European Food Safety Authority

DRAFT GUIDANCE DOCUMENT FOR THE RISK ASSESSMENT OF GENETICALLY MODIFIED PLANTS AND DERIVED FOOD AND FEED

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

The European Food Safety Authority (EFSA) invites comments from interested parties. Comments are to be submitted through the website of EFSA at the following URL: http://www.efsa.eu.int/cf/consultation.cfm. The consultation period will be closed on 30 April 2004.

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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) 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 present draft document provides detailed guidance for genetically modified plants (GM plants) and food and/or feed containing, consisting of or produced from these plants.

This document was compiled by the Scientific Panel on Genetically Modified Organisms (GMO Panel) of EFSA, consisting of the following members:

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The following *ad hoc* experts also contributed:

Gerhard Flachowsky (Panel on Additives and Products or Substances used in Animal Feed), Tony Hardy (Panel on Plant Health, Plant Protection Products and their Residues), Andreu Palou (Panel on Dietetic Products, Nutrition and Allergies) and Richard Phipps (University of Reading, UK).

The draft document is published on the EFSA website for a 3-week period of public consultation. On May 25, 2004, a stakeholder consultation will be held. The GMO Panel will consider all comments relating to the risk assessment of GMOs before preparing a revised guidance document. The GMO Panel will not consider issues related to risk management of GMOs (traceability, labelling, coexistence). Political and socio-economic issues are also outside the remit of the Panel. 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 for 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) N° 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 genetically modified (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.

I. INTRODUCTION

1. SCOPE OF THE DOCUMENT

This document provides guidance for the risk assessment of genetically modified (GM) plants[1] and/or derived food and feed submitted within the framework of Regulation (EC) No. 1829/2003 (EC, 2003a) on GM food and feed or Directive 2001/18/EC (EC, 2001a) on the deliberate release into the environment of genetically modified organisms[2] (GMOs). 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 authorisation criteria for both food and feed. The applicant has the choice of applying for the environmental risk assessment to be carried out at the same time as the safety assessment under the Regulation (EC) 1829/2003. The guidance document takes this into account and provides a framework for the full risk assessment of the genetic modification.
More specifically, this document provides detailed guidance to assist the applicant[3] in the preparation and the presentation of the application, according to Articles 5(8) and 17(8) of Regulation (EC) No. 1829/2003. This guidance document addresses the requirements of the Regulation (EC) No. 1829/2003 and is structured to meet the requirements of Annex IIIB of Directive 2001/18/EC and Annex II of the same Directive. Specific guidance on the presentation of the application can be found in the annexes to this document. This guidance does not consider issues related to risk management (traceability, labelling, coexistence).

This guidance document is an updated replacement of the 'Guidance document for the risk assessment of genetically modified plants and derived food and feed' of 6-7 March 2003, prepared for the EU Scientific Steering Committee by the Joint Working Group on Novel Foods and GMOs (EC, 2003d).

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[1] 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.

[2] Genetically modified organism means an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination (Directive 2001/18/EC). Techniques of genetic modification include 1) recombinant DNA techniques involving the incorporation of the DNA molecules into a host in which they are capable of continued multiplication; 2) direct introduction of DNA by e.g. micro-injection; 3) cell/protoplast fusion or hybridisation by methods that do not occur naturally. Techniques not considered to result in genetic modification are *in vitro* fertilisation, natural transformation and polyploidy induction (for more details see Directive 2001/18/EC, Annex I A).

[3] The term applicant is used hereafter as a generic reference to the official body submitting the application.

Food additives (Directive 89/107/EEC; EC, 1989), flavourings (Directive 88/388/EEC; EC, 1988) and feed additives (Regulation (EC) No 1831/2003; EC, 2003c) containing, or consisting of or produced from GM plants do not fall within the scope of this guidance document.

This guidance does not cover the contained use (Directive 90/219/EEC; EC, 1990a) or the deliberate release into the environment (Directive 2001/18/EC) of genetically modified micro-organisms (GMMs), 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 or consisting of GMMs, a parallel guidance document is under preparation by the GMO Panel. Similarly, guidance will be prepared for the safety assessment of the genetic modification where substances, such as food additives (Directive 89/107/EEC), flavourings (Directive 88/388/EEC) and feed additives (Regulation (EC) No 1831/2003) contain, or consist of or are produced from GMOs that fall within the scope of Regulation (EC) 1829/2003.

This guidance does not cover the deliberate release into the environment (Directive 2001/18/EC) of genetically modified animals, or the placing on the market of food and/or feed consisting of, containing or produced from genetically modified animals (Regulation (EC) No 1829/2003). Appropriate guidance will be prepared by the GMO Panel in the future.

Additional guidance also needs to be developed for the environmental risk assessment of GM plants used to produce medicinal products ('plant-made pharmaceuticals') for human and veterinary use (Regulation (EEC) No 2309/93; EC, 1993) as well as other non-food purposes (e.g. 'plant-made industrial compounds' and GM plants for phyto-remediation) through biotechnology.

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

Community food law (Regulation (EC) 178/2002)

The Regulation (EC) 178/2002 (EC, 2002c) lays down the general principles of food law and procedures in food safety. It defines food broadly, including animal feed and other agricultural inputs at the level of primary production. The Community food law establishes the European Food Safety Authority (EFSA) as an independent scientific source of advice, information and risk communication, nevertheless stating that the link between risk assessors and risk managers should be strengthened. The EFSA includes Scientific Panels, for example the GMO Panel. The EFSA should provide a comprehensive independent scientific view of the safety and other aspects of the whole food and feed supply chains. The Community food law defines 'food' as any substance or product, whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be ingested by humans. 'Food' includes drink and 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 Community food law also defines 'risk', 'risk analysis', 'risk assessment', 'risk management' 'risk communication' and 'hazard'. Articles 14 and 15 of the Community food law set the food and feed safety requirements, respectively.

GM food and feed regulation (Regulation (EC) 1829/2003)

Regulation (EC) 1829/2003 on genetically modified food and feed provides for the risk assessment of GMOs and derived food and feed to be carried out by EFSA 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 (EC, 2002c).

