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# UPDATED GUIDANCE DOCUMENT FOR THE RISK ASSESSMENT OF GENETICALLY MODIFIED PLANTS AND DERIVED FOOD AND FEED

**Draft document adopted in May 2008**

Prepared by the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority

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## ABOUT EFSA GUIDANCE

The GMO Panel regularly reviews its guidances in the light of experience gained, technological progress and scientific developments.

The EFSA Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Organisms and Derived Food and Feed, adopted by the GMO Panel on 24 September 2004, has been further completed with a chapter on General surveillance of unanticipated effects of the GM Plant as part of the post market environmental monitoring, which was adopted on 7 December 2005 and published in May 2006.

This guidance is now being updated by the GMO Panel in accordance with the experience gained during the risk assessment of the applications, the outcome of self tasking activities and additional guidance on stacked events. Further update of the Environmental risk assessment is foreseen in the next two years partly in response to the mandate from DG Environment of European Commission[1] and partly based on the outcome of EFSA's self tasking activities.



[1] (EFSA-Q-2008-262)

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# FOREWORD

Genetic modification, genetic engineering or recombinant-DNA technology, first applied in the 1970's, is one of the newest methods to introduce novel traits to micro-organisms, plants and animals. Unlike other genetic improvement methods, the application of this technology is strictly regulated. Before any Genetically Modified Organism (GMO) or product can be released into the EU market, it has to pass an approval system in which the safety for humans, animals and the environment is thoroughly assessed. The Regulation (EC) No 1829/2003 on genetically modified food and feed, which applies from April 18, 2004, provides that the European Food Safety Authority (EFSA) shall publish detailed guidance to assist the applicant in the preparation and presentation of the application for the authorisation of Genetically Modified (GM) food and/or feed. The assessment of the genetic modification itself complements, but does not replace, other requirements, as set in specific legislation (e.g. seed or other plant-propagating materials), that a product has to fulfill in order to be approved for the European market.

The EFSA Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Organisms (GMO Panel) and Derived Food and Feed, adopted by the GMO Panel on 24 September 2004, has been further completed with a new chapter 11.4 on General surveillance of unanticipated effects of the GM Plant as part of the post market environmental monitoring, which was adopted on 7 December 2005 (EFSA, 2006c).

This guidance is now being updated by the GMO Panel in accordance with the experience gained during the risk assessment of the dossiers, the outcome of self tasking activities and additional guidance on stacked events. Further update of the Environmental risk assessment is foreseen in the next two years partly in response to the mandate from DG Environment of European Commission[2] and partly based on the outcome of EFSA's self tasking activities.

The Guidance was developed by the GMO Panel of 2003-2006 of EFSA, consisting of the following members:

Christer Andersson, Detlef Bartsch, Hans-Joerg Buhk, Howard Davies, Marc De Loose, Michael Gasson, Niels Hendriksen, Colin Hill, Sirpa Kärenlampi, Ilona Kryspin-Sørensen, Harry Kuiper, Marco Nuti, Fergal O'Gara, Pere Puigdomenech, George Sakellaris, Joachim Schiemann, Willem Seinen, Angela Sessitsch, Jeremy Sweet, Jan Dirk van Elsas and Jean-Michel Wal.

The following ad hoc experts also contributed:

Gerhard Flachowsky, Tony Hardy, Andreu Palou and Richard Phipps.

[2] (EFSA-Q-2008-262)

The present draft document provides detailed update of this guidance by the GMO Panel of 2006-2009 of EFSA, consisting of the following members:

Hans Christer Andersson, Salvatore Arpaia, Detlef Bartsch, Josep Casacuberta, Howard Davies, Lieve Herman, Gijs Kleter, Marc de Loose, Niels Hendriksen, Sirpa Kärenlampi, Jozsef Kiss, Ilona Kryspin-Sørensen, Harry Kuiper, Ingolf Nes, Nickolas Panopoulos, Joe Perry, Annette Pöting, Joachim Schiemann, Willem Seinen, Jeremy Sweet, and Jean-Michel Wal.

The following ad hoc experts also contributed:

Boot Glandorf, Hans Jorg Buhk, Patrick du Jardin, Philippe Vain, Gerhard Flachowsky and Thomas Frenzel.

The draft updated document was published on 16 June 2008. EFSA will regularly review this guidance in the light of experience gained, technological progress and scientific developments. By establishing a harmonised framework for risk assessment, this document should provide useful guidance both for the applicants and risk assessors. A thoroughly prepared application and properly conducted risk assessment should facilitate the scientific evaluation of the product.

## TERMS OF REFERENCE

In accordance with Articles 5(8) and 17(8) of the Regulation (EC) No 1829/2003 on genetically modified food and feed, the European Commission has requested the European Food Safety Authority (EFSA), in a letter dated 27 October 2003 (ref. SANCO/D4/KM/cw/D/440551), to publish detailed guidance – before the date of application of the Regulation on GM food and feed which is 18 April 2004 – to assist the applicant in the preparation and the presentation of the application for authorisation of GM food and/or feed.

## MANDATE OF EFSA AND THE GMO PANEL

In accordance with Regulation (EC) No 178/2002 (EC, 2002c), EFSA shall provide scientific advice and scientific technical support for the Community's legislation and policies in all fields which have a direct or indirect impact on food and feed safety. It shall provide independent information on all matters within these fields and communicate on risks. EFSA shall contribute to a high level of protection of human life and health, and in this respect take account of animal health and welfare, plant health and the environment, in the context of the operation of the internal market.

The GMO Panel deals with questions on GMOs as defined in Directive 2001/18/EC (EC, 2001a), such as micro-organisms, plants and animals, relating to the deliberate release

into the environment and GM food and feed including their derived products (EFSA, 2002).

# I. INTRODUCTION

## 1. SCOPE OF THE DOCUMENT

This document provides guidance for the risk assessment of GM plants[3] and/or derived food and feed submitted within the framework of Regulation (EC) No 1829/2003 (EC, 2003a) on GM food and feed. The guidance also applies to feed intended for animals which are not destined for food production. When a product is likely to be used both for food and feed purposes, the application should fulfil the requirements for both food and feed. The document also provides guidance on the drawing up of Annex III B of the Directive 2001/18/EC on the deliberate release into the environment of GMOs (EC, 2001a) or in the preparation of the conclusion of environmental risk assessment as stated in Annex II paragraph D.2 of that Directive and in the set up of an environmental monitoring plan according to Annex VII, without prejudice to the Decisions 2002/623/EC (EC, 2002a), 2002/811/EC (EC, 2002b), 2002/812/EC (EC, 2002e) and 2003/701/EC (EC, 2003e) established within the framework of Directive 2001/18/EC. Therefore this document provides guidance for the full risk assessment of GM plants and derived food and feed. However, not all requirements of the guidance document may be applicable for all products (*e.g.* derived food and feed products, non-food/feed plants).

This Updated Guidance Document of the GMO Panel on the risk assessment of GM plants and/or derived food and feed will be a replacement of the 'Guidance document for the risk assessment of genetically modified plants and derived food and feed' of May 2006 (EFSA 2006).

This guidance document provides detailed guidance to assist the applicant in the preparation and the presentation of the application, according to Articles 5(8) and 17(8) of Regulation (EC) No 1829/2003. This document addresses the requirements of the Regulation (EC) No 1829/2003 and is structured according to the requirements set out in Articles 5(5)(a) and (b) and 17(5)(a) and (b) of the Regulation (EC) No1829/2003 for GMOs or food/feed containing or consisting of GMOs, i.e. taking into account Annexes IIIB, IID2 and VII of Directive 2001/18/EC. Specific guidance on the presentation of the application can be found in the Annexes to this document.

Food additives (EC, 2008, EC, 1989), flavourings (EC, 1988) and feed additives (EC, 2003c) containing, consisting of, or produced from GM plants fall within the scope of this guidance document.

[3] In the context of this document "genetically modified plants" are defined as genetically modified higher plants, (Gymnospermae and Angiospermae) in line with Directive 2001/18/EC.

This guidance does not consider issues related to risk management (traceability, labelling, co-existence). Socio-economic and ethical issues are also outside the scope of this guidance.

This guidance does not cover the deliberate release into the environment (Directive 2001/18/EC) of GMOs for experimental purposes (Part B notifications). Nor does it cover the contained use of genetically modified micro-organisms (GMMs) (Directive 90/219/EEC; EC, 1990a; EC, 1998), or the placing on the market of food and/or feed consisting of, containing, or produced from GMMs (Regulation (EC) No 1829/2003). For food and feed containing, consisting of or produced from GMMs, a parallel guidance document is provided by the GMO Panel (EFSA, 2006b).

## 2. LEGAL BACKGROUND FOR THE RISK ASSESSMENT OF GMOS, GM FOOD AND GM FEED AT COMMUNITY LEVEL

The EU Regulations, Directives and Decisions published in the Official Journal of the European Communities establish the procedures to be followed in seeking approval for GMOs as well as the requirements for the applications and are, therefore, always the primary source of advice.

### General food law (Regulation (EC) No 178/2002)

Regulation (EC) 178/2002 (EC, 2002c) lays down the general principles of food law and procedures in food safety including the tasks of EFSA. It defines food law broadly, including animal feed and other agricultural inputs at the level of primary production. In the general food law 'food' means any substance or product, whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be ingested by humans. 'Food' includes any substance intentionally incorporated into the food during its manufacture, preparation or treatment. 'Feed' means any substance or product, including additives, whether processed, partially processed or unprocessed, intended to be used for oral feeding to animals. The general food law defines 'hazard', 'risk', 'risk analysis', 'risk assessment', 'risk management' and 'risk communication'[4].

[4]
- 'Hazard' means a biological, chemical or physical agent in, or conditions of, food or feed with the potential to cause an adverse health effect.
- 'Risk' means a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard.
- 'Risk analysis' means a process consisting of three interconnected components: risk assessment, risk management and risk communication.
- 'Risk assessment' means a scientifically based process consisting of four steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation.
- 'Risk management' means the process, distinct from risk assessment, of weighing policy alternatives in consultation with interested parties, considering risk assessment and other legitimate factors, and, if need be, selecting appropriate prevention and control options.
- 'Risk communication' means the interactive exchange of information and opinions throughout the risk analysis process as regards hazards and risks, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, feed and food businesses, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

Articles 14 and 15 of the general food law set the food and feed safety requirements, respectively, in order to determine whether any food or feed is injurious to health.

### GM food and feed regulation (Regulation (EC) No 1829/2003)

According to Regulation (EC) No 1829/2003, GM food and feed should only be authorised for placing on the market after a scientific assessment of any risks which they might present for human and animal health and, as the case may be, for the environment. GM food and feed mean GMOs for food/feed use; food/feed containing or consisting of GMOs; food/feed produced from GMOs; and food containing ingredients produced from GMOs. Food products containing, consisting of, or produced from GMOs were previously regulated by Regulation (EC) No 258/97 on novel foods and novel food ingredients, which has been amended by Regulation (EC) No 1829/2003. For feed containing or consisting of GMOs, no specific Community legislation has been in place prior to the entering into force of this Regulation, the safety of GM feed being assessed under Directive 90/220/EEC (repealed by Directive 2001/18/EC). Articles 8 and 20 of Regulation (EC) No 1829/2003 establish transitional measures for existing products. Food and feed which have been lawfully placed on the EU market before 18 April 2004 continued to be allowed on the market, used and processed provided that they were notified to the Commission before 18 October 2004.

The Regulation requires that GM food/feed must not (a) have adverse effects on human health, animal health or the environment; (b) mislead the consumer/user; (c) differ from the food/feed which it is intended to replace to such an extent that its normal consumption would be nutritionally disadvantageous for the consumer/animals. In addition, GM feed must not harm or mislead the consumer by impairing the distinctive features of the animal products. Products can only be authorised by risk managers once the applicant has adequately demonstrated that the product satisfies these requirements. All these points have to be considered within the scientific risk assessment and applicants have to provide reliable, up to date and comprehensive data.

An application should be accompanied by the particulars specified by Article 5(3) and/or Article 17(3) of the Regulation for GM food and feed, respectively. The European Commission has established implementing rules for the application of these Articles, including rules concerning the preparation and the presentation of the application (Regulation (EC) No 641/2004; EC, 2004b).

The application shall be submitted to the national competent authority of a Member State, who makes it available to EFSA. EFSA then makes the application available to the other Member States and the Commission, and makes a summary of the application available to the public[5]. EFSA is responsible for the scientific assessment of the application. EFSA may ask the appropriate food/feed assessment body of a Member State to carry out a safety assessment of the food/feed in accordance with Article 36 of Regulation (EC) No 178/2002. EFSA may also ask a competent authority designated in

[5] http://www.efsa.europa.eu/EFSA/ScientificPanels/GMO/efsa_locale-1178620753812_GMOApplications.htm

accordance with Article 4 of Directive 2001/18/EC to carry out an environmental risk assessment. However, if the application concerns GMOs to be used as seeds or other plant-propagating material, EFSA shall ask a national competent authority under Directive (No) 2001/18 to carry out the environmental risk assessment that will be considered by EFSA during its final assessment.

From the receipt of a valid application, EFSA shall endeavour to comply with a time limit of six months to provide its opinion. The clock will be stopped whenever EFSA or the Commission's Community Reference Laboratory (CRL) seeks supplementary information from the applicant.

Taking into account the EFSA overall opinion, the Commission shall submit to the Standing Committee on the Food Chain and Animal Health a draft decision within three months of receipt of the overall opinion. A final decision shall be adopted in accordance with the Committee procedure. The authorisation is valid throughout the Community for a maximum of 10 years, after which a renewal of authorisation is required. The authorised product will have to comply with the provisions of Regulation (EC) No 1830/2003 concerning the traceability and labelling of GMOs and the traceability of food and feed products produced from GMOs (EC, 2003b). The authorised product shall be entered in a Community Register of GM food and feed, which is available to the public. Where appropriate, and based on the conclusions of the risk assessment, post-market monitoring requirements for the use of GM foods for human consumption or GM feeds for animal consumption may be imposed by the risk manager.

### Deliberate release of GMOs (Directive 2001/18/EC)

The principles regulating the deliberate release into the environment of GMOs are laid down in Directive 2001/18/EC (EC, 2001a) of the European Parliament and of the Council, which repeals Council Directive 90/220/EEC (EC, 1990b). This Directive puts in place a step-by-step approval process made on a case-by-case assessment of the risk to human/animal health and the environment before any GMOs can be released into the environment, or placed on the market as, or in, products. According to this Directive, the step-by-step principle means that the containment of GMOs is reduced and the scale of release increased gradually, but only if assessment of the earlier steps indicates that the next step can be taken.

Part B of the Directive deals with the deliberate release of GMOs for any other purpose than for placing on the market (e.g. field trials). For these releases, a notification must be submitted to the competent authority of the Member State within whose territory the release is to take place. The applicant may proceed with the release after receiving a written consent of the competent authority. A format for presenting the results of the release is established by Commission Decision 2003/701/EC (EC, 2003e).

Part C of the Directive stipulates the criteria to be fulfilled prior to the decision of placing on the market a GMO as, or in, products. The applicant must submit its application to the competent authority of the Member State where the GMO is to be placed on the market for the first time. The application must include a risk assessment. Annex III B of the Directive details the required information on which to base the risk assessment for higher plants. The principles for the environmental risk assessment, including aspects of

human and animal health, are laid down in Annex II of the Directive. Several supporting documents have been prepared to assist the applicant. Commission Decision 2002/623/EC (EC, 2002a) establishes guidance notes on the objective, elements, general principles and methodology of the environmental risk assessment referred to in Annex II to Directive 2001/18/EC. Council Decision 2002/811/EC (EC, 2002b) establishes guidance notes supplementing Annex VII to the Directive, describing the objectives and general principles to be followed to design the monitoring plan. Council Decision 2002/812/EC (EC, 2002e) establishes the summary information format. The EU Scientific Steering Committee published on March 2003 the 'Guidance document for the risk assessment of genetically modified plants and derived food and feed' prepared by the Joint Working Group on Novel Foods and GMOs (EC, 2003d). The guidance document of the GMO Panel and its updates replaced that guidance.

If the national competent authority gives a favourable opinion on the GMO, this Member State must inform the Commission and other Member States. If no objections are raised either by the Commission or by a competent authority, or if outstanding issues are resolved within the 105 days period, the assessor Member State grants an authorisation and the product may then be marketed throughout the Community. If, however, any objections are raised and maintained, a decision has to be taken at Community level. If an objection relates to risks of the GMO to human/animal health or to the environment, the Commission must then consult EFSA.

The Directive also introduces the obligation to propose a monitoring plan in order to trace and identify any direct or indirect, immediate, delayed or unforeseen effects on human/animal health or the environment of GMOs as, or in, products after they have been placed on the market[6]. The Directive also introduces a time limit for the authorisation, which cannot be given for more than 10 years. Authorisations can be renewed on the basis of an assessment of the results of the monitoring and of any new information regarding the risks to human/animal health and/or the environment.

### Interplay between Regulation (EC) No 1829/2003 and Directive 2001/18/EC

It is necessary for the environmental risk assessment to comply with the requirements referred to in Directive 2001/18/EC. In case of food and/or feed containing or consisting of GMOs, the applicant has the choice of either supplying an authorisation for the deliberate release into the environment already obtained under part C of Directive 2001/18/EC, without prejudice to the conditions set by that authorisation, or of applying for the environmental risk assessment to be carried out at the same time as the safety assessment under Regulation (EC) No 1829/2003.

### Interplay between Directive 2001/18/EC and Directive 91/414/EEC

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- 'Direct effects' refer to primary effects which are a result of the GMO itself and which do not occur through a causal chain of events.
- 'Indirect effects' refer to effects occurring through a causal chain of events, through mechanisms such as interactions with other organisms, transfer of genetic material, or changes in use or management.
- 'Immediate effects' refer to effects which are observed during the period of the release of the GMO.
- 'Delayed effects' refer to effects which become apparent either at a later stage or after termination of the release.

The risk assessment of plant protection products used directly in the cultivation of crop plants, including GM plants, falls within the scope of Directive 91/414/EEC (EC, 1991). The changes in management of the GM plants including, where applicable, changes in agricultural practices are considered under Directive 2001/18/EC.

### GM seeds and other plant-propagating material

GM varieties shall only be accepted for inclusion in a national catalogue according to Directive 2002/53/EC (EC, 2002f) and 2002/55/EC (EC, 2002g) after having been accepted for marketing in accordance with Directive 2001/18/EC (90/220/EEC) which ensures that all appropriate measures have been taken to avoid adverse effects on human/animal health or the environment of the release into the environment of the GM variety.

If the application concerns GM plants to be used as seeds or other plant-propagating material falling within the scope of Regulation (EC) 1829/2003 and the applicant has chosen to apply for the environmental risk assessment under the above mentioned Regulation, EFSA shall, in order to prepare its opinion, ask a national competent authority designated in accordance with Directive 2001/18/EC to carry out an environmental risk assessment.

When material derived from a plant variety is intended to be used in food or feed falling within the scope of Regulation (EC) No 1829/2003, the variety shall be accepted for inclusion in the common catalogue of varieties only if it has been approved in accordance with this Regulation.

Authorisations under Regulation (EC) No 1829/2003 should be without prejudice to the provisions of the Directives providing rules and the criteria for the acceptance of varieties and their official acceptance for inclusion in common catalogues and should not affect the provisions of the Directives regulating in particular the certification and the marketing of seeds and other plant-propagating materials.

### Additives and flavourings for use in foodstuffs

The authorisation of food additives is regulated by Directive 89/107/EC on the approximation of laws of the Member States concerning food additives authorised for use in foodstuffs intended for human consumption (EC, 1989). Flavourings are regulated by Directive 88/388/EEC on the approximation of the laws of the Member States relating to flavourings for use in foodstuffs and to source materials for their production (EC, 1988). In addition, food additives and flavourings containing, consisting of, or produced from, GMOs fall within the scope of Regulation (EC) 1829/2003 for the safety assessment of the genetic modification.

### Feed additives and certain products used in animal nutrition

The placing on the market of feed additives was authorised by Directive 70/524/EEC (EC, 1970) which, from 18 October 2004, was repealed by the Regulation (EC) No 1831/2003 on additives for use in animal nutrition (EC, 2003c) and the decision on detailed rules for its implementation (EC, 2008). In addition, feed additives containing,

consisting of, or produced from, GMOs fall within the scope of Regulation (EC) 1829/2003 for the safety assessment.

Directive 82/471/EEC concerning certain products used in animal nutrition (EC, 1982) provides for an approval procedure for feed materials produced using different technologies that may pose risk to human or animal health and the environment. If these products contain, consist of, or are produced from, GMOs they fall within the scope of Regulation (EC) No 1829/2003 instead.

Interplay between Regulation (EC) No 1829/2003 and legislation on additives and flavourings for use in foodstuffs, feed additives and certain products used in animal nutrition

Where a GM plant is used as the source of a product, the applicant should follow the specific legislation and the corresponding guidelines, if available. Guidelines are presently available for food additives (SCF, 1992; 2001a, b) and feed additives (EC, 2008, EC, 2001c; SCAN, 2001). To facilitate the assessment of the genetic modification, the applicant should follow the relevant parts of the present guidance document.

# II. PRINCIPLES AND STRATEGIES FOR RISK ASSESSMENT OF GENETICALLY MODIFIED ORGANISMS

## 1. INTRODUCTION

Identification, characterisation and handling of risk(s) should follow a structured approach, which is called risk analysis (risk governance), and which consists of three basic elements: ***risk assessment, risk management*** **and** ***risk communication*** *(EC 2000a, Codex Alimentarius 2001).*

- **Risk assessment** can be described as "a process of evaluation including the identification of the attendant uncertainties, of the likelihood and severity of an adverse effect(s)/event(s) occurring to man or the environment following exposure under defined conditions to a risk source(s)"(*EC 2000a).* A risk assessment comprises four steps: *hazard identification, hazard characterisation, exposure assessment and the* integrative *risk characterisation* (EC, 2000a, Codex Alimentarius, 2001). The information required to structure the risk assessment process is further detailed in Chapter IIIB-IIID. The risk assessment is a scientific exercise.

- **Risk management** is the process of weighing policy alternatives in the light of the result of a risk assessment(s) and of other relevant evaluations, and, if required, of selecting and implementing appropriate control options (including, where appropriate, monitoring/surveillance activities).

