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HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

**GUIDANCE DOCUMENT**

**FOR THE RISK ASSESSMENT OF GENETICALLY MODIFIED PLANTS  
AND DERIVED FOOD AND FEED**

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**Prepared for the Scientific Steering Committee by**

***The Joint Working Group on Novel Foods and GMOs***

**Composed of members of the Scientific Committees on  
Plants, Food and Animal Nutrition**

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## FOREWORD

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The Joint Working Group examined the comments and took them into account when preparing a revised document for submission to the meetings of the Scientific Steering Committee of 16/17 January and 6/7 March 2003.

## **I. INTRODUCTION**

### **1. Scope of the document**

This document is for the use of risk assessors and notifiers<sup>1</sup> who intend to apply for the commercial release of genetically modified plants and derived cultivars under existing Community legislation (Directive 2001/18/EC [Ref. 1]) and/or for the commercial authorisation of genetically modified (GM) food or feed, i.e. food or feed containing, consisting of or produced from genetically modified plants (Regulation (EC)258/97 on Novel foods [Ref. 2]; Proposal for a Regulation on GM food and feed [Ref. 3&4]).

This document does not cover genetically modified animals, or micro-organisms (including micro-organisms intended for use under containment conditions which are regulated by Directive 90/219/EEC [Ref. 5], as amended by Directive 98/81/EC [Ref. 6]), or medicinal products for human or animal use (which are regulated by Regulation 93/2309/EEC [Ref. 7]). The environmental assessment of GM plants used to produce medicinal products or other non-food products (e.g. cotton fibres, flowers) is covered in this document but additional guidance may be required, for example for long lived species such as trees. Issues such as containment or risk management are not within the scope of this document and thus the post-market monitoring of GM crops and derived food and feed is not addressed specifically.

### **2. Purpose of the document**

This document does not have any regulatory status, but elaborates on the information needed for the risk assessment of genetically modified plants and derived food and feed. It seeks to provide guidance to both notifiers and risk assessors and also aims to assist notifiers in the preparation of dossiers. The risk assessor or the regulator may require additional information on a case-by-case basis. Notifiers must adhere to the requirements laid down in the appropriate Directive or Regulation, including the guidance notes under Directive 2001/18/EC on environmental risk assessment [Ref. 8] and monitoring [Ref. 9]. It is not the purpose of this guidance document to prescribe specific protocols for the execution of experiments.

### **3. Legal background for the risk assessment of GMOs, GM food and GM feed at Community level**

The principles regulating the deliberate release into the environment of genetically modified organisms (GMOs) are laid down in Council Directive 2001/18/EC [Ref. 1].

Directive 2001/18/EC puts in place a step-by-step approval process made on a case-by-case assessment of the risk to human health and the environment before any GMOs or products containing GMOs could be released into the environment, or placed on the market. Annex II of the Directive also addresses aspects of animal health. The Directive introduces a time limit for the authorisation, which cannot be

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<sup>1</sup> The term notifier is used hereafter as a generic reference to the official body submitting the notification.

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 health and/or the environment. 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 health or the environment of GMOs as or in products after they have been placed on the market. Specific guidance notes have been established that supplement Annex VII of the Directive on the general principles to design a monitoring plan for the deliberate release of GMOs into the environment [Ref. 9].

Under Directive 2001/18/EC, a notifier who intends to market a GMO must submit an application to the competent authority of the Member State where the product is to be placed on the market. The application must include a risk assessment. The principles for the environmental risk assessment are laid down in Annex II of the Directive. Annex IIIB of the Directive has details of the required information on which to base the risk assessment for higher plants. If the national competent authority gives a favourable opinion on the GMO, this Member State must inform the European Commission and other Member States. If no objections are raised either by the Commission or by any other Member State, the assessor Member State grants an authorisation and the product may then be marketed throughout the Community. If, however, any objections are raised, a decision has to be taken at Community level. If an objection relates to risks of the GMO to human health or to the environment, the Commission must then consult the relevant Scientific Committee and Panels (Art. 28 of Directive 2001/18/EC [Ref. 1]; Art. 22.5. and Art. 28 of Regulation (EC)178/2002 [Ref. 10]).