Food products containing, consisting of or produced from GMOs were previously regulated by Regulation (EC) 258/97 on Novel Foods and Novel Food Ingredients, which has been amended by Regulation (EC) 1829/2003 from 18 April 2004. For feed containing or consisting of GMOs, no specific Community legislation has been in place prior to the entering into force of Regulation (EC) 1829/2003 and the safety of GM feed has been assessed under Directive 2001/18/EC.

The scope of Regulation (EC) 1829/2003 is GMOs for food/feed use; food/feed containing or consisting of GMOs; food/feed produced from GMOs; and food containing ingredients produced from GMOs, (referred to as GM food/feed). The Regulation states 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 will be authorised only when the applicant has adequately demonstrated that they satisfy the requirements.

An application should contain the particulars as specified by Article 5 and Article 17 of the Regulation. The Commission shall establish implementing rules for the application of these Articles, including rules concerning the preparation and the presentation of the application.

Under Regulation (EC) 1829/2003, an application for authorisation is sent to EFSA through a national competent authority. From the receipt of a valid application, EFSA will have a time limit of six months to carry out the safety assessment and to provide an opinion. Such time limit will be extended whenever EFSA seeks supplementary information from the applicant.

The other Member States and the Commission will be informed and EFSA will make the application available to them.

EFSA is responsible for the scientific risk assessment that takes into account any risk to human and animal health and, as the case may be, to the environment. However, if EFSA is not in a position to realise all the workload, EFSA may ask the appropriate food assessment body of a Member State to carry out an initial part of the safety assessment of the food or feed in accordance with Article 36 of Regulation (EC) No 178/2002.

On the basis of the opinion of EFSA, the Commission will draft a proposal for granting or refusing authorisation, which will be approved through qualified majority of the Member States within a Regulatory Committee. Regulation (EC) 1829/2003 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 authorised product will have to comply with the provisions of Regulation (EC) 1830/2003 concerning the traceability and labelling of GMOs and the traceability of food and feed products produced from GMOs and amending Directive 2001/18/EC (EC, 2003b). The authorised product shall be entered in a Community register of genetically modified food and feed, which will be made available to the public. 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 the use of genetically modified feeds for animal consumption may be imposed.

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

The principles regulating the deliberate release into the environment of GMOs are laid down in Council Directive 2001/18/EC (EC, 2001a), which repeals 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 health and the environment before any GMOs or products containing GMOs could be released into the environment, or placed on the market. The Directive 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 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.

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. 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 present guidance document is an updated replacement of that guidance.

Under Directive 2001/18/EC, an applicant 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, and also address aspects of human and animal health. 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 (Art. 28 of Directive 2001/18/EC (EC, 2001a)), now represented by the Scientific Panels of EFSA (Art. 22(5) and Art. 28 of Regulation (EC)178/2002 (EC, 2002c)).

Interplay between Regulation (EC) 1829/2003 and Directive 2001/18/EC

Under Regulation (EC) 1829/2003 and where the application concerns products containing or consisting of a GMO, the applicant will have 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 to submit the environmental risk assessment for review at the same time as the safety assessment under Regulation (EC) 1829/2003. In the latter case, it is necessary for the environmental risk evaluation to comply with the requirements

referred to in Directive 2001/18/EC and for the national competent authorities designated in accordance with article 4 of Directive 2001/18/EC to be consulted by EFSA. EFSA may also ask such competent authority to carry out an initial environmental risk assessment. In the case of GMOs to be used as seeds or other plant-propagating materials falling within the scope of Regulation (EC) 1829/2003, EFSA has the obligation to delegate the environmental risk assessment to a national competent authority. EFSA will conclude on the final assessment.

GM seeds

Regarding genetically modified seeds, Directive 98/95/EC (EC, 1998b) 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 to varieties derived by conventional breeding methods from the line concerned. If the variety is intended for food or feed crop production, it also must be authorised in accordance with Regulation (EC) 1829/2003 prior to inclusion in the Common Catalogue.

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 to these authorisation procedures, food additives and flavourings containing, consisting of or produced from GMOs also fall within the scope of Regulation (EC) 1829/2003 for the safety assessment of the genetic modification. Additives and flavourings for use in foodstuffs are however not covered by the scope of this document.

Products and additives used in animal nutrition

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. However, feed materials containing, consisting of or produced from GMOs fall within the scope of Regulation (EC) 1829/2003.

The placing on the market of feed additives is authorised by Directive 70/524/EEC concerning additives in feedingstuffs (EC, 1970). From 18 October 2004, the provisions of this Directive will be repealed by Regulation (EC) 1831/2003 on additives for use in animal nutrition (EC, 2003c). In addition to this authorisation procedure, feed additives containing, consisting of or produced from GMOs also have to undergo an authorisation procedure provided for by Regulation (EC) 1829/2003 before they can be placed on the market. However, feed additives are not covered by the scope of this document.

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)" (EC, 2000a). A risk assessment comprises hazard identification, hazard characterisation, exposure assessment and risk characterisation. A hazard is the potential of an identified 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 (EC, 2002a).

2. COMPARATIVE APPROACH

The risk assessment strategy for GMOs seeks to deploy appropriate methodologies and approaches to compare the GMO and derived products with their non-GM counterparts. The underlying assumption of this comparative assessment approach for GMOs is that traditionally-cultivated crops have gained a history of safe use for the normal consumer or animals as food or feed products. 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. This comparison is the *starting point* of the safety assessment which then focuses on the environmental or food/feed safety and nutritional impact of any intended or unintended differences identified.

It is obvious that the insertion of genes and other pieces of DNA from a donor organism into the host will result in a plant that is not identical to the parent and therefore the risk assessment in addition to focusing on intended modifications, concentrates on the outcomes of the transformation process using appropriate comparators. Thus the safety assessment of GMOs consists of two steps, i.e. a comparative analysis to identify differences, followed by an assessment of the environmental and food/feed safety or nutritional impact of the identified differences, including the intended differences.