- **Risk communication** is the interactive exchange of information and opinions throughout the risk analysis process concerning risk. It should involve not only risk assessors and risk managers, but also consumers and a wide range of other actual or potential stakeholders.

The terms hazard and risk are often interchangeably used, but have different meanings. The term **hazard** is associated with the **potential** of an agent or situation to cause an adverse effect(s)/event(s). It refers to an inherent property of that agent or situation. **Risk** is recognised as a function of the probability and severity of an adverse effect/event occurring to human and animal or the environment following exposure to a hazard, under defined conditions.

An extensive overview of risk assessment procedures is provided by the Scientific Steering Committee of the European Commission (SSC, 2000; 2003), and a detailed strategy for risk assessment of foods derived from GM plants has been described by the European Network on Safety Assessment of Genetically Modified Food Crops (ENTRANSFOOD, 2004), for chemicals in food and diet by Food Safety in Europe (FOSIE, 2002; 2003), and for environmental risk assessment by the EU (EC, 2002a).

Risk assessment of a GMO involves generating, collecting and assessing information on a GMO and its derived food/feed in order to determine its impact on human/animal health and the environment relative to non-GMO's, and thus its relative safety. In order to carry out the risk assessment sufficient scientific data must be available in order to arrive at qualitative/quantitative risk estimates. The final risk characterisation should result in informed qualitative, and if possible quantitative, advice to risk managers. It should explain clearly what assumptions have been made during the risk assessment, and what is the nature and magnitude of uncertainties associated with establishing these risks.

## 2. COMPARATIVE APPROACH FOR THE RISK ASSESSMENT OF GM PLANTS

The risk assessment strategy for GMOs seeks to deploy appropriate methods and approaches to compare the GMO and derived products with their non-GM comparators. The underlying assumption of this comparative assessment approach for GM plants is that traditionally cultivated crops have a history of safe use and familiarity for the normal consumer or animal and the environment. These crops can serve as a baseline for the environmental and food/feed safety assessment of GMOs. To this end the concepts of *familiarity* and *substantial equivalence* were developed by the OECD (OECD, 1993a; OECD, 1993b) and further elaborated by WHO/FAO (WHO/FAO, 2000) for the assessment of the environmental and food safety of GMOs, respectively. The risk assessment starts with the comprehensive characterisation of the intended effect of the genetic modification. This is followed by the comparative analysis of the molecular, agronomic and compositional characteristics of the organisms in question. This comparison is the *starting point* of the risk assessment which then focuses on the environmental or food/feed safety and nutritional impact of any intended or unintended differences identified.

### *2.1 Concept of familiarity*

The concept of familiarity is based on the fact that most GM plants are developed from crop plants, the biology of which is well researched. In a risk assessment it is appropriate to draw on this previous knowledge and experience and to use the non-GM crop as the comparator to the GM crop in order to highlight differences associated with the genetic modification and the subsequent management of the GM crop. Familiarity will also derive from the knowledge and experience available from conducting a risk analysis prior to scale-up of any new plant line or crop cultivar in a particular environment (OECD, 1993a), and from previous applications for similar constructs and traits in similar or different crops. The risk assessment should clearly identify any differences between the GM and non-GM plant, and focus on the significance and implications of these differences.

### *2.2 Concept of substantial equivalence or comparative safety assessment*

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

Where no comparator can be identified, a comparative safety assessment cannot be made and a comprehensive safety and nutritional assessment of the GM crop derived food/feed *per se* should be carried out. For instance, this could be the case where a trait or traits are introduced with the intention of modifying the composition of the plant significantly.

### *2.3* Intended and unintended effects

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

**Intended effects** are those that are targeted to occur from the introduction of the gene(s) in question and which fulfil the original objectives of the genetic modification process. Alterations in the phenotype may be identified through a comparative analysis

of growth performance, yield, disease resistance, etc. Intended alterations in the composition of a GM plant compared to the conventional comparator, *e.g.* the parent, may be identified by measurements of *single* compounds *e.g.* newly expressed proteins, macro- and micro-nutrients (*targeted* approach). Analytical methods used must meet specific quality and validation criteria.

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

# 3. ENVIRONMENTAL RISK ASSESSMENT AND MONITORING

The risk of environmental damage[7] (EC, 2004c; ACRE, 2002b) caused by a GM plant and its management requires evaluation in comparison with current non-GM

[7] According to Directive 2004/35/EC on environmental liability (EC 2004c), environmental damage relates to effects on

- protected species and natural habitats, which is any damage that has significant adverse effects on reaching or maintaining the favourable conservation status of such habitats or species. The significance of such effects is to be assessed with reference to the baseline condition, taking into account specific criteria listed in Annex I of this Directive;
- water, which is any damage that significantly adversely affects the ecological, chemical and/or quantitative status and/or ecological potential;
- land, which is any land contamination that creates a significant risk of human health being adversely affected as a result of the direct or indirect introduction, in, on or under land, of substances, preparations, organisms or micro-organisms.

The significance of any damage has to be assessed by reference to the conservation status at the time of the damage, the services provided by the amenities they produce and their capacity for natural regeneration. Significant adverse changes to the baseline condition should be determined by means of measurable data for which the Directive provides some more details. However, significant damage does not mean

- negative variations that are smaller than natural fluctuations regarded as normal for the species or habitat in question,
- negative variations due to natural causes or resulting from intervention relating to the normal management of sites, as defined in habitat records or target documents or as carried on previously by owners or operators,
- damage to species or habitats for which it is established that they will recover, within a short time and without intervention, either to the baseline condition or to a condition which leads, solely by virtue of the dynamics of the species or habitat, to a condition deemed equivalent or superior to the baseline condition.

comparators. Not all the requirements of the environmental risk assessment and monitoring may be applicable for all applications. Scientific information on environmental effects associated with the cultivation may not be required, *e.g.* if the scope of the application concerns import only.

Environmental risk assessment can be conducted in a tiered manner (Wilkinson *et al.*, 2003):

**Tier 1.** *Hazard identification*: The approach is to expose organisms to high levels of the GM plant and its products in order to determine potential adverse effects on target and non-target biota likely to be directly exposed to the GM plant and its products. These studies would normally be conducted under controlled laboratory or growth room conditions in order to quantify effects in relation to known exposure levels.

**Tier 2.** *Trophic layer effects*: the approach is to study the indirect effects of the GM plant on organisms not directly exposed to the GM plant but one or two steps removed in the food chain (*e.g.* predators and parasites of primary phytophagous or plant pathogenic organisms). These studies would also normally be conducted under controlled laboratory, growth room or glasshouse conditions in order to measure effects in relation to known exposure levels.

**Tier 3.** *Exposure Studies*: field trials are established, simulating the cultivation of the GM plant, in order to quantify actual levels of exposure of different biota and to determine likely ecological adverse effects due to the GM plant and its management, in comparison with equivalent non-GM materials and their management.

Tiers 1 and 2 identify the potential hazards while Tier 3 identifies the likely exposure levels so that the actual risk can be estimated.

*Monitoring:* It is recognised that an environmental risk assessment is framed within the scientific knowledge available at the time it was conducted. Thus, under current EU legislation, environmental risk assessments are required to identify areas of uncertainty or risk which relate to areas outside current knowledge and the limited scope of the environmental risk assessment. These include such factors as the impact of the large scale exposure of different environments when GM plants are commercialised, the impact of exposure over long periods of time and cumulative long-term effects. The legislation requires that plans for monitoring for these effects are presented in the application, if they are identified in the risk assessment.

The scientific knowledge and experiences gained from monitoring GM crops will in turn inform the risk assessment process. Thus the results of monitoring are opportunities to continually update environmental risk assessments in the light of any new knowledge.

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

## 4.1 Objectives of the different steps of the safety assessment

### *4.1.1. Hazard identification*

Hazard identification is defined as the identification of a risk source(s) capable of causing adverse effect(s)/event(s) to humans and/or the environment, together with a qualitative description of these effect(s)/event(s) (EC 2000a). Hazard identification is the first step in risk assessment and in case of GM plants is focussed on the identification of differences between the GM plant and its appropriate comparator. Identification of differences will determine which further studies should be carried out to characterise these differences with respect to possible impact on human/animal health and/or the environment.

### *4.1.2 Hazard characterisation*

The hazard characterization step is defined as t*he quantitative or semi-quantitative evaluation of the nature of the possible adverse health effects to humans and animals and/or the environment following exposure to a risk source(s* (EC, 2000a). This step is focussed on a possible quantification of the toxicological/nutritional potential of identified differences between the GM plant and derived food/feed and the non-GM comparator. Choice of the appropriate test model (animal species) and test material is considered and data are generated on the onset of adverse or nutritional effects, and the identification of possible dose response relationships.

### *4.1.3 Exposure assessment*

The aim of the exposure assessment is the quantitative estimation of the likely exposure of humans and animals to GM plant derived products (e.g. food/feed, pollen, new constituents). With regard to humans, an exposure assessment characterises the nature and size of the populations exposed to a source and the magnitude, frequency and duration of that exposure. For exposure assessment, it is necessary that every significant source of exposure is identified. In particular it is of interest to establish whether the intake of the GM plant derived products and new constituents are expected to differ from that of the conventional product which it may replace. In this respect specific attention will be paid to that GM food/feed which is aimed at modifying nutritional quality. This category of GM food/feed may require post-market monitoring to confirm the conclusion of the exposure assessment (see section D 7.5).

### *4.1.4. Risk characterisation*

The final risk characterisation of GM plants and derived food/feed is focused on the evaluation of all available data from hazard identification, hazard characterisation, and exposure/intake with respect to their safety and/or nutritional impact for humans/animals and the environment.

A comprehensive risk characterisation considers all the available evidence from several approaches including molecular analysis, agronomical and compositional analysis, toxicity and allergenicity testing, and environmental impact analysis with respect to potential adverse or nutritional effects of GM plants and derived food/feed on humans/animals or the environment.

It should explain clearly what assumptions have been made during the risk assessment in order to predict the probability of occurrence and severity of adverse effect(s)/event(s) in a given population and/or on the environment, and the nature and magnitude of uncertainties associated with establishing these risks. Uncertainties should be described, if occurring, for instance extrapolations from animal models to humans, including exposure route, exposure time (e.g. short-term to long-term), location (different sites of cultivation).

The risk characterisation should also indicate when a scientific risk assessment cannot be completed because of the lack of essential data or the availability of poor quality data. The final risk characterisation should result in informed qualitative, and where possible, quantitative guidance to risk managers.

### 4.2 Issues to be considered for the Risk Assessment of GM Plants

The risk assessment of GM plants and products should take account of the following:

- the characteristics of the donor and recipient organisms;
- the genetic modification and its functional consequences;
- the potential environmental impact;
- agronomic characteristics;
- the potential toxicity and allergenicity of gene products, plant metabolites and the whole GM plant;
- the compositional, nutritional characteristics;
- the influence of processing on the properties of the food or feed;
- the potential for changes in dietary intake;
- the potential for long-term nutritional impact;

# III. INFORMATION REQUIRED IN APPLICATIONS FOR GM PLANTS AND/OR DERIVED FOOD AND FEED[8]

The structure of this Section III is based on Annex III B of Directive 2001/18/EC, setting the legally required information in notifications concerning release of genetically modified higher plants (GMHPs) (Gymnospermae and Angiospermae). Article 5.5(a) of Regulation 1829/2003 stipulates that the technical dossier is required to to follow the structure of Annexes III and IV to Directive 2001/18/EC. This guidance was developed to support applicants in preparation and presentation of applications submitted under Regulation 1829/2003. The table in Annex VI correlates the requirements of the Regulation 1829/2003 and this guidance document.

## A. GENERAL INFORMATION

Information on the GM plant should be provided to specify the nature of the GM food(s) and feed(s) submitted for authorisation (Reg (EC) No 1829/2003, art 5(3)). The information should comprise:

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 EFSA
3. Title of the project
4. Scope of the application as defined in Annex II
5. Designation and specification of the GM plant and/or derived product
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.

[8] Not all the point included will apply in every case. In the case a provision does not apply for a certain application, reasons must be given for the omission of such data from the dossier.

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

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

- to identify the need for specific analyses *e.g.* the known occurrence in the family of specific toxins which are typically expressed at low levels in the unmodified recipient species, but which may be unintentionally increased following the genetic modification process.

- to evaluate all issues of potential concern, such as the presence of natural toxins, allergens or virulence factors.

Information is required under the following headings:

1. Complete name; (a) family name, (b) genus, (c) species, (d) subspecies, (e) cultivar/breeding line or strain, (f) common name. The most recent taxonomic classification should be used.

2. (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.

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

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

5. Geographical distribution and cultivation of the plant, including the distribution in Europe of the sexually compatible species.

6. 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.

7. 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.

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

9. Data on the past and present use of the recipient organism, e.g. history of safe use for consumption as food or feed, including information on how the plant is typically cultivated, transported and stored, whether special processing is required to make the plant safe to eat, and the plant's 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.

## C. INFORMATION RELATING TO THE GENETIC MODIFICATION

The requirements for molecular data are the same for applications under Directive 2001/18/EC for the placing on the market (Part C) and for the assessment of GM food and GM feed but may depend on the scope of the application.

Sufficient information should be provided on the genetic modification:

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

### *1. Description of the methods used for the genetic modification*

The applicant should provide information regarding:

(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 source of carrier DNA if used during the genetic transformation process;

### *2. Nature and source of vector used*

The applicant should provide:

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

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

### *3. Source of donor DNA, size and intended function of each constituent fragment of the region intended for insertion*

Information on the donor organism(s) and DNA sequence(s) should be provided to determine if the nature of the donor organism(s) or the DNA sequence(s) would trigger any safety issue.

***3.1. Information regarding the function of the DNA region(s) intended for insertion should comprise***:

(a) the complete sequence of the donor DNA used for the genetic transformation and indication of any alteration(s) to the donor sequence(s);

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

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

This information may not be required for sequence(s) not retained in the final event.

***3.2. Information regarding each donor organism should comprise***:

(a) classification and taxonomy;

(b) history of use regarding food and feed safety;

## D. INFORMATION RELATING TO THE GM PLANT

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

Applicants should provide information on the trait and the changes that it makes to the plant phenotype.

### *2. Information on the sequences actually inserted or deleted*

Information should be provided to assess whether unintended effects may be expected as a result of the insertion.

Applicants should provide information on:

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

(b) the organisation of the inserted genetic material at the insertion site and methods used for the characterisation;

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

(d) sub-cellular location(s) of insert(s) (nucleus, chloroplasts, mitochondria or maintained in a non-integrated form) and methods for its determination; segregation analysis following appropriate self- or cross-pollination should be used to confirm sub-cellular location of insert(s).

(e) sequence information including the location of primers used for detection; sequencing both 5' and 3' flanking regions of insert(s) should extend, wherever possible, into the host plant genome. This serves two primary functions. Flanking sequence data may identify insertion into, and interruptions of known ORFs[9] or regulatory regions and/or the potential for insertional events to produce novel chimeric proteins.

(f) identification of any ORFs newly created by the insertions with contiguous plant genomic DNA including those that could result in fusion proteins. If potential chimeric ORFs are identified bioinformatic analyses using up-to-date databases should be conducted to investigate the possibility for similarities with known toxins or allergens. Depending on the information gathered, further analyses may be needed to complete the information necessary for a comprehensive risk assessment.

## *3. Information on the expression of the insert*

Information should be provided:

- to demonstrate whether the intended effect of the modification has been achieved;

[9] **O**pen **R**eading **F**rames

- to demonstrate whether deliberate modifications made to the amino acid sequence of the expressed protein result in changes in its post-translational modification or affect sites critical for its structure or function.

Where events are combined by conventional crossing and where altered expression of the gene products (and/or phenotype) is viewed as a potential safety issue, further assessment will be required on a case-by-case basis, e.g. additional field trials, appropriate animal feeding studies and environmental studies.

The applicant has to provide the following information:

(a) Information on developmental expression of the insert during the life cycle of the plant;

The requirement for information on developmental expression should be considered on a case-by-case basis taking into account the promoter used, the intended effect of the modification and the potential for effects on non-target organisms. This type of information may be primarily relevant to environmental safety aspects. Data on expression levels from those parts of the plant that are used for food/feed purposes are considered necessary in all cases.

(b) Parts of the plant where the insert is expressed;

Applicants should be aware that the information on the expression in the plant of genetic elements from any part of the inserted DNA is required if a potential risk is identified. Where tissue-specific promoters have been used, information may be requested on expression of target genes in other plant parts relevant for risk assessment. Evidence should be provided to indicate that expression of the inserted gene(s) is as expected and stable in the tissues targeted.

(c) Potential creation of fusion proteins;

The creation of any new ORFs should be investigated by bioinformatic analysis in particular regarding the homology to known toxins and allergens.

(d) Methods used for expression analysis;

The methods used for the analysis of gene and protein expression must be provided.

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

Protein expression data should be related to the conditions in which the crop is grown and should be carried out in parallel with compositional analysis as specified in Section 7.1.2.

Depending on the nature of the insert, information on the RNA levels could also be required.

(f) With regard to the stacking of events by conventional crossing, data should 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 requested.

## *4. Genetic stability of the insert and phenotypic stability of the GM plant*

Information should be provided:

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

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

Applicants should provide data from multiple (normally five) generations (generative or vegetative propagation, respectively) for single events. Data should be analysed using appropriate statistical methods.

For stacked events comparisons between the insert structures in the original events and the GM stacks should be carried out on materials representative of those designed for commercial production, i.e. which will enter the environment and the food/feed chain.

To assess genetic stability of the event(s), applicants should use appropriate molecular approaches detailed in Section D.2.a.

## *5. Conclusions of molecular characterisation (Sections C and D1-4)*

The molecular characterisation should provide data on the expression and stability of the intended trait(s). This also applies to situations where events have been stacked by conventional breeding.

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

The molecular characterisation should specifically identify whether the event(s) raise(s) any issues regarding the potential for producing new toxins or allergens.

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

# 6. General recommendations

Risk assessment may be simplified for transgenic events in which presence of DNA not essential to achieve the desired trait is minimised (ACRE, 2001a, 2002a).

# 7. Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food/feed

## 7.1. Comparative analysis

The comparative analysis of composition and agronomic and phenotypic characteristics:

- represents, together with the molecular characterisation, the starting point to structure and conduct the risk assessment of a new GM plant and its derived products;
- identifies similarities and differences in composition, agronomic performance and phenotypic characteristics (intended and unintended alterations) between the GM plant and its most appropriate non-GM comparator which has a history of safe use;
- identifies similarities and differences in composition between derived food/feed product(s) and their comparator;

### 7.1.1. Choice of the comparator

In the case of vegetatively propagated crops, comparative analyses should include the non-GM near-isogenic variety used to generate the transgenic lines. In the case of crops that reproduce sexually, comparators would include appropriate non-GM lines with comparable genetic background. Since many crops used to produce food and feed are developed using back-crossing, it is important that in such cases, tests for morphological, agronomical and compositional similarity use the most appropriate controls and do not simply rely on comparisons with the non-GM material originally used for the genetic modification. In all cases the comparator should have a history of safe use. Information on the breeding scheme (pedigree) in relation to both the GM plant and the non-GM comparator and justification for the use of the selected comparator should be provided.

Where no appropriate comparator can be identified, a comparative safety assessment cannot be made and thus a comprehensive safety and nutritional assessment of the products derived from the GM crop should be carried out. For instance, this would be the

case where a trait or traits are introduced with the intention of bringing significant qualitative/quantitative changes in protein/metabolite profiles.

For the assessment of nutritionally improved GM foods/feed or derived ingredients a comparison may be made with non-GM foods/feed or ingredients of comparable composition, with a history of safe use, which are intended to be replaced/substituted.

Where events have been stacked by conventional crossing it is possible that the individual events have been assessed previously according to the EFSA Guidance document (EFSA, 2006a). To complete a risk assessment of GM stacks all information on the events which have already been risk assessed must be made available, e.g. as a web link. Where stacks contain events that have not been risk assessed a comprehensive evaluation of these events according to this document, including a comparison with appropriate non-GM parental material should be provided.

In the case of events stacked by conventional crossing the GMO Panel is aware that there is likely to be a move towards further increases in the numbers of events in GM stacks. As long as each event in the highest number of stacked events has been risk assessed, the risk assessment might also be applicable to stacks containing fewer of these events. Thus a single risk assessment for the highest number of stacked events could cover all combinations with fewer of these events. However, applicants need to take into account the potential impact of any reduction in the number of events involved and provide scientific reasons why specific data on the stacked events with a lower combination of events are not included.

The appropriate comparator for the stack could include a non-GM line as defined in the first paragraph of this section, the single parental GM lines or GM lines containing previously stacked events when the latter have been fully risk assessed. The applicant should provide detailed information justifying the choice of comparators.

The risk assessment of stacked events should follow the principles provided in the Guidance Document of the GMO Panel for the risk assessment of genetically modified plants containing stacked transformation events (EFSA, 2006a), although, on a case-by-case basis, not all components of this Guidance Document may be relevant. Conversely, additional information may be required. Where single events have been assessed, the risk assessment of stacked events should focus mainly on issues related to a) stability, b) expression of the events and c) potential interactions between the events.

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

(a) Principles of experimental design

Field trials used for production of material for the comparative assessment should be performed, focussing on the similarities and differences between two test materials: the genetically modified crop and its comparator, usually a near-isogenic non-GM line.

For each endpoint, the comparative assessment should involve two approaches: (i) a proof of difference, to verify whether the GM plant is different from its comparator 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 and its comparator are equivalent. In testing for difference the null hypothesis is that there is no difference between the GMO and its comparator against the alternative hypothesis that a difference exists. In testing for equivalence the null hypothesis is that the difference between the GMO and its comparator is at least as great as a specified minimum size (see explanation of equivalence limits below) against the alternative hypothesis that there is no difference or a smaller difference than the specified minimum between the GMO and its comparator. Rejection of the null hypothesis is required in order to conclude that the GMO and the comparator are unambiguously equivalent. The equivalence limits used for the test of equivalence must represent appropriately the range of background variation expected for commercial varieties with a history of safe use.

