SCIENTIFIC OPINION

Guidance on the risk assessment of genetically modified microorganisms and their food and feed products

EFSA Genetically Modified Organisms (GMO)²,³

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ABSTRACT

The EFSA Panel on Genetically Modified Organisms (EFSA GMO Panel) published its first Guidance Document for the risk assessment of genetically modified microorganisms and their derived products intended for food and feed use in October 2006. Since then, the EFSA GMO Panel has gained significant experience in assessing applications for marketing food and feed involving genetically modified microorganisms within the EU, which has enabled it to identify areas of the Guidance that needed clarification and issues that should be covered more in depth. In addition, the Panel has received and acknowledged input from different stakeholders, and considered recent developments in relevant European legislation. This revised document provides updated guidance for the preparation and presentation of applications for science-based risk assessment. It describes the steps to be taken and the issues to be considered when carrying out a comprehensive risk characterisation.

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KEY WORDS


1 On request from EFSA, Question No EFSA-Q-2009-521, adopted on DD Month YYYY.
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SUMMARY

The European Food Safety Authority (EFSA) asked the EFSA Panel on Genetically Modified Organisms (EFSA GMO Panel) to establish a self-tasking Working Group with the aim of updating the Guidance Document for the risk assessment of genetically modified microorganisms and their derived products intended for food and feed use. The aim of the Working Group was to provide updated guidance for the preparation and presentation of applications involving genetically modified microorganisms (GMMs).

To complete its mandate, the self-tasking Working Group: i) reviewed the existing Guidance Document, and identified areas of improvement, ii) considered input received from the Member States and applicants on the existing Guidance Document and iii) discussed and developed improvements for those areas.

The EFSA GMO Panel published its first Guidance Document for the risk assessment of genetically modified microorganisms and their derived products intended for food and feed use in October 2006. This revised document was adopted on (XX-XX-XXXY). The EFSA and the EFSA GMO Panel published the Guidance on the EFSA web site for public consultation prior to the final adoption of this document.

Updated guidance for the preparation of applications is given throughout the different chapters of the guidance document. Chapter I clarifies the scope of the document and the regulatory background for the risk assessment of GMMs and their food and feed products at the Community level. Chapter II describes the overall risk assessment strategy. Chapter III describes the steps to be taken and the issues to be considered when carrying out a comprehensive risk characterisation. These issues include general information, information relating to the recipient/parental, the donor(s) of the genetic material, the genetic modification and the final GMM, as well as information relating to the food and/or feed containing, consisting of, produced from and produced with GMMs. It also includes information on modification of the genetic traits or phenotypic characteristics of the GMM and evaluation of food and feed safety aspects of the GMM and derived products. Data on composition, toxicity, allergenicity, nutritional value and environmental impact provide, on a case-by-case basis, the cornerstones of the risk assessment process. The characterisation of risk may give rise to the need for further specific activities including post-market monitoring of the GMM and derived food and feed and/or for the environmental monitoring of the GMM. A table (Table 1) summarising the risk assessment requirements for the different categories of GMMs and their products is also provided.

4 Regulation (EC) No 1829/2003 defines food and feed ‘produced from GMOs’ as derived, in whole or in part, from GMOs, but not containing or consisting of GMOs. “Containing or consisting of GMMs” is understood as products in which GMMs capable of replication or of transferring genetic material are present. “Produced with GMMs” is understood as produced by fermentation using a GMM which is kept under contained conditions and is not present in the final product. Such products are not included in the scope of Regulation (EC) No 1829/2003 (EC, 2004).

5 According to Regulation (EC) No 1829/2003 “Derived (food and/or feed) products” is understood as products containing, consisting of or produced from GMMs, but not produced with GMMs.
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BACKGROUND AS PROVIDED BY THE EFSA GMO PANEL

In accordance with Articles 5(8) and 17(8) of the Regulation (EC) No 1829/2003 on genetically modified food and feed, EFSA shall publish detailed guidance to assist the applicant in the preparation and presentation of applications for the authorisation of genetically modified food and/or feed. Against this background, the Commission requested EFSA, in a letter dated 1 February 2005, to provide guidance on the scientific information necessary for the risk assessment of food and feed produced using genetically modified microorganisms (GMMs; Ref. SANCO/D4/KN/cw/D/440010 (2005)).

On 17 May 2006, the EFSA GMO Panel adopted a Guidance Document for the risk assessment of GMMs and their derived products intended for food and feed use (EFSA, 2006) that was published in October, 2006. The Guidance covers GMMs for food and feed use, food and feed containing or consisting of GMMs, food and feed produced from or containing food ingredients or feed materials produced from GMMs as well as substances such as additives, vitamins and flavourings produced by the GMMs. The EFSA GMO Panel will regularly review this guidance in the light of experience gained, technological progress and scientific developments.

Applicants wishing to pursue the marketing of GMMs under the scope of Regulation (EC) No 1829/2003 are advised to prepare and present their applications according to this GMM Guidance Document. In addition, the EFSA GMO Panel follows this Guidance Document for the assessment of applications for GMMs and their products for food and/or feed use, irrespective of whether or not they fall in the scope of Regulation (EC) No 1829/2003.

To date, a number of applications under Regulation (EC) No 1829/2003, as well as under other Regulations, all of them involving GMMs, have been assessed or are currently under assessment by the EFSA GMO Panel according to the GMM Guidance Document. During this period (October 2006 – today), the EFSA GMO Panel has gained significant experience, which has enabled it to identify areas of the Document that need to be subjected to clarification and issues that should be covered more in depth. In addition, the Panel has received and acknowledged input from different stakeholders (including applicants and Member States) on possible improvements of the present Guidance Document.

TERMS OF REFERENCE AS PROVIDED BY THE EFSA GMO PANEL

In the framework of this mandate, the EFSA GMO Panel proposed a self-tasking activity to update the Guidance Document for the Risk Assessment of genetically modified microorganisms and their derived products intended for food and feed use. In particular, the GMO Panel proposed:

1. to update its existing Guidance Document for the risk assessment of genetically modified microorganisms and their derived products intended for food and feed use (EFSA, 2006) with precise guidelines on the risk assessment of genetically modified microorganisms and their derived food and feed;
2. to consult applicants via EFSA on the draft Guidance;
3. to complete a public consultation of the draft Guidance;
4. to review the draft Guidance taking into account the results of the consultations.

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I. INTRODUCTION

SCOPE OF THE DOCUMENT

This document provides guidance for the scientific risk assessment of GMMs and food and/or feed containing, consisting of, produced from and produced with GMMs. In addition, it provides detailed guidance to assist in the preparation and presentation of applications to market GMMs and their products for food and/or feed use, according to Regulation (EC) No 1829/2003.

For the purpose of this guidance document, the GMMs covered include archaea, bacteria and eukarya. Eukarya includes filamentous fungi, yeasts, protozoa and microalgae. This document does not cover the use of tissue cultures of plant or animal cells, or viruses or viroids; nor does it cover issues related to risk management (traceability, labelling, etc.). Socioeconomic and ethical issues are also outside the scope of this guidance.

“Synthetic Biology” is an emerging interdisciplinary field of research which combines biology, molecular biology, chemistry, biotechnology, information technology, and engineering sciences. The objective of Synthetic Biology is to modify biological systems, for example minimal cells with a downsized genome, also including chemically synthesized components, typically synthetic DNA, for production of new living entities. At present it is mainly focused on fundamental research but the potential to utilize this technology for the development of microorganisms with importance in the food and feed areas is enormous. Guidance on the risk assessment of microorganisms generated by Synthetic Biology requires a separate document which takes into account the unique features of such organisms.

LEGAL BACKGROUND

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

Guidance documents or guidelines available for the safety assessment of specific GMM-derived products are referred to in Chapter II, Section. B.2.

General food law (Regulation (EC) No 178/2002)
Guidance on the risk assessment of GMMs and their food and feed products

Regulation (EC) No 178/2002\(^{12}\) lays down the general principles of food law and procedures in food safety including the tasks of EFSA. It establishes the principles of risk analysis and general food and feed safety requirements. The Regulation defines food law broadly, covering a wide range of provisions with a direct or indirect effect on the safety of food and feed, including provisions on animal feed and other agricultural inputs at the level of primary production. In the general food law ‘food’ means any substance or product, whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be ingested by humans. ‘Food’ includes any substance intentionally incorporated into the food during its manufacture, preparation or treatment. ‘Feed’ means any substance or product, including additives, whether processed, partially processed or unprocessed, intended to be used for oral feeding to animals. The general food law defines ‘hazard’, ‘risk’, ‘risk analysis’, ‘risk assessment’, ‘risk management’ and ‘risk communication’. Articles 14 and 15 of the general food law set the food and feed safety requirements, respectively, in order to determine whether any food or feed is injurious to health.

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

According to Regulation (EC) No 1829/2003, GM food and feed should only be authorised for placing on the market after a scientific assessment of any risks which they might present for human and animal health and for the environment. GM food and feed mean GMOs for food/feed use; food/feed containing or consisting of GMOs; food(feed) produced from GMOs; and food containing ingredients produced from GMOs.

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

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

The principles regulating the deliberate release\(^{13}\) into the environment of GMOs are laid down in Council Directive 2001/18/EC. 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 can be released into the environment, or placed on the market as, or in, products. The step-by-step principle means that the containment of GMOs is reduced and the scale of release increased gradually, but only if assessment of the earlier steps indicates that the next step can be taken.

Part B of the Directive deals with the deliberate release of GMOs for any other purpose than for placing on the market. For these releases, a notification must be submitted to the competent authority of the Member State within whose territory the release is to take place. The applicant may proceed with the release only when he has received a written consent of the competent authority.

Part C of the Directive deals with the placing on the market, i.e. making available to third parties, of GMOs as, or in, products. The applicant must submit an application to the competent authority of the


\(^{13}\) ‘Deliberate release’ means any intentional introduction into the environment of a GMO or a combination of GMOs for which no specific containment measures are used to limit their contact with and to provide a high level of safety for the general population and the environment; ‘organism’ means any biological entity capable of replication or of transferring genetic material (Article 2)
Member State where the GMO is to be placed on the market for the first time. The application must include a risk assessment. Annex IIIA of the Directive details the required information on which to base the risk assessment for organisms other than higher plants, e.g. GMMs. The principles for the environmental risk assessment, including aspects of human and animal health, are laid down in Annex II of the Directive. Several supporting documents have been prepared to assist the applicant. Commission Decision 2002/623/EC\(^\text{14}\) 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\(^\text{15}\) establishes guidance notes supplementing Annex VII to the Directive, describing the objectives and general principles to be followed to design the monitoring plan. Council Decision 2002/812/EC\(^\text{16}\) establishes the summary information format. EFSA published on October 2006 the ‘Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified microorganisms and their derived products intended for food and feed use’ (EFSA, 2006). The present guidance document is an updated replacement of that guidance.

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

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


The regulation and risk assessment of plant protection products fall within the scope of Directive 91/414/EEC\(^\text{17}\), to be replaced as of 14 June 2011 by Regulation (EC) No 1107/2009\(^\text{18}\). In case a micro-organism submitted to be authorised as plant protection product has been genetically modified, the dossier presented by the notifier in order to commence the assessment for the authorization of the product shall include the consent to the deliberate release into the environment in accordance with Directive 2001/18 and a copy of the environmental risk assessment carried out by the competent authority in accordance with that Directive. Therefore, a plant protection product which contains a GMM needs also to be assessed within the scope of Directive 2001/18/EC and shall be examined in accordance with that Directive, in addition to the assessment under Regulation (EC) No 1107/2009.

For the assessment to be performed in compliance with Directive 2001/18/EC the requirements and principles set in this guidance document apply. In the application submitted under Directive 2001/18/EC, it is advisable to include relevant information according to the data requirements laid down by Regulation (EC) No 1107/2009 in order to allow risk assessors to perform a comprehensive assessments of the risks linked to the expected use of that GMM.


