shall be replaced by the following:

"*Article 1*

This chapter provides detailed rules concerning applications for authorisation submitted in accordance with Articles 5 and 17 of Regulation (EC) No 1829/2003 except for those covered by Commission Regulation *[XXX/2009 – reference to this Regulation]*."

2) Articles 5 to 19 shall be deleted.

*Article 8*
*Amendments to Regulation (EC) No 1981/2006*

Regulation (EC) No 1981/2006 is amended as follows:

1) In Article 2, point (a) is replaced by the following:

"(a) 'full validation procedure' means :

(i) the assessment through a ring trial involving national reference laboratories of the method performance criteria set by the applicant as compliant with the document entitled 'Definition of minimum performance requirements for analytical methods of GMO testing' referred to

- in the case of GM plants for food or feed uses, food or feed containing or consisting of GM plants and food or feed produced from GM plants, in point 1.1 (B) of Annex IV to Regulation (EC) No *XXX*/2009;

- in all other cases, in point 1), B) of Annex I to Regulation (EC) No 641/2004"

and

(ii) the assessment of the repeatability and trueness of the method provided by the applicant;

2) In Article 3, paragraph 2 is replaced by the following:

"2. The CRL shall request the applicant to pay an additional contribution of EUR 60 000 where a full validation procedure of a method of detection and identification for a single GMO event according to the requirements laid down in the following provisions is required:

(a) Annex IV of Regulation (EC) No *XXX*/2009 , when the application is related to GM plants for food or feed uses, food or feed containing or consisting of GM plants and food or feed produced from GM plants, or

(b) point 1(B) of Annex I to Regulation (EC) No 641/2004, in all other cases."

That amount shall be multiplied by the number of GMO events to be fully validated.

The CRL shall reduce the amount of the additional contribution, in proportion of the costs saved:

(a) where the material needed to perform the full validation procedure is supplied by the applicant; and/or

(b) where the applicant provides data that refer to modules, such as DNA extraction protocols and species specific reference systems, already validated and published by the CRL."

*Article 9*
*Monitoring*

The Commission shall monitor the application of this Regulation and the publication of new or additional guidance from the European Food Safety Authority as well as the availability of updated guidance on products falling under the scope of Regulation (EC) No 1829/2003 which have not yet been covered by this Regulation.

Article 10
Transitional provisions

1. Until [*fixed date corresponding to 6 months after the publication in the Official Journal*], applicants may choose to submit applications under Regulation (EC) No 641/2004 in the version in force on [*fixed date corresponding to the publication in the Official Journal*].

2. For applications submitted until [*fixed date corresponding to 18 months after the publication in the Official Journal*] compliance with point 1.3.2. of Annex II shall be voluntary.

*Article 11*
*Entry into force*

This Regulation shall enter into force on the 20th day following that of its publication in the *Official Journal of the European Union*.

Done at Brussels,

*For the Commission*
*José Barroso President of the Commission*

## ANNEX I

## PREPARATION AND PRESENTATION OF APPLICATIONS

The application shall contain the following information:

### PART I: GENERAL INFORMATION

**1. Name and address of the applicant (company or institute);**

**2. Name, qualification and experience of the responsible scientist(s) and contact details of the responsible person for all dealings with the European Food Safety Authority (EFSA);**

**3. Designation and specification of the GM plant and derived product;**

**4. Scope of the application**

(a) GM food

- ☐ Food containing or consisting of GM plants
- ☐ Food produced from GM plants or containing ingredients produced from GM plants

(b) GM feed

- ☐ Feed containing or consisting of GM plants
- ☐ Feed produced from GM plants

(c) GM plants for food or feed uses

- ☐ Products other than food and feed containing or 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.

**5. 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.

**6. 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.

**7. 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

All the following requirements shall be provided in the application except where it is not justified by the scope (e.g. limited to derived products),

### 1. HAZARD IDENTIFICATION AND CHARACTERISATION

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

*(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 within the Union;*

*(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, such as 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)*

*(e) Additional information relating to the recipient or parental plants required for the environmental safety aspects:*

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

(b) Sexual compatibility with other cultivated or wild plant species;

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

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

(e) Geographical distribution within the Union of the sexually compatible species.

(f) 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;

(g) 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. General 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(s)

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*

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 protocols for 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. Potential risk associated with horizontal gene transfer*

*1.3.7. Effects of processing*

*1.3.8. Conclusion*

**1.4. Toxicology**

*1.4.1. 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. 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 and 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**

**6. ENVIRONMENTAL MONITORING PLAN**

**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 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 to Regulation (EC) No 1946/2003[12].

**PART IV: LABELLING**

The application shall include:

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

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

(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 languages of the Union as required by 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, SAMPLING AND IDENTIFICATION AND REFERENCE MATERIAL

The applicant shall provide methods for detection, sampling and identification as well as samples of the food/feed, their controls samples to the CRL. A copy of the completed form for submission of the samples to the CRL and a proof of sending to the CRL shall be provided in the application.

– Information as to the place where the reference material can be accessed shall be provided in the application.

– The applicant shall follow the following instructions in the preparation and the sending of the samples:

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

(b) The parcel shall be specified to contain "Free samples", and it shall include the list of all items and their storage instructions.

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

The information required by Annex III to Directive 2001/18/EC shall be provided where it is not covered by the requirements of other parts of the application.

## PART VII: SUMMARY OF APPLICATIONS

This part specifies the standardised form, which the summary of the dossier applications should follow. Depending on the scope of the application, some of the requested information may not be applicable. The summary shall not contain parts considered to be confidential in accordance with Article 30 of Regulation (EC) No 1829/2003.

### 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 Union (if the applicant is not established in the Union)

**1.3. Scope of the application**

(a) GM food

☐ Food containing or consisting of GM plants

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

(b) GM feed

☐ Feed containing or consisting of GM plants

☐ Feed produced from GM plants

(c) 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 another authorisation procedure within the Union?**

No ☐

Yes ☐ (in that case, specify)

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

Yes ☐

No ☐ (in that case, provide 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 Union under Part C of Directive 2001/18/EC?**

No ☐

Yes ☐ (in that case, specify)

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

No ☐

Yes ☐ (in that case, 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 GMO plants, food and/or feed containing or consisting of GMO plants, 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 Union

(ii) In export markets for EU 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 within the Union**

**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 within the Union 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*

(a) Nature and source of the vector used

(b) 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) Chromosomal 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 the insertion site

(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

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

(a) Toxicological testing of newly expressed proteins

(b) Testing of new constituents other than proteins

(c) Information on natural food and feed constituents

(d) Testing of the whole GM food/feed

## 6. ALLERGENICITY

(a) Assessment of allergenicity of the newly expressed protein

(b) Assessment of allergenicity of the whole GM plant or crop

## 7. NUTRITIONAL ASSESSMENT

(a) Nutritional assessment of GM food

(b) Nutritional assessment of GM feed

## 8. EXPOSURE ASSESSMENT – ANTICIPATED INTAKE/EXTENT OF USE

## 9. RISK CHARACTERISATION

## 10. POST-MARKET MONITORING ON GM FOOD/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

(a) Persistence and invasiveness

(b) Selective advantage or disadvantage

(c) Potential for gene transfer

(d) Interactions between the GM plant and target organisms

(e) Interactions of the GM plant with non-target organisms

(f) Effects on human health

(g) Effects on animal health

(h) Effects on biogeochemical processes

(i) Impacts of the specific cultivation, management and harvesting techniques

### 11.3. Potential interactions with the abiotic environment

## 12. ENVIRONMENTAL MONITORING PLAN

(a) General (risk assessment, background information)

(b) Interplay between environmental risk assessment and monitoring

(c) Case-specific GM plant monitoring (approach, strategy, method and analysis)

(d) General surveillance of the impact of the GM plant (approach, strategy, method and analysis)

(e) 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 Union 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

## I. INTRODUCTION

### 1. DEFINITIONS

For the purpose of this annex, the following definitions shall apply:

1. 'hazard identification' means 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 ;

2. 'hazard characterisation' means 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;

3. 'risk characterisation' means 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. SPECIFIC CONSIDERATIONS

### 2.1. Insertion of marker genes and other DNA not essential to achieve the desired trait

During the process of genetic modification of plants and other organisms, marker genes are 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. Such marker genes shall be carefully selected. In case of use of Antibiotic Resistance Marker genes, Article 4 (2) of Directive 2001/18/EC shall be respected. The applicant, when considering the submission of an application related to a GM plant with such type of gene, shall also consider the opinion of EFSA on the matter[13].