Background variation may have several sources: variation within a variety arises due to environmental factors and variation between varieties arises due to a combination of both genetic and environmental factors. In order to identify and estimate differences attributable only to genotypes it is essential to control environmental variability. Therefore, commercial varieties must 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. Test material (GM crop and comparator(s)) and commercial varieties must all be randomized to plots within a single field at each site, usually in a completely randomized or randomized block experimental design. It is important that the choice of sites for the trials represents as fully as possible the range of receiving environments where the crop will be grown; the choice must be justified explicitly. The choice of commercial varieties must be appropriate for the chosen sites and must be justified explicitly. Environmental variation is manifest at two scales: site-to-site and year-to-year: many years are required to capture adequately the full range of the year-to-year variation. Since the primary concern is not environmental variation per se, but whether potential differences between the test materials vary across environmental conditions, the approach recommended here defines a minimum number of sites for replication of the field trials, but allows flexibility in the number of years over which those trials are conducted. In the case that sites cover a very restricted geographic range, then replication of trials over more than one year is required.

The recommendations for replication within sites in this document recognize the need to maximize efficiency within available resources and it is expected to provide sufficient statistical power for a wide variety of endpoints with differing variability.

(b) Specific protocols for experimental design

At each site the test materials (GM crop and comparator(s)) must be identical. In addition, unless there is explicit justification, at each site there should be at least three appropriate commercial varieties of the crop that have a known history of safe use. In this document the number of test materials plus the number of commercial varieties is denoted by t. For example, if there are the GM crop, the near-isogenic comparator plus four commercial varieties, then t=6. In this document, the number of results to be

obtained for each test material and commercial variety at each site (the replication) is denoted as r. The minimum requirements for replication that follow were chosen to give an appropriate number of plots on the basis both of extensive experience with field trials and levels of degrees of freedom for desired precision in simple designed experiments. The minimum level of replication shall be an integer greater or equal to [15/(t-1)] +1. For example, if t=5 (the minimum value) then r, the replication, must be at least 5; if t=6 then r must 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 must be replicated at a minimum of eight sites, chosen to be representative of the range of likely receiving environments where the crop will be grown. The trials may be conducted in a single year, or spread over multiple years. The commercial varieties may vary between sites, but unless there is explicit justification there must be at least six different commercial varieties used over the entire set of trials.

The field trials must 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 conditions during growth and time of harvest, as well as the conditions during storage of the harvested material.

In the case of GM plants containing stacked events, unless previous risk assessments have confirmed that single events do not interact, additional comparisons with test materials consisting of GM parental lines is recommended. If previous risk assessments have confirmed that single events combined within a stack do not interact, then this stack may replace the single GM parental lines of the stack in the comparisons. In the case of herbicide tolerant GM plants, three test materials must be compared: GM plants exposed to the intended herbicide, the control treated with conventional herbicide(s) and GM plants treated with the same conventional herbicide(s); such a design allows assessment of whether the expected agricultural practice influences the expression of the studied endpoints.

(c) Statistical analysis

Analysis of data should be presented in a clear format, using standardised scientific units. The raw data and the programming code used for the statistical analysis must 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 its comparator is interpreted as a ratio on the natural scale. However, for other endpoints the logarithmic transformation may not be optimal and a natural scale or other scales may be more suitable.

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

The total variability in each endpoint observed in the field trials must be estimated and partitioned using an appropriate statistical model in order to derive confidence intervals for the observed difference between the GM crop and its comparator and to set equivalence limits (FDA, 2001) based on the variability observed among the commercial varieties. Confidence intervals are used both in proof of difference and proof of equivalence, whereas equivalence limits are used only in the latter.

A statistical mixed model, with fixed and random statistical effects, is recommended for estimation of the overall variation and definition of the contributions of the different factors (variance components) to the total observed variation. This mixed model will include but not be restricted to the following factors, each with a number of levels appropriate to the chosen experimental design: (i) test material (normally with two levels: GM crop and its comparator), (ii) a factor with two levels representing the difference between the means of the test materials and of the commercial varieties, (iii) commercial variety, (iv) blocks within sites, (v) site. Of these factors, (i) and (ii) must be treated as fixed effects; (iii) and (iv) as random effects; and (v) can be treated as a fixed or random effect on a case-by-case basis. Further information may be found in the report of EFSA self-task activity on statistical considerations for the safety evaluation of GMOs (EFSA, 2008 in preparation).

Full details must be given, for each endpoint analysed, listing: (i) the assumptions underlying the analysis, (ii) full specification of the model chosen, including indication of fixed and random effects, (iii) results of any test of interaction between the test materials and sites, (iv) degrees of freedom, (v) the estimated residual variation for each fixed source of variation, and appropriate variance components for the random factors, (vi) any other relevant statistics. The likely impact of other growing conditions not tested in the trial should be discussed.

The analysis proceeds by testing for difference and for equivalence applying the same mixed model described above to each endpoint. Specifically, for a particular endpoint the mean difference between the GM and its comparator is computed and a 90% confidence interval constructed around it. In addition, an upper and lower equivalence limit must be set for each endpoint, according to the variability observed between commercial varieties. It is recommended to calculate each equivalence limit as the estimated difference between the mean of all commercial varieties and the comparator plus or minus the product of 1.96 times the estimated standard deviation of the random effect for the commercial varieties in the mixed model. Upper and lower equivalence limits are assumed to be symmetrical, as expected for a normal distribution, around the point estimator of the mean difference between commercial varieties and the comparator.

All these calculated quantities should be displayed, for all the endpoints simultaneously, on a single graph or a few graphs. The graph should show the line of zero difference between the GM and its comparator and, for each endpoint: the lower and upper equivalence limits, the mean difference between the GM and its comparator and its confidence interval (see example below). 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 should be labelled with values that specify the change on the natural

scale. In the case of logarithmic transformation, changes of 2x and ½x will appear equally spaced on either side of the line of zero difference.

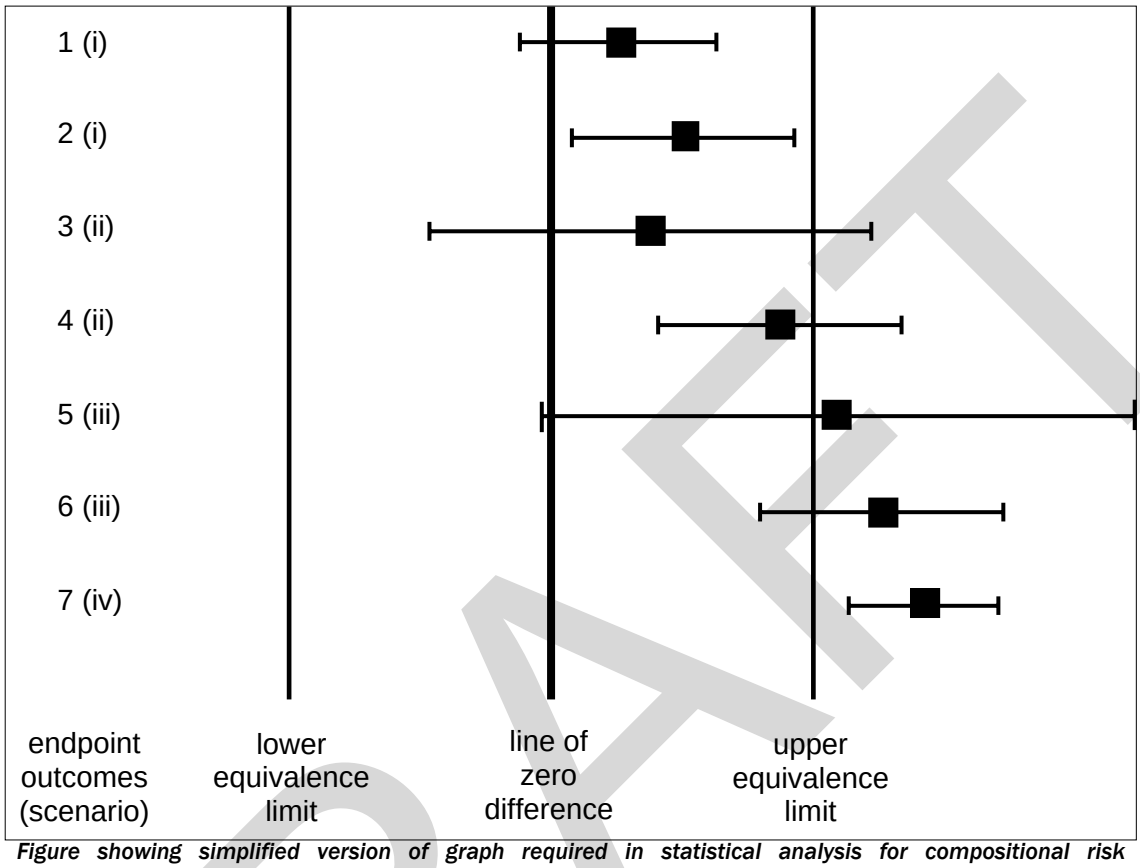


*Figure showing simplified version of graph required in statistical analysis for compositional risk assessment. Without loss of generality there are seven distinct outcomes for each endpoint when comparing the mean difference between the GM crop and its comparator, with its confidence interval, against: (i) the vertical line showing zero difference (for proof of difference), and (ii) the vertical lines showing equivalence limits (for proof of equivalence). Each of these possible outcomes is shown for seven imaginary endpoints: squares represent the mean differences; bars represent confidence intervals. For outcomes 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 control. Regarding equivalence, outcomes 1 and 2 correspond to scenario (i), see below; outcomes 3 and 4 correspond to scenario (ii), see below; outcomes 5 and 6 correspond to scenario (iii), see below; outcome 7 corresponds to scenario (iv), see below.*

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

Note that since the confidence interval graph is used also for the test of difference, then each difference test will have a 90% confidence level. Although 1 in 10 of these tests is

expected to yield a significant result by chance alone, the applicant is required to report and discuss all significant differences observed between the GM and its comparator, focussing on their biological relevance (see Chapter IV on risk characterization).

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

(i) the confidence interval for the difference between the GMO and its comparator lies entirely between the equivalence limits. The appropriate conclusion is that the GM is equivalent to its comparator.
(ii) the point estimate of the difference between the GMO and its comparator lies between the equivalence limits, but at least one of the ends of the confidence interval falls outside the equivalence limits. The appropriate conclusion is that there is probable equivalence between the GM and its comparator.
(iii) the point estimate of the difference between the GMO and its comparator lies outside the equivalence limits, but the confidence interval overlaps with at least one of the equivalence limits. The appropriate conclusion is that there is probable non-equivalence between the GM and its comparator.
(iv) the confidence interval for the difference between the GMO and its comparator lies entirely outside the equivalence limits. The appropriate conclusion is that there is non equivalence between the GM and its comparator.

In case of significant difference and/or lack of equivalence, further analysis is recommended to assess how the difference observed between the GM crop and its comparator varies across sites, possibly using a standard ANOVA approach. Whatever approach is adopted, full details must be given, for each endpoint analysed, listing: (i) the assumptions underlying the analysis, (ii) degrees of freedom, (iii) the estimated residual variation for each source of variation, and appropriate variance components, (iv) 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.

This merging of the results of both tests (proof of difference and proof of equivalence) allows any difference or lack of equivalence found to be placed in context and interpreted within a risk assessment framework (see section 7.2 on toxicology and Chapter IV on risk characterization for pertinent discussion).

### 7.1.3. Selection of material and compounds for analysis

Analysis of the composition is crucial when comparing the GM plant and/or derived food/feed product with its most appropriate non-GM comparator. The material to be used for the comparative assessment should be selected while taking into account the uses of the GM plant and the nature of the genetic modification. Analysis should normally be carried out on the raw agricultural commodity, as this usually represents the main point of entry of the material into the food/feed production and processing chain. Additional analysis of processed products (food/feed, food ingredients, feed materials, food/feed additives or food flavourings), may be required on a case-by-case

basis (see also Section III, D 7.6). The analyses should be carried out according to appropriate quality standards.

### 7.1.4. Comparative analysis of composition

The compositional analysis should be carried out on an appropriate range of compounds as well as newly expressed proteins (see Section D.3). In each case, proximates (including moisture and total ash), key macro- and micro-nutrients, anti-nutritional compounds, and natural toxins should be determined. Information on the key nutrients, anti-nutrients, and toxins as well as other secondary plant metabolites characteristic for specific crop plant species are provided in OECD consensus documents which may provide further guidance for compositional analysis (OECD a).

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

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

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

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

In case of nutritionally enhanced GM plants, intented effects can be confirmed by the method described in 7.1.2.

### 7.1.5. Comparative analysis of agronomic and phenotypic characteristics

Compositional analysis represents a key component of the comparative approach for identifying unintended effects during the risk assessment process. However, unintended effects may also manifest themselves through, for example, changes in susceptibility to biotic and abiotic stresses, through morphological and developmental changes or through modified responses to agronomic and crop management regimes. Therefore, the comparison between the GM plants and their most appropriate comparators should 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 should follow the specifications made under Section III, D 7.2.

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 must be assessed in field trials over at least one season. On a case-by-case basis, additional information on agronomic traits of the stacked events may be required from additional field trials.

### 7.1.6. Effect of processing

Food or feed produced from GM plants may include food ingredients (*e.g.* oil, flour, sugar, syrup, baked foods, beverages), feed materials (*e.g.* maize gluten feed, syrup, oil, starch, soya meal), food additives (*e.g.* lecithin), feed additives (e.g. enzymes, vitamins), flavourings, and certain products used in animal nutrition. These compounds can range from single compounds to complex mixtures. Genetic modification can target metabolic pathways resulting in changes in the concentration of non-protein substances or in new metabolites (*e.g.* nutritionally enhanced foods, functional foods).

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

The applicant should assess whether or not the processing and/or preserving technologies applied are likely to modify the characteristics of GM end product compared with its non-GM comparator. This would require the description of the different processing technologies in sufficient detail, paying special attention to the steps which may lead to significant changes in the product content, quality or purity. If the GM plant (or relevant parts of it) is considered safe for consumption, and there is no reason to suspect that the products would be any different from their traditional comparators, further toxicological tests with the processed products are normally not requested. This is also the case when the product is assessed separately and there is no

reason to suspect that it would be any different from its conventional comparator (e.g. oil from insect protected cottonseed). Depending on the product, information should be provided on the composition, level of undesirable substances, nutritional value and metabolism, as well as on the intended use.

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

### 7.1.7. Conclusion of the comparative analysis

The conclusion of the comparative analysis should clearly state:

- whether the GM plant and/or the processed product(s) is different from its non-GM comparator with respect to its composition and agronomic and phenotypic characteristics, except for the introduced trait(s);
- whether the GM plant is equivalent to its non-GM comparator with respect to its composition;
- characteristics for which the GM plant or its processed product(s) is not equivalent to its conventional comparator, except for the introduced trait(s) which should be considered as unintended effects. It should in particular be indicated whether these observations are in line with the information obtained from the molecular characterisation or whether these characteristics may be indicative of other effects. Additional targeted compositional analysis should be carried out when the observed alterations may be indicative of other metabolic modifications.
- Intended effects may be confirmed by applying the method as described in section 7.1.2 to identify differences.
- Whether, in the case of events stacked by traditional crossing, interactions between the combined events raise any additional safety concerns.

## *7.2. Toxicology*

The purpose of performing toxicological studies of single compounds, using animals and/or in-vitro systems, is to identify adverse effects of the test compounds and to identify the highest dose level(s) that do not result in adverse effects (No-Observed-Adverse-Effect level, NOAEL). From the NOAEL in 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 interindividual variations among humans. This internationally accepted approach is similar to that applied with testing chemicals in foods and is described in detail by FOSIE, the European project "Food Safety in Europe: Risk Assessment of Chemicals in Food and Diet" (FOSIE, Food and Chem Tox 40 (2002), 2/3,).

Regarding GM food/feed, 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, should be assessed.

Toxicological analysis should be performed:

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

- to demonstrate that unintended effect(s) of the genetic modification(s) that have been identified, or that may be assumed to have occurred based on the preceding comparative molecular, compositional or phenotypic analyses, have no adverse effects on human and animal health. For this purpose testing of single compounds and/or of whole GM food/feed may be considered.

The requirements of toxicological testing must be considered on a case-by-case basis and will be determined by the outcome of the comparative analysis, i.e. the differences identified between the GM product and its conventional comparator, including intended as well as unintended changes. In principle, the assessment must consider the presence of (i) newly expressed proteins (ii) the potential presence of other new constituents and/or (iii) possible changes in the level of natural constituents beyond normal variation. The specific information requirements and testing strategies are outlined in Sections 7.2.1 – 7.2.5.

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

### 7.2.1. Standardized Guidelines for Toxicity Tests

Internationally agreed protocols and test methods described by the OECD (OECD b) or in the most up-to-date European Commission Directive on dangerous substances (EC, 2002d) should be used for toxicity testing. Use of any methods that differ from such protocols should be justified. Studies should be carried out according to the principles of Good laboratory Practice (GLP) described in Council Directive 2004/10/EC (EC, 2004a) and be accompanied by a statement of GLP-compliance. A non-exhaustive list of

validated test protocols which may be used in a possibly adapted form for GMO toxicological testing is provided in the table 1 below (modified from FOSIE, 2002).

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

**Table 1** *OECD Guidelines for animal toxicity tests*

| No. | Subject | Note |
|---|---|---|
| 402 | Acute Dermal Toxicity | Updated Guideline, adopted 24 February 1987 |
| 406 | Skin Sensitisation | Updated guideline, adopted 17 July 1992 |
| 407 | Repeated Dose 28-day Oral Toxicity Study in Rodents | Updated guideline, adopted 27 July 1995 |
| 408 | Repeated Dose 90-Day Oral Toxicity Study in Rodents | Updated guideline, adopted 21 September 1998 |
| 410 | Repeated Dose Dermal Toxicity:21/28-Day | Original guideline, adopted 12 May 1981 |
| 415 | One-Generation Reproduction Toxicity | Original guideline, adopted 26 May 1983 |
| 416 | Two-Generation Reproduction Toxicity Study | Updated guideline, adopted 22 January 2001 |
| 417 | Toxicokinetics | Original guideline, adopted 4 April 1984 |
| 421 | Reproduction/Developmental Toxicity Screening Test | Original guideline, adopted 27 July 1995 |
| 424 | Neurotoxicity Study in Rodents | Original guideline, adopted 21 July 1997 |
| 451 | Carcinogenicity Studies | Original guideline, adopted 12 May 1981 |
| 452 | Chronic Toxicity Studies | Original guideline, adopted 12 May 1981 |
| 453 | Combined Chronic Toxicity/Carcinogenicity Studies | Original guideline, adopted 12 May 1981 |

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

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

### 7.2.2. Toxicological testing of newly expressed proteins

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

If specific testing is required it is essential that the tested protein is equivalent to the newly expressed protein as it is expressed in the GM plant. If, due to the lack of sufficient amount of test materials (*e.g.* plant proteins), a protein produced by micro-organisms is used, the structural, biochemical and functional equivalence of this microbial substitute to the newly expressed plant protein must be demonstrated. For example, comparisons of the molecular weight, the isoelectric point, 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 should be evaluated.

To demonstrate the safety of newly expressed proteins:

- A molecular and biochemical characterisation of the newly expressed protein is required, including determination of the primary sequence, molecular weight, studies on post-translational modifications and a description of the function. In the case of newly expressed enzymes, information on the enzyme activities is needed including the temperature and pH range for optimum activity, substrate specificity, and possible reaction products.

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

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

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

- Repeated dose toxicity studies using laboratory animals should be performed, unless reliable information can be provided which demonstrates the safety of the newly expressed protein (including its mode of action) and that the protein is not structurally and functionally related to proteins which have the potential to adversely affect human or animal health.

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

If the applicant considers that a decision on safety can be taken without conducting a repeated dosing study or that other tests are more appropriate, the applicant must state the reasons for this.

### 7.2.3. Testing of new constituents other than proteins

Identified new constituents other than proteins should be evaluated. This may include toxicological testing on a case-by-case basis, which includes an assessment of their toxic potency and occurrence in the GM food/feed. To establish their safety, information analogous to that described in the "Guidance on submissions for food additive evaluations by the Scientific Committee on Foods" (SCF, 2001a) and Directive 2001/79/ EC (EC, 2001b) should be provided. This implies the submission of information on a core set of studies and the consideration of whether or not any other type of study might also be appropriate. Normally, the core set includes information on metabolism/toxicokinetics, sub-chronic toxicity, genotoxicity, chronic toxicity, carcinogenicity and reproduction and developmental toxicity (for specific OECD guidelines for animal tests, see Table 1). Genotoxicity test protocols are given in the table below (Modified from the Report of the EFSA GMO Panel working group on Animal Feeding Trials, 2008):

**Table 2** *Genotoxicity tests as described by OECD guidelines (OECDb)*

| 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>*In vitro* mammalian cell micronucleus test |

### 7.2.4. Information on natural food and feed constituents

Natural food and feed constituents comprise a large variety of substances: macro- and micronutrients, anti-nutrients, and natural toxins as well as other secondary plant metabolites. If the intended or unintended effect of the modification is that the content of such natural food and feed constituents is altered beyond the natural variation, this paragraph applies.

To demonstrate the safety of the altered content of natural food and feed constituents a detailed risk assessment based on the knowledge of the physiological function and/or toxic properties of these constituents should be submitted. The result of this assessment would determine if, and to what extent, toxicological tests are required.

### 7.2.5. Toxicological testing of the whole GM food/feed

The risk assessment of the GM plant and derived food/feed is primarily based on molecular characterisation, comparative agronomic, phenotypic and compositional analysis, and the toxicological evaluation of the identified intended and unintended effects. Toxicological testing of the whole GM food/feed using animals should be carried out in case the composition of the GM plant is modified substantially, as may be the case with extensive genetic modifications targeted at (i) specific alterations in the metabolism leading to improved characteristics for human or animal nutrition and/or health, or (ii) improved responses to environmental stress conditions, like salt or metal tolerance, or drought resistance.

Furthermore, toxicological testing of whole GM food/feed should be considered if there are any indications or remaining uncertainties for the potential occurrence of unintended effects based on the preceding molecular, agronomical, phenotypical and/or compositional analysis.

***90-day toxicity study in rodents***

In case an animal toxicity study should be carried out with the GM plant derived food/feed, a subchronic, 90-day rodent feeding study should be considered. The design of such a study should be adapted from the OECD 90-day rodent toxicity study, Guideline 408 (OECD, 1998) Special attention must be paid to the selection of doses and the avoidance of problems of nutritional imbalance. The highest dose level should be the maximum achievable without causing nutritional imbalance. Stability of test diets and nutritional equivalence between control and test diets are other important aspects to consider. If designed and carried out properly such a study is of sufficient specificity, sensitivity and predictivity to act as a sentinel study in order to detect in a comparative manner toxicologically relevant differences as well as nutritional deficiencies/improvements that may be due to the expression of new substances, intended alterations in levels of natural compounds or unintented effects (Report of the EFSA GMO Panel working group on Animal Feeding Trials, 2008).