Risk assessments carried out under Directive 2001/18/EC address human health related to exposure to the GMO concerned, including incidental consumption; it does not address the use of GMOs and their products as food. GM food is currently regulated by Regulation (EC)258/97 on Novel Food and Novel Food Ingredients [Ref. 2]. The information necessary to support applications is described in the annex of the Commission Recommendation 97/618/EC [Ref. 11]. At present, the authorisation decision must be in accordance with Regulation (EC)258/97 and the authorisation procedure is as disclosed in the Directive 2001/18/EC. However, in July 2001, the European Commission adopted a proposal for a Regulation of the European Parliament and of the Council on genetically modified food and feed [Ref. 3]. An amended proposal was adopted in October 2002 [Ref. 4]. This draft Regulation lays down a Community procedure for the assessment, authorisation and supervision of GM food and feed. Based on a scientific risk assessment, this Regulation provides for a time limit of a maximum of 10 years for the Community authorisation on GMOs for food and/or feed use, food and/or feed containing or consisting of GMOs, as well as food and/or feed produced from or by GMOs. The proposed Regulation provides for such risk assessments to be carried out by the European Food Safety Authority established under the Regulation (EC)178/2002 laying down the general principles and requirements of food law and the procedures in matters of food safety [Ref. 10]. Where appropriate and based on the conclusions of the risk assessment, post-market monitoring requirements for the use of the genetically modified foods for human consumption or for animal consumption may be imposed. Separate guidance for this post-market monitoring remains to be developed.

Regarding genetically modified seeds, the Community legislation (Directive 98/95/EC, [Ref. 12]) specifies that those national authorities that have agreed to the use of a GM variety on their territory must notify this acceptance to the European Commission. The Commission must examine the information supplied by the Member State concerned and its compliance with the provision of the Community seed legislation. If such is the case, the variety concerned is included in the “Common Catalogue of varieties of Agricultural Plant Species”. The seed legislation requires that GM varieties must be authorised in accordance with Directive 2001/18/EC. Risk assessment for a given GM plant performed under Directive 2001/18/EC applies also for varieties derived by conventional breeding methods from the line concerned. If the varieties are intended for food crop production, it also must be authorised in accordance with the Novel Food Regulation (EC)258/97 prior to inclusion in the Common Catalogue. Specific conditions for the environmental risk assessments of GM plant varieties remain to be developed by the European Commission (White Paper on Food Safety [Ref. 13]).

#### **4. Presentation of Dossiers**

Each dossier should be a complete document containing all of the information required for a full risk assessment of the product(s) in question. Assessors should not be required to undertake any additional literature reviews, or assemble, or process data to evaluate the dossiers.

To facilitate easy access of information in dossiers, a detailed index should be prepared. Continuous numbering of pages and appendices is required.

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. Statistical analysis of data should be provided and the statistical power tested whenever necessary. Data presented in sections of the dossier should be clearly labelled whether in the form of tables, figures, photographs, analytical gels, etc. Such data can also be submitted electronically for clarity and to preserve the quality of the original data. In addition, the appropriate controls or reference points included should be clearly labelled and referenced. Data provided for risk assessment should be restricted to what is necessary for a comprehensive risk evaluation.

Data provided in support of an application should be of at least the quality expected of data submitted to a high-ranking peer-review journal. Particular attention should be paid to the sensitivity and specificity of methods employed and to the adequacy and appropriateness of controls.

## II. THE RISK ASSESSMENT STRATEGY

### 1. Defining risk assessment

Risk assessment can be defined 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)” [Ref. 14]. A risk assessment comprises hazard identification, hazard characterisation, exposure assessment and risk characterisation. A hazard is the potential of a risk source to cause an adverse effect. The sequential steps in risk assessment of GMOs identify characteristics which may cause adverse effects, evaluate their potential consequence, assess the likelihood of occurrence and estimate the risk posed by each identified characteristic of the GMOs [Ref. 8].

### 2. Comparative analysis

The risk assessment strategy first seeks to deploy appropriate methodologies and approaches to compare the GM crop or product with its non-GM counterparts. This comparison is the *starting point* of the safety assessment which then focuses on any intended or unintended differences identified. Established and validated protocols should be used throughout and the data analysed using appropriate statistical techniques. Limits of detection of the methods used should be stated.

It is obvious that the insertion of genes and other associated DNA from a donor organism into the host will result in a plant that is not completely identical to the parent. The risk assessment process therefore concentrates on the outcomes of the transformation process using appropriate comparators. To this end the concepts of *familiarity* and *substantial equivalence* were developed by the OECD [Ref. 15&16] and further elaborated by WHO/FAO [Ref. 17] for the assessment of the environmental safety of GMOs and the safety of genetically modified foods/feeds, respectively.

#### *Concept of familiarity*

The concept of familiarity is based on the fact that most genetically engineered organisms are developed from organisms such as crop plants the biology of which is well understood. It is not a risk/safety assessment in itself but familiarity allows the risk assessor to draw on previous knowledge and experience with the introduction of similar crops including GM crops into the environment. Familiarity comes from the knowledge and experience available for conducting a risk/safety analysis prior to scale-up of any new plant line or crop cultivar in a particular environment [Ref. 15].