The risk assessment may be facilitated if the presence of inserted DNA not essential to achieve the desired trait is minimised.

[13] ARM Statement of EFSA on the consolidated presentation of opinions on the use of antibiotic resistance genes as marker genes in genetically modified plants, the EFSA Journal (2009) 1108, 1-8

### 2.2. Risk assessment of GM food and feed containing stacked transformation events combined by conventional crossing

A risk assessment of the single events is a pre-requisite for the assessment of stacked events.

The assessment of GM food and feed containing more than two transformation events combined by conventional crossing shall cover all sub-combinations of these events. In such a case, the applicant shall either provide a scientific rationale justifying that there is no need for experimental data obtained for the concerned sub-combinations or provide the experimental data.

The risk assessment of the stacked events shall then mainly focus on issues related to

(a) stability of the inserts,

(b) expression of the events and

(c) potential synergistic or antagonistic effects resulting from the combination of the events.

## II. SCIENTIFIC REQUIREMENTS

### 1. HAZARD IDENTIFICATION AND CHARACTERISATION

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

The applicant shall provide comprehensive information relating to the recipient or (where appropriate) the parental plants:

– to evaluate all issues of potential concern, such as the presence of natural toxins or allergens;

– to identify the need for specific analyses.

For these purposes, 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 within the Union.

(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 and 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*

The applicant shall provide sufficient information on the genetic modification:

– to identify the DNA intended for transformation and related vector sequences potentially delivered to the recipient plant;

– to characterise 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 elements:

(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 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 shall be clearly indicated;

– 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

The applicant shall provide information on the donor organism(s) and on the DNA sequence(s) intended to be inserted 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.

When the introduced trait is herbicide tolerance, the applicant shall provide information on the mode of action and of the active substance and its metabolism in 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 shall 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 shall span the entire transgenic locus(i) as well as flanking sequences and include all appropriate controls. For the determination of copy number of the insert, complementary methods can also be used (e.g. real-time PCR)

(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 Open Reading Frames corresponding to any nucleotide sequence that contains a string of codons that is uninterrupted by the presence of a stop codon in the same reading frame (hereafter, referred to as ORFs) or regulatory regions. Bioinformatic analyses shall 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 insert(s). 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 (e.g. transcription analysis) may be needed to complete the risk assessment.

1.2.2.3. Information on the expression of the insert(s)

The applicant shall provide information:

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

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

For these purposes, the applicant shall provide the following information:

(a) Methods used for expression analysis 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(s) 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 used for food and feed purposes shall be provided 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 section 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 shall be obtained from field trials as specified in section 3.3.2 and be related to the conditions in which the crop is grown;

(g) When justified by the nature of the insert, (e.g. gene silencing through RNA interference) information on the expression of thetargeted gene(s) and on possible effects on other endogenous genes (to be selected by *in silico* analysis) shall be provided;

(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

The applicant shall provide information:

– 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.

For this purpose, applicants shall provide data from multiple (normally five) 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 original events and the GM stacks shall be carried out using plant materials representative of those designed for commercial production. The applicant shall provide adequate justification for its choice.

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

*1.2.3. 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) 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.

## 1.3. Comparative analysis

The comparative analysis of composition and agronomic as well as phenotypic characteristics shall constitute, together with the molecular characterisation, the starting point to structure and conduct the risk assessment of a new GM food and feed and its derived products. It shall aim 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 and feed and its conventional counterpart.

Where no appropriate conventional counterpart can be identified, a comparative safety assessment cannot be made and consequently a safety and nutritional assessment of the GM food and feed shall be carried out as for other novel foods that do not have conventional counterparts (e.g. where the GM food and feed is not closely related to a food and feed with a history of safe use or where a specific trait or specific traits are introduced with the intention of bringing complex changes in the composition of the GM food and feed.

### *1.3.1. Choice of the conventional counterpart and additional comparators*

In the case of vegetatively propagated crops, the conventional counterpart shall, in principle, be the near-isogenic variety used to generate the transgenic line.

In the case of crops that reproduce sexually, the conventional counterpart shall have a genetic background comparable to the GM plant. Since many crops used to produce food and feed are developed using back-crossing, a conventional counterpart with a genetic background that is as close as possible to the GM plant shall be selected.

In all cases, the applicant shall provide information on the breeding scheme (pedigree) in relation to both the GM plant and the conventional counterpart and an adequate justification for the use of the selected conventional counterpart. The history of safe use of the conventional counterpart shall be adequately supported by both qualitative and quantitative data[14]. In addition, the applicant may include 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 and in order to assess whether the expected agricultural practices influence the expression of the studied endpoints, 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 management regimes.

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[14] See, for example, Constable A, Jonas D, Cockburn A, Davi A, Edwards G, Hepburn P, Herouet-Guicheney C, Knowles M, Moseley B, Oberdörfer R, Samuels F. History of safe use as applied to the safety assessment of novel foods and foods derived from genetically modified organisms. Food Chem Toxicol. 2007 45, 2513-2525.

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. Description of the protocols for the experimental design

(a) 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 plant, its conventional counterpart (selected in accordance to section 1.3.1) and reference varieties: the objective shall be to determine whether the GM plant and/or derived food and feed is different from its conventional counterpart and/or equivalent to reference 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 difference, extent and pattern on exposure; and

(ii) a proof of equivalence to verify whether the GM plant is equivalent or not to reference varieties with a history of safe use, apart from the introduced trait(s). In testing for difference the null hypothesis shall 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 shall that the difference between the GMO and the set of reference varieties is at least as great as a specified minimum size (see section 1.3.2.2) against the alternative hypothesis that there is no difference or a smaller difference than the specified minimum between the GMO and the set of reference varieties. Rejection of the null hypothesis is required in order to conclude that the GMO and the set of reference 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 reference varieties with a history of safe use.

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 reference 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 material (GMO, conventional counterpart, reference varieties and additional comparator(s) where appropriate) shall be randomised to plots within a single field at each site, usually in a completely

randomised or randomised 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 explicitly justified. The choice of reference varieties shall be appropriate for the chosen sites and shall be justified explicitly. In the case that sites cover a very restricted geographic range, the applicant shall replicate the trials over more than one year.

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.

(b) Specific protocols for experimental design

Within each site the test materials (GM plants, conventional counterpart and additional comparator(s), where appropriate) shall be identical for all replicates. In addition, unless there is explicit justification for not doing so, at each site there shall be at least three appropriate reference varieties of the crop that have a known history of safe use. The number of distinct test materials plus the number of reference varieties shall be denoted by t. For example, if there are the GM plant, the conventional counterpart plus four reference varieties, then t=6. The number of results to be obtained for each test material and reference variety at each site (the replication) shall be denoted as r. 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 eight sites, chosen to be representative of the range of likely receiving environments where the plant will be grown. The trials may be conducted in a single year, or spread over multiple years. The reference varieties may vary between sites and at least six different reference varieties shall be used over the entire set of trials.