Similar principles are also applicable to biocidal products. The regulation and risk assessment of biocidal products fall within the scope of Directive 98/8/EC. In case a micro-organism submitted to be authorised as biocidal product has been genetically modified, a copy of the evaluation of the data concerning the assessment of the risks to the environment as established in Directive 2001/18/EC shall also be included by the applicant in the dossier submitted in the context of Directive 98/8/EC. For the assessment to be performed in compliance with Directive 2001/18, the requirements and principles set in this guidance document apply. In the application submitted under Directive 2001/18/EC, it is advisable to include relevant information according to the data requirements laid down by Directive 98/8/EC in order to allow risk assessors to perform a comprehensive assessment of the risks linked to the expected use of that GMM.

**Contained use of GMMs (Directive 2009/41/EC)**

The contained use of genetically modified microorganisms is regulated by Directive 2009/41/EC. Annex III describes in general terms the principles to be followed to perform the assessment of the contained uses as regards the risks to human health and the environment. Commission Decision 2000/608/EC provides technical guidance notes in order to facilitate the implementation and explanation of Annex III. GMMs kept under contained use do not fall under the scope of Regulation (EC) No 1829/2003 and Directive 2001/18/ECC. When GMMs, including those developed by self-cloning, and their derived food or feed are released, they fall under the scope of Regulation (EC) No 1829/2003 and Directive 2001/18/ECC and therefore are subject to this guidance.

**Food improvement package**

A common authorisation procedure is established for food enzymes, food additives and food flavourings by Regulation (EC) No 1331/2008. In addition, harmonised criteria and requirements concerning the assessment and authorisation of these products are laid down by Regulations (EC) 1332/2008, 1333/2008 and 1334/2008, respectively. A food additive, a food enzyme, or flavouring or a source material which falls within the scope of Regulation (EC) No 1829/2003 should also be authorised in accordance with that Regulation. This document provides the guidance for the preparation of an application under Regulation (EC) No 1829/2003.

**Additives for use in animal nutrition (Regulation (EC) No 1831/2003)**

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20 'Contained use' means any activity in which microorganisms are genetically modified or in which such GMMs are cultured, stored, transported, destroyed, disposed of or used in any other way, and for which specific containment measures are used to limit their contact with, and to provide a high level of safety for, the general population and the environment (Article 2).


Placing on the market of feed additives is authorised under Regulation (EC) No 1831/2003 on additives for use in animal nutrition\textsuperscript{26}. In addition, feed additives containing, consisting of, or produced from GMOs also fall within the scope of Regulation (EC) No 1829/2003 and have to be subject to an application under this Regulation.

II. PRINCIPLES AND STRATEGIES FOR RISK ASSESSMENT OF GENETICALLY MODIFIED MICROORGANISMS


DEFINITIONS IN RISK ANALYSIS

For the purposes of this guidance document, the definitions set out in Articles 2 and 3 of Regulation (EC) No 178/2002 of the European Parliament and the Council apply.

Identification, characterisation and handling of risk(s) should follow a structured approach. This risk analysis process consists of three interconnected elements: risk assessment, risk management and risk communication.

Risk assessment means a scientifically based process consisting of four steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation. In this process, which includes the identification of the attendant uncertainties, 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) is evaluated. The terms hazard and risk are often used interchangeably, but have different meanings. The term hazard means a biological, chemical or physical agent in, or conditions of, food or feed with the potential to cause an adverse health effect. It refers to an inherent property of that agent or condition. Risk means a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard.

Risk management means the process, distinct from risk assessment, of weighing policy alternatives in consultation with interested parties, considering risk assessment and other legitimate factors, and, if need be, selecting appropriate prevention and control options. The control options include, where appropriate, monitoring/surveillance activities.

Risk communication means the interactive exchange of information and opinions throughout the risk analysis process as regards hazards and risks, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, feed and food businesses, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

Risk assessment is a scientific exercise. An extensive overview of risk assessment procedures is provided by the Scientific Committee of EFSA (EFSA, 2009a) and for environmental risk assessment by the EU (EC, 2000). The information required to structure the risk assessment process of GMMs and GMM derived products is further detailed in Chapter III (Sections B-D) of this document. The risk assessment involves generating, collecting and assessing information on a GMM in order to determine its potential impact on human and/or animal health and the environment compared to the non-modified organism from which it is derived. To carry out the risk assessment, sufficient scientific and technical data must be available to arrive at qualitative and/or quantitative risk estimates.

CATEGORISATION OF THE GMMs AND DERIVED PRODUCTS FOR RISK ASSESSMENT PURPOSES

Due to the diversity of GMMs and their products covered in this guidance, the following categorisation is recommended to optimise the risk assessment. GMMs and their products intended for human and animal consumption range from a single compound used in food or feed through to pure cultures of viable GMMs. Amino acids or vitamins that have been purified by crystallisation would represent examples at one end of this spectrum and cultures of probiotic microorganisms or dairy starters at the other extreme. Four product categories of GMMs or their food and feed products are
distinguished in the light of the level of scrutiny required for risk assessment (see Figure 1 and Table 1 for different requirements):

**Category 1:** Chemically defined purified compounds and their mixtures produced with GMMs in which both GMMs and newly introduced genes have been removed. (e.g. amino acids, vitamins);

**Category 2:** Complex products produced
- with GMMs in which both GMMs and newly introduced genes have been removed (e.g. most enzyme preparations); or
- from GMMs in which both GMMs and newly introduced genes are no longer present (e.g. cell extracts);

**Category 3:** Products produced from GMMs in which GMMs capable of replication or of transferring newly introduced genes are not present; but in which newly introduced genes are still present (e.g. heat-inactivated starter cultures);

**Category 4:** Products consisting of or containing GMMs capable of replication or of transferring newly introduced genes (e.g. live starter cultures).

Depending of the Category and scope of the product, its characterisation (specification) and safety assessment will be undertaken according to relevant legislation (See Chapter I) for which different Guidance Documents or Guidelines apply (See page 23, Section 2).

**COMPARATIVE APPROACH**

The choice of comparator is a key consideration in the evaluation of the GMM and its products, and its use should be justified. Most GMMs developed for food or feed purposes belong to well-characterised microbial species with a history of safe use in food or feed, and the traits introduced are well characterised. The concept "Qualified Presumption of Safety" (QPS) has been introduced as a generic approach to the safety assessment of microorganisms used in food and feed and for the production of food or feed additives, among other products (EFSA, 2007). Provided that the taxonomic status of the microorganism is unequivocally established and a sufficient body of knowledge exists on its apparent harmlessness to humans, animals and environment, further safety assessment of the microorganism is either not necessary or will be limited to evidence that certain qualifications are met (e.g. lack of transmissible antimicrobial27 resistance or production of toxins). The list of QPS organisms is reviewed annually and updated (EFSA Panel on Biological Hazards (BIOHAZ), 2009). In the context of this Guidance Document, the QPS status can be used as a justification for the safety of the parental microorganism. If the parental has a QPS status, the risk assessment can focus on the changes introduced during the development of the GMM.

Even though the recipient/parental microorganism may not have the QPS status, previous knowledge helps to structure the risk assessment process. If components critical for safety have been identified in the recipient/parental, the presence or absence of such components in the GMM or its product (e.g. endogenous toxins, secondary metabolites) should be tested and, if present, their implications for humans, animals and the environment assessed.

The recipient may be derived from a microorganism for which safety has been assessed during previous modification steps. To make use of those safety assessments, it is necessary to clearly

27 Antibiotics are substances produced by or derived from a microorganism and which selectively destroy or inhibit the growth of other microorganisms. In contrast, antimicrobials are active substances of synthetic or natural origin which destroy microorganisms, suppress their growth or their ability to reproduce in animals or humans. As GMMs may contain genes coding for resistance to synthetic substances with inhibitory properties as well as to naturally-occurring inhibitory substances, the term ‘antimicrobials’ is used throughout this document.
indicate the relationship and differences between the recipient and the ancestral strains assessed for safety. The safety assessment of the GMM may then focus on those differences, together with those introduced during the development of the final GMM.

### INTENDED AND UNINTENDED EFFECTS

**Intended effects** are those genotypic and phenotypic changes that are targeted to occur due to the genetic modification, and that fulfil the objectives of the genetic modification.

**Unintended effects** are other consistent phenotypic changes in the GMM resulting from its genetic modification. Unintended effects could be due to genetic rearrangements, to changes in the expression of particular genes in the recipient genome, metabolic perturbations and pleiotropic effects. Such unintended effects may also result from the synthesis of new fusion proteins. Some unintended effects might be predicted or explained in terms of current knowledge of biology and of the integration of metabolic pathways.

Predicted intended and unintended effects should be analysed based on the most appropriate methodology. Unintended effects, including those which could not be predicted, are addressed in the safety and nutritional assessment of the product.
III. INFORMATION REQUIRED IN APPLICATIONS FOR GMMs AND/OR THEIR PRODUCTS

The structure of this Chapter III is based on Annex III A of Directive 2001/18/EC, setting the legally required information in notifications concerning releases of GMOs other than higher plants. Articles 5.5(a) and 17.5(a) of Regulation 1829/2003 stipulate that the application shall be accompanied by a complete technical dossier supplying the information required by Annexes III and IV to Directive 2001/18/EC and information and conclusions about the risk assessment carried out in accordance with the principles set out in Annex II to Directive 2001/18/EC. This guidance was developed to support applicants in preparation and presentation of applications submitted under Regulation 1829/2003.

In the case of food or feed containing, consisting of, produced from and produced with GMMs obtained by self-cloning, applicants should address all of the requirements needed for the risk assessment of GMMs and their products as described in this document.

Not all the points included will apply to every case. Unless otherwise specified, the applicant is advised to refer to Figure 1 (page 43) and Table 1 (page 44) for an indication of which data must be supplied for applications belonging to each Category. Reasons must be given for the omission of such data from the dossier.

A. GENERAL INFORMATION

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

- name and address of the applicant (company or institute);
- name, qualification and experience of the responsible scientist(s) and contact details of the person responsible for all dealings with EFSA;
- title of the project;
- scope of the application;
- designation and specification of the GMM and/or derived product, including its proprietary name, the generic and commercial names of the product, production strain, and the conditions of use;
- short description of the method of production and manufacturing.

B. HAZARD IDENTIFICATION AND CHARACTERISATION

Problem formulation including hazard identification. The risk assessment begins with problem formulation in which all important questions for the risk characterisation are identified. Problem formulation helps to make the risk assessment process transparent by explicitly stating the assumptions underlying the risk assessment.

In this document, problem formulation includes the identification of characteristics of the GMM or its product capable of causing potential adverse effects (hazards), of the nature of these effects, and of pathways of exposure through which the GMM or its derived product may adversely affect human or animal health, or the environment. Existing scientific information must be considered and knowledge gaps identified. Scientific uncertainties must be addressed.
In case of GMMs hazard identification is focused on the identification of differences between the GMM and appropriate comparators. The outcome of this exercise determines which further studies should be carried out to characterise these differences with respect to possible impact of the GMM and/or its product on human/animal health and the environment.

**Hazard characterisation.** Hazard characterisation is the quantitative, semi-quantitative or qualitative evaluation of the potential adverse effects on human health, animal health and the environment following exposure to a risk source(s). For human and animal health, this step is focused on possible quantification of the toxicological/nutritional potential of the GMM and/or food/feed derived from or produced with it. Whenever an appropriate non-GM comparator is available, a comparative approach can be applied (see Chapter II.).

1. Information relating to the GMM

1.1. Characteristics of the recipient or (when appropriate) parental organism

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

- to identify the need for specific analyses e.g. the known occurrence in related microorganisms (e.g. in the genus) of specific toxins which are typically expressed at low levels in the unmodified recipient/parental species, but which may be unintentionally increased following the genetic modification process;

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

The applicant should provide a comprehensive description of the recipient/parental microorganism. The history of all previous modifications should be described. The QPS status can be used to meet the information requirements. Information relating to the recipient or (when appropriate) the parental organism must include the following:

1.1.1. Scientific name, taxonomy and other names

The following taxonomic information needs to be provided: (a) genus, (b) species, (c) subspecies (if appropriate) (d) strain, (e) deposition number, (f) generic name, commercial name.