#### *Concept of substantial equivalence*

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 genetically modified food/feed [Ref. 16&11]. Application of this



concept may result in identification of similarities and potential differences between the GM crop, food/feed and their non-GM counterpart. The outcome of this comparative approach will further structure the safety assessment procedure, which may include additional toxicological and nutritional testing. Application of the substantial equivalence concept is a *starting* point for the safety assessment. It provides assurance that the GM food/feed may be as safe as the traditional counterpart, or that no comparison can be made because of the lack of an appropriate comparator. Analysis of substantial equivalence involves not only a comparison of the chemical composition between the new and the traditional food or feed, but also of the molecular, agronomical and morphological characteristics of the organism in question. Such comparisons should be made with GM and non-GM counterparts grown under the same regimes and environments. When the degree of equivalence is established as substantial, a greater emphasis is placed on the newly introduced trait(s). Where substantial equivalence does not occur, this does not necessarily identify a hazard. Where a trait or traits are introduced with the intention of modifying composition significantly and where the degree of equivalence cannot be considered substantial, then the safety assessment of characteristics other than those derived from the introduced trait(s) becomes of greater importance.

### 3. Issues to be considered

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

- the characteristics of the donor and recipient organisms;
- the genes inserted and expressed;
- the potential consequences of the genetic modification;
- the potential environmental impact following a deliberate release;
- the potential toxicity and allergenicity of gene products and metabolites;
- the compositional, nutritional, safety and agronomic characteristics;
- the influence of food processing on the properties of the food or feed;
- the potential for changes in dietary intake;
- the potential for long-term nutritional impact.

The risk assessment should take into account any potential impact of horizontal DNA transfer between plant or plant components and micro-organisms in relevant environments. Genes integrated in the GM plant should also be subjected to risk assessment with respect to the possible effects of ingestion of the protein expressed in plant parts.

Different outcomes of a genetic transformation event can be envisaged:

**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 transformation process.

**Unintended effects** are considered to be consistent differences between the GM plant and its appropriate control lines, which go beyond the primary expected effect(s) of introducing the target gene(s). They may be evident in the phenotype or composition of the GM plant when grown under the same conditions as the controls. Unintended effects can often be predicted or explained in terms of our current knowledge of plant

biology and metabolic pathway integration and interconnectivities. Additionally, molecular and biochemical analyses can be used to determine changes at the level of transcription and translation that could lead to unintended effects.

#### **4. General recommendations**

Risk assessment may be simplified if genes extraneous to the successful deployment of the target transformation event are not present in the GM plant. Whenever possible, notifiers are encouraged to develop, for commercial release, those transgenic lines in which only DNA essential to the modification of the trait in question is transferred to the plant [Ref. 18].

The choice of a particular marker gene should be given careful consideration in view of the amount of information required for risk assessment. At an early stage in the development of GM plants some strategies are available which can be considered best practice to reduce the potential identified risks and to avoid some unidentified risks in the environment [Ref. 19]. The overall aim is to reduce environmental exposure and the potential risks from the transgenes and their products. Three principle ways can achieve this:

- avoid or minimise the inclusion of superfluous transgenes or sequences;
- avoid or minimise superfluous expression of the transgene;
- avoid or minimise the dispersal of transgenes in the environment.

#### **5. Forthcoming developments**

Profiling technologies such as metabolomics, proteomics and transcriptomics are considered as emerging technologies to extend the breadth of comparative analyses and to identify the need for further risk assessment [Ref. 20]. Should new technologies be applied, then the expectation is that all approaches are properly validated and that statistical analyses have been performed to the highest standard [Ref. 21].

### III. SPECIFIC INFORMATION FOR RISK ASSESSMENT

#### 1. Molecular characterisation<sup>2</sup>

The requirements for molecular data are the same for applications under Directive 2001/18/EC for commercial purpose (so called Part C releases) and for the assessment of GM food and GM feed. Some data necessary for traceability may not be relevant for risk assessment.

##### *1.1 Information on the donor and recipient organisms*

Notifiers should provide information both on the organisms used as the DNA donor(s) for genetic modification and the recipient organism. This information should include the most recent taxonomic classification including the family, genus, species, subspecies, cultivar/breeding line or strain. Taxonomic information could be used 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. Information should be provided on all issues of potential concern, such as the presence of natural toxins, allergens or virulence factors. Data should be provided on the previous use of the donor and the recipient organism.

##### *1.2 Method used for the genetic modification*

The transformation protocol should be described in detail and relevant references for the transformation method should be provided. For *Agrobacterium*-mediated transformation, the strain designation of the *Agrobacterium* used during the transformation process must be provided, including an indication of if, and how, the Ti/Ri plasmid based vector was disarmed. For transformation methods that involve the use of helper plasmids, a detailed description of these plasmids should be given.

##### *1.3 Information on the DNA used in transformation*

A physical and genetic map should detail the position of all coding and non-coding sequences, origins of replication and transfer, and other plasmid elements together with the notifier's selected restriction sites for the generation of probes, and the position and nucleotide sequence of primers used in PCR<sup>3</sup> analysis. A table identifying each component, its size, its origin and its role should accompany the map. The complete sequence of the DNA used in the transformation should be given. The map/table should also indicate if there have been modifications that affect the amino acid sequence of the product of the introduced gene. Supporting documentation should be provided to allow adequate risk assessment of the changes made. If carrier

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<sup>2</sup> A full risk assessment will still be required where wholly synthetic genes are used.