When the GM plant is tested together with other GM plants of the same crop species (e.g. *Zea mays*) the production of material for the comparative assessment of these different GM plant 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 comparator(s) in the same randomised block. This shall be subject to two strict conditions:

(i) each of the appropriate comparator(s) shall always occur together with its particular GM plant in the same block; and

(ii) all the different GM plants and their comparator(s) and all the reference varieties used to test equivalence with those GM plants shall be fully randomized within each block.

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, to reduce the number of plots per block, by excluding some of the GM plants and their appropriate comparator(s) from each block. This shall be subject to two strict conditions:

(i) each of the appropriate comparator(s) shall always occur together with its particular GM plant in the same block and

(ii) all of the reference varieties shall appear in each of the incomplete blocks and be fully randomised with the GM plants and their comparator(s).

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 conditions during growth and time of harvest, as well as the conditions during storage of the harvested material.

Further information for the application of the requirements of this section is available in the EFSA report on "Statistical considerations for the safety evaluation of GMOs"[15].

1.3.2.2. 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 total variability of each endpoint observed in the field trials shall be estimated and partitioned using appropriate statistical models in order to derive two sets of confidence limits and to set a lower and upper equivalence limit based on the variability observed among the commercial varieties. One set of confidence limits is used in the test of difference; the other set and the equivalence limits are used in the test of equivalence.

A linear mixed statistical model (denoted model 1) shall be used for calculation of the confidence limits for both tests (difference and equivalence); a slightly different model (model 2) shall be used to estimate the equivalence limits to be used in the equivalence test.

Denote by $I$ an indicator variable (uncentered in the mixed model) such that $I$=1 for a field plot having any of the commercial varieties, and $I$=0 otherwise. Then the random factors for model 1 should include, but not necessarily be restricted to, those representing the variation: (i) between the test materials (a set that includes the GM crop, its conventional counterpart, each of the commercial varieties and any additional comparators); (ii) in the interaction between the test materials and *I;* (iii) between sites; and (iv) between blocks within sites. Model 2 should be identical to

[15] EFSA report on "Statistical considerations for the safety evaluation of GMOs" [NB: reference to be modified when report published]

model 1 except that the random factor representing the interaction between the test materials and *I* is omitted.

The fixed factor for both models shall have as many levels as there are test materials and represent the contrasts between the means of the test materials. The test materials are as defined above: the GM crop; its conventional counterpart; the set of commercial varieties; and any additional test materials. The set of commercial varieties shall be considered as a single level of the fixed factor. For the difference and equivalence tests, the component of the fixed factor of interest is the single degree-of-freedom contrast between, respectively, the GM crop and its conventional counterpart, and the GM crop and the set of commercial varieties.

Both the difference test and the equivalence test are implemented using the well-known correspondence between hypothesis testing and the construction of confidence limits. 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 of non-equivalence when the both confidence limits fall between the equivalence limits. The choice of 90% confidence limits corresponds to the customary 95% level for statistical testing of equivalence.

For each endpoint, calculation of the confidence limits, estimation of equivalence limits and associated statistical tests shall be performed as described below, using the following notation. Sample means are denoted by *m*, with subscripts G, C and R for the GM crop, its conventional counterpart and the set of commercial (reference) varieties, respectively. The variability encompassed in the standard error of the difference between the means of any two test materials, X and Y, calculated using model *i* ($i$ = 1,2), is denoted *sed*(XY;*i*). The 100*a*% point of Student's *t* distribution is denoted as *t*(*df*;*i*;*a*), where *i* denotes the model used and *df* is the appropriate number of degrees of freedom which is recommended to be calculated by the Kenward-Roger method. The least significant difference between the means of any two test materials, X and Y, using model *i,* shall be calculated as the product of *t*(*df*;*i;a*) and *sed*(XY;*i*), and is denoted *lsd*(XY;*i*;*a*).

For the difference test, the two-sided 90% confidence limits shall be calculated about $m_G$, as $m_G \pm lsd$(GC;1;95); the null hypothesis of equality between $m_G$ and $m_C$ shall be rejected and the test deemed statistically significant if $m_C$ falls outside these limits. For the equivalence test, the two-sided 95% equivalence limits shall be estimated as $m_R \pm lsd$(GR;2;97.5) and two-sided 90% confidence limits shall be calculated about $m_G$, as $m_G \pm lsd$(GR;1;95); the null hypothesis of non-equivalence shall be rejected and the test deemed statistically significant if and only if the confidence limits lie entirely inside the equivalence limits.

It is convenient to assess visually the quantities involved in the above tests for all the endpoints simultaneously, on a single graph or a few graphs. This may be done by shifting all relevant values for each particular endpoint to a scale that has $m_C$, the mean of the conventional counterpart for that endpoint, as its baseline zero value. Therefore, on this new scale, the values of the means of the GM crop, its conventional counterpart and the set of commercial varieties, become, respectively: $m_G - m_C$, 0, $m_R - m_C$.

The confidence limits for the difference test become: $m_G - m_C \pm lsd(GC;1;95)$, the equivalence limits $m_R - m_C \pm lsd(GR;2;97.5)$, and the confidence limits for the equivalence test $m_G - m_C \pm lsd(GR;1;95)$. To facilitate visual interpretation, instead of using the two sets of confidence limits in the graphs, only one, that for the difference test, shall be displayed. Without some adjustment, the confidence limits for the difference test would not give a valid visual representation for the equivalence test on the graph. This problem is overcome by making an adjustment to the displayed equivalence limits. After this adjustment the displayed confidence limits for the difference test may be used as a basis also for the visual representation of the equivalence test. In this way, one confidence limit may serve visually for assessing the outcome of both tests simultaneously. The adjustment of the equivalence limits consists of two steps: (1) scaling the basic equivalence limits, so that the confidence limits required for the difference and equivalence tests have the same width; and (2) an appropriate shift to facilitate display of the adjusted limits, together with $m_G$, on the scale that has $m_C$ as its baseline zero value. The adjusted equivalence limits for visual display shall be calculated by the formula:

$$(m_G - m_C) + \{[(m_R - m_G) \pm lsd(GR;2;97.5)]\ lsd(GC;1;95) / lsd(GR;1;95)\}$$

The graph shall show the line of zero difference between the GM and its conventional counterpart and, for each endpoint: the lower and upper adjusted equivalence limits, the mean difference between the GM and its conventional counterpart, and the confidence limits for this difference (see the set of possible outcomes for a single endpoint in Figure 1). When, in addition to the conventional counterpart, another test material is used as comparator, the mean difference between the GM and that comparator, its confidence limits and its adjusted equivalence limits shall be displayed on the same graph referred to above, for all such additional comparators, by referring this to the same zero baseline as defined by the conventional counterpart. 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.

It is a consequence of the simplified graphical display that confidence limits for the difference test were chosen as 90%, yielding a 10% size for the difference test, in which 1 in 10 of such tests is expected to yield a significant result by chance alone. Despite the expected proportion of spurious significant differences, the applicant shall report and discuss all significant differences observed between the GM crop, its conventional counterpart and, where applicable, any other test material, focusing on their biological relevance (see section 3. on Risk Characterization).