Whole feeding trials may be paralleled by experiments in in vitro and in vivo systems from animal and/or human origin, studying for instance gene expression profiles and/or potential cytotoxicity of newly expressed proteins or metabolites.

In the case of complex genetic modifications involving the transfer of multiple genes, the potential risk(s) of possible interactions between the expressed proteins, new metabolites and original plant constituents should be assessed. The outcome of the molecular analysis and knowledge of the mode of action of the newly expressed proteins may provide indications for possible synergistic interactions, as well as information on the response to combined administration of proteins to target organisms and regarding effects on the activity of target enzymes. Generally, feeding trials with this type of GM foods/feeds is requested in order to assess the impact of consumption on human and animal health. On a case-by-case basis this is also applicable to foods and feeds derived from GM plants obtained through conventional breeding of parental GM lines (stacked events).

***Additional animal studies with respect to reproductive, developmental or chronic toxicity***

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

In cases where structural alerts, indications from the subchronic study or other information on whole GM plant derived food and feed are available that suggest the potential for reproductive, developmental or chronic toxicity, the performance of such testing should be considered (Report of the EFSA GMO Panel working group on Animal Feeding Trials, 2008). OECD protocols for subchronic, reproductive, developmental and chronic toxicity testing can be adapted for the testing of whole GM plant derived food and feed (see table 1 and the Report of the EFSA GMO Panel working group on Animal Feeding Trials, 2008)

***Other animal studies to examine the safety and the characteristics of GM food/feed (see also sections 7.4.1 and 7.4.2)***

Supplemental information to 90-day toxicity tests 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). Because of their rapid weight gain such animals are sensitive to the presence of certain undesirable substances in their feed (ILSI 2003) Studies of this type are, however, limited to those materials suitable for inclusion in their diets and which can be nutritionally matched to a suitable control diet.

Livestock feeding studies with target animal species should be considered, on a case-by-case basis and be hypothesis driven. The focus should be on the safety of expressed products, 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 sections 7.4.1 and 7.4. 2 and the Report of the EFSA GMO Panel working group on Animal Feeding Trials, 2008)

***Interpretation of relevance of toxicity tests***

As noted in the EFSA GMO Panel's report on the conduct of animal trials with GM products (Report of the EFSA GMO Panel working group on Animal Feeding Trials, 2008), any effects observed in the animal trials should be evaluated by experts in order to identify relevant effects. The experts' experience will facilitate the interpretation of the observed effects with respect to potential consequences for the health of humans and animals and thus assess their relevance for the safety of food and feed derived from the GM product. This interpretation can be supported by additional information and considerations, including the examples discussed below.

Information on the background variability in a given parameter can be obtained from data from other animals of the same species/strain tested in the same or other experiments, or from internationally harmonized databases. If the change observed in a certain parameter falls within this background range of variability, it should still be further considered if there is a dose-response relationship, gender specificity, linkage with other changes, or any plausible cause.

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

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

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

With regard to the potential cause for an observed effect, it is also important to take the likelihood of causality 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.

Whole feeding trials may be paralleled by experiments in in vitro and in vivo systems from animal and/or human origin, studying for instance gene expression profiles and/or potential cytotoxicity of newly expressed proteins or metabolites.

In the case of complex genetic modifications involving the transfer of multiple genes, the potential risk(s) of possible interactions between the expressed proteins, new metabolites and original plant constituents should be assessed. The outcome of the molecular analysis and knowledge of the mode of action of the newly expressed proteins may provide indications for possible synergistic interactions, as well as information on the response to combined administration of proteins to target organisms and regarding effects on the activity of target enzymes. Generally, feeding trials with this type of GM foods/feeds is requested in order to assess the impact of consumption on human and animal health. On a case-by-case basis this is also applicable to foods and feeds derived from GM plants obtained through conventional breeding of parental GM lines (stacked events).

Any adverse effect(s) noted in individuals exposed to GM food/feed material as part of their professional activities e.g. farming, seed processing should be submitted by the applicant.

## *7.3. Allergenicity*

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

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

### 7.3.1. Assessment of allergenicity of the newly expressed protein

Allergenicity is not an intrinsic, fully predictable property of a given protein but is a biological activity requiring an interaction with individuals with a pre-disposed genetic background. Allergenicity therefore depends upon the genetic diversity and variability in atopic humans. Given this lack of complete predictability it is necessary to obtain, from

1594 several steps in the risk assessment process, a cumulative body of evidence which
1595 minimises any uncertainty with regard to the protein(s) in question.

1596 In line with the recommendations of the Codex *ad hoc* Intergovernmental Task Force on
1597 Foods Derived from Biotechnology (Codex Alimentarius, 2003), an integrated, stepwise,
1598 case-by-case approach, as described below, should be used in the assessment of
1599 possible allergenicity of newly expressed proteins.

1600 The source of the transgene must be considered carefully to make clear whether or not
1601 it encodes an allergen. Information should specify at what stage of the development of
1602 the plant and in what organs of the plant the allergenic protein may be expressed. When
1603 the introduced genetic material is obtained from wheat, rye, barley, oats or related
1604 cereal grains, applicants should assess the newly expressed proteins for a possible role
1605 in the elicitation of gluten-sensitive enteropathy or other enteropathies which are not IgE
1606 mediated.

1607 Where events have been stacked by conventional crossing an assessment of any
1608 potential for increased allergenicity to humans and animals should be provided.
1609 These potential effects may arise from additive, synergistic or antagonistic effects of
1610 the gene products. This assessment will clearly require a case-by-case approach.

1611 In every case the first step in the assessment should be a search for sequence
1612 homologies and/or structural similarities between the expressed protein and known
1613 allergens. Identification of potential linear IgE binding epitopes should be conducted by
1614 a search for homologous peptidic fragments in the amino acid sequence of the protein.
1615 The number of contiguous identical or chemically similar amino acid residues used in
1616 the search setting should be based on a scientifically justified rationale in order to
1617 minimise the potential for false negative or false positive results[10]. The use of different
1618 homology searching strategies based on the sequences available in relevant databases
1619 may identify several scenarios. These include a high degree of homology, with or
1620 without conservation of the allergenicity, or a low degree of homology with conservation
1621 of allergenicity (Mills *et al.*, 2003). To reduce the uncertainty of the conclusions that may
1622 be drawn from the search of sequence homology alone, efforts should be encouraged to
1623 improve the bioinformatic approach i) to improve and harmonise the algorithms that
1624 are used by the different applicants and ii) to develop databases which include
1625 information on the three dimensional structure and function of known allergens and of
1626 proteins belonging to protein families which include a high proportion of allergens.

1627 The second step for assessing the potential that exposure to the newly expressed
1628 proteins might elicit an allergic reaction in individuals already sensitised to cross
1629 reactive proteins, is based on *in vitro* tests that measure the capacity of specific IgE
1630 from serum of allergic patients to bind the test protein(s).

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

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

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

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

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

Specific (as well as targeted) serum screening requires a sufficient number and sufficient volumes of relevant sera from allergic humans. These might not always be available either because the allergy is not frequent or for other reasons. The use of existing models and the development and validation of new alternative models that can substitute for and/or complement the use of human biological material for evidence of cross reactivity and elicitation potency should be encouraged. 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.

Animal models are certainly also useful tools for the assessment of the sensitising potential of newly expressed proteins, *i.e.* their capacity to induce an allergic immune response with the synthesis of specific IgE in individuals that have never been exposed to those proteins nor to proteins that cross react with them. The development of animal models should be encouraged and, once validated, their use may increase the body of evidence to support a conclusion.

### 7.3.2. Assessment of allergenicity of the whole GM plant or crop

If the host of the introduced gene is known to be allergenic, any potential change in the allergenicity of the whole GM food should be tested by comparison of the allergen repertoire with that of the conventional non-GM variety.

It should be pointed out that these approaches should 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 use of modern analytical tools including profiling techniques, although still in development, may provide, in association with human and animal serum or cell-based assays, valuable additional information.

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

In addition, data 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, will provide useful information for the risk assessment process.

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

## 7.4. Nutritional assessment of GM food/feed

Nutritional evaluation should be provided:

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

- to demonstrate that unintended effects of the genetic modification that were identified during hazard identification or that may be assumed to have occurred based on the preceding molecular, compositional or phenotypic analyses (see sections 7.1), have not adversely affected the nutritional value of the GM food/feed.

- to assess, where events have been stacked by conventional crossing, potential changes in nutritional value that might arise from additive, synergistic or antagonistic effects of the gene products including compositional changes. This may be particularly relevant where the combined expression of the newly introduced genes has unexpected effects

on biochemical pathways. This assessment will clearly require a case-by-case approach.

Compositional analysis is the starting point and cornerstone for the nutritional assessment of food and feed material. Consensus documents prepared by OECD (OECD a) provide guidance for the minimum number of key components needed to be analysed for the respective food/feed plants. However, the analyses conducted should be determined on a case-by-case basis and may vary depending on the introduced trait.

### 7.4.1. Nutritional assessment of GM food

GM foods may have the potential to improve the nutritional status of individuals and populations and provide products with additional health benefits (enhanced functionality). GM foods also have the potential to introduce nutritional imbalances as a result of both expected and unexpected alterations in nutrients and other food components.

The nutritional assessment of GM foods should consider:

- composition of the GM foods with regard to the levels of nutrients and anti-nutrients (see compositional studies as described in Sections III, D 7.1.4)
- bioavailability and biological efficacy of nutrients in the foods taking into account the potential influences of transport, storage and expected treatment of the foods;
- anticipated dietary intake of the foods (see Section III, D 7.5) and resulting nutritional impact.

If the GM food has been assessed as compositionally equivalent to the non-GM comparators except for the introduced trait(s) (see Sections 7.1.2) no further studies to demonstrate nutritional equivalence are required, provided that the new trait(s) is not expected to influence the nutritional characteristics of the food.

Further nutritional testing should be carried out if the composition of the GM food has intentionally or unintentionally been modified substantially or if there are any indications for the occurrence of unintended effects based on the preceding molecular, compositional, agronomical and/or compositional analysis (see Sections 7.1). In these cases a subchronic (90-day) feeding study in rodents using the whole GM food is normally required to demonstrate whether any changes are of toxicological relevance (see Section 7.2.5). Since it starts with juvenile animals in rapid growth phase that are sensitive to effects on weight gain, this toxicity study also gives information on nutritional aspects. The necessity and design of further nutritional studies will depend on the outcome of this subchronic feeding study. Supplemental information regarding the nutritional value may be obtained from comparative growth performance studies conducted with other animal species, e.g. broiler chickens (see Section 7.2.5 and 7.4.2), addressing the nutritional assessment of GM feed (ILSI 2003, ILSI 2007).

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. Whereas the assessment of the intended benefits is not within the scope of this document, the potential risks of these GM foods have to be assessed. When animal feeding studies are performed, the choice of an appropriate comparator is of particular importance for the safety assessment (see section 7.1.1).

In cases where an altered bioavailability may raise concern and needs to be established, the level of the nutrient in the food should be determined, taking into account all the different forms of the compound. The methods to test for bioavailability should be selected on a case-by-case basis and depend 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.

### 7.4.2. Nutritional assessment of GM feed

Once compositional equivalence has been established in GM feeds modified for agronomic traits, nutritional equivalence can be assumed and has been demonstrated in many studies with food producing animals as recently reviewed (e.g. Report of the EFSA GMO Panel Working Group on Animal Feeding Trials, 2008). Routine livestock feeding trials generally add little to a nutritional assessment of feed from GM plants with agronomic traits. If such studies are necessary or recommended feed ingredients from a non-GM plant with comparable genetic background (e.g. an isogenic plant) should be compared with feed ingredients of the transgenic plant according to internationally recognized protocols and/or guidelines (e.g. ILSI 2003).

These target animal feeding studies should 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. For feedstuffs intended only for aquaculture, growth studies with aquatic species such as carp or other typical herbivores are preferable.

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

nutritional value, it might on a case-by-case basis be required to assess the nutritional profile of these foods.

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

## *7.5. Anticipated intake/extent of use*

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

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

The concentrations of the new 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 should be determined by appropriate methods. Expected intake of these constituents should be estimated taking into account the influences of processing, storage and expected treatment of the food/feed in question, e.g. potential accumulation or reduction. In cases where the genetic modification has resulted in an altered level of a natural constituent, or if a new constituent occurs naturally in other food/feed products, the anticipated change in total intake of this constituent should be assessed considering realistic as well as worst case intake scenarios.

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

Information should also be provided on any expected benefit and/or adverse reactions, as well as any scientific evidence on the efficacy of the GM food/feed for the intended effect at the level proposed.

### *7.6. Conclusion of the toxicological/nutritional and allergenicity assessment*

The conclusions of the toxicological/nutritional assessment of GM plant derived foods/feed should indicate:

- whether the GM derived food/feed is as safe and as nutritious as its non-GM comparators;
- whether the information provided and the testing strategy used to assess the intended and/or unintended changes of the GM food/feed are considered adequate;
- whether intended and/or unintended changes of the GM plant derived food/feed is likely to have adverse effects on human or animal health in the context of its intended uses and taking account of the anticipated exposure of derived food/feed;
- whether additional toxicological/nutritional studies are needed to assess the whole GM plant derived food /feed;
- whether introduction of the GM plant into the market is likely to influence the overall use of the respective crop and/or the intake of specific GM plant derived food/feed products;
- the potential for modified toxicity/nutritional value of the GM plant derived food/feed due to additive, synergistic or antagonistic effects of the gene products for events stacked by conventional crossing.

The conclusions of the allergenicity assessment should clearly indicate:

- whether the novel protein(s) is likely to be allergenic;
- whether the GM food/feed is likely to be more allergenic than the conventional comparator;

- if there is no comparator, then the allergenicity assessment should conclude on the likelihood of allergenicity of the novel GM food;

- the potential for modified allergencity due to additive, synergistic or antagonistic effects of the gene products for events stacked by conventional crossing;

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

## 7.7. Post-market monitoring of GM food/feed

Where appropriate a Post Market Monitoring (PMM) programme should be performed for GM food/feed. The appropriateness of performing a PMM is indicated by findings in the pre-market safety assessment. Furthermore, as pre-market risk assessment studies cannot fully reproduce the diversity of the populations who will consume the marketed product, the possibility therefore remains that unpredicted side effects may occur in some individuals of the population, such as those with certain disease states (*i.e.* allergic individuals), those with particular genetic/physiological characteristics or those who consume the products at high levels. Indeed, risk assessment also relies on an estimate of exposure to the food, which is variable and subject to uncertainty before the food is marketed. A PMM should therefore address the following questions: i) is the product use as predicted/recommended? ii) are known effects and side-effects as detected during the pre-market risk assessment as predicted? and iii) does the product induce unexpected side effects? (Wal *et al.*, 2003).

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

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

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

## 8. Mechanism of interaction between the GM plant and target organisms (if applicable)

The applicant should describe the expression and mode of action of any new traits (for example insect resistance, herbicide tolerance) present in the modified plant. The likely effects on the target organism and its population dynamics should be described. If more than one novel trait is present then interactions between the traits and their effects on target organisms should also be described. There should be a reference to Sections III, D 1 and 3 of this document where this information has already been given. The potential environmental implications of, for example, the development of resistance/tolerance by the target organisms are included in Section III, D 9.4 below.

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

It is important to determine whether the GM plant or hybrids formed with related plant species have changes in their environmental fitness. The assessments of potential changes in the interactions between the GM plant and the biotic environment (*e.g.* non-target organisms) are carried out on a case-by-case basis taking into account the biology of the transformed plant and, where gene transfer might occur, of any other recipient organisms, the characteristics and expression of the introduced genetic material, the properties and consequences of the genetic modification, the scale of release and gene transfer and the assessment of any risk to the receiving environment that might arise from the release of the GM plant.

Genes inserted in a GM plant should be evaluated for their potential impact on the environment. Where the GM plant contains more than one transgene or event, assessment should include consideration of the impact of interactions between transgenes. The assessment should also consider the consequences of low frequencies of gene transfer to related and unrelated organisms, and take into account any potential for enhanced gene transfer reported in Section III, D 6.

Examples of possible interactions between the GM plant and its biotic environment to be considered include:

(a) effects on the numbers and diversity of relevant populations of species in the receiving environment (plant, animal, microbe);

(b) altered susceptibility to pests and pathogens facilitating the dissemination of infectious diseases and/or creating new reservoirs or vectors;

(c) compromising prophylactic or therapeutic medical, veterinary, or plant protection treatments;

(d) effects on beneficial plant-microbial associations and biogeochemistry (biogeochemical cycles), particularly on microbial-mediated carbon and nitrogen recycling through changes in soil decomposition of organic material.

Data should be provided from field experiments in areas representative of those geographical regions where the GM plant will be grown commercially in order to reflect relevant meteorological, soil and agronomic conditions. Where data from field studies on other continents are supplied, the applicant should submit a reasoned argument that the data is applicable to European conditions.

Risk assessments should be carried out for each of the different environmental compartments that are exposed to the GM plant. Whether or not any parts of it will remain in the environment after harvest will depend on the specific plant, its management regime and agronomic practices. Where changes to environments are predicted, the nature and the extent of the changes should be described and related to those caused by equivalent non-GM plants. Where the changes differ from those of non-GM plants then an assessment of the relative harm to the receiving environment should be made.

If appropriate, an assessment of the potential impact of growing GM crops on wider biodiversity in the crop ecosystem would require the combination of several different approaches (ACRE, 2001b). However, since crop ecosystems are highly disturbed and dynamic areas, predicted changes in biodiversity may not necessarily be associated with environmental harm as defined in Directive 2004/35/CE (EC, 2004c). Comparisons should be made with existing crop systems and assessments of impact related to impacts of current non-GM crops.

## *9.1. Persistence and invasiveness*

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

The applicant should identify whether the GM plant differs from the parental or near isogenic non-GM plant in its biology. This should include information on biological features that affect fitness and environmental sensitivity (*e.g.*, multiplication, dormancy, survivability, dispersal, outcrossing ability, stress tolerance, and sensitivity to specific agents). The information provided should be linked to environmental risk assessment including interaction with other organisms and the environment (Sections III, D 8, 9 and 10).

If a GM plant or hybrids formed with related plant species become more persistent or invasive then they are more likely to have an environmental impact. An assessment is required of the likelihood of the GM plant becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats. The likely consequences of this increased persistence should be assessed.

Hybrids formed with related plant species are referred to Section III, D 9.5.

The applicant should refer to GM plant specific traits (see Section III, D 1), which may have an impact on increased persistence and spread both in natural and cultivated areas.

## *9.2. Selective advantage or disadvantage*

An assessment is required of any selective advantage or disadvantage conferred to the GM plant. If appropriate, comparisons should be made with the non-GM parent/relative grown in similar circumstances and with similar phenotypes that are available from conventional breeding.

Hybrids formed with related plant species are referred to Section III, D 9.5.

The applicant should, if appropriate, refer to data collected from representative field trials mentioned in Sections III, D 7.2 and 7.4, if they have relevance to environmental interactions concerning GM plant fitness. If no specific field data are provided, the applicant must discuss any consequences of selective advantage or disadvantage of the new trait(s) both in natural and cultivated areas.

## *9.3. Potential for gene transfer*

*(a) Plant to bacteria gene transfer:*

An assessment is required to assess the potential for plant to bacteria gene transfer and its consequences. The horizontal gene transfer from GM plants to bacteria with subsequent expression of the transgene is regarded as a rare event under natural conditions and especially in the absence of selective pressure, particularly if no homologous sequences are present (Nielsen *et al.*, 1997). The transfer is even less likely if the DNA inserted in the GM plant does not show homology with bacterial DNA (Gebhard and Smalla, 1998), as integration mostly occurs by homologous recombination. The inserted DNA should be evaluated for possible enhancement of gene transfer potential (*e.g.* presence of replication origins or genes/sequences that might enhance recombination). The potential impact (consequences) of such an event should be evaluated in Section III, D 7 for human and animal health and in Section III, D 9 for the environment, in particular in the light of possible long-term fixation of genetic material from GM crops in natural bacterial assemblages (Nielsen and Townsend, 2004). This may also have relevance for other microbial groups.

*(b) Plant to plant gene transfer:*

The transfer of genes from GM plants to other sexually compatible plants is a naturally occurring process (Ellstrand *et al.*, 1999). However, the gene(s) inserted may modify the potential for plant to plant gene transfer due to altered flower biology *e.g.* altered flowering period, attractiveness to pollinators, change in fertility. Thus, a risk

assessment should include an evaluation of any new change in the biology of the GM plant that might increase or decrease the potential for plant to plant gene transfer. Alternatively, experimental evidence that outcrossing frequency is unaffected should be provided.

An assessment is required of the potential for gene transfer to the same or other sexually compatible plant species under conditions of planting the GM plant and any selective advantage or disadvantage conferred to those plant species. Consideration should also be given to the fact that the gene flow characteristics of related species may differ from those of the transformed plant so that the potential for gene transfer might change.

The potential consequence arising from out-crossing to other plant cultivars should be considered and assessed for environmental risk. This will vary with species and traits. For example, the release of GM oilseed rape raises the issue of gene transfer, since this crop will readily cross-pollinate with nearby oilseed rape crops and may spontaneously hybridise also with some wild relatives. In cases where gene transfer cannot be limited between certain adjacent plants, the risk assessment should focus on the consequences of cross-pollination. The potential consequence arising from out-crossing to compatible wild species should be considered and assessed for environmental risk (Saeglitz and Bartsch, 2002). This will depend on non-GM sexually compatible plants being present in regions where the GM crops are being grown and which are available to receive pollen and produce fertile hybrids. The selective advantage of any transferred trait should be evaluated in different habitats where the selection pressures are likely to be different. For example, drought may be the main cause for the limited geographic distribution of a given plant species but where drought stress can be alleviated using a GM approach the ecological behaviour of the corresponding wild population may change after transgene introgression. On the other hand, transferred herbicide tolerance may be an advantageous trait in agricultural land but not in habitats where the herbicide is not applied.

The applicant should also refer to information provided in Sections III, D 9.1, 9.2 and 10, which may have an impact on increased persistence and spread both in natural and cultivated areas of sexually compatible plants and their wild relatives.