The taxonomical identification of the parental microorganism is important for the risk assessment of a GMM because it provides a reference which can be used to predict its relevant characteristics. This may advise on analyses of specific toxins, allergens or virulence factors that are typically expressed in the genus/species. Identification of a microorganism (strain) should be based on up-to-date methodologies and current knowledge about the genus and species.

Guidance for taxonomic identification of bacteria and archaea is provided by the OECD (2003a) and Bergey’s Manual (Bergey’s, online). For many genera of current interest in biotechnology, only approximations of species assignments can be made. In those cases, a designation to the lowest level permissible (usually genus or subgenus) is needed.

Information about the current classification of fungi can be obtained from the International Commission on the Taxonomy of Fungi (ICTF, online). Furthermore, information can be obtained from the Dictionary of the Fungi edited by the Center for Agricultural Biosciences International (CABI, 2008) and from the US Department of Agriculture Agricultural Research Service fungal database (USDA ARS, online).
1.1.2. Phenotypic and genetic markers

Phenotypic characteristics may include morphology, growth requirements, growth rates, temperature and pH ranges and optima, capacity for formation of spores, aerobic and/or anaerobic metabolism, antimicrobial resistance characteristics and occurrence of genotypic markers. Genotypic markers refer to e.g. auxotrophic mutations and genes coding for antimicrobial resistance.

1.1.3. Degree of relatedness between recipient and donor(s); description of identification and detection techniques

The relationship between the recipient and donor(s) should be described, when appropriate.

The technique used for identification of the recipient/parental organism, as well as the methods to detect the strain in all relevant environmental samples (e.g. food/feed/faecal samples) should be provided. The identification technique should be detailed and adequate to identify the recipient/parental organism unequivocally at the strain level.

1.1.4. Source and natural habitat of the recipient microorganism

Information should be provided on the source from which the recipient strain has been isolated. This includes information on the natural habitats of the species and its ecological role (e.g. plant pathogenicity, symbiotic relationships, intestinal adhesion, capacity to degrade recalcitrant compounds), if available.

1.1.5. Organisms with which transfer of genetic material is known to occur under natural conditions and presence of indigenous genetic mobile elements (vectors)

The inherent capability of the parental microorganism to exchange genes may influence the potential for horizontal gene transfer (OECD, 2010). Numerous microbial species are known as natural gene exchangers. This depends on the presence of the proper host machinery for transfer. Information based on a recent literature survey should be provided concerning:

- the inherent capability of the recipient/parental species to transfer or acquire DNA;
- the possible presence of plasmids and their host range (specificity);
- the possible presence of conjugative transposons, integrons and/or sex/mating factors, their sequence, frequency of mobilisation;
- the presence of genes that confer resistance/tolerance (e.g. to antimicrobials, heavy metals, toxins).

1.1.6. Information on the genetic stability of the recipient microorganism

The genetic stability of the recipient/parental microorganism should be verified to confirm its identity and phenotypic characteristics and stable performance. The correspondence of the recipient/parental strain and the GMM should be verified by a genetic fingerprinting technique appropriately reflecting the genomic similarities. If any safety studies refer to previous generations of the parental strain, the correspondence between the genotypes has to be established.

1.1.7. Pathogenicity, ecological and physiological traits
Guidance on the risk assessment of GMMs and their food and feed products

This should include any data relating to any impact on human or animal health or the environment, when appropriate. The following information is required:

a) A classification of hazard according to the current Community legislation concerning the protection of human health and/or the environment, and specifying to which risk group the microorganism belongs (Annex III of Directive 2000/54/EC28).

b) Information relating to pathogenicity, infectivity, toxigenicity, virulence, and allergenicity should be provided, as appropriate:

- Information should be provided on the history of safe use of the recipient/parental strain or any close relative, if available. Effects of any previous use or unintended presence (e.g. as a contaminant) in food or feed has to be considered. The whole human population, including vulnerable groups has to be taken into consideration.

- Information on pathogenicity should be provided for the recipient/parental strain, and also for related strains and species, if relevant.

- Information on the ability to colonise plants, animals (including invertebrates) or humans should be provided. In particular, applicants should provide information on the viability and ability of the recipient/parental microorganism to survive in the gastrointestinal tract of humans or animals consuming the GMM (Category 4).

- Information regarding probiotic or immunomodulatory properties should be provided, taking into account the whole human population, including immunocompromised individuals, infants and the elderly.

c) Information relating to the presence of introduced genes that encode antimicrobial resistance:

- The presence of introduced antimicrobial resistance genes should be analysed using appropriate methods, as provided by the most recent EFSA guidance (EFSA, 2008).

- To allow the evaluation of the potential for transfer of the introduced antimicrobial resistance genes to other organisms, information should be provided as to whether such genes are located on mobile genetic elements.

d) When appropriate (Category 4), any information relating to the involvement of the recipient/parental organism in environmental processes should be provided e.g. decomposition of organic matter, participation in soil nitrogen cycle, mobilisation of phosphate, colonisation of rhizospheres, production of plant growth promoting factors.

1.1.8. Description of history of use

Documented information of the parental strain regarding its previous use, or presence as a part of the natural microbiota, in food or feed should be provided. The information should include: typical cultivation conditions; type of use in food or feed; viability during the production process. In cases when the parental strain is part of the food or feed, its stability during the typical shelf life of the product and an estimation of the final human or animal exposure should be given. When the parental species has a QPS status, this should be indicated together with the information whether the strain used for the GMM construction fulfils the specific QPS qualifications (e.g., lack of transmissible

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antimicrobial resistance genes). With a non-QPS organism the previous history of use of the parental
strain of the GMM should be presented.

1.1.9. History of previous genetic modifications

A detailed description and risk assessment of any previous genetic modification should be provided.

1.2. Characteristics of the origin of the inserted sequences [donor organism(s)]

The inserted sequences in the GMM may be of different origins. The required information depending
on the source is detailed below. When the inserted DNA is a combination of sequences from different
origins, the pertinent information for each of the sequences should be provided.

Alternatively, the genome of the recipient microorganism may be modified in such a way that it does
not involve foreign DNA. An example of this would be the deletion of a gene. In these cases, the
characteristics of the donor should be provided only when the strain used is different from the
recipient.

Information on the donor organism has to be provided in order to:

- trace the source and function of the gene(s) to be inserted;
- evaluate the potential toxicity, virulence or allergenicity of the gene product. It is
  particularly important to provide information on issues related to pathogenicity, or any
  other traits that have the potential to affect human, animal or plant health or the
  environment.

1.2.1. DNA from defined donor organisms

A description of the donor organism should provide information on its identity and major biological
properties.

The description of the donor organism should include:

- (a) genus, (b) species, (c) subspecies (if appropriate) (d) strain (for microorganisms), (e)
  deposition number (for microorganisms), (f) generic name, commercial name.
  Up-to-date taxonomic identification should be provided (for microorganisms, see
  Section B.1.1.1.). Previous name(s) should also be indicated.

- In cases when unspecified DNA is expected to be associated with the genes to be
  transferred, further detailed information is required. This information should include the
  elements outlined above in 1.1.4 and 1.1.6. This information is not needed for
  microorganisms with a QPS status or plants and animals with a history of safe use as
  food or feed.

1.2.2. Synthetic DNA

Synthetic DNA sequences may be used to introduce gene(s) into organisms. In such cases, information
should be provided on:

- rationale and strategy for the design;
- similarities with natural sequences and function in natural organisms;
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1.2.3. Nucleic acids directly extracted from environmental samples

Nucleotide sequences obtained from nucleic acids extracted from environmental samples, possibly selectively amplified by PCR, may be used as a source for gene(s) to construct GMMs. In such cases, information should be provided on:

- the type of environmental sample;
- the DNA extraction and amplification/cloning procedure;
- rationale and strategy for the selection of the DNA sequence(s);
- similarities with sequences and function in taxonomically defined organisms;
- DNA sequence and a physical map of the functional elements;
- amino acid sequence(s) and function(s) of the encoded protein(s), including the role played in the metabolism and substrate specificity.

1.3. Description of the genetic modification

The genetic modification protocol should be described. When helper plasmids or carrier DNA are used, they should also be described. If carrier DNA is used, its source must be stated and a risk assessment provided. The information provided should allow for the identification of all genetic material potentially delivered to the recipient/parental microorganism.

1.3.1. Characteristics of the vector

The description of the vector(s) used for the construction of the GMM should include:

- the source and type (plasmid, phage, virus, transposon) of the vector used;
- a fully annotated sequence of the vector;
- a physical and genetic map detailing the position of all functional elements and other vector components, together with the restriction endonuclease sites selected for the generation of probes, and the position and nucleotide sequence of primers used in PCR analysis;
- a table identifying each component, properly annotated, such as coding and non-coding sequences, origin(s) of replication and transfer, regulatory elements, their size, origin and role, should accompany the map.

1.3.2. Information relating to the genetic modification

The genetic modification process should be described in detail. This should include:

- methods used to introduce, delete, replace or modify the DNA into the
recipient/parental, and methods for selection of the GMM;

- information on the location of the modified DNA. It should be indicated whether this DNA is located in the vector\textsuperscript{29}, in the chromosome or, for eukaryotic microorganisms, in organelles (mitochondria, chloroplasts, etc.).

- A description of the sequences actually inserted, deleted, replaced or modified, and in the case of insertions, the copy number of the inserts should be provided and accompanied by experimental data e.g. Southern analysis. In the case of deletion(s), the size and function of the deleted region(s) must be provided.

Whether any functional vector sequences not intended to remain in the GMM are truly absent (e.g. those encoding for antimicrobial resistance or origins of replication) should be tested experimentally. If a vector (mobile genetic element associated with the inserted DNA) is present in the final production strain, the following additional information should be provided based on literature data and/or experimental evidence:

- an estimate of the copy number of plasmids per cell;

- information relating to the host range;

- the frequency of mobilisation and the transfer capacity of the inserted vector(s) used for creating the genetic modification. Any information on the expected stability of the inserted vector in the recipient/parental microorganism, and on its capacity to transfer genetic material to other organisms should be provided. The method(s) used to determine the transfer capabilities of the inserted DNA should be provided. When the origin of replication of the vector has a broad host range, this should be taken into account in the evaluation of the stability and transfer capabilities of the vector.

1.4. Information relating to the GMM and comparison of the GMM with an appropriate comparator

1.4.1. Description of genetic trait(s) or phenotypic characteristics and, in particular, any new traits and characteristics which may be expressed or no longer expressed

This information is needed to identify any hazards resulting from the intended modification of the recipient/parental microorganism. Intended modifications are those that are targeted to occur due to the introduction of DNA sequences or inactivation of gene(s). The applicant is requested to deposit the GMM in a recognised culture collection and to provide the deposition number.

The genetic modification and the uses of the GMM should be described, together with intended changes in the metabolism of the microorganism:

- a description of the intended trait(s) and the anticipated changes to the phenotype of the microorganism is required; both qualitative and quantitative differences should be reported;

- the production of expected by-products or any side-effects should be described;

\textsuperscript{29} “Vector” is understood as the agent containing the introduced DNA sequence used as a vehicle to transfer such sequence into the transformed cell. If the introduced DNA remains in the vector, this should be indicated, as well as the subcellular localisation where the vector is present.
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- the microorganism selected as the comparator should closely represent the behaviour or characteristics of the GMM (OECD, 2003b). In most cases, the most appropriate comparator is the parental or recipient strain that is identical except for the introduced trait(s). Provided that systematics is properly applied, taxonomic relatedness may be used as a criterion to select a comparator other than the parental microorganism. In any case, the comparator should be a strain which has previously been evaluated for safety. Whenever possible, the comparator should have a QPS status. When the recipient strain does not have a history of safe use, the choice of a different strain as comparator must be justified; all the available information should be provided and evaluated on a case-by-case basis.