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

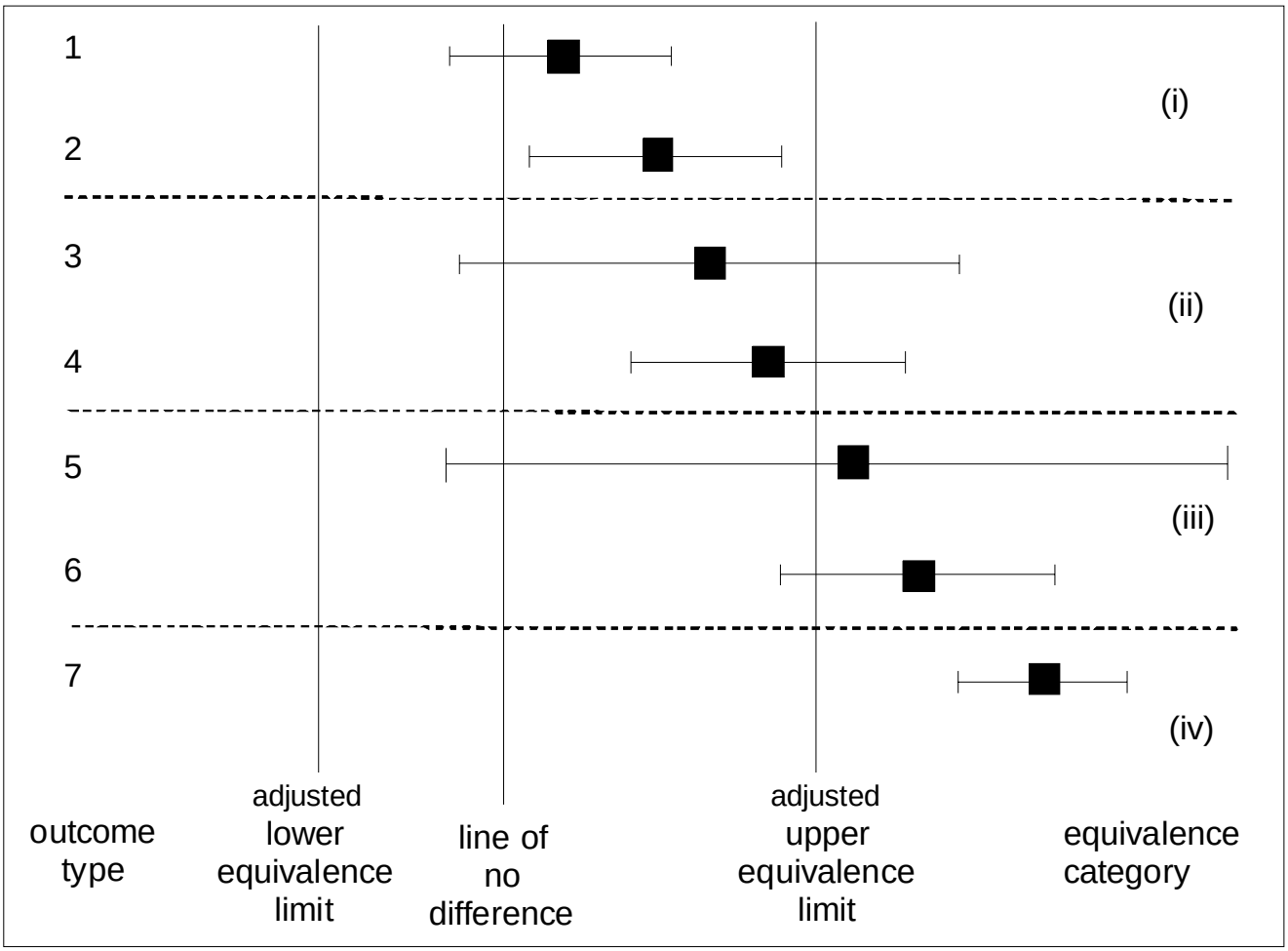


Figure 1. Simplified version of a graph for comparative assessment showing the 7 outcome types possible for a single endpoint. After adjustment of the equivalence limits, a single confidence limit (for the difference) serves visually for assessing the outcome of both tests (difference and equivalence). Here, only the upper adjusted equivalence limit is considered. Shown are: the mean of the GM crop on an appropriate scale (square), the confidence limits (whiskers) for the difference between the GM crop and its conventional counterpart (bar shows confidence interval), a vertical line indicating zero difference (for proof of difference), and vertical lines indicating adjusted equivalence limits (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 conventional counterpart. Regarding interpretation of equivalence, four categories (i) - (iv) are identified: in category (i) the null hypothesis of non-equivalence is rejected in favour of equivalence; in categories (ii), (iii) and (iv) non-equivalence cannot be rejected. See text for what appropriate conclusions may be drawn.

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 insufficient 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 significantly 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 (category (i), Figure 1): both confidence limits lie between the adjusted equivalence limits and the null hypothesis of non-equivalence is rejected. The appropriate conclusion is that the GM is equivalent to the set of commercial varieties.

- Outcome types 3 and 4 (category (ii), Figure 1): the mean of the GM crop lies between the adjusted equivalence limits, but the confidence interval bar overlaps at least one of the adjusted equivalence limits on the graph. Non-equivalence cannot be rejected but 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 (category (iii), Figure 1): the mean of the GM crop lies outside the adjusted equivalence limits, but the confidence interval bar overlaps with at least one of the adjusted equivalence limits. Non-equivalence cannot be rejected and 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 (category (iv), Figure 1): both confidence limits lie outside the adjusted equivalence limits. The appropriate conclusion is that the evidence analysed here demonstrates 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 for any particular endpoint, further analysis shall be done to assess whether there are interactions between any of the test materials and site, possibly using a simple 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.

Further information for the application of the requirements of this section is available in the EFSA report on "Statistical considerations for the safety evaluation of GMOs"[16].

[16] EFSA report on "Statistical considerations for the safety evaluation of GMOs" [http://www.efsa.europa.eu/cs/Satellite]

*1.3.3. Selection of material and compounds for analysis*

Analysis of the composition is crucial when comparing the GM food and feed 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. Unless duly justified, analysis shall be carried out on the raw agricultural commodity, as this usually represents the main point of entry of the material into the food and feed production and processing chain. Additional analysis of processed products (food and feed, food ingredients, feed materials, food and feed additives or food flavourings), shall be conducted where appropriate and on a case-by-case basis (see also section 1.3.6). The sampling, analysis and preparation of the tested material 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 1.2.2.3), the compositional analysis shall be carried out on an appropriate range of compounds. In each case, the applicant shall provide at least analysis on proximates (including moisture and total ash), key macro- and micro-nutrients, anti-nutritional compounds, natural toxins, and already identified allergens, as well as other secondary plant metabolites characteristic for specific crop plant species as referred to in the OECD consensus documents on compositional considerations for new plant varieties (OECD consensus documents)[17]. 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 shall 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. The applicant shall pay particular attention to key nutrients such as proteins, carbohydrates, lipids/fats, fibre, vitamins and minerals. 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.

The applicant shall also provide analysis on key toxins inherently present in the recipient plant which may adversely affect human/animal health depending on their toxic potency and levels. The concentrations of such compounds shall be assessed according to plant species and the proposed use of the food and feed product. Similarly, anti-nutritional compounds, such as digestive enzyme inhibitors, and already identified allergens shall be studied.

The characteristics of the introduced trait may trigger further analysis of specific compounds including metabolites of potentially modified metabolic pathways. The applicant shall consider when appropriate) the inclusion of compounds other than the key nutrients, key toxins, and anti-nutrients and allergens identified by the OECD consensus documents and justify the selection of these compounds.

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[17] OECD consensus documents on compositional considerations for new plant varieties being published in the Series on the Safety of Novel Foods and Feeds.

The same conditions apply for events stacked by conventional crossing. Where appropriate, additional compounds may be selected for analysis depending upon the introduced traits.

*1.3.5. Comparative analysis of agronomic and phenotypic characteristics*

The applicant shall provide a comparison between the GM plant and its conventional counterpart. This comparison shall enable the applicant to identify unintended effects during the risk assessment process and 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. Where appropriate, additional information on agronomic traits of the stacked events shall be provided from additional field trials.

*1.3.6. Potential risk associated with horizontal gene transfer*

The applicant shall assess any potential risk associated with horizontal gene transfer from the product to humans, animals and micro-organisms when intact and functional DNA remains in the GM food and feed.