Concept of familiarity

The concept of familiarity is based on the fact that most GMOs are developed from organisms such as crop plants, the biology of which is well researched. In a risk/safety 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 transformation and the subsequent management of the GM crop. Familiarity will also derive from the knowledge and experience available from conducting a risk/safety 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 crop, including its management and usage, and focus on the significance and implications of these differences.

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 (OECD, 1993b; EC, 1997b). Application of this concept, also denoted as comparative safety assessment (Kok and Kuiper, 2003), serves the purpose of identifying similarities and potential differences between the GM crop-derived food/feed and the non-GM counterparts, 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 and derived products in question, as well as their chemical composition. Such comparisons should be made between GM and non-GM counterparts grown under the same regimes and environmental conditions. The outcome of this comparative analysis will further structure the second part of the safety assessment procedure, which may include further specific toxicological and nutritional testing. This approach should provide evidence whether or not the GM crop derived food/feed is as safe as the traditional counterpart. Where no appropriate 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 would be the case where a trait or traits are introduced with the intention of modifying the composition of the plant significantly.

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;
- the expression of inserted genes;
- the potential consequences of the genetic modification;
- the potential environmental impact following a deliberate release;
- the potential toxicity and allergenicity of gene products, metabolites and the whole GM plant;
- the compositional, nutritional, safety and agronomic 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.

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. 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 organism compared to the parent, may be identified by measurements of *single* compounds e.g. newly expressed proteins, macro and micro-nutrients (*targeted* approach). Analytical detection methods used must meet specific quality and validation criteria.

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). 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 controls. 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 has occurred within, or in the proximity of, an endogenous gene. Furthermore a comparative and targeted analysis should be carried out of single compounds in the GM organism and its conventional counterpart and 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 and nutritional impact.

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, applicants 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 (ACRE, 2000).

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 (ACRE, 2001a). The overall aim is to reduce environmental exposure and the potential risks from the transgenes and their products. Three principle approaches can be considered to 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

To increase the chances of detecting unintended effects due to the genetic modification of organisms, profiling technologies such as transcriptomics, proteomics and metabolomics, extend the breadth of comparative analyses (EC, 2000b; Kuiper et al., 2003; Cellini et al., 2004; ILSI USA monograph, 2004). The utility and applicability of these technologies in the detection of altered gene and protein expression and metabolite composition in GM plants has been under scrutiny in specific research projects funded, for example, by EU FP5 (GMOCARE project[4]) and the UK Food Standards Agency (GO2 research programme[5]). The applicability of metabolomic techniques, such as gas chromatography coupled to mass spectrometry (GC-MS), and off-line liquid chromatography (HPLC) coupled to nuclear magnetic resonance (NMR), for the simultaneous analysis of a broad variety of metabolites in GM plants and their conventional counterparts has been demonstrated. These non-targeted approaches may be of particular relevance for GM food crops with specific metabolic pathways modified e.g. those leading to enhanced nutritional profiles, obtained through the insertion of single or multiple genes.

Further exploration of profiling approaches is needed with respect to the evaluation of specificity and sensitivity. Profiling methods are not aimed at replacing conventional analyses but may be useful to confirm and complete other data. The development of appropriate profiling databases is strongly recommended.

[4] http://www.entransfood.com/RTDprojects/GMOCARE

[5] http://www.foodstandards.gov.uk/science/research/NovelFoodsResearch/g02programme

III. INFORMATION REQUIRED IN APPLICATIONS CONCERNING RELEASES OF GENETICALLY MODIFIED PLANTS (GYMNOSPERMAE AND ANGIOSPERMAE)

A GENERAL INFORMATION

1. Name and address of the applicant (company or institute)
2. Name, qualification and experience of the responsible scientist(s)
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

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

Applicants 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.

Information is required under the following headings:

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

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.

1. Description of the methods 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 of *Agrobacterium* used during the transformation process must be indicated, including information or references on 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. If carrier DNA is used in a transformation event, its source must be stated and a risk assessment provided.

2. Nature and source of vector used

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 applicant's selected restriction sites for the generation of probes, and the position and nucleotide sequence of primers used in PCR analysis. A table identifying each component, its size, its origin and its role should accompany the map.

3. Size, source (name) of donor organism(s) and intended function of each constituent fragment of the region intended for insertion

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. A risk assessment of these changes needs to be provided.

D. INFORMATION RELATING TO THE GM PLANT

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

A description of the trait and the changes that it makes to the plant phenotype is required. Phenotypic modifications should be quantified in relation to the comparable untransformed plant. The targets of the trait should be identified as well as the sensitivity of non-targets. The purposes of the transformation and the uses of the GM crop should be described together with changes in the crop composition, management, cultivation, deployment, geographic range and end use.

2. Information on the sequences actually inserted or deleted

Applicants should provide information on:

(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. Inheritance patterns following appropriate self- or cross- pollination should be used to confirm that segregation is as predicted from the insert location. In the case of plastid integration, the absence of insert DNA in the nuclear genome must be demonstrated.

(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. Information on flanking sequences should be sufficient to allow identification of potential chimeric ORFs[6] generated at the junctions of the insert and the plant DNA.

[6] open reading frames

Where DNA from mitochondria or chloroplasts flanks the insert, as can occur with biolistic delivery methods, sequence data should extend to the nuclear genome of the parent plant. PCR amplification of the flanking sequences both adjacent to and across the insertion point in the parent plant could be used to demonstrate that this has been achieved. Applicants should indicate where the sequence of the insert in the plant does not match the sequence of the intended transformation event (plasmid sequence) and provide a risk assessment on any changes observed. If a chimeric ORF is identified from flanking sequence analysis then expression analysis should be performed to determine if there is transcription. Homology analysis should be conducted to establish the absence of any putative toxins or allergens encoded by the identified chimeric ORF. However, 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.

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

When events have been combined by the interbreeding of independent approved GM lines or by re-transformation of an existing approved GM line, the need for further molecular analysis will depend, on a case-by-case basis, on the nature of the genetic modifications involved. However, copy number and sufficient data from Southern hybridisations or PCR amplifications to demonstrate maintenance of insert structure is a minimum requirement for molecular characterisation with such events. Stability of copy number and insert size should be demonstrated where relevant. Further insert sequencing may be required on a case by case basis.