### *9.4. Interactions between the GM plant and target organisms*

An assessment is required of the potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GM plant and target organisms, such as predators, parasitoids and pathogens (if applicable). An example of this is provided by the EU Working Group on Bt who have developed risk assessments and protocols for evaluating the development of resistance in target insects to Bt toxins (SCP, 1999).

Data on the comparative susceptibility of the GM plant to pests and diseases compared with that of the non-modified plants are useful indicators of effects, together with

observations on agronomic performance during greenhouse and experimental field trials.

## *9.5. Interactions of the GM plant with non-target organisms*

An assessment is required of the possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GM plant with non-target organisms (also taking into account organisms which interact with target organisms), including impact on population levels of competitors, herbivores, symbionts (where applicable), predators, parasites and pathogens. An example of direct interaction approaches is provided by the Working Group on Bt (SCP, 1999).

Assessors should use a tiered approach to this risk assessment, first identifying potential hazards in controlled tests and then evaluating exposure in the field in order to estimate potential risks (see Section II, 3). If first tier tests do not identify sensitivity in exposed species then second and third tier test may not be required.

Impact should be assessed on non-target species (plant, animals and microbes) in the crop ecosystem (which may include pollinators, beneficial, predatory and phytophagous species), and, if appropriate, the aquatic environment. Studies should be designed in order that sufficient statistical power is obtained to detect possible effects on non-target organisms. Adequate statistical power can be achieved from the proper control of variation and replication, since power depends on sample size, the degree of random variation between experimental units and the chosen significance of the tests. An appropriate approach might be to select a desired level of statistical power and the size of effect to be detected, collect preliminary data to estimate within-treatment variability and then to calculate the required sample size for the proposed study. The duration of experiments to assess the risks to non-target organisms should be sufficient to reflect the pattern and duration of exposure that these organisms are likely to experience under field conditions (Perry *et al.*, 2003; Marvier, 2002). However, it is important that food chain effects due to reductions in target prey species, (*e.g.* declines in parasitoids populations) are differentiated from, for example, population declines due to the effects of GM toxin accumulation in food chains.

## *9.6. Effects on human health*

An assessment is required of the possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GM plant and persons working with, coming into contact with, or in the vicinity of the GM plant release(s). This assessment is particularly required for GM crops which are not destined for human or animal consumption and where impacts on human health may not have been so meticulously studied.

The applicant should refer to Section III, D 7, where this issue has already been addressed.

## 9.7. Effects on animal health

An assessment is required of the possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from exposure to or consumption of the GM plant and any products derived from it, if it is intended to be used as animal feed.

The applicant should refer to Section III, D 7, where this issue has already been addressed.

## 9.8 Effects on biogeochemical processes

An assessment is required of the possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GM plant and target and non-target organisms in the vicinity of the GM plant release(s).

The applicant should address, where appropriate, the potential impact on biogeochemical processes as these influence ecosystem function, *e.g.* in relation to soil microbial communities. Examples are $CO_2$-evolution, organic matter turnover, nitrogen fixation (Nannipieri *et al.*, 2003). Soil fertility strongly influences the growth and productivity of plants. As plant-associated (rhizosphere) and soil microbial communities perform the vital biotransformation that underpins soil fertility, any negative impact(s) on microbial participants in this key compartment would have to be carefully evaluated. This should be assessed on a case-by-case basis with particular reference to the nature of the introduced trait and the consequences of the genetic modification/alteration in the GM plant.

The risk assessment should aim to establish if direct or indirect effect(s) of the genetic modification in the GM plant have any long-term or sustainable deleterious effect on the recognised soil microbial communities and the associated functional activities that are responsible for maintaining soil fertility and plant productivity. The assessment should also address the fate of any (newly) expressed gene products and derivatives in those environmental compartments where they are introduced and which result in exposure of non-target organisms (*e.g.* in soil after the incorporation of plant material). Exposure should also be estimated to relevant soil biota (*e.g.* earthworms, micro-organisms, organic matter breakdown) in relation to the impact on decomposition processes. Risk assessment should also include an analysis to determine if a shift occurs in populations of deleterious organisms in the presence of the modified plant.

## 9.9. Impacts of the specific cultivation, management and harvesting techniques

An assessment is required of the possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting

techniques used for the GM plant where these are different from those used for non-GM plants.

The applicant should describe the intended commercial management regimes for the GM crop including changes in applications of plant protection products (pesticides and/or biocontrol agents), rotations and other plant management measures for the GM plant where these are different from the equivalent non-GM plant under representative conditions. The applicant should aim to assess the direct and indirect, immediate and delayed effects, of the management of the GM plant. This should include the biodiversity within the GM crop and adjacent non-crop habitats likely to be affected by the GM crop and its cultivation.

The extent of such studies will depend on the level of effect associated with a particular GM plant and on the quality and availability of the literature that is relevant to the particular risk assessment. For example, the published results of the UK's Farm Scale Assessments of genetically modified herbicide-tolerant crops (Squire *et al.*, 2003) may give information relevant to other herbicide-tolerant crops. However, it will be necessary to compare the relative efficacy of different herbicides and their management programmes on weed species in order to assess the impact of herbicide regimes on biodiversity.

The management and utilisation of a GM crop may vary from region to region and farm to farm. It may be difficult to predict the range of farming practices that will be deployed with the GM crop. The risk assessment should assess the consequences of this unpredictability of farm management and relate this to monitoring (see Section III, D 11.).

## *10. Potential interactions with the abiotic environment*

The assessments on potential changes in the interactions of the GM plant with the abiotic environment should be carried out on a case-by-case basis taking into account the biology of the recipient plant, the characteristics of the introduced genetic material, the properties and consequences of the genetic modification, the scale of release and the assessment of any risk to the receiving abiotic environment that might arise from the release of the GM plant.

Examples of possible interactions between the GM plant and its abiotic environment are:

(a) alteration of climatic conditions (*e.g.* altered production of greenhouse gases),

(b) altered sensitivity to, or tolerance of, climatic conditions (*e.g.* cold, heat, humidity),

(c) altered sensitivity to, or tolerance of, abiotic fractions of soil (*e.g.* salinity, mineral nutrients, mineral toxins),

(d) altered sensitivity to, or tolerance of, gases (*e.g.* $CO_2$, oxygen, $NH_3$),

(e) alteration of mineralisation (*e.g.* root exudates changing the soil pH).

Changes in the abiotic environment caused by any GMO may have impacts on the biotic environment so these consequences should be evaluated.

The applicant should refer to Section III, D 9, where this issue has already been addressed.

# *11. Environmental Monitoring Plan*

## 11.1. General

The Regulation (EC) No 1829/2003 introduces the obligation for applicants to implement, if appropriate, a GMO monitoring plan for Environmental Monitoring according to Annex VII of the Directive 2001/18/EC (Regulation (EC) No 1829/2003 Art. 5(5)(b) and Art 17(5)(b)) and a proposal for the post-market monitoring regarding use of the food and feed for human and animal consumption (Regulation (EC) No 1829/2003 Art. 5(3)(k) and Art. 17(3)(k). The latter is not described in any detail in the Regulation (EC) No 1829/2003. Section III, D 7.11 of this Guidance Document refers to the post-market monitoring of GM food/feed.
In reference to Directive 2001/18/EC the Environmental Monitoring is introduced in order to identify any direct or indirect, immediate and/or delayed adverse effects of GMOs, their products and their management to human health or the environment, after the GMO has been placed on the market.

Since the Regulation (EC) No 1829/2003 explicitly refers to Annex VII of Directive 2001/18/EC the structure and content of this environmental monitoring plan should be designed in accordance with the Council Decision 2002/811/EC supplementing Annex VII (strategy, methodology, analysis, reporting; EC, 2002b, see also ACRE, 2004; Wilhelm *et al.*, 2003).

An environmental monitoring plan is required for applications for placing on the market of GMOs or food/feed containing or consisting of GMOs conforming with Annex VII to Directive 2001/18/EC. It is explained in the Guidance notes supplementing Annex VII that the extent of the market release shall be taken into account. Thus, the monitoring plan should be targeted rather than considering every possible environmental aspect. Applications concerning only food/feed or ingredients (for example, imported into but not cultivated within the EU) will thus not normally be required to describe a detailed environmental monitoring plan if the applicant has clearly shown that environmental exposure is absent or will be at levels or in a form that does not present a risk to other living organisms or the abiotic environment.

Monitoring can be defined as the systematic measurement of variables and processes over time and assumes that there are specific reasons to collect such data, for example, to ensure that certain standards or conditions are being met or to examine potential changes with respect to certain baselines. Against this background, it is essential to

identify the type of effects or variables to be monitored, an appropriate time-period for measurements and, importantly, the tools and systems to measure them. Monitoring results, however, may lead to adjustments of certain parts of the original monitoring plan, or may be important in the development of further research. The Council Decision 2002/811/EC (EC, 2002b) provides no clear differentiation between the monitoring principles of either case-specific monitoring or general surveillance (Den Nijs and Bartsch, 2004). This Guidance document provides further assistance in the following sections.

## *11.2. Interplay between environmental risk assessment and monitoring*

Monitoring of effects: Foreseen and unforeseen

The environmental monitoring of the GM plant will have two aims: (1) to study any possible adverse effects of the GM plant identified in the formal risk assessment procedure, and (2) to identify the occurrence of adverse unforeseen effects of the GMO or its use which were not anticipated in the environmental risk assessment. Where there is scientific evidence of a potential adverse effect linked to the genetic modification, then case-specific monitoring should be carried out after placing on the market, in order to confirm the assumptions of the environmental risk assessment. Consequently, case-specific monitoring is not obligatory and is only required to verify the risk assessment, whereas a general surveillance plan must be part of the application. Applicants who are proposing to have no case-specific monitoring are encouraged to provide arguments in support of this position. These arguments should relate to the assumptions applicants have made in the environmental risk assessment, as well as to the lack of any identified adverse effects in tier 1, 2, or 3 tests (see Section II, 3 of this Guidance document).

Monitoring framework

Council Decision (2002/811/EC) (EC, 2002b) explicitly suggests that general surveillance should include long-term monitoring, to allow for unexpected effects that may occur after longer periods of environmental exposure.

Changes in the management and cultivation techniques of new GM crops may affect the environment *e.g.* through changes in agrochemical usage. Directive 2001/18/EC requires that the impacts of any such indirect effects, *e.g.* changes of cultivation methods, should be addressed by the monitoring plan based on the outcome of the environmental risk assessment.

The environmental monitoring plan should describe in detail the monitoring strategy, methodology, analysis, reporting and review as laid down in Council Decision 2002/811/EC. In this respect,

(a) **GM plant-based parameters** will depend on the particular GM plant, trait and environment combination. Key parameters to be observed may refer to species/ecosystem biodiversity, soil functionality, sustainable agriculture, or plant

health. Indicators should be measurable, appropriate, adequate in terms of statistical power, and comparable with existing baseline data.

(b) **background and baseline environmental data** *e.g.* soil parameters, climatic conditions, general crop management data *e.g.* fertilisers, crop protection, crop rotations and previous crop history should be collected, where appropriate, to permit the assessment of the relevant parameters listed under a).

## *11.3. Case-specific GM plant monitoring*

The main objective of case-specific monitoring is to determine the significance of any adverse effects identified in the risk assessment (see Sections III, D 8, 9 and 10). The assessment of risk should be based on Annex II of the Directive (2001/18/EC).

Case-specific monitoring should be targeted at those environmental factors most likely to be adversely affected by the GM plant which were identified in the environmental risk assessment. The scientific approach should be designed in order to test the specific hypothesis of expected adverse effects derived from the environmental risk assessment. The monitoring programme design should also reflect levels of exposure in different geographical regions and other specific site influences. Such monitoring may be carried out at a limited number of sites ('local monitoring'), where exposure is greatest and intensive recording and data collection can take place. This would be particularly appropriate when it is envisaged that there will be a phased or gradual introduction of the GM crop into a limited number of regions in various EU Member States. The scale of the monitoring should be increased as the area and range of the GM crop expands, and the crop is grown in more regions. The monitoring should consist of the systematic recording of relevant parameters at representative locations where there is significant and repeated growing of the GM crop. This might also be defined according to the extent of the cultivation of the GM crop, the occurrence of targeted pest species or particular climatic/eco-regions. The methods selected, the duration of the monitoring, the extent or number of areas and the parameters to be monitored will be determined on a case-by-case basis. Whilst the planning and execution of case-specific monitoring is under the applicant's responsibility, it may be appropriate for the applicant to involve public institutions to contribute to the agreed work.

## *11.4. General surveillance for unanticipated adverse effects*

The objective of general surveillance is to identify the occurrence of unanticipated adverse effects of the GM plants or its use on human health or the environment that were not anticipated in the environmental risk assessment. General surveillance applies where no adverse effect has been identified in the environmental risk assessment, but is always required in order to detect unanticipated adverse effects (EC, 2002b). Monitoring of potential adverse cumulative long-termeffects and areas of uncertainty identified in the environmental risk assessment are important objectives of monitoring (EC, 2002b) which should be considered initially within Case-Specific Monitoring. When there is a negligible degree of uncertainty in the environmental risk assessment then no

Case-Specific Monitoring is indicated. However, general surveillance is always required for monitoring any unanticipated adverse effects.

An effect can be defined as an alteration that results in values that fall outside the normal range, given the variation due to the constant changes in the agricultural practices, rural environment and associated biota in the European Union. A major challenge of general surveillance is determining whether:

- an unusual effect has been observed
- the effect is adverse and
- the adverse effect is associated with the GM plant or its cultivation.

The use of a range of monitoring systems to supply data and the ability to compare data from these different sources will help to indicate whether an effect is unusual and adverse.The identification of an adverse effect which is potentially linked to specific GM plants would trigger the need for a specific study to evaluate harm and determine cause.

An objective of the Directive 2001/18/EC (EC, 2001a) is to protect the environment including biodiversity, water and soil. The GMO Panel is of the opinion that one important task within general surveillance is to link monitoring to these environmental protection goals. Recently, EU Directive 2004/35/EC on environmental liability with regard to the prevention and remedying of environmental damage (EC, 2004c) defined environmental damage as a measurable adverse change in a natural resource or measurable impairment of a natural resource service which may occur directly or indirectly.

Within a broader concept of environmental issues, unanticipated adverse effects on human health have also to be addressed in the monitoring plan presented by the applicant. The scope of monitoring for unanticipated adverse effects on human health is defined, according to Directive 2001/18/EC, as monitoring for unanticipated adverse effects that may result from handling of the GM plant.

It might prove very difficult to design monitoring (including general surveillance) for unanticipated adverse effects on human health. However, knowing that the release of GM plants needs to be considered in context of their interaction with other environmental components, monitoring for health effects could be considered in conjunction with human population screening methods currently used by public health organisations (for assessing such elements as incidences of allergic reactions) and as part of the suggested plant production and farm questionnaires.

### 11.4.1 Approach and principles of general surveillance

Applications concerning food/feed uses and import and processing do not require scientific information on possible environmental effects associated with the cultivation of the plant. The extent of general surveillance for these GM plants will depend on the level of environmental exposure. Therefore the GMO Panel differentiates between

general surveillance plans as part of applications for import/processing and applications for cultivation.

#### *11.4.1.1. Approach and principles for GM plants intended for import and processing only*

General surveillance plans as part of applications for import and processing will need to take account of the modified characteristics specific to the GM plants in question, their intended use and the receiving environment (EC, 2002b). The extent of the general surveillance plan will depend on the level of environmental exposure, the establishment, persistence and spread of the GM plant and does not require scientific information on possible environmental effects associated with the cultivation of the plant. The applicant has to show that environmental exposure will be at levels or in a form that does not present a risk to other living organisms or the abiotic environment (see section 11.1 of the Guidance document).

In the case of non-viable GM material (e.g. derived products not containing any living GMOs) and according to Directive 2001/18/EC, the applicant does not have to provide any environmental monitoring plan (including general surveillance).

In the case of imported GM products containing viable propagating material, general surveillance plans should consider that if substantial loss, spillage and establishment is possible, appropriate management systems should be in place to restrict environmental exposure.

The EFSA GMO Panel has assessed general surveillance plans as part of applications for import and processing of maize and oilseed rape (e.g. EFSA, 2003, 2004c, 2004d, 2005a, 2005b, 2005c). Monitoring plans of GMOs applications submitted Regulation (EC) No 1829/2003, for which an opinion in accordance with Articles 6.5 and 18.5 has been published, are available on EFSA web page[11].

#### *11.4.1.2. Approach and principles for GM plants intended for cultivation*

General surveillance plans as part of applications for cultivation will need to take account of the full environmental effects of the GM plant including its cultivation.

The GMO Panel is of the opinion that general surveillance is a general overseeing of the geographical regions where GM plants are grown without having any specific hypothesis on adverse effects on human health or the environment. As general surveillance is not hypothesis-driven, it is not conducted using directed experimental approaches (see also ACRE, 2004; Sanvido et al., 2005). However, robust scientific methodology should be applied wherever possible in order to evaluate empirical knowledge. This especially refers to defining sample sizes, sampling and recording methods, in order to produce statistically valid data for determining causes and effects.

[11]http://www.efsa.europa.eu/EFSA/ScientificPanels/GMO/efsa_locale-1178620753812_GMOOpinions455.htm

Existing surveillance systems should be used where practical (e.g. routine farm recording systems) and any 'unusual' effect, not occurring in similar situations within conventional cropping, should be recorded (e.g. effects on soil).

The establishment, persistence and spread of a GM plant is not an environmental hazard in itself. Similarly, dispersal of pollen and seeds and gene flow per se are not environmental hazards and thus the focus of general surveillance should be on recording any unanticipated consequences of the cultivation of the GM plant, such as unforeseen weediness, invasiveness or changes in plant population dynamics or populations of biota associated with the GM plants. However, an unanticipated adverse effect is most likely to occur where the level of environmental exposure is highest. Thus, an evaluation of how and where the GM plant will be grown and the associated environmental exposure is considered a good starting point in any general surveillance plan.

General surveillance of the impact of GM plant should

- be applicable, in a proportionate and cost-effective manner, for monitoring the GM plant in a range of representative environments, reflecting the range and distribution of farming and environments exposed to the GM plants and its cultivation. If unusual effects on human health or the environment are reported, more focussed in-depth studies should be carried out in order to determine cause and relationship with GM plants. Such additional studies would be Case-Specific Monitoring studies as they would require an experimental approach to confirm the specific hypothesis that an observed effect is associated with the GM plant,
- complement available general environmental monitoring. The higher the ecological integration and scale (from the individual to a population, from single farm to regions) the more difficult it is to distinguish potential effects of the GM plants from other factors. Initially, general surveillance should focus on each event individually. Additionally, when several GM plants have been commercialised, the interactions between these GM plants and their management may need to be considered where appropriate.

The EFSA GMO Panel has assessed general surveillance plans as part of applications for cultivation (e.g. EFSA, 2005d, 2005e). Monitoring plans of GMOs applications submitted under Regulation (EC) No 1829/2003, for which an opinion in accordance with Articles 6.5 and 18.5 has been published, are available on EFSA web page[12].

### 11.4.2 Main elements of general surveillance

The applicant should:

- define the methods and approaches that will be used to conduct general surveillance of regions where the GM plant occurs,

[12] http://www.efsa.europa.eu/EFSA/ScientificPanels/GMO/efsa_locale-1178620753812_GMOOpinions455.htm

- refer to introduction, stewardship and exploitation plans for the GM plant, and
- make proposals for the time period, area covered, and the frequency of monitoring.

#### *11.4.2.1. Existing monitoring systems*

Applicants will have developed plans for the introduction, marketing, management and stewardship of the GM plant. The GMO Panel is of the opinion that applicants should include these into the monitoring plans, where appropriate, as they will contain some data of relevance to the implementation of the monitoring plan.

General surveillance should, when compatible, make use of established routine surveillance practices such as monitoring of agricultural plants, variety/seed registration, plant protection, plant health and soil surveys as well as ecological monitoring and environmental observations (EC, 2002b).

Many of the existing monitoring systems and networks collecting environmental data are unlikely to always provide data of relevance that may be used in monitoring impacts of GM plants. The design of the existing monitoring programs, the targets (e.g. birds, plant protection, etc.), the time, frequency and scale of data collection, sampling, analysis and reporting methods may not suit the monitoring of GM plants because they have been designed for other purposes. Moreover, the existing monitoring systems will differ from country to country and it may not be feasible or practicable to modify existing surveillance systems in order to make them suitable for general surveillance of GM Plants. Thus applicants may not consider existing networks to be sufficiently useful sources of information for monitoring. There may be a need for additional environmental surveys and to amend the monitoring objectives of existing monitoring systems (see also Sanvido *et al.*, 2004, 2005).

Because existing monitoring systems can be of variable quality and consistency, it is important that the consistency and reliability of surveys utilised in general surveillance is evaluated in order to ensure long-term coherence and reliability of data collection and data quality. In addition, as environmental surveys will differ between networks, methods for integrating data from different origins should be evaluated.

Knowing the limitations of existing monitoring systems, it is important for the applicant to describe the processes and criteria that will be used for selecting and evaluating existing monitoring systems for supplying data related to the unanticipated adverse effects of GM plants in the general surveillance.

Specifically the applicant should

- describe which observations could be monitored through existing monitoring schemes,
- identify the type of existing monitoring systems that would be appropriate for this in the countries where the GM plant will be grown (e.g. monitoring of agricultural cultivars and plant protection surveys),

- describe the criteria and generic approach used to evaluate existing monitoring networks and how appropriate networks will be selected,
- describe how arrangements for collecting, collating and analysing data will be made,
- identify which category of additional surveys could be required to contribute to the general surveillance (e.g. public institutions, farm associations) in selected regions or Member States,
- describe how formal agreements, procedures and communication will be established with the Commission and Member States or other third parties before commercial market introduction, although detailed arrangements may not have been agreed at the time of the application.

According to Council Decision 2002/811/EC the responsibility for each step in the monitoring plan should be clearly assigned by the applicant. Where third parties are employed or contracted to conduct monitoring studies, the nature of their involvement should be detailed.