<sup>3</sup> The **Polymerase Chain Reaction** is used to amplify sequences of DNA by repeated replication using a pair of oligonucleotide primers that bind to either strand of the target sequence at each extremity. Repetitive replication of the DNA is achieved by the use of a thermostable DNA polymerase.

DNA is used in a transformation event, its source must be stated and a risk assessment provided.

#### ***1.4 Information on the sequences actually inserted/deleted***

Notifiers should provide information on:

- the copy number of all detectable inserts, both complete and partial;
- the organisation of the inserted genetic material at the insertion site including relevant sequence data of the inserted material and of the surrounding region. Sufficient of the sequences flanking the insert should be determined to identify the formation of potential chimeric ORFs<sup>4</sup> generated at the junctions of the insert and the plant DNA. If a chimeric ORF is identified then expression analysis should be performed to determine if there is potential transcription. Homology analysis should be conducted to establish the absence of any putative toxins or allergens encoded by the identified chimeric ORF. Potential effects arising from the insertion cannot be characterised by molecular techniques alone but requires a broader consideration including compositional and phenotypic analysis. This may be particularly relevant where known ORFs or regulatory regions are disrupted by the insertion event, but will require a case-by-case approach.
- all sequence information (in electronic format) including the location of primers used for detection.

The above information will also be required when genes have been stacked by the interbreeding of GM lines containing transformation events approved through the regulatory process. The need for further risk assessment will depend, on a case-by-case basis, on the nature of the genetic modifications.

#### ***1.5 Information on the expression of the insert***

Notifiers 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. Such requests may be made even where the gene is under the control of a bacterial promoter. 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. Any expression of potential fusion proteins should be determined. Bioinformatic analysis can be deployed to help identify potential novel fusion proteins. Expression analysis could then be used to detect novel transcripts if identified through the bioinformatic analysis. The selection of appropriately validated analytical approaches is an issue for the applicant. Applications to date have commonly used approaches such as Northern blotting<sup>5</sup> and RT-PCR<sup>6</sup>. The sensitivities of techniques employed will vary and lack of a distinct

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<sup>4</sup> open reading frames

<sup>5</sup> Northern blotting involves the extraction of RNA from a cell population and separation of molecules of different sizes by electrophoresis followed by detection of sequences that hybridize with a complementary probe sequence.

<sup>6</sup> RT-PCR is a very sensitive method for detecting mRNA. It involves making a cDNA copy of the RNA using reverse transcriptase. This is followed by PCR amplification using the cDNA as the target for the amplification reaction.

transcript signal does not necessarily indicate that the corresponding protein is not accumulated.

Immunochemical determination by ELISA<sup>7</sup> has proven adequate for determining the levels of novel gene products in the genetically modified plants, while Western blotting<sup>8</sup> provides additional information on the molecular weight of the gene product. Where ELISA tests are routinely used to quantify the expression level of the target protein, it is imperative that the specificity of the antibodies developed are validated. For example, where crude plant extracts are used as test material there may be non-specific cross-reaction with protein other than the target protein.

Expression analysis will also be required when traits are stacked by the interbreeding of GM lines containing transformation events approved through the regulatory process. The need for further risk assessment will depend on the nature of the genetic modifications (case-by-case basis).

### ***1.6 Information on inheritance and stability***

Notifiers should provide data subjected to statistical analysis from a representative number of generations (vegetative or generative propagation) that demonstrate the inheritance pattern and the stability of the sequences inserted and expression of corresponding proteins.

## **2. Comparative analysis**

### ***2.1 Choice of the comparator***

In the case of vegetatively propagated crops, comparative analyses should include the parental variety used to generate the transgenic lines. In the case of crops that reproduce sexually, comparators would include appropriate non-GM lines of comparable genetic background. Since many crops used to produce food and feed are developed using back-crossing, it is important that in such cases, substantial equivalence testing uses the most appropriate controls and does not simply rely on comparisons with original parental material. For example, specific male pollinator lines may be used in the generation of the final product. In all cases, evaluation of the extent of equivalence will be greatly enhanced by additional, valid comparisons, with published data on the performance and composition of commercial varieties of the crop species in question and which have a known history of safe use. Such data could indicate that the GM lines fall within the variation reported for the species in question. Where traits are stacked by the interbreeding of GM lines, the appropriate comparator will be the equivalent non-GM hybrid. Where this is not possible (e.g. in vegetatively propagated crops) the GM parent lines are appropriate comparators.