3. Information on the expression of the insert

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

This type of information may be relevant to environmental safety aspects, whereas analysis of the plant materials actually used for food/feed is considered more relevant for human or animal safety assessment.

(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. Such requests may be made even where the gene is not under the control of a plant 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.

(c) Expression of potential fusion proteins.

The potential creation of newly expressed fusion proteins should be investigated by bioinformatic analysis and the demonstrated absence of any harmful fusion proteins.

An investigation of newly expressed transcripts may be appropriate.

(d) Methods used for expression analysis

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

4. 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 8, 9 and 10).

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

Applicants should provide statistically analysed data, from a representative number of generations (vegetative or generative propagation), to demonstrate the inheritance pattern and stability of the trait(s) introduced (including the expression of corresponding proteins under representative environmental conditions).

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

(a) Plant to bacteria gene transfer:

The transfer of genes from GM plants to bacteria is considered to be unlikely if no homologous sequences are present (Nielsen et al., 1997), However, due to homologous recombination, the risk of gene transfer and subsequent integration and expression may be enhanced by the presence of bacterial sequences within the GM plant insert DNA (Gebhard and Smalla, 1998) and thus this should be minimized. 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 7 for human and animal health and in section 9 for the environment.

(b) Plant to plant gene transfer:

The transfer of genes from GM plants to other sexually compatible plants is considered to be a naturally occurring process under certain circumstances (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. extended 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. The potential consequence arising from out-crossing should be assessed in section 9.3.

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

Genes inserted in a GM plant should be evaluated for their potential impact on human and animal health. This evaluation should include the potential (albeit a rare occurrence) for a plant to bacteria or plant to plant transfer event to take place. It should also take into account any capacity for enhanced gene transfer reported in section 6. Thus, on a case- by- case basis, specific experimental data on gene transfer and its consequences may be required.

7.1 Comparative assessment

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, tests for morphological, agronomical and chemical similarity use the most appropriate controls and do not simply rely on comparisons with original parental material. For example, non-GM parental 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 quality assessed 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 events are combined by the interbreeding of GM lines, the appropriate comparator will be the non-GM equivalent. Where this is not possible (e.g. in vegetatively propagated crops) the GM parent lines are appropriate comparators.

7.2 Field trials

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

(a) 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 normally 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.

(b) 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 presented separately, and 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).

(c) the baseline used for consideration of natural variations.

Quality assessed 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 applicant and/or compiled from the literature. The databases used for comparison should be specified. Special attention must 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 between the identified difference and the genetic modification process. Modifications that fall outside normal ranges of variation will require further evaluation to determine any biological significance.

7.3 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 of 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 (a minimum list) which may provide further guidance for compositional analysis to establish the extent of compositional equivalence (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 soil content of minerals might influence the content in plants. 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 affect, adversely, 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 important identified allergens should be studied. Compounds other than key nutrients, key toxicants, and important anti-nutrients and allergens may be included in the analyses on a case-by-case basis.

Knowledge of the introduced trait may trigger studies of specific compounds. 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.

7.4 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.

7.5 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.

7.6 Effect of the production and processing

For processed and/or preserved foods/feeds derived from GM sources the applicant should assess whether or not the technologies applied are likely to modify the characteristics of the GM product compared with its non GM counterpart. On a case –by-case basis experimental data may be required. 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 newly expressed protein(s) in the final product. Processing includes for example silage making, oilseed extraction, refining and fermentation.

7.7 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.

Information on known or anticipated human exposure to other sources of analogous GM food/feed and from other routes of exposure to new gene products and constituents, including amount, frequency and other factors influencing exposure, should be provided. On the basis of the available consumption data, the anticipated average and maximum intake of the GM food/feed 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. Information should 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. 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 taking into account the influences of processing, storage and expected treatment of the food/feed in question.

7.8 Toxicology

Toxicology studies evaluating 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. Should specific studies be required to address the efficacy, nutritional value or wholesomeness of GM feed, e.g. long term feeding trials on target species, the information gained could also be used for additional assurance of the safety of the GMO in the case of human consumption.

The requirements of toxicological testing in the safety assessment of food/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 differences identified between the GM product and its conventional counterpart, including available information on intended changes. Thus, the toxicological testing 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 new proteins expressed as result of the genetic modification, the potential presence of other new constituents and/or possible changes in the level of natural constituents beyond normal variation. These potential deviations from the conventional counterparts may require different toxicological approaches and varying degrees of testing.

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.

Those toxicological studies which are carried out should be conducted using internationally agreed protocols. Test methods described by the OECD (OECD b) or in the most up-to-date European Commission Directives on dangerous substances are recommended (EC, 2002d). 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, 2004) and accompanied by a statement of GLP-compliance.

7.8.1 Safety evaluation of newly expressed proteins

The studies required to investigate the 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 might not be required.

To demonstrate the safety of newly expressed proteins the following information is needed:

A molecular and biochemical characterisation of the newly expressed 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 newly expressed 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) and the methodology 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 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 characterized and evaluated with regard to the hazards linked to their biological activity.

In the case of newly expressed proteins with an insufficient data base and, in particular, if the available data suggest the existence of any cause for concern, specific toxicity studies should be carried out.

Repeated dose toxicity studies 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 28-day oral toxicity study with the newly expressed protein in rodents should be performed according to OECD guideline 407 (OECD, 1995). Depending on the outcome of the 28-days toxicity study, additional targeted investigations may be required including investigation of the 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.

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 is used which was produced by micro-organisms, the structural and functional equivalence of the 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.

7.8.2 Testing of new constituents other than proteins

Identified new constituents other than proteins should be evaluated according to the traditional toxicological approach 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, 2001) and Directive 2001/79/EC (EC, 2001b) is needed. 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.

7.8.3 Information on natural food and feed constituents

Natural food and feed 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. In case of constituents with a physiological or biochemical function (macro- and micro-nutrients), an integrated toxicological and nutritional evaluation is required (see section 7.10).