#### *11.4.2.2. Use of GMO-focussed monitoring systems*

In addition to using existing monitoring systems, applicants are encouraged to develop new and more focused monitoring systems especially at the production level. Questionnaires, directed at farms where GM plants are grown, are considered a useful method to collecting first hand data on the performance and impact of a GM plant and for comparing it with conventional plants (ACRE, 2004; Sandivo *et al.* 2005; Wilhelm *et al.*, 2004a,b). Experience from other established surveillance and monitoring systems (*e.g.* the approach used for consumer and pharmaceutical surveillance systems) could be used in designing questionnaires. Special emphasis should be given to the statistical design of such questionnaires. Issues of human health (e.g. due to exposure and handling of GM plants) may also be integrated into farm questionnaires.

As appropriate, the applicants should

- inform growers, seed suppliers or other stakeholders about the GM plant and the need to supply data on seed sales, areas sown, plant management, etc.
- be pro-active in developing reporting systems so that farmers (or their agents and advisors) intending to purchase genetically modified seeds will be fully informed about the GM plant, the importance of the monitoring programme and the reporting of unanticipated effects during and after the cultivation of the GM plant,
- describe the number of farmers/growers involved, the area covered, the reporting methods and the suitability of the data collected for statistical analysis,
- establish independent audits to ensure the independence and integrity of all monitoring data,
- indicate the likely frequency of inspections.

Farm questionnaires should

- be designed to ensure the statistical validity and representativeness of the collected data, including the proportion of fields growing the GM plant in a region and the number of questionnaires required to achieve statistical power in the data collected,
- be designed to generate data on the agronomic management of GM plants as well as data on impacts on farming systems and the farm environment,
- use a field or group of fields growing the GM plant as the basic unit for monitoring,
- observe the field/fields in subsequent years for any unusual residual effects,
- be user friendly but also information rich,
- be constructed to encourage independent and objective responses from farmers, land managers and others involved with the GM plant or its products.

Questionnaires adapted to agronomists or other stakeholders working on the farms growing the GM plants may also be useful sources of information. Focussed questionnaires and interviews are generally accepted by respondents. Professional interviewers may be an additional help.

Examples of farm questionnaires have been developed by Wilhelm *et al.*, (2004a,b) and some farm questionnaires have already been assessed by the GMO Panel (EFSA, 2005d, 2005e).

Farm questionnaires should be distributed, completed and collated annually via an arranged reporting system (*e.g.* farm questionnaire forms or online systems). These should be analysed by the applicant and reports submitted at the agreed time intervals (usually annually) to appropriate Competent Authorities. The results of the farm questionnaires will allow the applicant to record the implementation of recommended management and stewardship of the GM plant (*e.g.* good agricultural practice, hazard analyses, critical point compliance) and to identify unanticipated adverse effects.

### 11.4.3 Importance of a baseline

There is a need for general surveillance plans using both existing and novel monitoring systems to be able to compare impacts of GM plants and their cultivation with those of conventional plants. The baseline is the current status quo e.g. current conventional cropping or historical agricultural or environmental data. Direct comparison with non-GM plant reference areas should be used if available, but reference can also be made to the historical knowledge and experiences of the "observer" (*e.g.* farmers, inspectors, wildlife surveyors) in relation to the situation prior to the introduction of the GM plant (see initiative developed by FAO, 2005). It will be important to inform observers to report any unusual events and not to attempt to anticipate impacts.

There is also a need to take into account the fact that the GM event will occur in a changing genetic background of new varieties which may have an impact independent of the GM event and thus it is the event that needs to be monitored in any variety.

### 11.4.4 Data quality, management and statistical analyses

The design of the monitoring programme will influence the quality and usefulness of resulting data, hence efforts should be made to ensure that data from all the monitoring systems used can be statistically analysed (Wilhelm *et al.* 2003, 2004a,b). Meta-analyses of different datasets might be useful. If relationships between datasets can be identified, it will contribute to the credibility of monitoring.

The general surveillance plan should

- take account of the scale of commercialisation as well as the historical baseline knowledge in different areas to be monitored,
- consider the geographical areas to be studied and which existing environmental monitoring programmes could be useful for inclusion,
- consider national cultivation registers of GM plants (including co-existence measures) as they can provide useful data,
- describe the generic approach used for data collection, management and exploitation within general surveillance (*e.g.* data from existing networks and questionnaires),
- describe how any unusual adverse effects related to GM plants will be identified, including details of the statistical approach,
- include a comprehensive description of the techniques to be used for data analysis and statistical analysis, including the requirements for statistical significance,
- provide a detailed description of the operational handling of data from different sources into a 'general surveillance database',
- describe the approach to categorise the data (*e.g.* influencing factor, monitoring character) and the method for pooling the results and matching them with data on GM cultivation in time and space,
- contain data from Case-Specific Monitoring that might complement the general surveillance data.

## *11.5. Reporting the results of monitoring*

Following the placing on the market of a GMO, the applicant has a legal obligation to ensure that monitoring and reporting are carried out according to the conditions specified in the consent. The applicant is responsible for submitting the monitoring reports to the Commission, the competent authorities of the Member States, and where appropriate to EFSA. Applicants should describe the methods, frequency and timing of reporting in their monitoring plan.

Although no timeframe for reporting is specified in Council Decision 2002/811/EC (EC, 2002b), reports, allowing for case-specific adaptations, preferably should be submitted

- annually confirming that monitoring has been carried out according to the given consent together with a summary of major preliminary results that are important for a short-term feedback on the environmental risk assessment ('annual reports'), and

- periodically (*e.g.* every third year) covering longer periods in which observations and data collected are reported and analysed in detail and which therefore provide more comprehensive reports that are important for a longer term feedback on the environmental risk assessment ('comprehensive report').

The comprehensive monitoring report should include in more detail the results of any relevant monitoring by third parties, including the farmers/growers, seed companies, independent surveyors, local, regional and national environmental surveyors. In addition, the applicant should evaluate these results and incorporate full analysis and conclusions in the submitted monitoring report. If appropriate, the applicant should provide access to raw data for stimulating scientific exchange and co-operation.

Flow of information on the cultivation of GM plants:

Where GM plants are grown the following procedures should be complied with:

(a) All GM seeds must be labelled with the variety, and should also contain information on the construct, the supplier's name and address, full instructions on any specific cultivation requirements, and reporting procedures for any incidents, including the address of the Consent Holder for the marketing of the seeds.

(b) The farmer/grower is required to declare the variety, sowing date, amount of cultivated crops and exact geographic location to the national cultivation register according to Directive 2001/18/EC - Art 31 (3b).

(c) The farmer should record all relevant cropping and management data for that GM crop and these data should be available for inspection.

Flow of information in instances where GM plants are thought to have caused unusual or adverse effects:

If adverse effects have been detected in areas where GM plants are grown or where there is a suspicion that the GM plants may be associated with an incident, the following procedures should be complied with:

(a) Farmers should follow the procedure for reporting established by the applicant at the time of purchase of the GM seeds and provide information to the information point specified therein of any unusual observations without delay.

(b) The applicant shall immediately take the measures necessary to protect human health and the environment, and inform the competent authority thereof. In addition, the applicant shall revise the information and conditions specified in the application.

(c) The applicant may inform external organisations (*e.g.* public institutions), asking them to immediately communicate any adverse effects they may detect to a specified information point.

(d) The applicant could carry out a preliminary examination in order to verify whether a GM plant-related effect has really occurred and provide the competent authority with a report on the result of its preliminary investigations, including an assessment of potential harm.

(e) If information becomes available to the competent authority which could have consequences for the risks of the GMO(s) to human health or the environment it shall immediately forward the information to the Commission and the competent authorities of the Member States.

(f) Where adverse effects on the environment are observed, further assessment should be considered to establish whether they are a consequence of the GM plant or its use, as such effects may be the result of environmental factors other than the placing on the market of the GM plant in question. The competent authority should inform the Commission of the reported observation and, together with the applicant and scientific institutions or experts investigate the causes and consequences of the reported incident. The competent authority should submit a report to the Commission and EFSA on the extent of any environmental damage, remedial measures taken, liability and recommendations for the future use/management of the GM plant.

### *11.6. Review and adaptation*

Monitoring plans should not be viewed as static. It is fundamental that the monitoring plan and associated methodology are reviewed at appropriate intervals and may need to be modified and adapted depending on the results of the monitoring information collected. The monitoring plan might also be adapted based on an assessment of the appropriateness and cost effectiveness of the monitoring plan. Implementation of the revised monitoring plan remains the responsibility of the applicant unless otherwise determined by the competent authority.

## *12. ERA of GM plants containing transformation events combined by conventional breeding*

In the case of GM plants containing transformation events combined by conventional breeding the environmental risk assessment should take into account the evaluation of the individual events and additional data from molecular characterisation and comparative compositional analysis of the stacked events when determining potential interactions between genes or between gene products. The environmental risk assessment should evaluate any interactions between the stacked events which could result in modified environmental effects of the GM plant. In particular the combination of transgenes may result in changes in expression levels which may lead to a significant biological impact that may need to be assessed. However, it should be noted that expression levels may vary significantly also in the individual events. The guidelines below set out certain minimum requirements for the provision of information. If possible

adverse effects have been identified through experimentation or if there are scientific reasons to believe they might exist then further data should be provided or information given.

***Invasiveness and selective advantage or disadvantage***

Comparison between plants containing the stacked events and the most appropriate comparators during one representative growing season and multiple geographical locations representative of the various environments in which the GM plants will be cultivated are necessary. Additional field data may be required if changes are observed in i.e. behaviour, fitness, reproduction, survivability or dissemination.

***Interactions between the stacked events and target organisms***

In order to evaluate/identify possible altered efficacy of biocidal gene products to target organisms in the stacked events as compared to the individual events, the potential impact on target organisms should be assessed in one year field trials initially. If biologically relevant changes are observed, additional studies might be required.

***Interactions between the stacked events with non-target organisms***

Stacked biocidal events may have different effects on non-target organisms when compared with the individual events. Therefore there is a need to focus on changes in sensitivity of non target organisms and/or specificity of biocidal gene products. To test the hypothesis that such combined events do not interact, a minimum of one year field trials are required. Where appropriate, further laboratory tests on a range of relevant non-target organisms representing ecological functions, using plant material containing the combined events may be required.

***Impacts of the specific cultivation, management and harvesting techniques***

Differences in the specific cultivation, management and harvesting techniques between plants containing the stacked events and the parental lines, and any environmental impacts of such differences, should be evaluated and, where appropriate, supported by relevant data.

***Environmental Monitoring Plan***

The general principles of the Post-Market Environmental Monitoring (PMEM) as described in the Guidance Document of the GMO Panel are retained for applications concerning stacked events. Case-specific monitoring should take into account the results of the environmental risk assessment, plus any monitoring already proposed or established for individual events previously approved. Consideration should be given to any additional environmental exposure or other effect due to the combination of events identified in the environmental risk assessment. General surveillance should proceed as for any other GM crop and take account of any general surveillance plans already proposed or established for individual events previously approved.

# IV. INTEGRATIVE RISK CHARACTERISATION OF GM PLANTS REGARDING FOOD/FEED SAFETY AND ENVIRONMENTAL IMPACT

## 1. INTRODUCTION

The risk assessment process consists of four steps *i.e.* hazard identification, hazard characterisation, exposure assessment, and culminates in the final integrative risk characterisation.

Risk characterisation is defined as: "The quantitative or semi-quantitative estimate including attendant uncertainties, of the probability of occurrence and severity of adverse effect(s)/event(s) in a given population under defined conditions based on hazard identification, hazard characterisation and exposure assessment" (SSC, 2000). This chapter describes how the risk characterisation step should be carried out and gives examples of issues to be addressed.

Where the total scientific information is insufficient, inconclusive, or uncertain, or where there are indications that the possible effects on human/animal health and the environment may be potentially dangerous and inconsistent with the chosen level of protection, the precautionary approach may be invoked (EC, 2000b). Application of the precautionary approach is distinct from the normal conservative scientific approach in the assessment of data based on safety or extrapolation factors. Application of the precautionary approach is the responsibility of the risk manager and not of the risk assessor and will therefore not be dealt with in this Chapter.

## 2. HOW TO CARRY OUT THE RISK CHARACTERISATION

Risk analysis starts with defining the proper questions which should be addressed during the risk assessment, *i.e.* identification of potential risks of cultivation of GM plants and/or human/animal consumption of derived food/feed. Problem formulation should involve risk managers, risk assessors and stakeholders *e.g.* producers, growers, environmental and consumer groups. For instance, cultivation areas, exposure routes and intake, target populations (humans/animals/environment) and health end-points should be identified for the GM plant and its derived foods/feed and existing knowledge on the use of the non-modified parent plant and derived foods/feed should be collected.

The final risk characterisation of GM plants and derived foods/feed is focused on data from hazard identification and hazard characterisation, using laboratory and target animal studies, environmental studies (laboratory scale, greenhouse) and field trials, and on exposure/intake data. A *comprehensive* risk characterisation should be carried out, *i.e.* considering all the available evidence from several approaches including molecular analysis, agronomical and compositional analysis, toxicity and allergenicity testing, and environmental impact analysis. The risk characterisation may give indications for the requirement of specific activities for post-market monitoring of GM food/feed and for environmental monitoring of GM plants.

The risk characterisation should provide evidence whether the hazard identification and subsequent characterisation is complete. It is essentially an *iterative* process. Integration and evaluation of data from hazard characterisation and exposure assessment may indicate that appropriate risk estimation can be made, or that further data should be generated in order to complete the risk characterisation. For instance if an increased intake of a GM derived food/feed by humans or animals may be expected further data on toxicity at extended dose ranges may have to be generated. The absence of data essential for the risk assessment and the quality of existing data should be discussed. It should be clear from the discussion how this body of information has been taken into account when the final risk estimation is determined.

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

Estimation of uncertainties in experimental data should be handled by proper statistical analysis, while quantification of uncertainties in assumptions (*e.g.* extrapolation of data from animals to humans, extrapolation from environmental laboratory studies to complex ecosystems) may be more difficult, but should be highlighted.

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

# 3. ISSUES TO BE CONSIDERED FOR RISK CHARACTERISATION

Risk characterisation of GM plants should be carried out in a holistic manner as stated above and on a case-by-case basis depending on the type of genetic modification, taking into considerations cultivation practice of the GMO and use of the derived foods/feed for human/animal consumption. Below a number of issues are described for consideration in the risk characterisation step. The list of issues is by no means exhaustive.

## Molecular characterisation

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

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

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

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

**Comparative analysis**

An important issue to be evaluated is whether the comparative analysis between the GM crop and the traditionally grown crop with respect to agronomic, morphological and compositional characteristics has been carried out appropriately according to current guidelines and what evidence is available that the conventional crop can be taken as a reference for safe environmental cultivation and human/animal use.

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

The intended/unintended effects of the genetic modification are expected to 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 all or most conditions can be an indicator of such an effect. Whilst sporadic differences or laci o may reflect the inherent variability known to occur in the GM plant and the non-GM comparator 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 7.1.2, the following background data may be considered to put them into context with respect to their potential relevance for the human/animal health, and the environment:

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

  Commonly considered is the range of levels observed for the compounds known to occur in the comparator and in conventional varieties with a history of safe use in food and feed. This variability may be caused by differences that are genotype-dependent, environmentally dependent, or caused by genotype x environment interactions. . In addition, the range of levels observed in a broad spectrum of food and feed representative for the human and animal diet may be

taken into account. The rationale for considering this variability in the safety assessment is that it reflects the levels of the specific compound to which consumers may be exposed.

- *Information of variation of constituents from databases.*

  The databases used for comparison should be specified. When using literature data, however, databases must be adequately assessed for their quality (*e.g.* type of material analyzed, analytical method used). No formal statistical analysis should be carried out, but ranges as well as mean values should 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. It should be noted that several environmental factors such as soil composition and fertilization might influence levels of compounds in plants and should be taken into account when comparing analytical data from field studies with literature data.

Based upon one or more of the considerations above, it can be established whether the differences and/or lack of equivalence observed can 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.

Another important issue to be addressed is whether unintended effects of potential significance have been missed. Where the occurrence of unintended effects cannot be excluded, strategies to assess the potential human/animal health and environmental implications should be explained.

**Food/feed safety in relation to intake**

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

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

Data on the characteristics of the compounds including potential biological effects in humans and animals, and effects in the environment should be considered. If the compounds have known adverse health effects and maximum levels for the presence of

these compounds in the plant or derived products were laid down in specific legislation, these maximum levels should 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), should be considered in relation to the anticipated intake. In cases where the compound has been safely consumed in food, the intake levels of consumers from a conventional diet can implicitly be considered as safe.

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

In cases where more complex genetic modifications are produced, *e.g.* via transfer of multiple genes in a single construct, re-transformation of pre-existing GM lines, and trait stacking through conventional breeding of GM parents, strategies for the assessment of any risk(s) associated with possible interactions between the newly expressed proteins, new metabolites and original plant constituents should be discussed. A holistic approach for the assessment should be demonstrated considering all available information on *e.g.* the mode of action of the newly expressed proteins, the molecular and compositional/agronomical characteristics of the GM plant, and where applicable on the outcome of animal toxicity studies and feeding trials. Where animal feeding trials are not performed an explanation should be provided as to why these were not considered necessary.

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

With respect to intake estimations of GM plant derived foods for humans, the applied methodologies should be evaluated with respect to uncertainties associated with the prediction of long-term intake. Specific attention should 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 should 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 should be discussed.

### Environmental impact

Predicting impacts of GM plants on complex ecosystems which are continually in flux is difficult and largely based on experiences with other introductions and an understanding of the robustness of ecosystems. It is recognised that an environmental risk assessment

is limited by the nature, scale and location of experimental releases, which biospheres have been studied and the length of time the studies were conducted. Probabilistic methods could be used to determine ranges of plausible values rather than single values or point estimates, which are subsequently combined in order to quantify the uncertainty in the end result. These methods could provide a powerful tool to quantify uncertainties associated with any steps in the risk assessment.

Among others issues to be addressed are whether or not sound predictions can be made of the stability of introduced and expressed traits in the GM plant under representative environmental conditions, whether the potential manifestation of adverse environmental effects can be predicted in the long term, and whether extrapolation of data from small to large-scale use is possible.

Scientific knowledge and experience gained from growing GM crops during the monitoring and provisional approval periods for GM crops will also inform the risk assessment process and are opportunities to continually update environmental risk assessments in the light of any new knowledge.

## 4. THE RESULT OF RISK CHARACTERISATION

The final risk characterisation should result in informed qualitative, and where possible, quantitative guidance to risk managers. It should explain clearly what assumptions have been made during the risk assessment in order to predict the probability of occurrence and severity of adverse effect(s)/event(s) in a given population and/or on the environment, and the nature and magnitude of uncertainties associated with establishing these risks.

It should be clearly indicated when a scientific risk assessment *cannot* be completed because of the lack of essential data or the availability of poor quality data.

The risk characterisation should include considerations:

- whether cultivation of GM plants is as safe for the environment as the cultivation of non-GM plants;
- whether consumption of foods/feed derived from GM plants is as safe for humans/animals as the conventional comparators;
- whether specific conditions for GM crop cultivation, may be required;
- regarding the scientific basis for different options to be considered for risk management, including post market monitoring.

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Annex I

# EFSA GUIDANCE TO APPLICANTS ON THE PRESENTATION OF APPLICATIONS FOR THE REQUEST OF AUTHORISATION OF GENETICALLY MODIFIED PLANTS AND/OR DERIVED FOOD AND FEED

24 September 2004

## Introduction

This annex provides guidance on the presentation of applications for the placing on the market of genetically modified plants and/or derived products introduced under Community legislation (on *genetically modified (GM) food and feed*[13] and on *the deliberate release into the environment of genetically modified organisms*[14] *(GMOs)*) to be evaluated by the GMO Panel of EFSA. This annex will be regularly updated in view of the experience that EFSA and the GMO Panel will develop with the handling of GMO applications.

## Application for the authorisation of GM Plants and/or derived food and feed

An application for the authorisation of a GMO and/or derived product submitted within the framework of Regulation (EC) No 1829/2003 should preferably be presented in English and should consist of the particulars as specified by Articles 5 (3) and 17 (3) of that Regulation and as further detailed in Regulation (EC) No 641/2004[15].

In the case of an application relating to a GMO for food or feed use, references to "food" or "feed" shall be interpreted as referring to food or feed containing, consisting of or produced from the GMO according to Articles 5 and 17 (4) of Regulation (EC) No 1829/2003 in respect of which an application is made.

[13] Regulation (EC) No 1829/2003 on genetically modified food and feed, OJ L 268, 18.10.2003, p. 1.

[14] Directive 2001/18/EC on the deliberate release into the environment of GMOs and repealing Council Directive 90/220/EEC, OJ L 106, 17.4.2001, p. 1

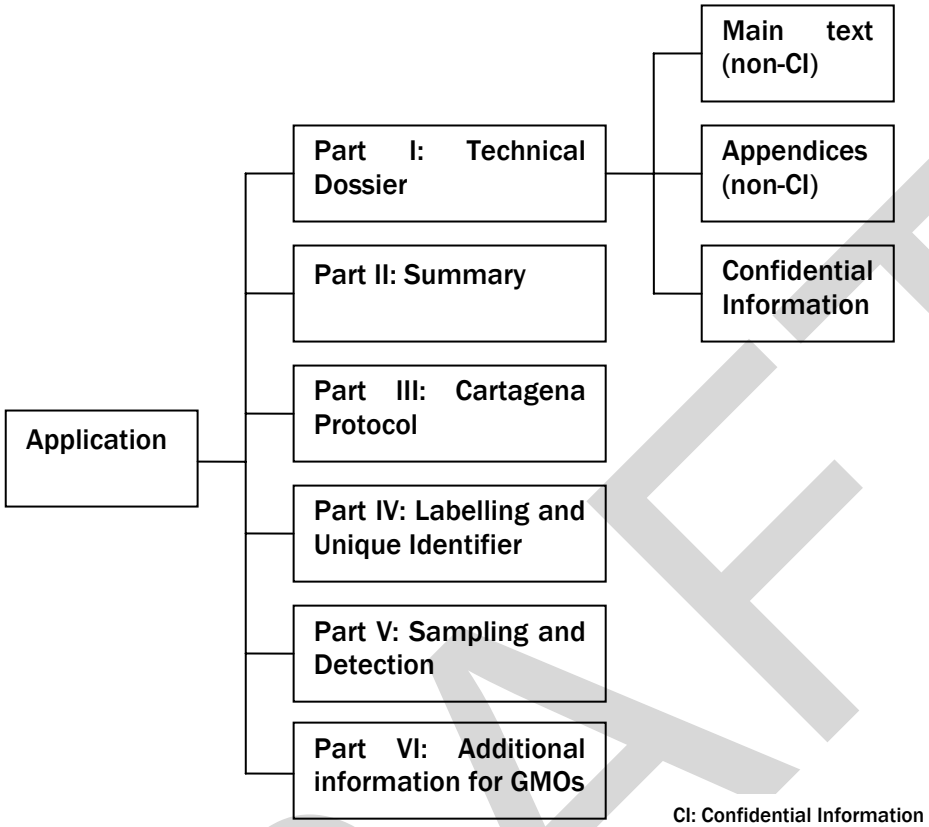
[15] Regulation (EC) No 641/2004 on detailed rules for the implementation of Regulation (EC) No 1829/2003 of the European Parliament and of the Council as regards the application for the authorisation of new genetically modified food and feed, the notification of existing products and adventitious of technically unavoidable presence of genetically modified material which has benefited from a favourable risk evaluation, OJ L 102, 7.4.2004, p. 14.