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<sup>7</sup> Enzyme-Linked Immuno Sorbent Assays use antibodies that are tagged with enzymes to amplify the signal from a positive interaction between specific antigens and antibodies, increasing many times the sensitivity of the test.

<sup>8</sup> Western blotting involves the extraction of proteins from a cell population and separation of molecules of different sizes by electrophoresis followed by immunological detection of the protein of interest by its reaction with a specific, labelled antibody.

## **2.2 Field trials**

Protocols of field trials performed with genetically modified and control crops must be specified and documented with respect to:

*number of locations, growing seasons, geographical spreading and replicates;*

The basic set of data should be obtained from a comparison of the GM plant and an appropriate control line grown in the same field under comparable conditions. This comparison should cover more than one growing season and multiple geographical locations representative of the various environments in which the GM plants will be cultivated. The number of replicates at each location should reflect the inherent variability of the plant.

*statistical models for analysis, confidence intervals;*

Experimental design should be rigorous and analysis of data should be presented in a clear format. Field trial data should be analysed statistically, using appropriate statistical tools. A completely randomised design, for example, could indicate whether the experimental factors (location, year, climatic conditions, plant variety) interact with one another. The confidence intervals used for statistical analysis should be specified (normally 95%, with possible adjustment according to the hazard of the constituent to be compared).

*the baseline used for consideration of natural variations.*

Data demonstrating the natural range in component concentrations found in non-GM counterparts should be provided to enable additional comparisons with the GM plant in question. Data may be generated by the notifier or compiled from literature. The databases that were used for comparison have to be specified. Special attention has to be paid to the comparability of the analytical methods used to create the data. Ranges as well as mean values should be reported and considered.

Statistically significant differences in composition between the modified crop and its traditional counterpart grown and harvested under the same conditions should trigger further investigations as to the relationship with the genetic modification process. Modifications that fall outside normal ranges of variation will require further evaluation to determine any biological significance.

## **2.3 Selection of compounds for analysis**

Analysis of the composition of the GM plant/food/feed is crucial when comparing the product with its non-GM counterparts. Analysis should be carried out on the raw agricultural commodity, such as grain, as this usually represents the main point of entry of the material into the food/feed chain. Analysis on specific derived products should be required only on a case-by-case basis and when justified scientifically.

In each case, key macro- and micro-nutrients, toxicants, anti-nutritional compounds, and other constituents (including moisture and total ash) should be determined. Examples of the key nutrients, anti-nutrients and toxicants characteristic for plant species and information on the extent of natural variation are provided in OECD consensus documents which may provide further guidance for compositional analysis to establish the extent of compositional equivalence [Ref. 22].

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 in nutritionally significant levels and/or that 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 intention 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 toxicants are those compounds, inherently present, whose toxic potency and levels may harm human/animal health. The concentrations of such compounds should be assessed according to plant species and the proposed use of the food/feed product [Ref. 23]. Examples would include digestive enzyme inhibitors and those anti-nutritional, potentially toxic, or allergenic compounds recognised as being normally present, or newly introduced as a result of the genetic modification. Compounds other than key nutrients and toxicants may be included in analyses on a case-by-case basis.

Knowledge of the introduced trait may suggest the possibility of effects beyond that specifically intended. For example, if the introduction of a gene that confers herbicide tolerance is functionally equivalent to an existing gene involved in aromatic amino acid synthesis, analysis of the protein content and amino acid composition would be prudent.

If changes relative to the comparator and/or any commercial varieties included in field trials are found, then any downstream metabolic and toxicological consequences should be examined. Where appropriate, published ranges for parameters measured can be taken into account

#### ***2.4 Agronomic traits***

Compositional analysis represents a key component of the risk assessment process. However, unintended effects may also manifest themselves through, for example, changes in susceptibility to important pests and diseases, through morphological and developmental changes or through modified responses to agronomic and crop management regimes.

### **3. Environmental risk assessment**

Environmental risk assessments are 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 evaluation of any risk to the receiving environment that might arise from the release of the GMO.

Examples of possible interactions between the GM plant and its environment including potential impact on other organisms are:

- effects on the dynamics of populations of species in the receiving environment and the genetic diversity of each of these populations;
- altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or creating new reservoirs or vectors;
- compromising prophylactic or therapeutic medical, veterinary, or plant protection treatments, for example by transfer of genes conferring resistance to antibiotics used in human or veterinary medicine;
- effects on biogeochemistry (biogeochemical cycles), particularly carbon and nitrogen recycling through changes in soil decomposition of organic material.

#### ***3.1 Geographical relevance of data***

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 notifier should submit a reasoned argument that the data is applicable to European conditions.

#### ***3.2 Impact on wild plants***

The potential consequence arising from out-crossing to compatible wild species should be considered and assessed for environmental risk. This will depend on sexually compatible plants being present and available outside the crop to receive pollen and produce fertile hybrids. Selection pressure in non-crop habitats that is required to maintain the selective advantage of any transferred trait should be identified. For example, transferred herbicide tolerance may not be an advantageous trait in habitats where the herbicide is not applied.