7.8.4 Testing of the whole GM food/feed

If the composition of the GM plant is modified substantially, or if there are any indications for the potential occurrence of unintended effects, based on the preceding molecular, compositional or phenotypic analysis, not only new constituents, but also the whole GM food/feed should be tested.

The testing programme should include at least a 90-day toxicity study in rodents. Special attention must be paid to the selection of doses and the avoidance of problems of nutritional imbalance. At least two dose levels of the GM and parental test food should be included in the diet. The highest dose level should be the maximum achievable without causing nutritional imbalance, whilst the lowest level should approximate the anticipated human intake. Stability of test diets and nutritional equivalence between control and test diets are other important aspects to consider (König et al., 2004).

To detect unintended effects it is recommended that comparative growth studies are conducted with young rapidly growing animal species such as the broiler chick. Because of their rapid weight gain, broilers are sensitive to the presence of certain 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. In a recent ILSI report (ILSI, 2004) the sensitivity of the model has, however, been questioned.

The choice of the control diet in testing whole GM food/feed or components derived from the GM crop that is compositionally different, should be based on the composition of the traditional food/feed of ingredient which is intended to be substituted. For instance in case of food/feed derived from GM crops that contain new or altered oils, oils should be used as controls which are intended to be replaced. In case of whole GM foods/feed or derived products that contain enhanced levels of specific biologically active compound(s), the test design should include the GM food/feed or derived product, the parent food/feed or derived product, and the GM test material spiked with the enhanced active compound at relatively high dose levels that would induce a biological effect. The latter control diet would be informative on whether specific matrix effects may be expected and on the sensitivity of the test system. 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 (see for more information SAFOTEST project[7]).

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.

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. This is also applicable to foods and feeds derived from GM plants obtained through traditional breeding of parental GM lines (combined events). 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 are requested in order to assess the impact of consumption on human and animal health.

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.

7.9 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. 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.

7.9.1 Assessment of allergenicity of the newly expressed protein

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

[7] http://www. entransfood.com

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

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

In every case the first step in the assessment should be a search for sequence homologies and/or structural similarities between the expressed protein and known allergens. 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 number of contiguous identical or chemically similar amino acid residues used in the search setting should be based on a scientifically justified rationale in order to minimise the potential for false negative or false positive results[8]. Using different homology searching strategies based on the sequences available in relevant databases may identify several scenarios. These include a high degree of homology, with or without conservation of the allergenicity, or a low degree of homology with conservation of allergenicity (Mills et al., 2003). To reduce the uncertainty of the conclusions that may be drawn from the search of sequence homology alone, efforts should be encouraged to improve the bioinformatic approach i) to improve and harmonize the algorithms that are used by the different applicants and ii) to develop data bases which include information on the three dimensional structure and function of known allergens and of proteins belonging to protein families which include a high proportion of allergens.

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

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 positive IgE responses 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.

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[8] It is recognized 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.

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 also essential 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. Several animal models are developed and their use should be encouraged in order to increase the body of evidence to support a conclusion.

7.9.2 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.

Development of modern analytical tools including profiling techniques (see section II, 5) is encouraged in association with human and animal serum or cell-based assays. These are certainly promising and efficient tools which could be used to detect new proteins or peptide fragments with allergenic potential in whole GM crops and in (processed) GM foods.

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.

7.10 Nutritional assessment of GM food/feed

7.10.1 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:

(a) nutrient composition (see compositional studies as described in section 2);

(b) biological efficacy of nutrient components in the foods;

(c) 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 extreme 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 biomolecules, 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 7.11).

7.10.2 Nutritional assessment of GM feed

Compositional analysis is the starting point and cornerstone for the nutritional assessment of feed material. Consensus documents prepared by OECD (OECD a) provide excellent guidance for the analyses needed and the analyses conducted should be determined on a case-by-case basis and may vary depending on the introduced trait. It should be noted that there are significant differences in composition of conventionally bred varieties and thus the compositional analysis of GM crops must be assessed

against the background of natural variability in the conventional counterpart(s). Attention is drawn to the crop composition database ILSI (2003b) as a key source for such data and to ILSI (2004), which addresses the issue of nutritional assessment of GM feeds.

Once compositional equivalence has been established in GM feeds modified for agronomic input traits, nutritional equivalence can be assumed (Clark and Ipharraguerre, 2001; Flachowsky and Aulrich, 2001), since routine long-term livestock feeding studies generally add little to a nutritional assessment. In the case of crops modified for agronomic input traits with combined events the need for long-term feeding studies should be assessed on a case-by-case basis.

In the case of GM crops with improved nutritional characteristics, livestock feeding studies with target species should be conducted on a case-by-case basis to study the nutritional benefits that might be expected and to provide further safety assurance. These 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 and should be conducted according to internationally agreed standard protocols (ILSI, 2003a). For feedstuffs intended only for aquaculture, growth studies with fish species such as carp or other typical herbivore fishes may be preferable to an extrapolation from results obtained with land-animals.

Studies of this type are, however, limited to those materials suitable for inclusion in the diets and which can be nutritionally matched to a suitable control diet.

When studies are conducted, the following guidelines are proposed:

(a) In the case of GM crops modified for improved bioavailability of nutrients, livestock feeding studies with target species should be conducted to determine the bioavailability of individual nutrients in the GM crop and a range of conventional varieties.

(b) 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. lysine), an appropriate control diet using its nearest genetic counterpart should be formulated by supplementing it with the specific nutrient to the extent of the change effected in the GM crop. It is also suggested that a number of other commercially relevant varieties should be included in the study.

(c) In the case of co-products (e.g. oilseeds meals) from which the modified ingredient has been extracted, these can be compared with those derived from an appropriate counterpart and other commercial varieties on the basis that they are essentially free from the modified component.

(d) In the case where nutritional components are to be deposited in food derived from animals receiving GM feed resources specific tests for content should be conducted.

7.11 Post-market monitoring of GM food/feed

Where appropriate a Post Market Monitoring (PMM) programme should be performed for GM food. 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 reliable and validated flow of information between the different stakeholders which may relate GM food consumption to any (adverse) effect on health.