1.4.2. Structure and amount of any vector and/or donor nucleic acid remaining in the GMM

A genetic map(s) indicating the organisation of the genetic elements in the inserted DNA should be provided, and the copy number(s) of the recombinant DNA sequence(s) estimated. This should be analysed by using appropriate methods, e.g. Southern analysis. The presence of any vector and/or donor DNA not intended to be inserted in the GMM should also be documented. This is especially important when antimicrobial resistance markers are present in the vector or donor DNA.

1.4.3. Stability of the genetic traits in the GMM

The genotype and phenotype of a GMM should be stable over the intended period of production and intended use of the organism in food or feed. The stability of the GMM should be demonstrated using representative batches, normally five. Methods used to demonstrate the stability of the GMM should be provided. These methods could include Southern analysis that targets the recombinant DNA and/or genetic fingerprinting that gives information about genetic rearrangements.

1.4.4. Rate and level of expression of the new genetic material and activity of the expressed proteins

The rate and level of expression of the inserted genetic material should be determined. The methods used for expression analysis and their sensitivity should be described.

- The conditions and level of expression of the inserted gene(s) should be provided. The information on expression levels should be derived using representative, normally five, batches.

- The location of the recombinant protein(s) in the GMM (e.g. intracellular, cell wall-associated, secreted) should be provided under the conditions envisaged during the use of the GMM in food or feed.

- When the final product is an enzyme, its activity and specificity should be given.

1.4.5. Description of identification and detection techniques

The techniques used for the identification and detection of the inserted sequence(s) and vector should be detailed.

1.4.6. Information on the ability to transfer genetic material to other organisms
The potential to transfer recombinant DNA from GMMs to other microorganisms needs to be characterised.

The following information should be provided:

- The presence of sequences within the recombinant DNA that could enhance gene transfer or integration of the introduced trait into the genome of other microorganisms.
- The presence of extrachromosomal replicons and elements carrying the recombinant DNA.
- Information on the potential host-range of the replicon.
- The presence of genes in the GMM that could provide selective advantage to the recipient microorganisms in case they were transferred unintentionally.

1.4.7. History of previous uses or environmental releases of the GMM, where appropriate

The applicant should provide any information on previous uses or releases of the GMM, including literature references or other documentation. Emphasis should be placed on information that relates to possible impacts on human or animal health or the environment.

1.4.8. Safety for humans and animals

DNA inserted in or deleted from the GMM can result in differences in the metabolic activity, colonisation capacity, and other trait(s). This could result in both intended and potentially unintended effects that may affect human or animal health. The following particulars should therefore be provided:

- Information on any changes in the GMM which may result in potential toxic, allergenic or other harmful effects on human or animal health, e.g. the possible stimulation or de-repression of endogenous toxin production;
- Information on the potential for DNA transfer to take place; such information should also take into account any capacity for enhanced gene transfer to occur; thus, on a case-by-case basis, specific experimental data on gene transfer and its consequences may be required;
- If the GMM remains viable in the final food or feed, information on the viability and residence time of the GMM in the alimentary tract of the target host species should be provided; this is particularly important if the viability of the GMM is affected by the genetic modification;
- Information on any impact that the GMM may have on the microbiota of the human or animal gastrointestinal tract.

2. Information relating to the product

Applicants should indicate in which of the four categories of GMMs or their products, as defined under Chapter II, the product belongs.

Only limited information focusing on the production system is required to perform a risk assessment on purified compounds (Category 1). For categories 2 and 3, it will be necessary to understand the processes by which the GMM has been inactivated in the product and the degree to which traces of
Guidance on the risk assessment of GMMs and their food and feed products

recombinant DNA may be detected in the product. If applicable it should be confirmed that the product does not contain viable but non-culturable cells (VBNC). The most intense scrutiny is reserved for products containing viable GMMs (Category 4), whether as food ingredient or feed material, or as a pure culture to be used for fermentation of food/feed.

Information relating to the GMM and/or product derived from or made with it should include a description of its main characteristics and its intended use(s). A description of the contained and food/feed fermentation process and the preparation of the product should be detailed. Comparison of the product with an appropriate comparator should be carried out. Any differences in the chemical composition, physical characteristics and nutritional properties or other traits that might affect human or animal health or the environment should be assessed.

Depending of the Category and scope of the product, its characterisation (specification) and safety assessment will be undertaken according to relevant legislation (See Chapter I) for which different Guidance Documents or Guidelines apply:

- Amino acids and enzymes used as feed additives (Categories 1 and 2, respectively) are assessed according to the Commission Regulation (EC) No 429/2008\(^{30}\) on detailed rules for the implementation of Regulation (EC) No 1831/2003 as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. According to the nature and use of the product, one or more Guidance Document of the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) may be applicable (EFSA, online). For these applications, Section B.2.2. of this Chapter will apply.

- Biomasses used as feed materials (Category 3 or 4) are assessed according to the Regulation (EC) No 1829/2003 as regards the preparation and the presentation of applications. The risk assessment of the product should be made following the Council Directive 83/228/EEC\(^{31,32}\) on the fixing of guidelines for the assessment of certain products used in animal nutrition. For these applications, Section B.2.2. of this Chapter will apply.

- Enzymes added to food for a technological purpose of manufacturing, processing, preparation, treatment, packaging, transport or storage of foods (Category 2) fall under the scope of Regulation (EC) No 1332/2008 and Regulation (EC) No 1331/2008. Applications for these products should follow the Guidance of the EFSA Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids (CEF) on the Submission of a Dossier on Food Enzymes for Safety Evaluation by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids (EFSA, 2009b). For these applications, Section B.2.2. of this Chapter will apply.

- Food additives (Categories 1 or 2) fall under the scope of Regulation (EC) No 1333/2008 and Regulation (EC) No 1331/2008. Applications should follow the Guidance on Submissions for Food additive Evaluations by the Scientific Committee on

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\(^{32}\) Guidance for the risk assessment of biomasses used as feed materials by the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) is in preparation.
Food (EC, 2001)\textsuperscript{33}. For these applications, Section B.2.2. of this Chapter will apply.

- Food flavourings (Categories 1 or 2) fall under the scope of Regulation (EC) No 1334/2008 and Regulation (EC) No 1331/2008. Applications for these products should follow the EFSA Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids (CEF) Guidance on the Data Required for the Risk Assessment of Flavourings to be used in or on Foods (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), 2010). For these applications, Section B.2.2. of this Chapter will apply.

- Food consisting of, containing or produced from GMMs other than the ones mentioned above should be assessed according to the principles laid down in Regulation (EC) 1829/2003 (EFSA, 2003a) and should follow Sections B.2.1.- B.2.4. of this Chapter. Examples would be foods produced with GMM starters such as wines, beers, fermented milks, cheeses, bakery products, cured meats and pickled vegetables, as well as foods produced by filamentous fungi or different surface cultures and GMM-derived biomasses directly used as foods or food ingredients.

Information should be provided on the GMM and/or derived product and its effects on the food containing the GMM and/or derived product. If relevant, any data on reaction products resulting either from the GMM or derived products with food constituents, should be considered. Information on possible adverse effects on nutrients is required to allow the safety assessment.

2.1. Information relating to the production process

The stages of the production process of the GMM (fermentation, cultivation) should be detailed. A flowchart showing the key stages is recommended.

2.2. Information relating to the product preparation process

Information relating to the preparation of the product should include the description of techniques used to remove GMM cells and to purify the product. The relevant parameters should be provided (e.g. temperature/time combinations, pH) (please refer to Section B.2.2.3.)

2.2.1. Information on the removal of the GMM cells from the product

Removal of the GMM is required in products belonging to Categories 1 and 2. The technique used to remove microbial cells from the product should be detailed and the removal of the microbial cells from the product should be experimentally verified.

- The reliability and efficacy of the removal procedure should be established. The removal of the production strain should be established using a recognised method for the enumeration of viable cells. The procedure has to ensure the detection of stressed cells by including a resuscitation step. Resuscitation should be done in cultivation media with a minimal selective pressure and/or by providing a longer incubation time compared to the normal culturing of viable organisms.

- At least three independent batches of product preparations should be sampled, each analysed in triplicate. A proper sampling method should be chosen and documented.

\textsuperscript{33}Guidance for the risk assessment of food additives by the EFSA Panel on food additives and nutrient sources added to food (ANS) is in preparation.
For example, an analytical sample can be prepared by pooling at least 10 different individual samples taken from the same preparation batch.

- The detection should consider specificity against the background microbiota of the sample.

2.2.2. Information on the inactivation of the GMM cells and assessment of the presence of remaining physically intact cells

Irreversible inactivation of the GMM leading to its inability to replicate is required for products belonging to Category 3. Information on the technique used to inactivate the microbial cells is required when the GMM has not previously been removed from the product and the product is considered free from viable cells. There is considerable variability in the resistance of microorganisms to inactivation agents and methods. For this reason, the efficacy of any technique used to inactivate GMMs should be established for the specific GMM within the product. Often cells that cannot be recovered in artificial culture can still be able to interact with humans, animals or the environment. For example, heat-inactivated probiotic bacteria have been demonstrated to have effects on the immunological functions of the host. Therefore, the possible presence of physically intact cells, VBNC and stressed cells should be analysed. The VBNC state is well described for some bacteria and may also exist in certain yeasts. The risk posed by the different viability stages should be deduced from the specific properties of the microorganism (its pathogenicity, its ability to cause opportunistic infections, its known or anticipated interactions with the host microbiota).

- The technique used to inactivate the microbial cells should be described in detail, justified and all physicochemical parameters should be provided. The reliability, sensitivity, and efficacy of the technique used to inactivate the specific GMM should be established, taking into consideration the current literature.

- The absence of viable GMM cells should be verified by means of a recognised method for detecting viable microorganisms, targeted to the GMM.

- The absence of stressed cells should be verified by including a resuscitation step in the culturing method (see point 2.2.1).

- The possible presence of VBNC should be analysed by non-culturing techniques. For this, epifluorescence techniques (microscopy, flow cytometry) may be used with dyes targeting different metabolic functions as well as RT-PCR targeting mRNAs for which short half-life has been confirmed (e.g. after the addition of antimicrobials that prevent new mRNA synthesis).

- The possible presence of physically intact dead cells should be analysed by non-culturing techniques. For this, epifluorescence techniques (microscopy, flow cytometry) may be used.

- At least three independent batches of product preparations should be sampled, each analysed in triplicate. A proper sampling method should be chosen and documented (see point 2.2.1).

2.2.3. Information on the possible presence of recombinant DNA

Information on the possible presence of recombinant DNA is required in products belonging to Categories 1, 2 and 3. If recombinant DNA corresponding to full-length coding sequences is found, the likelihood of gene transfer must be assessed (See Section B.3.). All the methods should be documented in detail.
• All DNA present in the product should be extracted. Therefore, a cell lysis step should be introduced in the DNA extraction procedure when the product belongs to Categories 2 or 3; this is not applicable to products belonging to Category 1. Special attention should be given to the detection of DNA present in microorganisms that are resistant to lysis, like spore-forming organisms. To verify the efficacy of the lysis step, the GMM must be added in different dilutions before DNA extraction.

• Control DNA should be added to the sample in different dilutions until DNA extinction before commencing the DNA extraction process, in order to check the limit of detection of recombinant DNA in the sample.

• The presence of DNA should be assessed using a PCR-based method. The reliability, efficacy and sensitivity of the DNA detection method should be documented. Positive and negative controls must be included to ensure functional PCR and to exclude PCR inhibition. As control DNA, total DNA of the GMM must be used. Should PCR inhibition be encountered when testing the product, samples taken before formulation may be used.

• At least one functional gene has to be targeted. Because DNA degradation can be sequence-dependent, all functional genes, if of concern, inserted into the GMM should be targeted specifically. The PCR should span the full length of the coding sequences but should not exceed it.

• At least three independent batches of product preparations should be sampled, each analysed in triplicate. A proper sampling method should be chosen and documented (see point 2.2.1).

2.3. Description of the product

2.3.1. Designation of the product

The identity of the product according to its principal function (i.e. specification of the Category of product to which it belongs), the name, the chemical definition, the chemical name, synonyms, trade names and abbreviations, if any, should be provided.