Where applications submitted in a Member State under other Community legislation[16] are transformed into an application under Article 46 of Regulation (EC) No 1829/2003, the original application shall be updated and revised according to the requirements of Regulation (EC) No 1829/2003 and to the EFSA guidance on GM plants and derived food and feed. As the case may be, the initial assessment report of the rapporteur Member State, as well as the response of the applicant to Member States' questions shall be made available to EFSA. The questions/answers should be grouped by subject (Molecular Characterisation, Food/Feed Safety, and Environmental Risk Assessment), and where appropriate, refer to the page-number in the dossier to easily trace-back the issue.

The **application** should consist of **six parts: Technical dossier, Summary, Cartagena Protocol, Labelling and Unique Identifier, Sampling and Detection, and Additional information for GMOs**. With regard to the electronic version (see 'Practical specifications' in this annex for further details on electronic versions), the applicant should use the following folder/subfolder structure:



[16] Regulation concerning novel foods and novel food ingredients, OJ L 43, 14.2.1997, p. 1; Directive on the deliberate release into the environment of GMOs and repealing Council Directive 90/220/EEC, OJ L 106, 17.4.2001, p. 1; Directive concerning certain products used in animal nutrition, OJ L 213, 21.7.1982, p. 8; Directive concerning additives in feedingstuffs, OJ L 270, 14.12.1970, p. 1.




3326

3327 ***PART I: TECHNICAL DOSSIER***

3328

3329 • The technical dossier should contain all necessary information for the risk
3330 assessment and should be structured according to the format of Annex III as
3331 proposed in the EFSA guidance document on GM plants and derived food and feed.
3332 Following Annex III and taking into account the detailed considerations from the
3333 Guidance document to each topic, the technical dossier should comprise the
3334 complete information required by Regulation (EC) No 1829/2003 (Articles 5 and 17
3335 (3) (a), (b), (d), (e), (h), (k). In the case of GMOs or food containing or consisting of
3336 GMOs, the technical dossier should also comprise the information required by
3337 Articles 5 and 17 (5) (a), (b). Applications submitted within the framework of
3338 Directive 2001/18/EC have to respect the technical requirements and formats set
3339 up by this Directive. Given the fact that such application may lead to a consultation
3340 of the GMO Panel according to Article 28 of the Directive, the application should
3341 preferably also be compiled according to this EFSA guidance document.

- In the case of GMOs and/or food or feed containing or consisting of GMOs, the application shall fulfil the requirements of Directive 2001/18/EC as specified by Articles 5 and 17 (5) (a) and (b). Alternatively, where the placing on the market of the GMO has been authorised under Part C of Directive 2001/18/EC, a copy of the authorisation decision shall be provided.

- Each technical dossier should be a complete stand-alone document containing all of the information required for a full risk assessment of the product(s) in question. Assessors should not be required to consider other applications on the same GMO, to undertake any additional literature reviews, or assemble, or process data to evaluate the dossiers.

- A copy of the studies as referred to in Articles 5 and 17 (3) (e) of Regulation (EC) No 1829/2003 should be included as appendices to the main text of the technical dossier. A summary of the data and cross-references to these studies should be made in the main text. The application shall clearly state which parts of the application are considered to be confidential in accordance with Article 2 (3) of Regulation (EC) No 641/2004, together with a verifiable justification in accordance with Article 30 of Regulation (EC) No 1829/2003. Confidential information (CI) that is part of the technical dossier should be submitted as a separate file under Part I of the application.

- To facilitate easy access of information in dossiers, information should be presented in conformity with the format proposed in this document and a detailed index should be prepared.

- Care should be taken to ensure that all parts of the dossier are fully legible. Particular attention is drawn to the presentation of experimental data including tables, physical maps and blots. Note that summary data is not sufficient and the raw data should be provided. A summary of data is however preferable in the main text of the technical dossier supposed that reference is made to the appendices of the technical dossier containing the full data. Data presented in sections of the dossier should be clearly labelled whether in the form of tables, figures, photographs, analytical gels, etc. and the quality of the original data should be preserved. In addition, the appropriate controls or reference points included should be clearly labelled and referenced. Statistical analysis of data should be provided and the statistical power tested where appropriate.

- Not all the points included in the guidance document will apply to every case. In the case a provision of the guidance document does not apply for a certain application, reasons must be given for the omission of such data from the dossier. It is to be expected that individual applications will address only the particular subset of considerations which is appropriate to individual situations. The level of detail required in response to each subset of considerations is also likely to vary according the scope of the application.

- Data provided in support of an application should be of at least the quality expected of data submitted to a peer-review journal. Particular attention should be paid to the

sensitivity and specificity of methods employed and to the adequacy and appropriateness of controls.

***PART II: SUMMARY***

Part II of the application should consist of the summary of the dossier as specified by Articles 5 and 17 (3) (l). The summary of the dossier shall be preferably presented in English in an easily comprehensible and legible form and follow the structure of the EFSA guidance on GM plants and derived food and feed as specified in Annex IV.

The summary should not contain parts which are considered to be confidential as this will be published on the EFSA website.

***PART III: CARTAGENA PROTOCOL***

Part III of the application shall apply only to applications concerning GMOs for food/feed use, or in the case of food/feed containing or consisting of GMOs. In these cases, Part III of the application should specify, in supplying the information required under Articles 5 and 17 (3) (c) of Regulation (EC) No 1829/2003, whether the information included in the application may be notified as such to the Biosafety Clearing-House under the Cartagena Protocol on Biosafety to the Convention on Biological Diversity (the Cartagena Protocol) approved by Council Decision 2002/628/EC[17].

If the application may not be notified as such, Part III shall include the information which complies with Annex II to Cartagena Protocol and which may be notified to the Biosafety Clearing-House by the Commission as provided for in Article 44 of Regulation (EC) No 1829/2003 in a separate and clearly identified document.

***PART IV: LABELLING AND UNIQUE IDENTIFIER***

Part IV of the application should comprise a proposal for labelling in accordance with Articles 12-14 and Articles 24-26 of Regulation (EC) No 1829/2003. In the case of GMOs, food and/or feed containing or consisting of GMOs (Articles 5 and 17 (5)), a proposal for labelling has to be included complying with the requirements of Article 4, B (6) of Regulation (EC) No 1830/2003 and Annex IV of Directive 2001/18/EC.

[17] The Cartagena Protocol was concluded, on behalf of the European Community, by Council Decision 2002/628/EC, OJ L 201, 31.7.2002, p. 48.

In supplying the information required under Articles 5 and 17 (5) (a) of Regulation (EC) No 1829/2003, a proposal for a unique identifier for the GMO in question, developed in accordance with Commission Regulation (EC) No 65/2004[18], should be given.

According to Article 3 (1) (d) of Regulation (EC) No 641/2004, a proposal for labelling in all official Community languages should be provided, where a proposal for specific labelling is needed in accordance with Articles 5 and 17 (3) (f) (g) of Regulation (EC) No 1829/2003.

### *PART V: SAMPLING AND DETECTION*

Methods for detection, sampling (including references to existing official or standardised sampling methods) and identification of the transformation event and, where applicable, for the detection and identification of the transformation event in the food/feed and/or in foods/feeds produced from it should be included in Part V in accordance with Articles 5 and 17 (3) (i) of Regulation (EC) No 1829/2003 and in accordance with Annex I to Regulation (EC) No 641/2004;

Samples of the food or feed and their control samples which are to be submitted in accordance with Articles 5 and 17 (3) (j) of Regulation (EC) No 1829/2003 should be in accordance with the requirements set out in Annexes I and II to Regulation (EC) No 641/2004. The application should be accompanied by information concerning the place where the reference material developed in accordance with Annex II of Regulation (EC) No 641/2004 can be accessed.

A format to provide information on GM detection methods and related samples can be found on the website of the Community Reference Laboratory (http://gmo-crl.jrc.it).

For practical reasons, the methods for detection and sampling and the samples of the food and/or feed and control samples should be sent directly to the Joint Research Centre (JRC). A copy of the completed form, as found in Annex V, and proof of sending to the JRC, should be provided in Part V of the application.

### *PART VI: ADDITIONAL INFORMATION FOR GMOs AND/OR FOOD/FEED CONTAINING OR CONSISTING OF GMOs*

In the case of GMOs and/or food and/or feed containing or consisting of GMOs in accordance with Articles 5 and 17 (5), Part VI of the application should include the information required by Annex IV of Directive 2001/18/EC where the information of Annex IV is not yet covered by the requirements of Parts I to V of this annex. For example, labelling information that is required by Annex IV of Directive 2001/18/EC

[18] 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, OJ L 10, 16.1.2004, p. 5.

should be covered by Part IV of the application and a cross-reference should be made from Part VI to Part IV of the application.

**Table with cross-references between the different parts of the application as specified by the Annexes of the guidance document and Regulation (EC) No 1829/2003**

| Guidance document: specifications for the format of an application | Regulation (EC) No 1829/2003 |
|---|---|
| **Part I: Technical Dossier** | Articles 5&17 (3) (a) (b) (d) (e) (h) (k) ; Articles 5&17 (5) (a) (b) |
| **Part II: Summary** | Articles 5&17 (3) (l) |
| **Part III: Cartagena Protocol** | Articles 5&17 (3) (c) |
| **Part IV: Labelling** | Articles 5&17 (3) (f) (g); Articles 5&17 (5) (a) ; Articles 12-14 and Articles 24-26 |
| **Part V: Sampling and Detection** | Articles 5&17 (3) (i) (j) |
| **Part VI: Additional information for GMOs and/or food/feed containing or consisting of GMOs** | Articles 5&17 (5), more specifically, Annex IV of Directive 2001/18/EC |

**Practical specifications**

One paper copy and one copy in electronic format (CD-ROM) of the application should be sent by registered post through the national Competent Authority (1829/2003-applications) or through the Commission (2001/18/EC-applications) to the scientific coordinator of the GMO Panel:

European Food Safety Authority
Scientific Coordinator GMO Panel
Largo N. Palli 5/A
43100 Parma
Italy

After an application has been considered to be valid by EFSA, this will be acknowledged to the applicant. The applicant will then be asked to send EFSA by registered post the requested amount of paper copies and copies in electronic format (CD-ROM) of the valid application.

EFSA has to make the application available to the Member States and to the Commission as required by Articles 5 and 17 (2) (b) of Regulation (EC) No 1829/2003. For this purpose, EFSA will use a secure electronic system (GMO EFSAnet) to make the electronic version of applications available to them.

The electronic version of the application should be certified by written statement of the applicant as being identical to the paper version. Common electronic formats should be used, such as "MS Word" or "Adobe Acrobat Reader". A print-out of the table of contents should accompany the CD-ROM, clearly indicating the different files and were they can be found. Cross-references should be made between the print-out and the electronic file names by describing the content for each file name. The files should be searchable using the search facilities of standard software packages. To improve navigation through the files, the use of bookmarks and hypertext links is strongly encouraged. In general, bookmarks and hypertext links should be provided for each item listed in the index and main text including tables, figures, publications, other references and appendices.

Confidential information has to be clearly indicated and should be separated from the other parts of the application.

The application in itself can not be confidential. Sections considered as confidential by the applicant should be kept to a minimum. Applicants are encouraged to make publicly available a maximum of the information submitted, for example by posting on the Internet the contents of the application.

The applicant should keep additional paper and electronic copies readily available in cases EFSA (GMO Panel) would require them.

The application will be considered valid if it fulfils the requirements as specified in the EFSA guidance document and accompanying annexes. Applications that are not submitted in English will cause a delay in the assessment process. EFSA may ask the applicant to translate those parts of the dossier not submitted in English and to confirm conformity of any translated text with the original.

# Annex II

## SCOPE OF THE APPLICATION

It should be specified whether **applications for authorisation** submitted in accordance with Articles 5 and 17 of Regulation (EC) No 1829/2003 are:

- New applications that have not been submitted before 18 April 2004 under other Community legislation (Regulation (EC) No 258/97, Directive 2001/18/EC or Directive 82/471/EEC)

- Applications that were submitted under other Community legislation which are transformed or supplemented in accordance with Article 46 of Regulation (EC) No 1829/2003.

The **scope** of the application shall cover one or more of the following categories:

**1 Food***

1.1 GM plants for food use

1.2 Food containing or consisting of GM plants**

1.3 Food produced from GM plants or containing ingredients produced from GM plants**

**2 Feed***

2.1 GM plants for feed use

2.2 Feed containing or consisting of GM plants**

2.3 Feed produced from GM plants**

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

** Where the application concerns a substance, the use and placing on the market of which is subject, under other provisions of Community law, to its inclusion on a list of substances registered or authorised to the exclusion of others, this must be stated in the application and the status of the substance under the relevant legislation must be indicated.

# 3 GM plants for environmental release

## 3.1 Import and processing

## 3.2 Seeds and plant propagating material for cultivation in Europe

# Annex III[19]

## FORMAT OF TECHNICAL DOSSIERS

### INFORMATION REQUIRED IN APPLICATIONS FOR GM PLANTS AND/OR DERIVED FOOD AND FEED

### A. 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 EFSA
3. Title of the project
4. Scope of the application as defined in Annex II
5. Designation and specification of the GM plant and/or derived product
6. Where applicable, a detailed description of the method of production and manufacturing
7. Where appropriate, the conditions for placing on the market the food(s) or feed(s) produced from it, including specific conditions for use and handling

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

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

[19] Annex III will be updated after the main document is finalised

2. (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.

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

4. Dissemination; (a) ways and extent (for example an estimation of how viable pollen and/or seeds declines with distance) of dissemination, (b) special factors affecting dissemination, if any.

5. Geographical distribution and cultivation of the plant, including the distribution in Europe of the compatible species.

6. 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.

7. 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.

## C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Description of the methods used for the genetic modification

2. Nature and source of vector used

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

## D. INFORMATION RELATING TO THE GM PLANT

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

2. Information on the sequences actually inserted or deleted

(a) The copy number of all detectable inserts, both complete and partial

(b) In the 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 including sequence data of the inserted material and of the flanking 5' and 3' regions.

(e) All sequence information (in electronic format) including the location of primers used for detection.

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

(c) Expression of potential fusion proteins.

(d) Methods used for expression analysis

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

5. Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food/feed

5.1. Comparative assessment

5.2. Production of material for comparative assessment

(a) Number of locations, growing seasons, geographical spread and replicates

(b) Statistical models for analysis, confidence intervals

(c) The baseline used for consideration of natural variations

5.3. Selection of material and compounds for analysis

5.4. Agronomic traits

5.5. Product Specification

5.6. Effect of processing

5.7. Anticipated intake/extent of use

5.8. Toxicology

*5.8.1. Safety assessment of newly expressed proteins*

*5.8.2. Testing of new constituents other than proteins*

*5.8.3. Information on natural food and feed constituents*

*5.8.4. Testing of the whole GM food/feed*

5.9. Allergenicity

*5.9.1. Assessment of allergenicity of the newly expressed protein*

*5.9.2. Assessment of allergenicity of the whole GM plant or crop*

5.10. Nutritional assessment of GM food/feed

*5.10.1. Nutritional assessment of GM food*

*5.10.2. Nutritional assessment of GM feed*

5.11. Post-market monitoring of GM food/feed

6. Mechanism of interaction between the GM plant and target organisms (if applicable)

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

7.1. Persistence and invasiveness

7.2. Selective advantage or disadvantage

7.3. Potential for gene transfer

7.4. Interactions between the GM plant and target organisms

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

7.6. Effects on human health

7.7. Effects on animal health

7.8. Effects on biogeochemical processes

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

# Annex IV

## FORMAT[20] OF THE SUMMARY OF APPLICATIONS FOR GENETICALLY MODIFIED PLANTS AND/OR DERIVED FOOD AND FEED

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

### A. GENERAL INFORMATION

### 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 |

### 2. Applicant

| |
|---|
| a) Name of applicant |
| b) Address of applicant |
| c) Name and address of the person established in the Community who is responsible for the placing on the market, whether it be the manufacturer, the importer or the distributor, if different from the applicant (Commission Decision 2004/204/EC Art 3(a)(ii)) |

[20] This format of summary is based on Part II of Council Decision 2002/812/EC of 3 October 2002 establishing pursuant to Directive 2001/18/EC of the European Parliament and of the Council the summary information format relating to the placing on the market of genetically modified organisms as or in products (Official Journal of the European Communities L280: 37-61), and is adapted according to the current guidance document.

**3. Scope of the application**

☐ GM plants for food use

☐ Food containing or consisting of GM plants

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

☐ GM plants for feed use

☐ Feed containing or consisting of GM plants

☐ Feed produced from GM plants

☐ Import and processing (Part C of Directive 2001/18/EC)

☐ Seeds and plant propagating material for cultivation in Europe (Part C of Directive 2001/18/EC)

**4. Is the product being simultaneously notified within the framework of another regulation (*e.g.* Seed legislation)?**

| | |
|---|---|
| Yes ☐ | No ☐ |
| If *yes*, specify | |

**5. Has the GM plant been notified under Part B of Directive 2001/18/EC and/or Directive 90/220/EEC?**

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

**6. Has the GM plant or derived products been previously notified for marketing in the Community under Part C of Directive 2001/18/EC or Regulation (EC) No 258/97?**

| | |
|---|---|
| Yes ☐ | No ☐ |
| If *yes*, specify | |

3734 **7. Has the product been notified in a third country either previously or**
3735 **simultaneously?**

| | |
|---|---|
| Yes ☐ | No ☐ |
| If *yes*, specify | |

3736

3737 **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 |
| c) Intended use of the product and types of users |
| d) Specific instructions and/or recommendations for use, storage and handling, including mandatory restrictions proposed as a condition of the authorisation applied for |
| e) Any proposed packaging requirements |
| f) A proposal for labelling in accordance with Articles 13 and Articles 25 of Regulation ((EC) No 1829/2003. In the case of GMOs, food and/or feed containing or consisting of GMOs, a proposal for labelling has to be included complying with the requirements of Article 4, B(6) of Regulation (EC) No 1830/2003 and Annex IV of Directive 2001/18/EC |
| g) Unique identifier for the GM plant (Regulation (EC) No 65/2004; does not apply to applications concerning only food and feed produced from GM plants, or containing ingredients produced from GM plants) |
| h) If applicable, geographical areas within the EU to which the product is intended to be confined under the terms of the authorisation applied for. Any type of environment to which the product is unsuited |

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3739 **9. Measures suggested by the applicant to take in case of unintended release or**
3740 **misuse as well as measures for disposal and treatment**

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|---|
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## B. INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

### 1. Complete name

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

### 2 a. Information concerning reproduction

| |
|---|
| (i) Mode(s) of reproduction |
| (ii) Specific factors affecting reproduction |
| (iii) Generation time |

### 2 b. Sexual compatibility with other cultivated or wild plant species

| |
|---|
| |

### 3. Survivability

| |
|---|
| a) Ability to form structures for survival or dormancy |

| |
|---|
| b) Specific factors affecting survivability |

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3753 **4. Dissemination**

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

3754

3755 **5. Geographical distribution and cultivation of the plant, including the distribution**
3756 **in Europe of the compatible species**

| |
|---|
| |

3757

3758 **6. In the case of plant species not normally grown in the Member State(s),**
3759 **description of the natural habitat of the plant, including information on natural**
3760 **predators, parasites, competitors and symbionts**

| |
|---|
| |

3761

3762 **7. Other potential interactions, relevant to the GM plant, of the plant with**
3763 **organisms in the ecosystem where it is usually grown, or used elsewhere,**
3764 **including information on toxic effects on humans, animals and other**
3765 **organisms**

| |
|---|
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## C. INFORMATION RELATING TO THE GENETIC MODIFICATION

### 1. Description of the methods used for the genetic modification

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| |

### 2. Nature and source of the vector used

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

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| |

## D. INFORMATION RELATING TO THE GM PLANT

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

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### 2. Information on the sequences actually inserted or deleted

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|---|
| 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) |

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| 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 |

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3783 **3. Information on the expression of the insert**

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| 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 |

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3785 **4. Information on how the GM plant differs from the recipient plant in**

| |
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| a) Reproduction |
| b) Dissemination |
| c) Survivability |
| d) Other differences |

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3787 **5. Genetic stability of the insert and phenotypic stability of the GM plant**

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

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

## 7. Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food/feed

### 7.1 Comparative assessment

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| Choice of the comparator |

### 7.2 Production of material for comparative assessment

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|---|
| a) Number of locations, growing seasons, geographical spread and replicates |
| b) The baseline used for consideration of natural variations |

### 7.3 Selection of material and compounds for analysis

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### 7.4 Agronomic traits

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### 7.5 Product specification

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### 7.6 Effect of processing

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### 7.7 Anticipated intake/extent of use

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### 7.8 Toxicology

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| 7.8.1 Safety assessment of newly expressed proteins |
| 7.8.2 Testing of new constituents other than proteins |
| 7.8.3 Information on natural food and feed constituents |
| 7.8.4 Testing of the whole GM food/feed |

### 7.9 Allergenicity

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| 7.9.1 Assessment of allergenicity of the newly expressed protein |
| 7.9.2 Assessment of allergenicity of the whole GM plant or crop |

## 7.10 Nutritional assessment of GM food/feed

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| 7.10.1 Nutritional assessment of GM food |
| 7.10.2 Nutritional assessment of GM feed |

## 7.11 Post-market monitoring of GM food/feed

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## 8. Mechanism of interaction between the GM plant and target organisms (if applicable)

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

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| 9.1 Persistence and invasiveness |
| 9.2 Selective advantage or disadvantage |
| 9.3 Potential for gene transfer |
| 9.4 Interactions between the GM plant and target organisms |
| 9.5 Interactions of the GM plant with non-target organisms |

| |
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| 9.6 Effects on human health |
| 9.7 Effects on animal health |
| 9.8 Effects on biogeochemical processes |
| 9.9 Impacts of the specific cultivation, management and harvesting techniques |

## 10. Potential interactions with the abiotic environment

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## 11. Environmental monitoring plan (not if application concerns only food and feed produced from GM plants, or containing ingredients produced from GM plants and if the applicant has clearly shown that environmental exposure is absent or will be at levels or in a form that does not present a risk to other living organisms or the abiotic environment)

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|---|
| 11.1 General (risk assessment, background information) |
| 11.2 Interplay between environmental risk assessment and monitoring |
| 11.3 Case-specific GM plant monitoring (approach, strategy, method and analysis) |
| 11.4 General surveillance of the impact of the GM plant (approach, strategy, method and analysis) |

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| 11.5 Reporting the results of monitoring |

## 12. Detection and event-specific identification techniques for the GM plant

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## E. INFORMATION RELATING TO PREVIOUS RELEASES OF THE GM PLANT AND/OR DERIVED PRODUCTS

### 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) |

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

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|---|
| 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 |

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3833 **3. Links (some of these links may be accessible only to the competent authorities**
3834 **of the Member States, to the Commission and to EFSA):**

| |
|---|
| a) Status/process of approval |
| b) Assessment Report of the Competent Authority (Directive 2001/18/EC) |
| c) EFSA opinion |

| |
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| d) Commission Register (Commission Decision 2004/204/EC[21]) |
| e) Molecular Register of the Community Reference Laboratory/Joint Research Centre |
| f) Biosafety Clearing-House (Council Decision 2002/628/EC[22]) |
| g) Summary Notification Information Format (SNIF) (Council Decision 2002/812/EC) |

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[21] Commission Decision of 23 February 2004 laying down detailed arrangements for the operation of the registers for recording information on genetic modifications in GMOs, provided for in Directive 2001/18/EC of the European Parliament and of the Council. Official Journal of the European Communities L 65: 20 – 22.