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 predicted? and iii) does the product induce unexpected side effects? (Wal et al., 2003).

Given the practical difficulties in performing a PMM, it should be required only in specific cases where there is no traditional comparator. Those cases could include GM (functional) foods with altered nutritional composition and modified nutritional value and/or with specific health claims. This could be the case for a GM food proposed as an alternative or as a replacer to a traditional food. Because of its specific properties, the intake of this GM food might be increased compared to the intake of the traditional counterpart, which could result in a significant impact on the long term nutritional and health status of some individuals of the population.

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

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 tolerance) present in the modified plant. There should be a reference to sections B 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 9.4 below.

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

The evaluations 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 evaluation 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. This evaluation should include consideration of 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 6.

Possible interactions between the GM plant and its biotic environment include:

(a) effects on the population dynamics and genetic diversity of 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 any parts of it will remain in the environment after harvest, will depend on the specific plant, its management regime and agronomic practices.

9.1 Persistence and invasiveness

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 applicant should refer to GM plant specific traits (see section D1), 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.

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

9.3 Potential for gene transfer

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.

The potential consequence arising from out-crossing to other plant cultivars should be considered and assessed for environmental risk. This will vary with species. 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. 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.

The applicant should also refer to information provided in sections 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. If appropriate, an assessment of the potential impact of growing GM crops on wider biodiversity in the crop ecosystem requires the combination of several different approaches (ACRE, 2001b).

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).

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. If appropriate, an assessment of the potential impact of growing GM crops on wider biodiversity in the crop ecosystem requires the combination of several different approaches (ACRE, 2001b), as considered further in section 9.5.

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.

Impact should be assessed on non-target species 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). If appropriate, an assessment of the potential impact of growing GM crops on wider biodiversity in the crop ecosystem requires the combination of several different approaches (ACRE, 2001b).

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).

The applicant should refer to section 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 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 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, 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.

If appropriate, an assessment of the potential impact of growing GM crops on wider biodiversity in the crop ecosystem requires the combination of several different approaches (ACRE, 2001b). The applicant should describe the appropriate 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.

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 Evaluations 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.

10. Potential interactions with the abiotic environment

The evaluations 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 evaluation 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 or tolerance to climatic conditions (e.g. cold, heat, humidity),

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

(d) altered sensitivity or tolerance to gases (e.g. CO_2, oxygen, NH_4),

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

11. Environmental Monitoring Plan

11.1 General

Directive 2001/18/EC introduces an obligation for applicants to implement GMO monitoring plans 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. Monitoring as detailed in Articles 13, 19 and 20 of Directive 2001/18/EC and in this GM Food and Feed Guidance Document refers to environmental monitoring, which takes place after the granting of a consent to place a GMO on the market. Article 13(2)(e) of the Directive requires applicants to submit a monitoring plan as part of their notifications. The structure and content of this environmental monitoring plan should be in accordance with the guidelines of Annex VII, Directive 2001/18/EC (strategy, method, analysis, reporting; Wilhelm et al. 2003).

An environmental monitoring plan is required for applications where the natural or cultivated environment will be exposed to GM plant propagules or GM plant products. 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 may, however, be important in the development of further research.

The environmental monitoring of the GM plant will have two focuses: (1) the possible effects of the GM plant, identified in the formal risk assessment procedure, and (2) unforeseen effects. 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 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. A published Guidance Note (2002/811/EG) (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.

The ultimate goal of the environmental monitoring plan should be to determine whether the data collected during case specific monitoring and General Surveillance identify specific effects due to commercialisation of the GM plant which alter the balance between the advantages of the introduction and any negative consequences, in both managed and natural environments, compared with current farming practices or other alternatives.

11.2 Case-specific GM plant monitoring

Guidance Note 2002/811/EC provides no clear differentiation between the monitoring principles of either case-specific monitoring or general surveillance (Den Nijs and Bartsch 2004). The main objective of case-specific monitoring is to determine the significance of any adverse effects identified in the risk assessment (see chapters 8, 9 and 10). 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. Such monitoring can be carried out at a limited number of sites, where exposure is greatest and intensive recording and data collection can take place. This specific and intensive, scientific measurement and data collection may often have an experimental approach. The monitoring program design further depends on levels of exposure in different geographical regions and other specific management influences. The evaluation of risk should be based on Annex II of the Directive (2001/18/EC).

11.3 General Surveillance of the impact of the GM plant

The objective of General Surveillance is to identify unforeseen adverse effects of the GM plant or its use, on human health and the environment, that were not predicted in the risk assessment. General Surveillance should be less experimental than case-specific

monitoring, largely based on routine observations and should be conducted over a wider range of sites and environments with a range of parameters observed at a low intensity. If unusual observations are reported, more focussed in-depth studies can be carried out. Existing surveillance systems should be used where practical e.g. routine farm recording systems, and any "abnormal" effects not usually occurring in similar situations with conventional cropping should be recorded. However, direct comparison with non-GM crop reference areas is not always necessary. Reference can be made to the historical knowledge and experiences of the "observer" (e.g. farmers, inspectors, botanical surveyors) in relation to the situation prior to the introduction of the GM plant.

General Surveillance should not be a substitute for general environmental monitoring by Member States. The higher the ecological integration and scale (from the individual to a population, from single farms to regions) the more difficult it is to distinguish potential effects of GM plant from other factors. Initially, General Surveillance should focus on each transgenic plant and type individually. Ultimately, when several GM plants have been commercialised, the interactions between these GM plants and their management regimes should be examined. Environmental monitoring at a regional level may be considered primarily to be a governmental task and additional to the monitoring requirements for the applicant following placing on the market. In the Directive 2001/18/EC, the possibility of additional surveillance by government authorities is described in Item 44 of the Conciliation Committee. The applicant should be aware of all relevant surveys and monitoring in areas where the GM plants will be grown and should refer to the results of this monitoring in reports to the Competent Authority and the Commission.