2.3.2. Intended use and mode of action

The intended use of the product and its mode(s) of action, where applicable, should be described. Any other potential uses should also be specified.

2.3.3. Composition

Compositional data are essential for the safety and nutritional assessment of the GM product (GMM and/or GMM-derived product). The qualitative and, where appropriate, quantitative composition of the GM product, should be provided. The extent of batch-to-batch variation should be determined. At least three representative batches should be included in each analysis and, in cases of any introduced changes in the production process, the effects on the composition should be assessed.

For products belonging to categories 3 and 4, as well as for food products belonging to category 2, the analysis should include the key nutrients, antinutrients, and other substances typical of the product (organic acids, alcohols, flavour components, etc.) as well as impurities. If relevant conventional products exist, they should be used as comparators for the corresponding GM products.
2.3.4. Physical properties

The applicant should describe the physical state (liquid, solid) of the product. The most appropriate physical properties including, e.g. shape, density, viscosity, surface tension and solubility, should be provided. The physical traits to be described should be defined for each product on a case-by-case basis. Methods used for the determination of these parameters should be described.

2.3.5. Technological properties

The technological attributes of the product should be specified for its intended use and for any other potential uses. The stability of the product, or activity, and the shelf-life should be defined for the conditions in which it is to be used, where appropriate. Methods used for the determination of these properties, their accuracy, reliability and efficiency should be described.

2.4. Considerations of the GM product for human health

These considerations are mainly relevant to products belonging to categories 3 and 4, but in some cases also for food products of category 2. The first step in the risk assessment of the GM product should be the comparison of the GMM with its appropriate comparator (See section 1.4.1.). This comparison should focus principally on the differences in the metabolic profiles between the GMM and its appropriate comparator growing in the same matrix and in the same product, taking into account the possible changes in the production process due to the use of the GMM. Given the complexity of many food matrices, this in should often, in practice, be combined with the compositional analysis of the product. In case no appropriate comparator is available, the safety assessment per se should be carried out.

In cases, when the parental organism of the GMM does not have a history of use in the particular application, traditional products can still be used as comparators to identify possible compositional changes and to assess their safety implications.

A totally novel type of product with no traditional counterpart, would require the most extensive safety evaluation.

Genes inserted in a GMM should be evaluated for their potential impact on human health, and the assessment should include the potential for a microorganism to transfer genetic material to other organisms and also to disseminate antimicrobial resistance genes which may have deleterious consequences for human health. Thus, specific experimental data on gene transfer and its consequences may be required on a case-by-case basis (See Section B.3).

2.4.1. Toxicology

The toxicological impact of any changes in the GMM and/or derived product resulting from the genetic modification should be assessed. The need for toxicological testing should be considered based on the outcome of the molecular and comparative analysis (see Sections B.1. and B.2.), i.e. the differences identified between the GMM and/or derived product and its comparator, including intended as well as unintended changes. The risk assessment should focus on the metabolites produced by the GMM during the fermentation process and in the food. The risk assessment of food should also take into account any significant changes in the production process resulting from the use of the GMM.

Toxicological studies, if needed, should be conducted using internationally agreed protocols and test methods described by the OECD (OECD, 1995). Use of any methods that differ from such protocols should be justified. Studies should be performed according to the principles of Good Laboratory
Practice (GLP) described in Directive 2004/10/EC\textsuperscript{34} and be accompanied by a statement of compliance with GLP.

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.

### 2.4.2. Assessment of proteins expressed by the newly introduced genes

Proteins expressed by the newly introduced genes have to be evaluated. The studies required to investigate the toxicity of a protein should be selected on a case-by-case basis, depending on the knowledge available with respect to the source of the protein, its function and activity and its history of consumption by humans and/or animals. In the case of proteins expressed in the GMM, when the parental microorganism has a QPS status (or otherwise established history of safe use), and the new proteins have a history of safe consumption, specific toxicity testing as outlined below is not required.

If sufficient test materials cannot be extracted either from the GMM or from the product, a protein from an alternative source should be used. The structural, biochemical and functional equivalence of the substitute protein to the protein expressed by the recombinant genes in the GMM must be demonstrated.

To assess the safety of proteins expressed by the recombinant genes, the following information is needed:

- molecular and biochemical characterisation of the protein(s) expressed by the newly introduced gene(s), including the sequence, molecular weight, post-translational modifications, and a description of the function. Information on the potential effect(s) on the metabolism of the GMM should also be provided;

- a search for homology to proteins should be conducted. Identified similarities should be evaluated, paying special attention to toxic proteins; the database(s) and the methodology used to carry out the search should be specified;

- the influence of processing and storage conditions of the food on the activity of the protein should be considered on a case-by-case basis; this is particularly important when the protein is excreted or released in significant amounts in the food.

When bioinformatic or other data (history of use and characteristics of the protein) suggest any concern, a repeated dose animal toxicity study with the protein expressed by the newly introduced gene may be considered depending on the use. Depending on the outcome of this study, further investigations may be required.

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

New constituents other than proteins as well as expected unintended changes due to the modification of specific metabolic pathways should be evaluated. This may include toxicological testing on a case-by-case basis. To establish the safety of new constituents, information analogous to that described in the Guidance on submissions for food additive evaluations by the Scientific Committee on Foods\textsuperscript{12}

(EC, 2001) 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.

2.4.4. Testing of the whole GM product

If no corresponding conventional product exists, or if there are any indications from the risk assessment for further testing of the GM product, toxicological testing should be considered. The testing programme should include a subchronic (90-day) feeding study in rodents (OECD, 1998). At least two dose levels of the GM product and comparator (if available) should be included in the diet. The highest dose level should be the maximum achievable without causing nutritional imbalance.

Additional toxicological studies may also be necessary, depending on the findings of the animal feeding study and the potential exposure.

For products under categories 3 and 4, particular attention should be paid to potential interaction(s) with the gut microbiota and the evaluation of any effect on the digestive physiology and human immune response.

2.4.5. Allergenicity

2.4.5.1. Introduction

Allergy is an adverse reaction that, by definition, is mediated by the immune system. Food allergies of regulatory concern involve IgE antibodies. Gluten intolerance (i.e. celiac disease) is of an autoimmune nature, and IgE antibodies are not involved. This Section deals both with the risk with regard to de novo induction of allergy (sensitisation), and with elicitation of allergic reactions in already allergic individuals (provocation) exposed to products derived from GMMs.

The food constituents that are responsible for allergenicity are in nearly all cases proteins or peptides. In a few cases low molecular weight substances and carbohydrates may also cause allergy. There are no structural or general functional characteristics that allow the identification of a protein as an allergen. Therefore, nearly all methods for the identification of allergens in a novel product are based on comparison with allergens that are already known. Only known allergens or ‘new’ allergens showing sufficient similarity to known allergens will be detected. An entirely ‘new’ allergen, be it a novel protein construct or a ‘natural’ protein which has not been used in man before, can only be detected to be an allergen if it is found to induce allergy in man or animals.

Allergic sensitisation: The specific conditions for allergic sensitisation are not well known, and this also applies to dose-response relationships. No threshold doses for allergic sensitisation have been determined. Similarly, available evidence has not been considered sufficient to allow the determination of allergen thresholds for the provocation of allergic reactions in sensitised individuals (EFSA Panel on Genetically Modified Organisms (GMO), 2010).

The integrated process which is described below covers both food and respiratory allergy risk.

2.4.5.2. Proteins expressed by the newly introduced genes

At present, there is no definitive test that can predict the allergic response in humans to a protein expressed by a newly introduced gene. Allergenicity is not an intrinsic, fully predictable property of a given protein. Rather, it is a biological activity requiring an interaction with pre-disposed individuals. Given the lack of predictability, it is necessary to obtain a cumulative body of evidence that minimises any uncertainty with regard to the protein(s) in question. In line with the recommendations of EFSA (EFSA Panel on Genetically Modified Organisms (GMO), 2010) and the Codex ad hoc
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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 proteins expressed by the newly introduced genes.

- Attention should be given to the choice of the expression host, since post-translational modifications allowed by different hosts (i.e. eukaryotic vs. prokaryotic systems) may have an impact on the allergenic potential of the protein.

- Source of the protein: The source of the transgene must be considered carefully as to whether or not it may encode a known allergen. It should be documented whether it is a known allergenic source, if it is a source with good evidence for non-allergenicity, or a source with little information regarding allergenicity. In cases when the introduced genetic material is obtained from wheat, rye, barley, oats or related cereal grains, applicants should assess the proteins expressed by the newly introduced genes for a possible role in the elicitation of gluten-sensitive enteropathy.

- Amino acid sequence homology comparison between the protein expressed by the newly introduced gene and known allergens: A search for sequence homologies and/or structural similarities and motifs between the expressed protein(s) and known allergens must be performed. The quality of the databases used should be considered. 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. Improvement and harmonisation of the algorithms that are used should be sought. A local alignment method with a known allergen with a threshold of 35% sequence identity over a window of at least 80 amino acids is considered a minimal requirement (EFSA Panel on Genetically Modified Organisms (GMO), 2010).

- Specific serum screening: An important procedure for assessing the potential that exposure to the protein expressed by the newly introduced gene 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). Rather than pooled sera, individual sera from well-characterised patients should be used.

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

  o If the source is not known to be allergenic, but if there are consistent indications of sequence homology to a known allergen, specific serum screening should be conducted with sera from patients sensitised to this allergen in order to confirm or exclude IgE cross-reactivity between the protein expressed by the newly introduced gene and this allergen.
Specific 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.

- Resistance to pepsin digestion: Stability to digestion by proteolytic enzymes has long been considered a characteristic of allergenic proteins. Although it has been established that no absolute correlation exists (Fu et al., 2002; EFSA Panel on Genetically Modified Organisms (GMO), 2010), resistance of proteins to pepsin digestion is still proposed as an additional criterion to be considered in an overall risk assessment. The use of well standardised methodology is essential. If 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 protein expressed by the newly introduced gene being allergenic. It may also be useful to compare intact, pepsin-digested and heat-denatured proteins for IgE binding. Since the protein(s) encoded by the newly introduced gene(s) will be present in the product as part of a complex matrix, the impact of the possible interaction between the protein and other components of such matrix, as well as the effects of the processing, should be considered by additional in vitro digestibility tests. In addition, the digestibility in infants as well as in individuals with impaired digestive functions may be assessed using different conditions in the digestibility tests.

- Although additional tests including in vitro cell based assays or in vivo tests on animal models have not been validated so far, they may be considered useful to provide additional information e.g. on the potential of the product for de novo sensitisation.

2.4.5.3. Assessment of allergenicity of the whole GM product

The potential of compounds present in the food matrix, other than those originated by the expression of the newly introduced genes, to increase the overall allergenicity (e.g. known allergens) should be considered.

The approach should be selected on a case-by-case basis depending on the available information on the allergenic potential of the source and/or the host. Modern analytical tools including protein profiling techniques may be used in association with human and animal serum or cell-based assays.

2.4.5.4. Adjuvanticity

Adjuvants are substances that, when co-administered with an antigen, increase the immune response to the antigen and therefore might increase as well the allergic response. In cases when known functional aspects of the protein expressed by the newly introduced gene or structural similarity to known strong adjuvants may indicate possible adjuvant activity, the possible role of GMMs as adjuvants should be considered/discussed. Moreover, the presence of other compounds in the GM product may affect its adjuvanticity, and this should be assessed.

2.4.6. Nutritional assessment

Identification of compositional changes in key nutrients and antinutrients is the starting point for the nutritional assessment of a GM product. The nutritional assessment should consider the anticipated dietary intake and the resulting nutritional impact.

The identification of consumer groups with exceptional consumption patterns should also be a part of the nutritional assessment of GM products. In particular, if the GM product is targeted for a certain consumer group (infants, the elderly, individuals with food allergy) or for some special purpose...
(weight control, functional food, dietary supplement), the effects on the overall nutrition should be carefully assessed.

- When compositional equivalence of the GM product to a corresponding product is demonstrated, no further studies are required.