*1.3.7. Effects of processing*

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. The applicant shall provide a 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.

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). 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.

When appropriate, depending on the product, information shall be necessary on the composition, level of undesirable substances, nutritional value and metabolism, as well as on the intended use.

When appropriate, depending on the nature of the newly expressed protein(s), it shall 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.

*1.3.8. 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 to the reference varieties, taking into account natural variation;

(b) whether compositional characteristics of the GM food and feed are, taking into account natural variation, different to the characteristics of its conventional counterpart and/or equivalent to the reference varieties, except for the introduced trait(s);

(c) characteristics for which the GM plant or the GM food and feed are different to the characteristics of its conventional counterpart and/or 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 be performed:

(a) to 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.

(b) to demonstrate that the intended effect(s) of the genetic modification has no adverse effects on human and animal health.

(c) to demonstrate that unintended effect(s) of the genetic modification(s) 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 applicant shall consider the need of toxicological testing based on the outcome of the molecular and comparative analysis referred to in section 2.2 and 2.3, *i.e.* the differences identified between the GM product and its conventional counterpart, including intended as well as unintended changes. For the purposes of this Regulation the applicant shall take into account 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.

As regards GM food and feed produced from GM plants, further toxicological tests with the processed products shall not be provided under the condition that the applicant provides an assessment of the GM plant (or relevant parts of it) demonstrating its safety and provided that there are no indications that the processed GM food and feed would be any different from their respective conventional counterpart. The applicant shall provide adequate justification in this regard. 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 (e.g. oil from insect protected cottonseed).

In case the applicant considers that a decision on safety can be taken without conducting some of the tests recommended in this chapter and/or that other tests are more appropriate it 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 shall 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 shall be provided.

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 and 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.

The applicant shall use internationally agreed protocols and test methods for toxicity testing as described by the OECD (see Tables 1 and 2 of section 1.7). Adaptations of these protocols or use of any methods that differ from such protocols shall be justified.

*1.4.1. Testing of newly expressed proteins*

The applicant shall provide an evaluation of all newly expressed proteins. 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. As regards proteins expressed in the GM plant, in the case where the history of safe use for consumption as food and feed of both the plant and the newly expressed proteins is duly documented[18], specific toxicity testing as provided for in the following paragraphs of this section shall not be required.

[18] See, in particular, Constable A, Jonas D, Cockburn A, Davi A, Edwards G, Hepburn P, Herouet-Guicheney C, Knowles M, Moseley B, Oberdörfer R, Samuels F. History of safe use as applied to the safety assessment of novel foods and foods derived from genetically modified organisms. Food Chem Toxicol. 2007 45, 2513-2525.

Where specific testing is required, the tested protein shall be 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 structure, molecular weight (e.g. using mass spectrometry), 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. The potential interaction with other plant constituents shall also 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 and feed. The influences of temperature and pH changes shall 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.

(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) A repeated dose 28-day oral toxicity study with the newly expressed protein in rodents. When appropriate depending on the outcome of the 28-day toxicity study, further targeted investigations shall be provided, including 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 and feed and is therefore discouraged.

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

*1.4.2. Testing of new constituents other than proteins*

The applicant shall provide a safety assessment of identified new constituents other than proteins. This shall include, on a case-by-case basis an evaluation of their toxic potency and of the need of toxicological testing as well as a determination of their concentration in the GM food and feed. To establish the safety of new constituents having no history of safe use for consumption in food and feed, the applicant shall provide information analogous to that described in the Guidance on submissions for food additive evaluations by the Scientific Committee on Foods" 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[19]. This shall include the submission of information on a core set of studies such as on metabolism/toxicokinetics, sub-chronic toxicity, genotoxicity, chronic toxicity, carcinogenicity and reproduction and developmental toxicity , accompanied by any other appropriate type of study. (for specific OECD guidelines for animal tests, see Table 1 of section 1.7). Genotoxicity test protocols are provided for in Table 2 of section 1.7.

*1.4.3. Information on natural food and feed constituents*

The present section shall apply only in the case where the intended or unintended effect of the modification would result in an alteration of the content of such natural food and feed constituents beyond the natural variation.

To demonstrate the safety of the altered content of natural food and feed constituents such as macro- and micronutrients, anti-nutrients, and natural toxins as well as other secondary plant metabolites, the applicant shall submit a detailed risk assessment based on the knowledge of the physiological function and/or toxic properties of these constituents. The result of this assessment shall determine if, and to what extent, the applicant shall provide toxicological tests.

*1.4.4. Testing of the whole GM food and feed*

The applicant shall primarily base its risk assessment of the GM plant and derived food and feed 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 and feed shall be carried out.

1.4.4.1. 90-day feeding study in rodents

The applicant shall adapt the OECD 90-day rodent toxicity study for the purposes of conducting the 90-day rodent feeding study for assessment of the safety and nutritional properties of the GM food and feed (see Table 1 of section 2.7). The aim of the study shall be to establish whether the GM food and feed is as safe and

[19] OJ L 133, 22.5.2008, p. 1.

nutritious as its traditional comparator, and to demonstrate the absence of unintended effects of toxicological concern.

Further information on the interests and limitations as regards the 90-day rodent feeding study is available in the Report of the EFSA GMO Panel Working Group on Animal Feeding Trials[20].

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

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 of GM plants that have been modified substantially include 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. In the case of insertion of multiple genes or gene cassettes, the applicant shall pay attention to the fact that 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 the applicant shall also consider that 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 or 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 applicant shall include in the testing program 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 and feed like for instance the production of new toxins.

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[20] EFSA, 2008 Report of the EFSA GMO Panel Working Group on Animal Feeding Trials, 2008. Safety and nutritional assessment of GM plants and derived food and feed. The role of animal feeding trials. Food and Chemical Toxicology 46 (2008) S2–S70

These indications are in particular obtained from the 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 that may indicate possible interruptions of known ORF 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 shall be evaluated with respect to the possible impact on the safety and/or nutritional properties of the GM plant, in particular those situations where differences of composition between the GM plant and its conventional counterpart have been observed and where equivalence cannot unambiguously be established. The applicant shall assess the information on the type and function of the test compound, 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 and feed shall be performed.

*(c) Indications of potential adverse interactions in stacked events*

In the case of stacked events, possible interactions between the expressed proteins, new metabolites and original plant constituents shall be assessed. Indications for possible adverse interactions shall be obtained from (i) the outcome of the molecular characterization, (ii) the knowledge of the mode of action of the newly expressed proteins, (iii) information on the response to combined administration of the proteins, 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

Given that 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, where appropriate, conduct testing of the whole food and feed beyond a 90-day rodent feeding study.

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

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

Supplemental information to 90-day feeding studies in rodents on the possible occurrence of 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).

Studies of this type are 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 by the applicant, 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).

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 with respect to potential consequences for the health of humans and animals and to assess their relevance for the safety of food and feed derived from the GM product. This evaluation may be supported by additional information and considerations. Information on the background variability in a given parameter may be obtained by the applicant from data from other animals of the same species/strain tested in the same or other experiments, or from internationally harmonised databases. If the change observed in a certain parameter falls within this background range of variability, further considerations shall be provided from the applicant with respect to a dose-response relationship, gender specificity, linkage with other changes, or any plausible cause.

The applicant shall in particular consider dose-response relationships in parameters that have been changed (i.e. commensurate increases in changes at increased doses) since they provide a strong indication for an effect of the tested compound. 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.

The applicant shall also identify possible inter-relationships between observed changes in single parameters which may strengthen the indication 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) potential adverse effects identified in other parts of the safety assessment have been confirmed or discarded;

(b) 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;

(c) 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;

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

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

The applicant shall evaluate the result of the toxicological assessment in the light of anticipated intake of the GM food and feed (see section 2).