#### ***3.3 Impact on non-modified crops***

The potential consequence arising from out-crossing to other crop cultivars should be considered and assessed for environmental risk. This will vary with crop. 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 with some wild relatives. In cases where gene transfer cannot be prevented between certain adjacent crops of, for example, oilseed rape or maize, the risk assessment should focus on the consequences of cross pollination even at very low frequency.



### ***3.4 Impact on organisms and ecological processes***

Risk assessments should be carried out for each of the different functional environmental compartments that are exposed to the GM plant. Whether any parts of it will remain in the environment after harvest, will depend on the specific crop and its management regime or agronomic practices. Soil fertility strongly influences the growth and productivity of plants. As 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 the agronomically relevant processes of soil fertility and plant productivity. The assessment should also address the fate of any (newly) expressed substance(s) 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). 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. Exposure should also be estimated to soil organisms and decomposition function (e.g. earthworms, micro-organisms, leaf litter breakdown) in relation to potential transfer to soil micro-fauna and impact on degradation.

Impact should be assessed on non-target arthropods (including pollinators, beneficial and predatory arthropods), grazing birds and mammals or, if appropriate, the aquatic environment. Such studies should include laboratory, greenhouse or field exposure experiments set up in such a way that enough statistical power is obtained to be able to observe possible negative impacts on non-target organisms. This risk assessment should take account of where in the plant and to what degree the inserted genes are expressed and therefore the extent to which non-target organisms are exposed either directly or indirectly.

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.

An assessment of the potential impact of growing GM crops on wider biodiversity in the crop ecosystem requires the combination of several different approaches [Ref. 24]. The notifier should describe the appropriate commercial management regime for the crop including changes in pesticide applications, rotations and other crop protection measures where different from the equivalent non-GM crop under representative conditions. The notifier should aim to assess the direct and indirect, immediate and delayed effects, of the management of the GM crop on all affected habitats. This should include the biodiversity within the GM crop and adjacent non-crop habitats. The necessary scale of such studies will depend on the level of risk associated with a

particular GM crop and on the quality and extent of the available literature that is relevant to the particular risk assessment.

#### **4. Food/feed safety assessment**

In addition to the molecular characterisation of the genetically modified plants and the necessary comparative compositional data to assess the extent of equivalence, further information is needed for the safety assessment of material intended for use as human food or animal feed.

##### ***4.1 Product Specification***

Specification of the origin and the composition of the GM plant and GM food/feed is needed to ensure the identity between the product tested/evaluated and the product to be marketed. In the design of the specification, parameters most relevant for the characterisation of the product from a safety and nutritional point of view should be considered. Information on the availability of specified reference material should be submitted.

##### ***4.2 Effect of the production process***

For processed foods/feeds derived from GM sources a description of the production process should be provided which should comprise a general outline of the processing steps and a detailed description of the conditions applied (description of physical, chemical and biochemical parameters). It is important to assess if, and to what extent, the processing steps lead to the concentration or to the elimination, denaturation and/or degradation of DNA and the novel protein(s)<sup>9</sup> in the final product.

##### ***4.3 Anticipated intake/extent of use***

An estimate of the expected intake is necessary for the safety evaluation of GM food/feed and to evaluate nutritional significance. Information should be provided on the intended function, the dietary role of the product, and the expected level of use. On the basis of the available consumption data, the anticipated average and maximum intake of the GM food should be estimated. 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.

The concentrations of the new gene products and constituents produced, or modified by the intended 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 exposure to these constituents should be estimated

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<sup>9</sup> In the context of this document “novel proteins” are proteins newly expressed as a consequence of the genetic modification.

taking into account the influences of processing, storage and expected treatment of the food/feed in question.

#### **4.4 Toxicology**

The toxicological requirements for food and feed derived from GM plants must be considered on a case-by-case basis and will be determined by the outcome of the assessment of the biological significance of any differences identified between the GM product and its conventional counterpart. This would not only include studies on newly expressed proteins but also the consequences of any genetic modification (e.g. gene silencing or over-expression of an endogenous gene). In principle, the safety assessment must consider the presence of proteins expressed as result of the genetic modification, the potential presence of other novel constituents and/or possible changes in the level of natural constituents beyond normal variation. These potential deviations from the conventional counterparts require different toxicological approaches.

There may be circumstances, when the notifier 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 notifier must state the reasons for not submitting the required studies or for carrying out studies other than those mentioned below.

Those toxicological studies which are carried out should be conducted using internationally agreed protocols. Test methods described by the OECD [Ref. 25] or in the most up to date European Commission Directives on dangerous substances are recommended [Ref. 26]. 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 87/18/EEC and accompanied by a statement of GLP-compliance [Ref. 27].