11.4 Parameters to be used in a monitoring plan

(a) **Background 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 to permit the assessment of the relevant parameters listed under b):

(b) **GM plant-based parameters depending on the specific combination of GMO/trait/environment:**

General surveillance methods will depend on the particular GM plant, trait and environment combination. The key parameters to be observed should include 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.

11.5 Implementing General Surveillance

<u>Existing surveillance systems</u>

In conjunction with the exploitation plan for the GM plant, the applicant should define the infrastructures that will be established and exploited in order to conduct General Surveillance of regions where the GM plant is grown. The applicant should identify existing surveillance systems which are already monitoring one or more of the relevant

parameters/elements. This should include arrangements for collecting and collating data.

The applicant should also identify which additional surveys will be asked to contribute to the General Surveillance (for example, public institutions, cultivation associations) in Member States. Although detailed arrangements may not have been agreed at the time of the application, the applicant should agree the formal procedures with the Commission and Member States before commercial market introduction. For example, when the GM cultivar is registered in the EU variety catalogue.

Involving Farmers/Growers of GM crops and suppliers of GM crop seeds

Applicants can obtain useful information directly from growers and seed suppliers of GM crops and should involve them in supplying data on seed sales, areas sown, crop management etc. Applicants should also be pro-active in developing reporting systems so that farmers intending to purchase genetically modified seeds will be involved in reporting unusual occurrences during and after the cultivation of the GM crop. The applicant should describe the number of farmers/growers involved, the reporting methods and the suitability of the data collected for statistical analysis.

Local Surveillance

Applicants may consider that an intensive local surveillance is more appropriate than a more extensive General Surveillance described above. 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. This consists of the systematic recording of a larger number of relevant parameters at a limited number of 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. A “split field design" (adjacent/parallel cultivation of GM and non-GM crop varieties under similar management conditions) may be appropriate for local surveillance during the early years of marketing release. However, the lack of availability of non-transgenic, isogenic varieties and the lack of statistical power due to the small number of locations may reduce the sensitivity of these experiments. The methods selected, the duration of the monitoring and the extent or number of areas, will be determined by the specific case and the parameters to be monitored.

Whilst the planning and conduct of local surveillance will be the applicant's responsibility, it will be acceptable if the applicant involves public institutions in carrying out some or all of the agreed work.

11.6 Reporting the results of monitoring

Following the placing on the market of a GMO, the applicant under Article 20(1) of the Directive 2001/18/EC, 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 and the competent authorities of the Member States. Information should also be made publicly available in

line with the requirements of Article 20(4) of the Directive. Applicants should describe the methods, frequency and timing of reporting in their monitoring plan.

Although no time frame for reporting is specified in the Guidance Note (2002/811/EC), reports should be submitted annually and summary reports covering longer periods in which observations and data are collected should be submitted at appropriate intervals during the monitoring period.

The monitoring report should include 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.

<u>Flow of information on the cultivation of GM plants:</u>

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 Dir. 2001/18/EG - 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.

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

If 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 agreed at the time of purchase of the GM seeds and provide information to the seed supplier/Consent Holder of any unusual observations without delay.

(b) The applicant should notify any relevant information immediately to the Member State Competent Authority and to both the Commission and EFSA.

(c) If unusual effects are detected by external organisations (e.g. public institutions), these must be communicated to the Consent Holder/ Seed supplier, the Member State Competent Authority and to the Commission and EFSA immediately.

(d) The Consent Holder/Seed supplier must carry out a preliminary examination of the report in order to verify whether a GM plant-related effect has really occurred and within a defined period (e.g. one month or dependent on the event) and should

provide the Competent Authority with a report on the result of its preliminary investigations, including an assessment of potential harm.

(e) Either directly upon receipt of the information or at the latest upon receipt of the Consent Holder's report, the Competent Authority should decide whether further authority action is required. If further action is required the Competent Authority should inform the Commission of the reported observation and, together with the applicant and professionally competent institutions or experts, should investigate the causes and consequences of the reported incident. The Competent Authority should submit a full report to the Commission and EFSA to include the extent of any environmental damage, remedial measures taken, liability and recommendations for the future use/management of the GM plant.

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Annex I

EFSA GUIDANCE TO APPLICANTS ON THE PRESENTATION OF APPLICATIONS FOR THE REQUEST OF AUTHORISATION OF A GENETICALLY MODIFIED ORGANISM AND/OR DERIVED PRODUCTS

April 2004

Introduction

This annex provides guidance on the presentation of applications for the placing on the market of genetically modified organisms (GMOs) and/or derived products introduced under Community legislation (on *genetically modified (GM) food and feed*[9] and on *the deliberate release into the environment of GMOs*[10]) 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 a GMO and/or derived product

1. An application for the authorisation of a GMO and/or derived product submitted within the framework of Regulation (EC) 1829/2003 should preferably be presented in English and should consist of the following elements as specified by Articles 5 and 17 of that Regulation:

   1.1. The technical dossier should be compiled according to the legislative requirements and according to the format proposed in the EFSA guidance document on GM plants and derived food and feed and as specified in Annex III. Applications submitted within the framework of Directive 2001/18/EC, and for which the GMO Panel has to be consulted according to Article 28 of the Directive, should also be compiled according to this EFSA guidance document.

   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.

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

[10] 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

To facilitate easy access of information in dossiers, information should be presented to conform with the format proposed in this document and 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. Note that summary data is not sufficient. 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.

Not all the points included in the guidance document will apply to every case. 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.

1.2. 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.

1.3. 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").

1.4. Either an analysis, supported by appropriate information and data, showing that the characteristics of the food or feed are not different from those of its conventional counterpart, having regard to the accepted limits of natural variations for such characteristics and to the criteria specified in Article 13(2)(a) of Regulation (EC) 1829/2003, or a proposal for labelling the food in accordance with Article 13(2)(a) and (3) of Regulation (EC) 1829/2003;

1.5. Either a reasoned statement that the food or feed does not give rise to ethical or religious concerns, or a proposal for labelling it in accordance with Article 13(2)(b) of Regulation (EC) 1829/2003;

1.6. 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;

1.7. 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 in accordance with the implementing rules[11] of Regulation (EC) No. 1829/2003;

1.8. Samples of the food or feed and their control samples, and information as to the place where the reference material can be accessed shall be made available to the Community Reference Laboratory (CRL) as specified in Article 32 of Regulation (EC) 1829/2003. A proof that the methods for detection and sampling and the samples of the food and control samples were sent to the CRL should be provided;

1.9. Where appropriate, a proposal for post-market monitoring regarding use of the food for human consumption or the feed for animal consumption;

2. 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 in respect of which an application is made.