**1.5. Allergenicity**

For the purposes of this Regulation, the applicant shall focus its assessment on the individuals who have a genetic predisposition to develop allergic reactions when exposed to food and feed (and pollen) derived from GMOs. The applicant shall focus its assessment on those individuals as regards sensitisation or elicitation of an allergic reaction.

The majority of the constituents responsible for allergenicity of food and feed as well as of pollen 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 including 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*

Given that 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 and that it depends upon the genetic diversity and variability in atopic humans, the applicant shall provide a cumulative body of evidence which shall minimise any uncertainty with regard to the protein(s) in question. In order to provide this body of evidence, the applicant shall follow an

integrated, stepwise, case-by-case approach in line with the recommendations of the Codex ad hoc Intergovernmental Task Force on Foods Derived from Biotechnology[21] and that allows the assessment of possible allergenicity of newly expressed proteins

The applicant shall provide a thorough characterisation of the source of the transgene enabling to identify whether it has the potential to encode for an allergen. The provided information shall specify at which stage of the development of the plant and in which 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.

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. The use of different homology searching strategies based on the sequences available in relevant databases may identify several scenarios, including a high degree of homology, with or without conservation of the allergenicity, or a low degree of homology with conservation of allergenicity.

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, shall be based on *in vitro* tests that measure the capacity of specific IgE from serum of allergic patients to bind the test protein(s).

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 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 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.

If the source is not known to be allergenic but there are consistent indications of sequence homology to a known allergen, the specific serum screening shall be

[21] Codex Alimentarius, 2003. Codex principles and guidelines on foods derived from biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organisation, Rome.

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 shall be 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, resistance of proteins to pepsin digestion is still proposed as an additional criterion to be considered in an overall risk assessment. In 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. 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. Animal models may also be 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 and feed 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. The applicant may use modern analytical tools including profiling techniques. These tools 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 including pollen of GM plants (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.

*1.5.3. Conclusion of the allergenicity assessment*

The conclusion of the allergenicity assessment shall indicate:

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

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

When there is a likelihood of allergenicity in one of the above mentioned cases, the GM food and feed shall be further characterised in the light of its anticipated intake (see section 2) and the applicant shall propose appropriate conditions for placing on the market, including labelling.

**1.6. Nutritional assessment**

The applicant shall provide nutritional evaluation:

(a) to demonstrate that introduction of the GM food and 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 and feed, as well as potential alterations in the total diet of the consumer; and,

(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, in accordance with sections 1.2 and 1.3, have not adversely affected the nutritional value of the GM food and 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 by the applicant. This may be particularly relevant where the combined expression of the newly introduced genes has unexpected effects on biochemical pathways.

The nutritional assessment of GM food and feed shall consider:

(a) the composition of the GM food and feed with regard to the levels of nutrients and anti-nutrients (see compositional studies as described in section 1.3);

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

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

When the comparative analysis has identified compositional characteristics of the GM food and 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 to the nutritional equivalence between the GM food and feed and its conventional counterpart, no further studies shall be 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.

When the applicant is required to provide a 90-day feeding study in rodents using the whole GM food and feed in accordance with section 1.4.4.1, the applicant shall also consider the information on nutritional aspects that is available since this study starts with juvenile animals in rapid growth phase that are sensitive to effects on weight gain. Further information in this respect is available in the Report of the EFSA GMO Panel Working Group on Animal Feeding Trials[22].

*1.6.1. 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 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, addressing the nutritional assessment of GM feed. 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.

[22] EFSA, 2008 Report of the EFSA GMO Panel Working Group on Animal Feeding Trials, 2008. Safety and nutritional assessment of GM plants and derived food and feed. The role of animal feeding trials. Food and Chemical Toxicology 46 (2008) S2–S70

*1.6.2. 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 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 plants 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 plant compared to its conventional counterpart and a range of conventional varieties. In the case of GM plants 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 plant. 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 shall be chosen.

When appropriate tests with various experimental designs shall be provided to demonstrate that the nutritionally improved GM plant fulfils the expected nutritional value. The exact experimental design and statistical feed approaches of feeding experiments in food producing animals to test the nutritional value of GM feed modified for enhanced nutritional characteristics shall depend on the targeted animal species, type of plant trait(s) studied and the size of the expected effect. The experimental diets shall 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 shall vary with the target species used in the study, but shall include feed intake, body weight, animal performance and bioavailability of nutrients.

Further information for the application of the requirements of this section is available in the Report of the EFSA GMO Panel Working Group on Animal Feeding Trials[23].

*1.6.3. Conclusion of the nutritional assessment*

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

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[23] EFSA, 2008 Report of the EFSA GMO Panel Working Group on Animal Feeding Trials, 2008. Safety and nutritional assessment of GM plants and derived food and feed. The role of animal feeding trials. Food and Chemical Toxicology 46 (2008) S2–S70

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

The applicant shall evaluate the result of the nutritional assessment in the light of anticipated intake of the GM food and feed (see section 2).

### 1.7. Standardised guidelines for toxicity tests

The applicant shall use for toxicity testing internationally agreed protocols and test methods described by the OECD (see Tables 1 and 2). Use of any methods that differ from such protocols shall be justified. 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.

The performance of test protocols depends on the type of GM food and 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 were developed for the assessment of risks at the workplace. (See previous sections of this Annex).

Table 1: Non-exhaustive list of validated test protocols for chemicals as described by OECD guidelines and which may be used in a possibly adapted form for GMO toxicological testing

| No. OECD | Title |
|---|---|
| 407 | Repeated Dose 28-day Oral Toxicity Study in Rodents |
| 408 | Repeated Dose 90-Day Oral Toxicity Study in Rodents |
| 410 | Repeated Dose Dermal Toxicity:21/28-Day |
| 415 | One-Generation Reproduction Toxicity |
| 416 | Two-Generation Reproduction Toxicity Study |
| 417 | Toxicokinetics |
| 421 | Reproduction/Developmental Toxicity Screening Test |
| 424 | Neurotoxicity Study in Rodents |
| 451 | Carcinogenicity Studies |
| 452 | Chronic Toxicity Studies |
| 453 | Combined Chronic Toxicity/Carcinogenicity Studies |
| 402 | Acute Dermal Toxicity |

| | |
|---|---|
| 406 | Skin Sensitisation |

Table 2: Genotoxicity tests as described by OECD guidelines[24]

| 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:<br><br>In vitro mammalian cell micronucleus test |

## 2. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

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

On the basis of representative consumption data for products derived from the respective conventional plants, the applicant shall estimate the anticipated average and maximum intake of the GM food and feed. Probabilistic methods may be used to determine ranges of plausible values rather than single values or point estimates. If possible, the applicant shall identify and consider particular sections of the population with an expected high exposure and shall within the risk assessment. Any

[24] OECD, 1995. OECD Guidelines for the Testing of Chemicals.

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 applicant shall determine by appropriate methods 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. Expected intake of these constituents shall be estimated taking into account the influences of processing, storage and expected treatment of the food and 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 and feed products, the anticipated change in total intake of this constituent shall be assessed considering realistic as well as worst case intake scenarios.

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

## 3. RISK CHARACTERISATION

### 3.1. Introduction

The applicant shall base its risk characterisation of GM plants and derived foods/feed on data from hazard identification, hazard characterisation, and on exposure/intake data. He shall ensure that the risk characterisation is comprehensive by considering all the available evidence from several analysis including molecular analysis, phenotypic, agronomical and compositional analysis, toxicity and allergenicity testing. The applicant shall consider indications resulting from the risk characterisation that may require specific activities for post-market monitoring of GM food and feed.

In performing his risk characterisation, the applicant shall demonstrate that the hazard identification and subsequent characterisation are complete. The applicant shall discuss the quality of existing data and information. The discussion shall clearly indicate how this body of information has been taken into account in the determination of the final risk characterisation.