[22] Council Decision of 25 June 2002 concerning the conclusion, on behalf of the European Community, of the Cartagena Protocol on Biosafety. Official Journal of the European Communities L 201: 48 – 49.

# Annex V

## SUBMISSION OF SAMPLES TO THE EUROPEAN COMMISSION- DG JOINT RESEARCH CENTRE

Submission of samples of the food/feed and their control samples referred to in Articles 5(3)(j) and 17(3)(j) of Regulation (EC) No 1829/2003 for applications for authorisation in accordance with Articles 5 and 17 of that Regulation and Article 4(1) and Annexes I and II of Regulation (EC) No 641/2004:

"European Commission - DG Joint Research Centre
Institute for Health and Consumer Protection
Unit "Biotechnology and GMOs"
Unit Head Mr Guy Van den Eede
TP 331 Via Fermi 1
I-21020
Ispra (VA), ITALY"

Reference: Date:

The undersigned (name) hereby submits samples of the food/feed and their control samples referred to in Articles 5(3)(j) and 17(3)(j) of Regulation (EC) No 1829/2003 for requests for applications for authorisation in accordance with Articles 5 and 17 of that Regulation and Article 4(1) and Annexes I and II of Regulation (EC) No 641/2004, for the following product:

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

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

EFSA: GMO@efsa.eu.int

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

Yours faithfully,

Signature:

Enclosures: samples, control samples

INSTRUCTIONS AND INFORMATION

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

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

►A copy of this letter should be included in Part V of the application as specified in Annex I of the EFSA Guidance on GM Plants and derived food and feed

►Regulation (EC) No 1829/2003 on genetically modified food and feed (OJ L 268, 18.10.2003, p. 1)

►Regulation (EC) No 641/2004 on detailed rules for the implementation of Regulation (EC) No 1829/2003 (OJ L 102, 7.4.2004, p. 14)

►http://www.efsa.eu.int

►http://europa.eu.int/comm/food/index_en.htm

## Acknowledgement of receipt

Submission of samples of the food/feed and their control samples referred to in Articles 5(3) (j) and 17(3)(j) of Regulation (EC) No 1829/2003 for applications for authorisations in accordance with Articles 5 and 17 of that Regulation and Article 4(1) and Annexes I and II of Regulation (EC) No 641/2004

Please write your return address below:

Reference:

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

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

Name of the food and/or feed:

Trade name (where applicable):

Short description:

Date: (dd/mm/yyyy)

Signature: Guy Van den Eede, Head of Unit

Stamp :

3938 # Annex VI

3939 ## CORRELATION TABLE COMPARING THE REQUIRED INFORMATION ACCORDING TO
3940 ## REGULATION (EC) 1829/2003
3941 ## AND THE GUIDANCE DOCUMENT (GD)

3942 If the product contains or consists of GMO, specific information has to be included as stipulated
3943 under Art. 5 of Regulation (EC) 1829/2003 referring to annexes II, III, IV, and VII of Directive
3944 2001/18/EC (grey shading). For feed (Art. 17) the same correlation system is valid. Differences
3945 between the GD and the legal requirements are underlined.

| | Text Regulation or Directive | Guidance document Annex | Guidance document section in Chapter III | Correlating parts in Annexes of the Guidance Document | Dossier |
|---|---|---|---|---|---|
| **1829/2003** | | | | | |
| **Art. 5(3)** | | | | | |
| (a) | the name and the address of the applicant; | Annex III | A.1 | Name and address of the applicant (company or institute) | Part I |
| (b) | the designation of the food, and its specification, including the transformation event(s) used; | Annex III | A.5 | Designation and specification of the GM plant and/or derived product | Part I |
| (c) | where applicable, the information to be provided for the purpose of complying with Annex II to the Cartagena Protocol on Biosafety to the Convention on Biological Diversity (hereinafter referred to as the Cartagena Protocol); | Annex I | | see Annex I, Part III | Part III |
| (d) | where applicable, a detailed description of the method of production and manufacturing; | Annex III. | A.6. | Where applicable, a detailed description of the method of production and manufacturing | Part I |
| (e) | a copy of the studies, including, where available, independent, | Annex I in general | | remark: Annex III B from 2001/18 was starting point for GD and respective Annexes | Part I |

| | Text Regulation or Directive | Guidance document Annex | Guidance document section in Chapter III | Correlating parts in Annexes of the Guidance Document | Dossier |
|---|---|---|---|---|---|
| | peer-reviewed studies, which have been carried out and any other material which is available to demonstrate that the food complies with the criteria referred to in Article 4(1); | | | | |
| (f) | either an analysis, supported by appropriate information and data, showing that the characteristics of the food are not different from those of its conventional comparator, having regard to the accepted limits of natural variations for such characteristics and to the criteria specified in Article 13(2)(a), or a proposal for labelling the food in accordance with Article 13(2)(a) and (3); | Annex I | | see Annex I, Part IV | Part IV |
| (g) | either a reasoned statement that the food does not give rise to ethical or religious | Annex I | | see Annex I, Part IV | Part IV |

| | Text Regulation or Directive | Guidance document Annex | Guidance document section in Chapter III | Correlating parts in Annexes of the Guidance Document | Dossier |
|---|---|---|---|---|---|
| | concerns, or a proposal for labelling it in accordance with Article 13(2)(b); | | | | |
| (h) | where appropriate, the conditions for placing on the market the food or foods produced from it, including specific conditions for use and handling; | Annex III | A.7 | same text as regulation 1829/2003 | Part I |
| (i) | methods for detection, sampling (including references to existing official or standardised sampling methods) and identification of the transformation event and, where applicable, for the detection and identification of the transformation event in the food and/or in foods produced from it; | Annex I | | see Annex I, Part V | Part V |
| (j) | samples of the food and their control samples, and information as to the place where the reference material can be accessed; | Annex I | | see Annex I, Part V | Part V |
| (k) | where appropriate, a | Annex III | D.7.11 | Post-market monitoring of GM food/feed | Part I |

| | Text Regulation or Directive | Guidance document Annex | Guidance document section in Chapter III | Correlating parts in Annexes of the Guidance Document | Dossier |
|---|---|---|---|---|---|
| | proposal for post-market monitoring regarding use of the food for human consumption; | | | | |
| (l) | a summary of the dossier in a standardised form. | Annex I | | see Annex I, Part II | Part II |
| | | | | | |
| **Art. 5(5)** | **Food/feed containing or consisting of GMO.** | | | | |
| (a) | reference to Annexes II, IIIB, and IV of 2001/18 **or** where the GMO is already authorised → copy of authorisation decision | | | | |
| (b) | monitoring plan according to Annex VII of 2001/18 | | | | |
| | | | | | |
| **2001/18** | | | | | |
| **Annex II** | | **AnnexIII** | **D.9** | **Potential changes in the interactions of the GM plant with the biotic environment resulting from the genetic modification** | |
| D.2.1 | Likelihood of the GMHP becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats. | Annex III | D.9.1 | Persistence and invasiveness | Part I |
| D.2.2 | Any selective | Annex III | D.9.2 | Selective advantage or | Part I |

| | Text Regulation or Directive | Guidance document Annex | Guidance document section in Chapter III | Correlating parts in Annexes of the Guidance Document | Dossier |
|---|---|---|---|---|---|
| | advantage or disadvantage conferred to the GMHP. | | | disadvantage | |
| D.2.3 | Potential for gene transfer to the same or other sexually compatible plant species under conditions of planting the GMHP and any selective advantage or disadvantage conferred to those plant species. | Annex III | D.9.3 | Potential for gene transfer | Part I |
| D.2.4 | Potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GMHP and target organisms, such as predators, parasitoids, and pathogens (if applicable). | Annex III | D.9.4 | Interactions between the GM plant and target organisms | Part I |
| D.2.5 | Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP with non-target organisms, (also taking into account organisms which interact with | Annex III | D.9.5 | Interactions of the GM plant with non-target organisms | Part I |

| | Text Regulation or Directive | Guidance document Annex | Guidance document section in Chapter III | Correlating parts in Annexes of the Guidance Document | Dossier |
|---|---|---|---|---|---|
| | target organisms), including impact on population levels of competitors, herbivores, symbionts (where applicable), parasites and pathogens. | | | | |
| D.2.6 | Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMHP and persons working with, coming into contact with or in the vicinity of the GMHP release(s). | Annex III | D.9.6 | Effects on human health | Part I |
| D.2.7 | Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any products derived from it, if it is intended to be used as animal feed. | Annex III | D.9.7 | Effects on animal health | Part I |
| D.2.8 | Possible immediate and/or delayed effects on biogeochemical | Annex III | D.9.8 | Effects on biogeochemical processes | Part I |

| | Text Regulation or Directive | Guidance document Annex | Guidance document section in Chapter III | Correlating parts in Annexes of the Guidance Document | Dossier |
|---|---|---|---|---|---|
| | processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s). | | | | |
| D.2.9 | Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs. | Annex III | D.9.9 | Impacts of the specific cultivation, management and harvesting techniques | Part I |
| | | | | | |
| **Annex III B** | | | | | |
| | **A. GENERAL INFORMATION** | | | **A. GENERAL INFORMATION** | |
| A.1 | Name and address of the notifier (company or institute) | Annex III | A.1 | Name and address of the applicant (company or institute) | Part I |
| A.2 | Name, qualifications and experience of the responsible scientist(s) | Annex III | A.2 | Name, qualification and experience of the responsible scientist(s) and contact details of the responsible person for all dealings with EFSA | Part I |
| A.3 | Title of the project | Annex III | A.3 | Title of the project | Part I |
| | | | | | |
| | **B. INFORMATION RELATING TO (A) THE RECIPIENT** | | | **B. INFORMATION RELATING TO THE RECIPIENT OR (WHERE** | |

| | Text Regulation or Directive | Guidance document Annex | Guidance document section in Chapter III | Correlating parts in Annexes of the Guidance Document | Dossier |
|---|---|---|---|---|---|
| | **OR (B) (WHERE APPROPRIATE) PARENTAL PLANTS** | | | **APPROPRIATE) PARENTAL PLANTS** | |
| B.1 | Complete name:<br>(a) family name<br>(b) genus<br>(c) species<br>(d) subspecies<br>(e) cultivar/breeding line<br>(f) common name. | Annex III | B.1 | Complete name;<br>(a) family name,<br>(b) genus,<br>(c) species,<br>(d) subspecies,<br>(e) cultivar/breeding line or strain,<br>(f) common name | Part I |
| B.2 (a) | Information concerning reproduction:<br>(i) mode(s) of reproduction<br>(ii) specific factors affecting reproduction, if any<br><br>(iii) generation time. | Annex III | B.2 (a) | Information concerning reproduction:<br>(i) mode(s) of reproduction<br>(ii) specific factors affecting reproduction, if any<br>(iii) generation time. | Part I |
| B.2 (b) | Sexual compatibility with other cultivated or wild plant species, <u>including the distribution in Europe of the compatible species</u>. | Annex III | B.2 (b) | (b) Sexual compatibility with other cultivated or wild plant species. | Part I |
| B.3 | Survivability:<br>(a) ability to form structures for survival or dormancy<br><br>(b) specific factors affecting survivability, if any. | Annex III | B.3 | Survivability;<br>(a) ability to form structures for survival or dormancy,<br>(b) specific factors if any affecting survivability. | Part I |
| B.4 | Dissemination:<br>(a) ways and extent (for | Annex III | B.4 | Dissemination;<br>(a) ways and extent (for example and estimation | Part I |

| | Text Regulation or Directive | Guidance document Annex | Guidance document section in Chapter III | Correlating parts in Annexes of the Guidance Document | Dossier |
|---|---|---|---|---|---|
| | example an estimation of how viable pollen and/or seeds declines with distance) of dissemination,<br><br>(b) specific factors affecting dissemination, if any. | | | of how viable pollen and/or seeds declines with distance) of dissemination,<br>(b) special factors affecting dissemination, if any. | |
| B.5 | Geographical distribution of the plant | Annex III | B.5 | Geographical distribution and cultivation of the plant, including the distribution in Europe of the compatible species - compare 2001/18 B.2. (b) | Part I |
| B.6 | 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. | Annex III | B.6 | 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. | Part I |
| B.7 | Other potential interactions, relevant to the GMO, of the plant with organisms in the ecosystem where it is usually grown, or elsewhere, including information on toxic effects on humans, animals and other | Annex III | B.7 | 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. | Part I |

| | Text Regulation or Directive | Guidance document Annex | Guidance document section in Chapter III | Correlating parts in Annexes of the Guidance Document | Dossier |
|---|---|---|---|---|---|
| | organisms | | | | |
| | | | | | |
| | **C. INFORMATION RELATING TO THE GENETIC MODIFICATION** | | | **C. INFORMATION RELATING TO THE GENETIC MODIFICATION** | |
| C.1 | Description of the methods used for the genetic modification. | Annex III | C.1 | Description of the methods used for the genetic modification | Part I |
| C.2 | Nature and source of the vector used. | Annex III | C.2 | Nature and source of vector used | Part I |
| | | | | | |
| | | | | | |
| | **D. INFORMATION RELATING TO THE GENETICALLY MODIFIED PLANT** | | | **D. INFORMATION RELATING TO THE GM PLANT** | |
| D.1. | Description of the trait(s) and characteristics which have been introduced or modified. | Annex III | D.1 | Description of the trait(s) and characteristics which have been introduced or modified | Part I |
| D.2 | Information on the sequences actually inserted/deleted: | Annex III | D.2 | Information on the sequences actually inserted or deleted | Part I |
| D.2 (a) | size and structure of the insert and methods used for its characterisation, including information on any parts of the vector introduced in the GMHP or any carrier or foreign DNA remaining in the GMHP; | Annex III<br><br>Annex III | D.2 (d)<br><br>D.2 (e) | the organisation of the inserted genetic material at the insertion site including sequence data of the inserted material and of the flanking 5' and 3' regions.<br><br>all sequence information including the location of primers used for detection. | Part I |
| D.2 (b) | in case of | Annex III | D.2 (b) | in the case of deletion(s), | Part I |

| | Text Regulation or Directive | Guidance document Annex | Guidance document section in Chapter III | Correlating parts in Annexes of the Guidance Document | Dossier |
|---|---|---|---|---|---|
| | deletion, size and function of the deleted region(s); | | | size and function of the deleted region(s) | |
| D 2 (c) | copy number of the insert; | Annex III | D.2 (a) | the copy number of all detectable inserts, both complete and partial | Part I |
| D.2 (d) | location(s) of the insert(s) in the plant cells (integrated in the chromosome, chloroplasts, mitochondria, or maintained in a non-integrated form), and methods for its determination. | Annex III | D.2 (c) | chromosomal location(s) of insert(s) (nucleus, chloroplasts, mitochondria or maintained in a non integrated form) and methods for its determination. | Part I |
| D.3 | Information on the expression of the insert: | Annex III | D.3 | Information on the expression of the insert | Part I |
| D.3 (a) | information on the developmental expression of the insert during the lifecycle of the plant and methods used for its characterisation; | Annex III<br><br>Annex III | D.3<br><br>D.3 | (a) Information on developmental expression of the insert during the life cycle of the plant.<br>(d) Methods used for expression analysis | Part I |
| D.3 (b) | parts of the plant where the insert is expressed (for example roots, stem, pollen, etc.). | Annex III | D.3 | (b)Parts of the plant where the insert is expressed | Part I |
| D.4 | Information on how the genetically modified plant differs from the recipient plant in:<br>(a) mode(s) and/or rate of reproduction;<br>(b) | Annex III | D.4 | Information on how the GM plant differs from the recipient plant in: reproduction, dissemination, survivability. | Part I |

| | Text Regulation or Directive | Guidance document Annex | Guidance document section in Chapter III | Correlating parts in Annexes of the Guidance Document | Dossier |
|---|---|---|---|---|---|
| | dissemination;<br>(c) survivability. | | | | |
| D.5 | Genetic stability of the insert and phenotypic stability of the GMHP. | Annex III | D.5 | Genetic stability of the insert and phenotypic stability of the GM plant | Part I |
| D.6 | Any change to the ability of the GMHP to transfer genetic material to other organisms. | Annex III | D.6 | Any change to the ability of the GM plant to transfer genetic material to other organisms<br>(a) Plant to bacteria gene transfer<br>(b) Plant to plant gene transfer | Part I |
| D.7 | Information on any toxic, allergenic or other harmful effects on human health arising from the genetic modification. | Annex III | D.7 | Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food/feed | Part I |
| D.8 | Information on the safety of the GMHP to animal health, particularly regarding any toxic, allergenic or other harmful effects arising from the genetic modification, where the GMHP is intended to be used in animal feedstuffs. | Annex III<br><br>Annex III<br><br><br><br><br><br>Annex III<br><br>Annex III<br><br>Annex III<br><br>Annex III<br><br>Annex III | D.7.1<br><br>D.7.2<br><br><br><br><br><br>D.7.3<br><br><br>D.7.4<br><br>D.7.5<br><br>D.7.6<br><br>D.7.7<br><br>D.7.8 | Comparative assessment<br><br>Production of material for comparative assessment<br>(a) number of locations, growing seasons, geographical spread and replicates<br>(b) statistical models for analysis, confidence intervals<br>(c) the baseline used for consideration of natural variations<br><br>Selection of material and compounds for analysis<br><br>Agronomic traits<br><br>Product Specification<br><br>Effect of processing<br><br>Anticipated intake/extent | Part I |

| | Text Regulation or Directive | Guidance document Annex | Guidance document section in Chapter III | Correlating parts in Annexes of the Guidance Document | Dossier |
|---|---|---|---|---|---|
| | | Annex III<br><br>Annex III<br><br>Annex III<br><br>Annex III | D.7.9<br><br>D.7.10<br><br>D.9.6<br><br>D.9.7 | of use<br><br>Toxicology:<br>(a) Safety assessment of newly expressed proteins<br>(b) Testing of new constituents other than proteins<br>(c) Information on natural food and feed constituents<br>(d) Testing of the whole GM food/feed<br><br>Allergenicity:<br>(a) Assessment of allergenicity of the newly expressed protein<br>(b) Assessment of allergenicity of the whole GM plant or crop<br><br>Nutritional assessment of GM food/feed<br><br>Effects on human health<br><br>Effects on animal health | |
| D.9 | Mechanism of interaction between the genetically modified plant and target organisms (if applicable). | Annex III | D.8 | Mechanism of interaction between the GM plant and target organisms (if applicable) | Part I |
| D.10 | Potential changes in the interactions of the GMHP with non-target organisms resulting from the genetic modification. | Annex III<br><br>Annex III | D.9<br><br>D.9.5 | Potential changes in the interactions of the GM plant with the biotic environment resulting from the genetic modification<br>Interactions of the GM plant with non-target organisms | Part I |
| D.11 | Potential interactions with the abiotic environment. | Annex III | D.9.8 | Effects on biogeochemical processes | Part I |

| | Text Regulation or Directive | Guidance document Annex | Guidance document section in Chapter III | Correlating parts in Annexes of the Guidance Document | Dossier |
|---|---|---|---|---|---|
| D.12 | Description of detection and identification techniques for the genetically modified plant. | Annex I | | see Annex I, Part V | Part V |
| D.13 | Information about previous releases of the genetically modified plant, if applicable. | Annex III<br><br>Annex III | D.7.2<br><br>D.7.4 | Production of material for comparative assessment<br>(a) number of locations, growing seasons, geographical spread and replicates<br>(b) statistical models for analysis, confidence intervals<br>(c) the baseline used for consideration of natural variations<br>Agronomic traits | Part I |
| | | | | | |
| **Annex IV** | **Additional Information** | Annex I | | see Annex I, Part VI | Part VI |
| | | | | | |
| **Annex VII** | **MONITORING PLAN**<br>This Annex describes in general terms the objective to be achieved and the general principles to be followed to design the monitoring plan referred to in Articles 13(2), 19(3) and 20. It will be supplemented by guidance notes to be developed in accordance with the procedure laid down in Article 30(2).<br>See also COUNCIL | Annex III | D.7.11.1 - D.7.11.5 | Addressed in Annex I, Part I | Part I |

| | Text Regulation or Directive | Guidance document Annex | Guidance document section in Chapter III | Correlating parts in Annexes of the Guidance Document | Dossier |
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| | DECISION of 3 October 2002 (2002/811/EC) | | | | |

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