3. In the case of GMOs or food or feed containing or consisting of GMOs, the application shall fulfil the requirements of Directive 2001/18/EC. 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.

4. 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.

5. Where applications submitted in a Member State under other Community legislation[12] are transformed into an application under Article 46 of Regulation (EC) 1829/2003, the original application shall be updated and revised according to the requirements of Regulation (EC) 1829/2003 and to the EFSA guidance on GM plants and derived food and feed. As the case may be, the initial assessment report

[11] Commission Regulation 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 or technically unavoidable presence of genetically modified material which has benefited from a favourable risk evaluation (adopted on 25 February 2004, to be published in the Official Journal).

[12] 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.

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.

Practical specifications

Four paper copies and 25 copies in electronic format (CD-ROM) of the technical dossier 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
European Food Safety Authority
Provisional address:
Rue de Genève 10 (office G-10, 6/36)
B-1140 Brussels (Evere)
Belgium

The electronic version of the application should be certified as being identical to the one on paper. 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.

Confidential business information should be clearly indicated and separated for the other parts of the application. Confidential business information could for instance be provided on a separate password protected CD-ROM (for which also 25 copies have to provided).

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.

Annex II

SCOPE OF THE APPLICATION

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

1 Food

1.1 GM plants for all uses in food, including the use of the GM plant for cultivation, import and processing

1.2 GM plants for all uses in food, including for import and processing only

1.3 Food containing or consisting of GM plants

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

2 Feed

2.1 GM plants for all uses in feed, including the use of the GMO for cultivation, import and processing

2.2 GM plants for all uses in feed, including for import and processing only

2.3 Feed containing or consisting of GM plants

2.4 Feed produced from GM plants

3 GM plants for environmental release

3.1 Import and processing

3.2 Cultivation in Europe

Annex III

FORMAT OF TECHNICAL DOSSIERS

INFORMATION REQUIRED IN APPLICATIONS CONCERNING RELEASES OF GENETICALLY MODIFIED PLANTS (GYMNOSPERMAE AND ANGIOSPERMAE)

A. GENERAL INFORMATION

1. Name and address of the applicant (company or institute)
2. Name, qualification and experience of the responsible scientist(s)
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

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

1. Complete name; (a) family name, (b) genus, (c) species, (d) subspecies, (e) cultivar/breeding line, (f) common name
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 and 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. Size, source (name) of donor organism(s) 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. Information on how the GM plant differs from the recipient plant in: reproduction, dissemination, survivability

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

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

   (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

7.2 Field trials

   (c) number of locations, growing seasons, geographical spreading and replicates

   (d) statistical models for analysis, confidence intervals

   (e) the baseline used for consideration of natural variations

7.3 Selection of compounds for analysis

7.4 Agronomic traits

7.5 Product Specification

7.6 Effect of the production and processing

7.7 Anticipated intake/extent of use

7.8 Toxicology

   7.8.1 Safety evaluation 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

   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

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

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

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

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

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

11. Environmental Monitoring Plan

11.1 General

11.2 Case-specific GM plant monitoring

11.3 General Surveillance of the impact of the GM plant

11.4 Parameters to be used in a monitoring plan

11.5 Implementing General Surveillance

11.6 Reporting the results of monitoring

Annex IV

FORMAT[13] 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) 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))

3. Scope of the application

[13] 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.

☐ Cultivation (Part C of Directive 2001/18/EC)

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

☐ Use as food/food ingredient (Regulation 1829/2003)

☐ Use as feed/feed material (Regulation 1829/2003)

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) 258/97?

Yes ☐	No ☐
If *yes*, specify	

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

Yes ☐	No ☐
If *yes*, specify	

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) Any proposed labelling requirements in addition to those required by Community law (Annex IV of Directive 2001/18/EC; Regulation 1829/2003 art. 13 and 25)
g) Unique identifier for the GM plant (Regulation (EC) 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

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

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

1. Complete name

a) Family name
b) Genus
c) Species
d) Subspecies
e) Cultivar/breeding line
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

4. Dissemination

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

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

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

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 the vector used**

3. **Size, source (name) of donor organism(s) 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 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

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

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

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

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

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

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

Choice of the comparator

7.2 Field trials

a) number of locations, growing seasons, geographical spreading and replicates
b) the baseline used for consideration of natural variations

7.3 Selection of compounds for analysis

7.4 Agronomic traits

1 **7.5 Product specification**

2 **7.6 Effect of the production and processing**

3 **7.7 Anticipated intake/extent of use**

4 **7.8 Toxicology**

7.8.1 Safety evaluation 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

5 **7.9 Allergenicity**

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

6 **7.10 Nutritional assessment of GM food/feed**

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

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

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

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
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

11. Environmental monitoring plan (not if application concerns only food and feed produced from GM plants, or containing ingredients produced from GM plants)

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

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

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)

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

a) Release country
b) Authority overseeing the release
c) Release site
d) Aim of the release
e) Duration of the release
f) Aim of post-releases monitoring
g) Duration of post-releases monitoring
h) Conclusions of post-release monitoring
i) Results of the release in respect to any risk to human health and the environment

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5 **3. Links (some of these links may be accessible only to the competent authorities**
6 **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
d) Commission Register (Commission Decision 2004/204/EC[14])
e) Molecular Register of the Community Reference Laboratory/Joint Research Centre
f) Biosafety Clearing-House (Council Decision 2002/628/EC[15])
g) Summary Notification Information Format (SNIF) (Council Decision 2002/812/EC)

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[14] 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.

[15] 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.