Any adverse effect noted in individuals exposed professionally should be submitted by the notifier.

##### **4.4.1 Testing of novel proteins**

To demonstrate the safety of novel proteins the following information is needed:

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

A search for homology to proteins known to cause adverse effects, e.g. protein toxins, should be conducted. A search for homology to proteins exerting a normal metabolic or structural function can also contribute valuable information. The database(s) used to carry out the search should be specified.

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

Data concerning the resistance of the novel protein to proteolytic enzymes (*e.g.* pepsin) should be obtained, *e.g.* by *in vitro* investigations using appropriate and validated tests. Stable breakdown products should be characterized and evaluated with regard to the hazards linked to their biological activity.

The studies required to investigate the toxicity of the novel protein should be selected on a case-by-case basis, depending upon the knowledge available with respect to the protein's function/activity and any history of its prior human consumption.

In the case of novel proteins with insufficient data base and, in particular, if the available data suggest the existence of any cause for concern, a repeated dose study should be performed, using laboratory animals capable of reacting rapidly to any induced physiological or metabolic disturbances, for example, young rapidly growing animals. Normally, this is a 28-day oral toxicity study according to OECD guideline 407 (1995)<sup>10</sup>. Additional targeted investigations should be conducted if the novel protein is suspected to act on specific organs or tissues including interactions with receptors of the endocrine, reproduction or nervous system.

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

It is essential that the tested protein is equivalent to the novel 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 is used which was produced by micro-organisms, the structural and functional equivalence of the microbial substitute to the novel plant protein has to 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.

#### *4.4.2 Testing of novel constituents other than proteins (novel metabolites)*

Other identified novel constituents other than proteins should be evaluated according to the traditional toxicological approach on a case-by-case basis. For establishing their safety, information analogous to that described in the “Guidance on submissions for

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<sup>10</sup> An acute, single dose test with a 14-day observation period, is inadequate to detect possible toxicity arising from repeated dosing. Furthermore, this test does not provide information on the dose-response relationship and is designed to examine only a few endpoints (mortality, morbidity, clinical observation and gross necropsy) and not the broad range of endpoints required to be investigated in repeated dose studies, such as haematology, clinical chemistry, urine analysis, organ weights and histopathological examination of organs and tissues.

food additive evaluations by the Scientific Committee on Foods” [Ref. 28] is needed. This implies the submission of information on a core set of studies and the consideration of whether any other type of study might also be appropriate. Normally, the core set includes information on metabolism/ toxicokinetics, subchronic toxicity, genotoxicity, chronic toxicity/carcinogenicity and reproduction and developmental toxicity.

#### *4.4.3 Information on natural food constituents*

Natural food constituents comprise a large variety of substances: macro- and micronutrients, secondary plant metabolites as well as natural toxicants and antinutritional factors. If the content of such natural food constituents is increased beyond the natural variation, a detailed safety 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.

#### *4.4.4 Testing of the whole GM food/feed*

If the composition is modified substantially, or if there are any uncertainties on the equivalence to a traditional counterpart, not only novel constituents, but also the whole GM food/feed should be tested.

For foods and products that can be used for both food and feed purposes, the testing programme should include at least a 90-day feeding study in rodents. Special attention must be paid to the selection of doses and the avoidance of problems of nutritional imbalance. Additional toxicological studies may also be necessary, depending on the potential exposure, the nature and extent of deviation from traditional counterparts and the findings of the feeding study.

For feed use only, where the modification is expected to substantially change composition and/or bioavailability then a suitable comparator for feeding studies is unlikely to be available. In these cases feeding studies with the more important target species should be made to demonstrate wholesomeness for the animal.

### **4.5 Allergenicity<sup>11</sup>**

Allergy is an adverse reaction (in this context to foods) which, by definition, is immune-mediated and particularly involves IgE antibodies. This section deals with the risks to genetically predisposed (i.e. atopic) individuals when exposed to foods derived from GM plants with regard to sensitisation or to elicitation of an allergic reaction.

The potential allergenicity of a protein is not a completely predictable parameter and will depend upon the genetic diversity and variability of specific IgE response in

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<sup>11</sup> This assessment strategy is focused on IgE mediated responses and is not applicable for assessing whether newly expressed proteins are capable of inducing gluten-sensitive or other enteropathies. See Codex documentation, Ref. 27. However, in case the introduced genetic material is obtained from wheat, rye, barley, oats or related cereal grains it should be assessed if the novel proteins play a possible role in the elicitation of gluten-sensitive enteropathy.

atopic humans. Given this lack of complete predictability it is necessary to obtain, from several steps in the risk assessment process, a cumulative body of evidence which minimises any uncertainty with regard to the protein in question.