- If significant changes in the composition of nutrients and/or antinutrients have been identified in the GM product, their nutritional relevance should be assessed based on current knowledge and taking into account the anticipated intake.

- If no corresponding conventional product exists, the estimation of the expected dietary intake is particularly relevant. Information on the anticipated intake and extent of use of the GM product will be required and the nutritional consequences should be assessed at average and at extreme levels of daily intake to find out, whether the nutrient intakes are likely to be altered by the introduction of such products into the food supply.

- In addition to the nutrient content, the bioavailability of nutrient components in the product should be considered.

3. Potential environmental impact of GMMs and their products

A precondition for authorisation of GMMs and their products is their environmental risk assessment with the objective of identifying their potential environmental damage and the likelihood of its occurrence. Environmental damage is defined as a measurable adverse change in a natural resource or measurable impairment of a natural resource service which may occur directly or indirectly. In the specific case of this guidance document, the most relevant environments are those which are exposed to the GMM or to their products e.g. human and animal gut, terrestrial ecosystems where solid waste or manure is applied, or aquatic ecosystems receiving waste water.

Possible adverse health effects posed by the handling or unintentional use of the product or the GMM itself should be assessed. Potential routes of environmental exposure to humans should be identified. The applicant should determine the route(s) by which the product and/or the microorganism may be disseminated, for example via air (aerosols, dust, etc.), water or other routes (e.g. physical contact). When the sources and routes of exposure are identified, it should be established whether the product or the GMM would have the ability to be taken up by the human body. If one or more routes of exposure and relevant routes of entry are identified, the possibility of adverse health effects should be evaluated. In case of potential adverse effects, quantitative methodologies relevant for human exposure assessment should be adopted in the environmental monitoring (EC, 2003).

Depending on the Category to which the product is assigned (see Chapter II), the level of environmental exposure will be different and therefore the information that is required varies as outlined below.

3.1. Environmental risk assessment (ERA) of products belonging to categories 1 and 2.

Environmental exposure of the GMM is negligible since no viable GMMs and recombinant genes originating from them are present in Category 1 and Category 2 products. Since contaminations with trace amounts of GMM or recombinant genes cannot always be excluded, consideration of potential environmental damage is required. This can be based on the nature of the DNA sequences incorporated and must take into account the use of the product. Requirements to assess environmental

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effects e.g. ecotoxicological properties of products themselves falling under these categories are legislated elsewhere (see Section B.2.) and not covered here.

3.2. ERA of products produced from GMMs in which GMMs capable of replication or of transferring newly introduced genes are not present; but in which newly introduced genes are still present (Category 3)

Since the lack of viability (potential to replicate) of the GMM must have been demonstrated (see Section B.2.2.2.), the environmental risk assessment for products of this Category should be focused on recombinant DNA. The probability of transfer of the recombinant DNA to other microorganisms and possible consequences of such horizontal gene transfer events for the environment must be assessed. Important information related to such an assessment should consider the following aspects:

- Quality and location of recombinant DNA. Recombinant DNA can be located in chromosome, or on plasmids or other mobile genetic elements. Furthermore, the length of the recombinant DNA may vary. Even for cell-free DNA, these aspects may have an influence on the potential for horizontal gene transfer and further dissemination and therefore they should be included in the ERA.

- Diversity of environments into which the recombinant DNA may be released. An environmental risk assessment should first consider the diversity of environments and the amount and/or concentration of recombinant DNA that may appear in such environments as a consequence of its intended use, waste disposal and accidental spillage. Examples of different environments are: the gastrointestinal tract of humans or animals receiving the product as food or feed; faeces; manure; waste water; surface waters; or soil. Factors influencing transmissions may include movement of air and water, drainage systems, or handling of products and livestock.

- Stability of recombinant DNA in relevant environments. Environments anticipated to receive considerable amounts of recombinant DNA must be characterised for their effect on DNA persistence. Processes and components contributing to the degradation of DNA (e.g. pH values, DNases or microbial activities) or their stabilisation (e.g. ambient temperatures, adsorption on clay minerals) should be identified and evaluated.

- Presence of indigenous microorganisms as potential recipients of DNA by horizontal gene transfer. Environmental microorganisms, especially bacteria, may be capable to acquire cell-free recombinant DNA from the environment, incorporate it into their own genome, and express the recombinant trait. This process of natural transformation can potentially occur in all environments inhabited by microorganisms. The probability of natural transformation very much depends on the specific environment and must therefore be assessed. This assessment could include considerations about the suspected density of microbial cells and presence of bacteria known to become competent for natural transformation. Information about bacterial species capable of natural transformation and their requirements to develop competence can be obtained from the literature or databases (Lorenz and Wackernagel, 1994).

- Consequences of horizontal gene transfer. As a worst case scenario, the novel properties of microorganisms with the acquired recombinant genes should be assessed (on a theoretical basis and consulting relevant literature) for providing selective advantages in specific environments or under specific environmental conditions. This assessment should also evaluate the possibility that the transfer of recombinant DNA may cause adverse effects related to human, animal or plant pathogenicity, or interference with ecosystem functions.
3.3. ERA of products consisting of or containing GMMs capable of replication or of transferring genetic material (Category 4)

This Category requires the most detailed environmental risk assessment. This assessment needs to consider whether the GMM is capable to survive (persist) and proliferate in specific environments, the possibility that the GMM may cause adverse health or environmental effects and the possibility that the recombinant DNA is transferred to and expressed in other organisms and/or other environments. Furthermore, as described in detail for Category 3, see above, the risk assessment needs to evaluate the fate and effects of cell-free DNA originating from the GMM. More specifically, the following aspects must be addressed.

ERA concerns the following aspects: i) competitive advantage of the GMM or natural recipients receiving recombinant DNA, ii) potential synergistic, antagonistic or other effects with indigenous microorganisms, iii) possible effects of the GMM or any indigenous organism receiving and expressing the recombinant gene on humans, animals and plants, iv) risk of interference with ecosystem functions (e.g., biogeochemical processes).

- Characterisation of GMM receiving environments. The environments most likely to receive the GMM as a consequence of their intended application, waste disposal or accidental release should be indicated. In addition, neighbouring environments possibly receiving the GMM by dispersal should be considered. Quantities of GMM that may be released to the specific environments should also be considered.

- Potential of GMM to survive (persist) and proliferate in receiving environments. Information is needed about the ecological range of a GMM. The natural ecosystems of the species or its close relatives should be indicated. Information on the environmental material from which the parental strain or other members of the species have been isolated, or from the kind of materials it may be expected to be isolated, should be given. Physiological properties (pH range, temperature optimum, potential carbon and nitrogen sources, potential electron acceptors, requirements for growth factors, intrinsic antimicrobial resistances, stress resistance, etc.) that are known to allow the GMM to compete and survive in specific environments should be considered. This also includes the capacity of an organism to form survival structures (e.g. spores), or enter into a VBNC state.

- Risk assessment of interactions of GMM with chemical and biological factors in a given environment, including indigenous microorganisms, plants and animals. If environments in which the GMM can survive have been identified, it is important to assess potential GMM related effects on abiotic properties and interactions with other organisms. Relevant abiotic factors may include the pH value or concentrations of nutrients. Interactions with other microorganisms may include competition for nutrients and production of toxic compounds or other secondary metabolites. It should be analysed whether the GMM may displace other organisms and whether such a displacement would have effects on soil functions, e.g., degradation of organic material or pesticides, nutrient cycling, disappearance of plant growth promoting bacteria, or other beneficial microorganisms. If the GMM might cause adverse effects on other microorganisms or the disruption of vital ecological processes, it is essential to assess the consequences of these effects by providing data from appropriate case-specific studies designed to consider relevant ecological interactions.

- Consideration and evaluation of factors contributing to the degradation or stabilisation of recombinant DNA in relevant environments. As described for Category 3 above, this includes an evaluation of specific environmental conditions and how they affect the stability of the recombinant DNA (e.g., pH value, presence of DNases, adsorptive surfaces).
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• Consideration of mechanisms which may allow the GMM to transfer recombinant DNA to environmental microorganisms:

  o Prokaryotes: Conjugation, natural transformation and transduction are the three known gene transfer processes between bacteria and their consideration is relevant for evaluating the probabilities of gene transfer from GMM to environmental microorganisms. Genetic factors required for conjugation can be located both on plasmids and the chromosome. Host ranges of conjugative elements can vary considerably and should be taken into account to evaluate the potential environmental dissemination of the recombinant genes. Since conjugation is regarded as the most likely and efficient gene transfer mechanism, GMM with conjugative elements such as plasmids need a specific and very thorough analysis. Transduction, the bacteriophage-mediated process, normally occurs only within a narrow host range and is therefore probably only relevant for risk assessment of GMM which have close relatives in a receiving environment. Natural transformation requires the presence of competent recipient cells and, for recombination, homologous sequences in recipients (see above Category 3). Information about the likelihood that each of the possible gene transfer mechanisms would have for a GMM can therefore be derived from the molecular nature of the genetic modification, neighbouring genes, presence of plasmids and other mobile elements and information about the existence of relevant bacteriophages.

  o Eukaryotes: For fungi, protozoa and microalgae mating is usually the process by which gene transfer takes place. The genetic factors required for mating are located in the chromosomes. Mating takes place between haploid individuals of the same species with sexual compatibility, and involves cell fusion, karyogamy, recombination, meiosis and sporulation. Plasmids and other extranuclear genetic elements can be transferred during mating. On the other hand, some fungal species are able to transfer genetic material by parasexual mechanisms (heterokaryosis), involving cell fusion and mitotic cross-over without meiotic events. Hence, when the GMM is an eukaryote, its ability to mate or undergo heterokaryosis, and the possible presence of compatible individuals in the recipient environments should be determined. If the GMM is a diploid or a polyploid, its ability to form viable spores must be taken into account.

• Presence of potential recipients for recombinant DNA in relevant environments and assessment of the probability of horizontal gene transfer. The probability of horizontal gene transfer also correlates with the abundance and diversity of potential recipients in a given environment (see also requirements for Category 3). Depending on the suspected gene transfer mechanism, this analysis may focus on the presence of potential recipients for conjugation, on closely related species which may be accessible to phage released by the GMM, or the presence of microorganisms accessible by natural transformation.

• Consideration of environmental and health consequences of a potential horizontal gene transfer. This analysis should assess worst case scenarios that could result from any microorganism present in a specific environment acquiring a competitive advantage by expressing the traits encoded by the recombinant DNA of the GMM. This could be, for example, transfer from a non-pathogenic to a pathogenic microorganism, from non-persisting GM yeast to mycorrhizal fungi, or from a gut bacterium to a bacterium colonising plant roots or the gut of soil invertebrates, or to microorganisms contributing to important ecosystem functions in soils, e.g. by providing key enzymatic activities in the biogeochemical cycling of carbon or nitrogen.
• Effects of GMMs on plants. When appropriate, exposure of relevant plants to the GMM should be evaluated and potential harmful effects should be assessed. Furthermore, if appropriate, it should be assessed whether the GMM can stimulate the growth of certain plant species and affect their growth characteristics after transmission to a specific environment.

• Effects of GMMs on animals. When appropriate, exposure of relevant animals (including vertebrates and/or invertebrates) to the GMM and its products or derivatives should be evaluated, and potential harmful effects should be assessed.

C. EXPOSURE ASSESSMENT/CHARACTERISATION

The aim of the exposure assessment is the quantitative, semi-quantitative or qualitative evaluation of the likely exposure of humans, animals and the environment to a GMM and/or its products. With regard to humans, exposure assessment characterises the nature and size of the populations exposed to a source and the magnitude, frequency and duration of that exposure. For exposure assessment, it is necessary that every significant source of exposure is identified. In particular it is of interest to establish whether the intake of the food or feed consisting of, containing or produced from the GMMs is expected to differ from that of the conventional product which it may replace. In this respect specific attention will be paid to the GMM and/or derived products aimed at modifying the nutritional quality. Such products may require post-market monitoring to confirm the conclusion of the exposure assessment (see Section E).