The applicant shall provide estimations of the uncertainties associated to each test as well as to the different stages of the risk assessment. He shall quantify them through proper statistical methods as much as possible. Distinction shall be made between uncertainties that reflect natural variations in biological parameters (including variations in susceptibility in populations), and possible differences in responses between species.

Depending on the issue to be addressed and the available data, the applicant shall perform a qualitative and, where possible, quantitative risk characterisation. The conditions for the estimated risk, and associated uncertainties, shall be as precise as

possible. For instance, expressions like 'no/negligible/acceptable/significant risk' shall 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

When appropriate and depending on the type of genetic modification, the applicant shall carry out a risk assessment of GM plants in an integrative manner in accordance with section 3.1 above. This risk assessment shall be performed on a case-by-case basis depending on the modified plant and the type of genetic modification, the cultivation practices of the GM plant and uses of the GM food and feed. The applicant shall take into account the different issues considered in hazard identification and characterisation and exposure steps. The outcomes of these issues shall be considered by the applicant together in the risk characterisation step. The list of issues provided in this section shall not be exhaustive.

#### *3.2.1. Molecular characterisation*

Evaluation of the characteristics and previous use of the donor and the recipient organism shall be 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 by the applicant in relation to the intentional and possibly unintentional insertion and expression of gene sequences.

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

#### *3.2.2. Comparative analysis*

The applicant shall demonstrate that the comparative analysis between the GM plant and its conventional counterpart with respect to agronomic, morphological and compositional characteristics has been carried out according to this Regulation. The selection of the conventional counterpart and additional comparators shall be justified in particular with respect to their history of safe use.

In performing his comparative safety assessment, the applicant shall identify possible differences between the GM plant and its conventional counterpart. 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, the applicant shall perform an analysis of the uncertainties associated with the comparative analysis.

Unintended effects may result in differences or lack of equivalence 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 most conditions maybe an indicator of such an effect. Whilst sporadic differences or lack of equivalence 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 1.3.5, the applicant shall consider whether to provide the following background data and shall put these observations 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.

The applicant shall consider the range of levels observed for the compounds known to occur in the conventional counterpart and in reference 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 given 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 applicant shall specify the databases used for comparison and adequately assess them 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 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 and feed safety in relation to intake*

The applicant shall evaluate the data generated to estimate possible risks to human/animal health associated with the consumption of GM plant derived foods/feed 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 and feed are 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 applicant shall provide adequate justification as regards 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 and feed as well as the absence of specific studies (e.g. on reproductive and developmental toxicity) if applicable. Moreover when feeding trials with whole GM food and 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).

The applicant shall consider data on the characteristics of the new compounds present in the GM plant including potential biological effects in humans and animals. 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 shall be considered as safe.

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

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, the applicant shall discuss strategies for the assessment of any risk(s) associated with possible interactions between the newly expressed proteins, new metabolites and original plant constituents**.** 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, the applicant shall provide an explanation as to why these were not considered necessary.

The applicant shall evaluate data provided to assess the allergenic potential of newly expressed proteins in GM plants with respect to introduction of new allergenic proteins into the food and 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 plant derived food. In particular the choice of the test models shall be justified with respect to specificity, predictability and validation status.

With respect to intake estimations of GM foods, the applicant shall evaluate the applied methodologies 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 provided.

### 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:

– The GM food and feed has no adverse effects on human and animal health ;

– The 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 food does not mislead the consumer;

– The GM feed does not harm or mislead the consumer by impairing the distinctive features of the animal products; and,

– 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 not of a proposal for labelling in the application, in accordance with Articles 5(3)(f) and 17(3)(f) of Regulation (EC) No 1829/2003.

## ANNEX III

## POST-MARKET MONITORING OF GM FOOD/FEED –

The applicant shall submit a proposal for Post Market Monitoring (PMM):

– when the safety assessment carried out in accordance with Article 4 of this Regulation concludes that it is not possible to address remaining uncertainties by collecting additional information within the framework of the safety assessment and;

– when these uncertainties are too high with respect to the following aspects:

(i) the food/feed consumption is difficult to predict or it is necessary to ensure that specific recommendations of uses are followed by the consumer;

(ii) the relevance and intensity of effects and side-effects detected during the pre-market risk assessment are difficult to be predicted, and;

(iii) potential side effects are identified but can not be studied in the framework of the safety assessment.

The applicant shall in particular consider the submission of a proposal for a PMM when the GM (functional) food/feed has altered nutritional composition and its nutritional value differs from the conventional food/feed that it would replace.

The applicant shall design the PMM to collect reliable information with respect to one or several of the three aspects outlined above. The PMM shall be based on strategies aiming at collecting relevant information from specific stakeholders including consumers and on a reliable and validated flow of information between the different stakeholders. The applicant shall also submit more specific strategies when estimations of individual intakes of a specific food item or intakes of particular age groups have to be collected. The applicant shall ensure that collected information shall allow to detect indications on whether any (adverse) effect on health may be related to GM food and feed consumption. The applicant shall provide adequate justification and a thorough description of the selected design of the proposed PMM including aspects related to the analysis of the collected information.

# ANNEX IV

# VALIDATION OF METHODS OF DETECTION, SAMPLING, IDENTIFICATION AND REFERENCE MATERIAL

## 1. INTRODUCTION

1. For the purposes of implementing Articles 5(3)(i) and 17(3)(i) of Regulation (EC) No 1829/2003, this Annex shall provide requirements on the performance characteristics of the submitted method as well as technical provisions on the type of information for the applicant to present so as to verify that these requirements are met. This includes information about the method as such and about the method testing carried out by the applicant.

2. The applicant shall also consider further information about the operational procedures of the validation process that is made available by the CRL, assisted by the European network of GMO laboratories.

## 2. DEFINITIONS

For the purpose of this annex, the following definitions shall apply:

1. 'reference material' (RM) means reference material as referred to in Articles 5(3)(j) and 17(3)(j) of Regulation (EC) No 1829/2003 and corresponds to any material or substance, one or more of whose property values are sufficiently homogenous and well-established to be used for calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials;

2. 'certified reference material' (CRM) means reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes its traceability to an accurate realisation of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence;

3. ‘method performance requirements’ means the minimum performance criteria that the method shall demonstrate upon completion of a validation study carried out by the CRL according to internationally accepted technical provisions and this in order to certify that the method validated is fit for the purposes of enforcing Regulation (EC) No 1829/2003.

## 3. METHOD VALIDATION

### 3.1. Information about the method

A. The method shall refer to all the methodological steps needed to analyse the relevant material in accordance with Articles 5(3)(i) and 17(3)(i) of Regulation (EC) No 1829/2003.

For a particular material this shall include the methods for DNA extraction and the subsequent quantification in a polymerase chain reaction (PCR) system. In such a case, the whole process from extraction up to the PCR-technique (or equivalent) shall constitute a method. The applicant shall provide information about the whole method.

B. The applicant shall be allowed to refer to existing methods for a certain module(s), if available and appropriate such as a DNA extraction method from a certain matrix. In such a case, the applicant shall provide experimental data from an in-house validation in which the method module has been successfully applied in the context of the application for authorisation.

C. The applicant shall demonstrate that the method fulfils the following requirements.

1. The method shall be event-specific and thus shall only be functional with the GMO or GM based product considered and shall not be functional if applied to other events already authorised; otherwise the method cannot be applied for unequivocal detection/identification/quantification. This shall be demonstrated with a selection of non-target transgenic authorised events and conventional counterparts, in the case of GM plants. This testing shall include closely related events, where relevant, and cases where the limits of the detection are truly tested.