The intrinsic allergenicity of the foreign protein(s) encoded by the introduced gene(s) must clearly be considered. Moreover, the consequences of any possible unintended effects of the genetic modification on the endogenous allergenic potential of the plant or plant product should also be considered on a case-by-case basis. For example, unintended qualitative or quantitative changes could occur in the pattern of allergenic proteins naturally present in the conventional plant or product.

#### *4.5.1 Assessment of allergenicity of the newly expressed protein*

In line with the recommendations of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology [Ref. 29] and the Codex *ad hoc* Intergovernmental Task Force on Foods Derived from Biotechnology [Ref. 30], an integrated, stepwise, case-by-case approach, as described below, should be used in the assessment of possible allergenicity of newly expressed proteins.

In every case a search for sequence homologies and/or structural similarities between the expressed protein and known allergens should be made as the first step in the assessment. Identification of potential linear IgE binding epitopes should be conducted by a search for homologous peptidic fragments in the amino acid sequence of the protein. The size of the contiguous identical or chemically similar amino acid search should be based on a scientifically justified rationale in order to minimise the potential for false negative or false positive results.

If the source of the introduced gene is considered allergenic, but no sequence homology 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 the source is not known to be allergenic but if a sequence homology to a known allergen is demonstrated, the specific serum screening should be conducted with sera from patients sensitised to this allergen.

If the source of the gene/protein is not known to be commonly allergenic and no sequence homology to a known allergen is demonstrated, or if the result of the specific serum screening of a newly expressed protein from a source known to be allergenic is equivocal, additional tests should be performed. These include pepsin resistance tests or targeted serum screening.

#### *4.5.2 Further analyses*

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 [Ref. 31], resistance of proteins to pepsin digestion is still proposed as an additional criterion to be considered in an overall risk assessment. In the case of resistance of a protein to degradation in the presence of pepsin 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 [Ref. 29] 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. If no relevant serum is available the expressed protein should be analysed for evidence of cross-reactivity and/or sensitising potential using other tests such as appropriate animal models or search for T-cell epitopes, structural motifs, etc. Complementary data on the biological origin and function and structural features of the newly expressed protein may also be provided in order to increase the body of facts to support a conclusion.

#### *4.5.3 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/feed 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.

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.

## **5. Nutritional assessment of GM food**

The development of GM foods has the potential to improve the nutritional status of individuals and populations and provide products with 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 evaluation of GM foods should consider:

- nutrient composition (see compositional studies as described in section 2);
- biological efficacy of nutrient components in the foods;
- assessment of dietary intake and nutritional impact

When substantial equivalence to an existing food is demonstrated, the only further nutritional assessment will deal with the impact of the introduction of the GM food on general human dietary intake patterns. Information on the anticipated intake/extent of use of the GM food will be required and the nutritional consequences should be assessed at average and at upper levels of daily intake. The influences of non-nutrient components of the GM food should also be considered.

Specific additional requirements should be applied to those GM foods aimed at modifying nutritional quality. In this case additional detailed studies on specific metabolites, tailored according to the genetic modification(s), would be required.

The introduction of a significant nutritional change in a food may require post-market assessment to determine if the overall diet has been altered and to what degree (see section I.3).

## **6. Nutritional assessment of GM feed**

In cases where composition of the GM plant differs significantly from the non-GM counterpart, a full range of physiological-nutritional studies should be carried out on a case-by-case basis with representative target animals. These studies could include digestibility, balance experiments or the determination of the nutritive value.

For feeds, it is recommended that comparative growth studies are conducted with a fast growing livestock species such as the broiler chick. Because of their rapid weight gain, broilers are particularly sensitive to the presence of toxic elements in their feed. Studies of this type are, however, limited to those materials suitable for inclusion in broiler diets and which can be nutritionally matched to a suitable control diet.

For feedstuffs intended only for aquaculture, extrapolating results from a growth study made with a fish species such as the catfish, may be preferable to an extrapolation from results obtained with broilers. Similarly, in the presence of a known toxicant, feeding studies may be restricted only to those livestock known to tolerate the compound. For example, gossypol prevents the use of cottonseed meal in animals other than ruminants. In this case milk production parameters, also recognised as relatively sensitive indicators of body condition, might substitute for growth rate.

## **7. Animal products**

The safety of products derived from animals fed a diet containing GM plant material and consumed by humans should be considered. However, it is considered unlikely that a potential gene transfer from GM plant material to animal cells will result in the expression of heterologous proteins which might be present in animal products or that intact newly expressed proteins will be absorbed. Consequently, it is not considered necessary to test routinely for the presence of introduced genes or their products unless their characteristics suggest cause for concern.

Proteins introduced into the GM plant and known to modify plant metabolism may alter the nature or concentration of metabolites which may have toxicological implications for the animal and/or consumers of animal products. In such cases further studies should be performed with respect to the toxicological implications for the animal and/or consumers of animal products. Guidance on these issues is provided in the relevant sections of this document.



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