D. RISK CHARACTERISATION

Risk characterisation is the quantitative, semi-quantitative or qualitative estimate, including attendant uncertainties, of the probability of occurrence and severity of adverse effect(s) or event(s) in a given population under defined conditions. It combines the outcomes of hazard identification, hazard characterisation and exposure assessment.

Risk characterisation should explain clearly what assumptions have been made during the risk assessment in order to predict the probability of occurrence and severity of adverse effect(s)/event(s) in a given population and/or on the environment. Any uncertainties inherent in the different stages of the risk assessment should be highlighted and quantified as much as possible. Estimation of uncertainties in experimental data should be handled by proper statistical analysis.

The conditions for the estimated risk, and associated uncertainties, should be as precise as possible. For instance, expressions like ‘no/negligible/acceptable/significant risk’ need, if possible, further numerical quantification in terms of probability of exposure and/or occurrence of adverse effects.

1. Issues to be considered

The risk characterisation of a GMM and its products is focused on the evaluation of all available evidence from hazard identification, hazard characterisation, and exposure/intake with respect to their safety and/or nutritional impact for humans/animals and the environment. The evidence includes the outcome from molecular analysis, microbiological and biochemical analysis (including antimicrobial susceptibility), compositional analysis, toxicity and allergenicity assessment, and environmental impact analysis with respect to potential adverse or nutritional effects of the GMM and/or its products.

Risk characterisation of GMMs should be carried out on a case-by-case basis depending on the Category of the GMM and/or product (see Chapter II), on the genetic modification, on the production process and on the expected use of the derived food or feed for human or animal consumption. Below a number of issues are described for consideration in the risk characterisation.
1.1. Information relating to the GMM

Evaluation of both the molecular characteristics and previous use of the recipient/parental and, when appropriate, of the donor organism is a key element to identify the need for specific analyses e.g. occurrence of specific metabolites in the recipient/parental microorganism which may be unintentionally increased as result of the genetic modification.

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

1.2. Information relating to the GM product

In the food safety assessment, applicants should take the following information into account: the compositional analysis, the molecular characteristics and mode of action of the protein expressed by the newly introduced gene(s) or the changes in metabolic pathways, the expected consumption of GM food (or feed) and, when applicable, toxicity studies and feeding trials. For intake estimations of foods derived from GMMs, the methodologies applied should be evaluated with respect to uncertainties associated with the prediction of long-term intake. Specific attention should be paid to those GM foods that are aimed at modifying nutritional quality or intended for specific consumer groups. Post-market monitoring should be especially considered when the GM food has an altered nutritional composition from the conventional food that it would substitute. If the performance of post-market monitoring is deemed necessary, the reliability, sensitivity and specificity of the proposed methods should be discussed.

The data generated should be evaluated with respect to:

- the expression of new proteins or presence of novel metabolites;
- significantly altered expression of original microbial proteins or levels of metabolites in GMM and their derived food.

If single constituents and/or whole GM food were found to induce adverse effects in specific studies, following information should be presented:

- dose response relationships, delayed onset of adverse effects;
- risks for certain groups in the population;
- use of uncertainty factors in extrapolation of animal data to humans.

The characteristics of the novel or altered compound(s), including potential biological effects in humans should be considered, including the effects of the processing (e.g. potential accumulation/depletion in food). Reference values, if defined, for acceptable or tolerable levels of intake, such as Acceptable Daily Intake (ADI) or Tolerable Upper Intake Level (TUL), should be considered in relation to the anticipated intake. In cases where the compound has a history of safe use in food, the intake levels from a conventional diet can implicitly be considered as acceptable.

Data provided to assess the allergenic potential of protein expressed by the newly introduced gene in GMMs must be evaluated with respect to a possible induction of allergy or provocation of allergic reactions in susceptible individuals.
1.3. Environmental impact

Predicting impacts of GMMs and derived food or feed on complex ecosystems can be difficult due to continuous flux and spatial heterogeneities in ecosystems creating a myriad of potential microbial habitats in which interactions between GMMs and their products with the indigenous organisms and other abiotic components can take place. It is recognised that an environmental risk assessment cannot provide data of a GMM or their products which would cover all potential environmental habitats and conditions. Consideration of environmental impact (damage) should therefore focus on environments in which exposure is most likely or in which, when relevant, viable GMMs could potentially proliferate. The likelihood of transmission of viable GMMs in the environment and their survival and persistence, as well as the possibility of transfer of recombinant DNA to other organisms are the key points to be considered. Experience with the introductions of other microorganisms or GMMs of similar properties (species, ecophysiology, genetic modification) and an understanding of the resilience of the relevant ecosystems can be helpful for estimating the environmental impact. For specific products with no viable GMMs or recombinant DNA, risk estimates may be based on knowledge about the environmental impact of comparable non-GM products.

The assessment of environmental impacts is not finished with the approval of a GMM or their product (as relevant for this guidance document). It is a crucial component of the required post-market monitoring. In addition to monitoring, environmental impact analysis also requires a continuous consideration of new knowledge provided by the scientific literature in order to anticipate potential novel environmental risks and/or modify recommendations for risk management measures.

2. Conclusions from the risk characterisation of GMMs and derived food/feed

The risk characterisation must conclude on:

- whether placing on the market of a GMM and its derived products is safe for the environment;
- whether consumption of food or feed derived from GMMs is safe for humans or animals.

The conclusions should explain the assumptions made during the risk assessment and the nature and magnitude of uncertainties associated with establishing these risks.

The risk assessment may identify issues that require management. In those cases, risk management strategies should be proposed considering the scientific basis of the different options (e.g. for post-market monitoring).

E. POST-MARKET MONITORING REGARDING USE OF THE GM PRODUCT FOR FOOD OR FEED

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.

Post market monitoring (PMM) is a risk management tool which allows the evaluation of uncertainties identified during the risk assessment. Such uncertainties may relate to the likelihood or severity of a hazard, or to long-term effects of such a hazard. This PMM should be case specific. A PMM should address the following questions (Hepburn et al., 2008):

- is the use of the product as predicted or recommended?
- are known effects and side-effects as predicted?
- does the product induce unexpected side-effects?
The applicant should submit a proposal for PMM when uncertainties exist with respect to the relevance and intensity of effects and side-effects identified during the pre-market risk assessment are difficult to predict. The applicant should also consider the submission of a proposal for a PMM when the GM food has an altered nutritional composition from the conventional food that it would replace.

The applicant should submit specific data collection strategies, when estimations of individual intakes of a specific GM product or intakes of any particular consumer group have to be made. The applicant should ensure that any such information shall indicate as whether any adverse health effects may be related to GM food consumption.

F. POST-MARKET ENVIRONMENTAL MONITORING (PMM) OF GM PRODUCTS

1. General

Regulation (EC) No 1829/2003 introduces an obligation on applicants to implement a GMO monitoring plan for Environmental Monitoring according to Annex VII of the Directive 2001/18/EC (Regulation (EC) No 1829/2003 Art. 5(5)(b) and Art 17(5)(b)) and a proposal for the PMM regarding use of the food and feed for human and animal consumption (Regulation (EC) No 1829/2003 Art. 5(3)(k) and Art. 17(3)(k)). The latter is not described in any detail in the Regulation (EC) No 1829/2003. Section E of this Chapter refers to the PMM of GM food or feed.

In reference to Directive 2001/18/EC, the post-market environmental monitoring is introduced in order to identify any direct or indirect, immediate and/or delayed adverse effects of GMOs, their products and their management to human health or the environment, after the GMO has been placed on the market.

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

A PMEM is required for applications for placing on the market of GMOs or food or feed containing or consisting of GMOs conforming with Annex VII to Directive 2001/18/EC. As a consequence, GMM products under Category 4 need to comply with the Guidance notes supplementing Annex VII, which explain that the extent of the market release shall be taken into account. GMM products under Category 3 should be considered for monitoring for environmental risks identified (similar to Case-specific monitoring, see Section F.2) with appropriate components as laid out in Annex VII (consequences of horizontal gene transfer). GMM products under categories 1 and 2 do not need PMEM.

Monitoring may be defined as the systematic measurement of variables and processes over time and it assumes that there are specific reasons to collect such data, for example, to ensure that certain standards or conditions are being met or to examine potential changes with respect to certain baselines. Against this background, it is essential to identify the type of effects or variables to be monitored, an appropriate time-period for measurements and, importantly, the tools and systems to measure them. Monitoring results, however, may lead to adjustments of certain parts of the original monitoring plan, or may be important in the development of further research. This Guidance document provides further assistance in the following Sections.

The PMEM of the GMM (Category 4) will have two focuses: (1) the possible effects of the GMM, identified in the formal risk assessment procedure, and (2) identification of the occurrence of adverse unforeseen effects of the GMM or its use that were not anticipated in the environmental risk assessment. When there is scientific evidence of a potential adverse effect linked to the genetic modification, then case-specific monitoring should be carried out after placing on the market, in order to confirm the assumptions of the environmental risk assessment. Consequently, case-specific
monitoring is not obligatory and is only required to verify the risk assessment, whereas a general
surveillance plan must be part of the application. Applicants who are proposing to have no case-
specific monitoring are encouraged to provide arguments in support of this position. These arguments
should relate to the assumptions and conclusions applicants have made in the environmental risk
assessment.

Monitoring of potential adverse cumulative long-term effects is an important objective of monitoring.
Potential adverse cumulative and/or long-term effects of the GMM identified in the risk assessment
should be considered initially within case-specific monitoring.

2. Case-specific GM monitoring

The main objective of case-specific monitoring is to determine the significance of any potential
adverse effect identified in the risk assessment (see Section D.). The assessment of risk should be
based on Annex II of the Directive 2001/18/EC.

Case-specific monitoring should be targeted at those environmental factors most likely to be adversely
affected by the GMM that were identified in the environmental risk assessment. The scientific
approach should be designed in order to test the specific hypothesis of potential adverse effects
derived from the environmental risk assessment. In order to monitor potential risks identified in the
risk assessment, environmental hotspots may be identified, in which the effect is most likely to occur.
The monitoring should consist of the systematic recording of relevant parameters at representative
locations and hotspots. The methods selected, the duration of the monitoring, the extent and the
parameters to be monitored should be determined on a case-by-case basis.

3. General surveillance of the impact of the GMM

General surveillance is always routinely applied even in circumstances in which no adverse effect has
been identified in the risk assessment. It is required in order to detect unforeseen or unanticipated
adverse effects.

A major challenge of general surveillance is determining whether:

i. an observed effect is unusual;

ii. an unusual effect is adverse; and

iii. the adverse effect is associated with the GMM or its use.

The use of a range of monitoring systems to supply data and the ability to compare data from these
different sources will help to indicate whether an effect is unusual and adverse. The identification of a
novel adverse effect (after step ii) would trigger the need for a specific study to evaluate harm and
determine cause in relation to step iii.

3.1. Approach and principles

The objective of general surveillance is to identify the occurrence of unforeseen adverse effects of the
GMM or its use on human and animal health and the environment that were not predicted in the risk
assessment. An effect is defined as a difference that is outside the normal variation expected in a
particular environment and it should be determined whether the effect could be adverse.

In many cases, unforeseen effects of a GMM can only be addressed by looking at general aspects
(such as ecosystem functioning on a broad scale). It will be impossible to address all receiving
environments, and therefore the applicant should focus on those environments where the exposure is
greatest. The applicant needs to consider assessing possible changes in ecosystem functioning and
provide a strategy to detect these changes.
General surveillance plans should be developed for all GMMs that have the potential to enter and survive in the environment. Existing surveillance systems should be used where practical (e.g. routine recording systems), and any ‘unusual’ observations, not occurring in similar reference situations, should be recorded.

The establishment and persistence of a GMM is not an environmental hazard in itself. Similarly, dispersal and transfer of the recombinant genes to other organisms *per se* are not hazards and the focus of general surveillance should be on recording any unanticipated consequences of the GMM establishment and spread. Thus, an evaluation of the potential receiving environments and the exposure will be a good starting point in any general surveillance plan.