2. The method shall be applicable to samples of the food or feed, to the control samples and to the reference material, which is referred to in Articles 5(3)(j) and 17(3)(j) of Regulation (EC) No 1829/2003.

3. The applicant shall take into consideration the following documents for the development of the detection method:

– Foodstuffs -- Methods of analysis for the detection of genetically modified organisms and derived products - General requirements and definitions: ISO 24276:2006,

– Foodstuffs -- Methods of analysis for the detection of genetically modified organisms and derived products - Nucleic acid extraction: ISO 21571:2005,

– Foodstuffs -- Methods of analysis for the detection of genetically modified organisms and derived products Quantitative nucleic acid based methods: ISO 21570:2005,

– Foodstuffs -- Methods of analysis for the detection of genetically modified organisms and derived products Protein based methods: ISO 21572:2004,

– Foodstuffs -- Methods of analysis for the detection of genetically modified organisms and derived products Qualitative nucleic acid based methods: draft European standard ISO 21569:2005.

4. The method shall also take into consideration the more detailed requirements set out in the common criteria set by the CRL and ENGL for minimum performance requirements for analytical methods for GMO testing[25].

D. For the purpose of implementing Articles 5(3)(i) and 17(3)(i) of Regulation (EC) No 1829/2003, the applicant shall provide:

(a) in the case of an application for authorisation covering GM food or feed containing or consisting of a GMO, the event-specific quantitative detection method of the GM material; and,

(b) in the case of an application for authorisation covering GM food or feed produced from a GMO where the genetically modified material is detectable, the event-specific quantitative detection method in the GM foods or feeds produced from the GMO. The applicant shall discuss the validity and limitations of the detection methods in the various types of foods and feeds (the various matrixes) that are expected to be placed on the market.

E. The applicant shall provide a complete and detailed description of the method. The following points shall be clearly addressed:

1. Scientific basis: The applicant shall provide an overview of the principles of how the method works. This overview shall include references to relevant scientific publications.

2. Scope of the method: The applicant shall indicate the matrix(es) (e.g. processed food, raw materials), the type of samples and the percentage range to which the method may be applied.

3. Operational characteristics of the method: The required equipment for the application of the method shall be clearly mentioned, with regard to the analysis *per se* and the sample preparation. Further information of any specific aspects crucial for the application of the method shall also be mentioned here.

4. Protocol: The applicant shall provide a complete optimised protocol of the method. The protocol shall present all the details as required to transfer and apply the method independently in other laboratories. Further guidance regarding the submission of this information is provided by the CRL.

5. The prediction model (or alike) needed to interpret results and to make inferences shall be described in full details. Instructions for the correct application of the model shall be provided.

25 http://gmo-crl.jrc.ec.europa.eu/doc/Min_Perf_Requir_Analyt_methods_131008.pdf

### 3.2. Information about the method testing carried out by the applicant

A. The applicant shall provide all the available and relevant data of the method optimisation and testing carried out. These data and results shall be presented, where possible and appropriate, by using the performance parameters as referred to under section 1.2. The applicant shall also provide a summary of the testing carried out and the main results as well as all the data including the outliers.

B. The applicant shall ensure that the provided information demonstrates the robustness of the method for inter-laboratory transferability. For this purpose, he shall provide the results of the testing of the method by at least one laboratory that is independent from the laboratory which has developed the method.

C. The applicant shall provide the following information required about the method development and the method optimisation:

1. primer pairs tested (in the case of a PCR-based test), including a justification as to how and why the proposed primer pair has been selected;

2. stability testing, which shall be established through the submission of experimental results from testing the method with different plant varieties;

3. specificity, which shall be established through the submission of the full sequence of the insert(s), together with the base pairs of the host flanking sequences so as to enable the CRL to assess the specificity of the proposed method by running homology searches in a molecular database.

D. The applicant shall, besides the information requested in the previous sections of this annex, provide the following information regarding the testing:

– participating laboratories, time of the analysis and outline of the experimental design, including the details about the number of runs, samples, replicates etc.,

– description of the laboratory samples (e.g. size, quality, date of sampling), positive and negative controls as well as reference material, plasmids and alike used,

– description of the approaches that have been used to analyse the test results and outliers,

– any particular points observed during the testing,

– references to relevant literature or technical provisions used in the testing.

### 3.3. Samples of the food and feed and their control samples

In view of implementing Articles 5(3)(j) and 17(3)(j) of Regulation (EC) No 1829/2003, the applicant shall, together with the information specified under sections 1, 2 and 3 of this Annex, also provide samples of the food and feed and their control samples of a type and amount to be specified by the CRL for the specific application for authorisation.

## 4. REFERENCE MATERIAL

The reference material shall be produced under ISO Guide 34 (laying down the general requirements for the competence of reference material producers) by a producer accredited to ISO Guide 34.

The applicant shall provide information as regards the place where the reference material can be accessed. This shall be accompanied by adequate information demonstrating that the availability of the reference material will be maintained throughout the period of validity of the authorisation.

The reference material shall be produced from a single genotype. Breeding information describing the parental origin of the genetic modification shall be provided.

For verification and value assignment, a method that has been properly validated (see ISO/IEC 17025:5.4.5) shall be used. Uncertainties have to be estimated according to the ISO Guide to the Expression of Uncertainty in Measurement (GUM). Main characteristics of these internationally accepted technical provisions are provided in the following sections.

1. GM RM containers:

   – GM RM container (bottles, vials, ampoules, etc.) shall be tight and contain not less than the stated amount of material,

   – samples shall have appropriate homogeneity and stability,

   – the commutability of the GM RM has to be assured,

   – packaging shall be appropriate to the purpose,

   – labelling shall be of good aspect and quality.

2. Homogeneity testing:

   – between-bottle homogeneity shall be examined;

   – any possible between-bottle heterogeneity shall be accounted for in the overall estimated RM uncertainty. This requirement shall apply even when no statistically significant between-bottle variation is present. In this case, the method variation or the actual calculated between-bottle variation (whichever is larger) shall be included in the overall uncertainty;

3. Stability testing:

   – Stability shall be positively demonstrated by appropriate statistical extrapolation for the GM RM shelf-life to be within the stated uncertainty; the uncertainty related to this demonstration is part of the estimated RM uncertainty. Assigned values are valid only for a limited time and shall be subject to a stability monitoring.

4. Batch characterisation:

   The methods used for verification and for certification shall:

   – be applied under metrologically valid conditions,

   – have been properly technically validated before use,

   – have precision and accuracy compatible with the target uncertainty.

   Each set of measurements shall:

   – be traceable to the stated references, and

   – be accompanied by an uncertainty statement whenever possible.

   Participating laboratories shall:

   – have the required competence for the execution of the task,

   – be able to achieve traceability to the required stated references,

   – be able to estimate its measurement uncertainty,

   – have in place a sufficient and appropriate quality assurance system.

5. Final storage:

   – To avoid a posteriori degradation, all samples shall be stored under conditions designated for the final storage of the GM RM before measurements are started,

   – Otherwise, they shall be transported from door to door keeping them at all times under such storage conditions for which it has been demonstrated that there is no influence on the assigned values.

6. Establishment of a certificate for CRMs:

   – A certificate complemented by a certification report shall be established, containing all information relevant to and needed by the user. The certificate and report shall be made available when the GM CRM is distributed,The certified value of the quantity of GM material shall be given in mass fraction. It may be complemented by a value in GM-DNA copy number in relation to target taxon specific copy numbers calculated in terms of haploid genomes.

– The certificate shall mention the parental origin of the genetic modification (either paternal and maternal or only paternal or only maternal) as verified experimentally during the certification.

– Certified values (e.g. quantity of GM material expressed in mass fraction shall be traceable to stated references and be accompanied by an expanded uncertainty statement valid for the entire shelf-life of the GM CRM.