General surveillance should be conducted using robust science based strategies and methodologies. This especially refers to defining sample sizes, sampling and recording methods, in order to produce statistically valid data for relating causes and effects.

Background and baseline data, e.g. relevant environmental parameters, climatic conditions, general application management data should be collected, when appropriate, to permit the assessment of the relevant parameters. If unusual observations on human or animal health and the environment are reported, more focussed in-depth studies should be undertaken in order to determine cause and relationship with the GMM.

The methods and approaches for the monitoring of unforeseen adverse effects of the GMM and its use for human health and the environment should be appropriate, proportionate and cost-effective.

### 3.2. Main elements of General Surveillance

The applicant should:

- define the methods and approaches that will be used to conduct general surveillance;
- refer to use and possible spread of the GMM;
- make proposals for the time, environments addressed, and the frequency of monitoring.

### 4. Monitoring systems

General surveillance with respect to the use and handling of GM products and/or GMMs could, when compatible, make use of established surveillance practices (e.g. industry health monitoring systems). Use of an existing monitoring system just because it exists might not always be appropriate, and in many cases, it will be very difficult to relate observed effects to the release of a GMM.

In addition to existing monitoring networks, applicants are encouraged to develop new and more focused monitoring systems e.g. by questionnaires. In some cases user surveys might be a useful approach to collecting first hand data on the impact of a GMM on receiving environments. There should be emphasis on the statistical design and representativeness of these surveys.

At present there are no suitable large-scale surveillance and monitoring systems suitable for the identification of possible adverse health effects posed by the handling or use of GMMs in humans. Experience in designing surveys and their statistical analysis is available from other established surveillance and monitoring systems (e.g. those used for consumer and pharmaceutical surveillance systems).
5. Reporting the results of monitoring

Following placement on the market of a GMM, the applicant has a legal obligation to ensure that monitoring and reporting are carried out according to the conditions specified in the consent. The applicant is responsible for submitting the monitoring reports to the Commission, the competent authorities of the Member States, and when appropriate to EFSA. Applicants should describe the methods, frequency and timing of reporting in their monitoring plan. Applicants are requested to comply with Council Decision 2009/770/EC\(^{36}\) concerning the reporting format.

Figure 1: Flow diagram showing the approach to the categorisation and risk assessment

- Information relating to the GMM (Section B.1.)
- Information relating to the production process (Section B.2.1.)
- Information relating to the product preparation process (Section B.2.2.)
- Description of the product (Section B.2.3.)
- Considerations of the GM product for human health (Section B.2.4.)
- Environmental risk assessment (ERA) of GMM-derived products belonging to categories 1 and 2 (Section B.3.1.)
- ERA of products consisting of or containing GMMs not capable of replication, or products which contain newly introduced genes (Category 3) (Section B.3.2.)
- Environmental risk assessment of products consisting of or containing GMMs capable of replication or of transferring genetic material (Section B.3.3.)
- Post-market monitoring regarding use of the GM product for food or feed (Section E)
- Post-market environmental monitoring of GM products (Section F)
**G. SUMMARY OF THE RISK ASSESSMENT REQUIREMENTS**

A summary of the information required for applications for the placing of GMMs and their derived food and feed products on the market is provided in Table 1.

This table contains the items required to the risk assessment of GMMs and derived food and feed products according to Chapter III, with cross-references to the different sections of the text. It provides a simple and immediate list of the requirements for an application. However, the applicant should always refer to the main text of this guidance to address the requirements for the submission of an application in sufficient detail.

**Table 1: Information required for applications for the placing of GMMs and their derived food and feed products**

<table>
<thead>
<tr>
<th>Characteristics of the recipient or parental microorganism</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
<th>Chapter, Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Scientific name, taxonomy and other names</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>III. B.1.1.1.</td>
</tr>
<tr>
<td>2. Phenotypic and genetic markers</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>III. B.1.1.2.</td>
</tr>
<tr>
<td>3. Degree of relatedness between recipient and donor(s), Description of identification and detection techniques</td>
<td>X(a)</td>
<td>X(a)</td>
<td>X(a)</td>
<td></td>
<td>III. B.1.1.3.</td>
</tr>
<tr>
<td>4. Source and natural habitat of the recipient microorganism</td>
<td>X(b)</td>
<td>X(b)</td>
<td>X(b)</td>
<td></td>
<td>III. B.1.1.4.</td>
</tr>
<tr>
<td>5. Organisms with which transfer of genetic material is known to occur under natural conditions and presence of indigenous genetic mobile elements</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>III. B.1.1.5.</td>
</tr>
<tr>
<td>7. Pathogenicity, ecological and physiological traits</td>
<td>X(b)</td>
<td>X(b)</td>
<td>X(b)</td>
<td></td>
<td>III. B.1.1.7.</td>
</tr>
<tr>
<td>8. Description of its history of use</td>
<td>X(b)</td>
<td>X(b)</td>
<td>X(b)</td>
<td></td>
<td>III. B.1.1.8.</td>
</tr>
</tbody>
</table>

---

\(a\) Information not required in case of self-cloning with the same strain  
\(b\) Information not required if proposed QPS status is authorised
## Characteristics of the origin of the inserted sequences (donor organism(s))

<table>
<thead>
<tr>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
<th>Chapter, Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. DNA from defined donor organisms</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2. Synthetic DNA</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>3. Nucleic acids directly extracted from environmental samples</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

## Description of the genetic modification

<table>
<thead>
<tr>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
<th>Chapter, Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Characteristics of the vector</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2. Information relating to the genetic modification</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

## Information relating to the GMM and comparison of the GMM with its appropriate comparator

<table>
<thead>
<tr>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
<th>Chapter, Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Description of the genetic trait(s) or phenotypic characteristics and any new trait which can be expressed or no longer expressed</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2. Structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified microorganism</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>3. Stability of the microorganism in terms of genetic traits</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>4. Rate and level of expression of the new genetic material and activity of the expressed proteins</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>5. Description of identification and detection techniques</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>6. Information on the ability to transfer genetic material to other organisms</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>7. History of previous uses or environmental releases of the GMM</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>8. Safety for humans and animals</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

## Information relating to the production process

<table>
<thead>
<tr>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
<th>Chapter, Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>III. B.2.1.</td>
</tr>
</tbody>
</table>
## Guidance on the risk assessment of GMMs and their food and feed products

<table>
<thead>
<tr>
<th>Information relating to the product preparation process</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
<th>Chapter, Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Information on the removal of the GMM cells from the product</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>III. B.2.2.1.</td>
</tr>
<tr>
<td>2. Information on the inactivation of the GMM cells and assessment of the presence of remaining physically intact cells</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>III. B.2.2.2.</td>
</tr>
<tr>
<td>3. Information on the possible presence of recombinant DNA</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>III. B.2.2.3.</td>
</tr>
</tbody>
</table>

### Description of the product

<table>
<thead>
<tr>
<th>Description of the product</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
<th>Chapter, Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Designation of the product</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>III. B.2.3.1.</td>
</tr>
<tr>
<td>2. Intended use and mode of action</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>III. B.2.3.2.</td>
</tr>
<tr>
<td>3. Composition</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>III. B.2.3.3.</td>
</tr>
<tr>
<td>4. Physical properties</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>III. B.2.3.4.</td>
</tr>
<tr>
<td>5. Technological properties</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>III. B.2.3.5.</td>
</tr>
</tbody>
</table>

### Considerations of the GM product for human health

<table>
<thead>
<tr>
<th>Considerations of the GM product for human health</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
<th>Chapter, Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Toxicology</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>III. B.2.4.1.</td>
</tr>
<tr>
<td>2. Risk assessment of proteins expressed by the newly introduced genes</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>III. B.2.4.2.</td>
</tr>
<tr>
<td>3. Testing of new constituents other than proteins</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>III. B.2.4.3.</td>
</tr>
<tr>
<td>4. Testing of the whole GM product</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>III. B.2.4.4.</td>
</tr>
<tr>
<td>5. Allergenicity</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>III. B.2.4.5.</td>
</tr>
</tbody>
</table>

### Environmental risk assessment (ERA) of GMM-derived products belonging to categories 1 and 2

<table>
<thead>
<tr>
<th>Environmental risk assessment (ERA) of GMM-derived products belonging to categories 1 and 2</th>
<th>Category 1</th>
<th>Category 2</th>
<th>Chapter, Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>X</td>
<td></td>
<td>III. B.3.1.</td>
</tr>
</tbody>
</table>

### ERA of products produced from GMMs in which GMMs capable of replication or of transferring newly introduced genes are not present; but in which newly introduced genes are still present (Category 3)

<table>
<thead>
<tr>
<th>ERA of products produced from GMMs in which GMMs capable of replication or of transferring newly introduced genes are not present; but in which newly introduced genes are still present (Category 3)</th>
<th>Category 1</th>
<th>Chapter, Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td></td>
<td>III. B.3.2.</td>
</tr>
</tbody>
</table>

---

c Only for food containing, consisting of or produced from GMMs falling under Regulation (EC) No 1829/2003 and under III.B.2.1. to III.B.2.4. of the present document (see Section III.B.2.).
### Guidance on the risk assessment of GMMs and their food and feed products

<table>
<thead>
<tr>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
<th>Category 4</th>
<th>Chapter, Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERA of products consisting of or containing GMMs capable of replication or of transferring genetic material</td>
<td></td>
<td></td>
<td>X</td>
<td>III. B.3.3.</td>
</tr>
<tr>
<td>Post-market monitoring regarding use of the GM product for food or feed</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>III. E.</td>
</tr>
<tr>
<td>Post-market environmental monitoring of GM products</td>
<td>X</td>
<td>X</td>
<td></td>
<td>III. F.</td>
</tr>
</tbody>
</table>
REFERENCES

ACRE (Advisory Committee on Releases to the Environment), 2004. Guidance on Best Practice in the Design of Post-market monitoring Plans in Submissions to the Advisory Committee on Releases to the Environment. ACRE Guidance Note 16.


Guidance on the risk assessment of GMMs and their food and feed products


GLOSSARY AND ABBREVIATIONS

AD1 Acceptable Daily Intake
EC European Commission
EFSA European Food Safety Authority
ERA Environmental Risk Assessment
GLP Good Laboratory Practice
GM Genetically-Modified
GMM Genetically-Modified Micro Organism
GMO Genetically-Modified Organism
ORF Open Reading Frame
PCR Polymerase Chain Reaction
PMM Post-Market Monitoring
QPS Qualified Presumption of Safety
SCF Scientific Committee on Foods
TUL Tolerable Upper Intake Level
VBNC Viable But Non-Culturable

Adjuvant: Substances that, when co-administered with antigens, increase the immune response to the antigen and therefore might also increase an allergic response.

Allergy: An adverse reaction directed against substances foreign to the body, which is mediated by the immune system.

Antibiotic: A substance produced by, or derived from a microorganism, that selectively destroys or inhibits the growth of other microorganisms.

Antimicrobial: An active substance of synthetic or natural origin which destroys microorganisms, suppresses their growth or their ability to reproduce in animals or humans, excluding antivirals and antiparasites.

Contained use: any activity in which microorganisms are genetically modified or in which such GMMs are cultured, stored, transported, destroyed, disposed of or used in any other way, and for which specific containment measures are used to limit their contact with, and to provide a high level of safety for, the general population and the environment.
**Deliberate release:** Any intentional introduction into the environment of a GMO or a combination of GMOs for which no specific containment measures are used to limit their contact with and to provide a high level of safety for the general population and the environment.

**Food and feed containing or consisting of GMMs:** Food and feed in which GMMs capable of replication or of transferring genetic material genes are present.

**Food and feed produced from GMMs:** Food and feed (including food and feed ingredients such as additives, flavourings and vitamins) derived, in whole or in part, from GMMs, but not containing or consisting of GMMs.

**Food and feed produced with GMMs:** Food and feed (including food and feed ingredients such as additives, flavourings and vitamins) produced by fermentation using a GMM which is kept under contained conditions and is not present in the final product.

**Genetically modified organisms:** (Micro)organisms in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.

**Hazard:** A biological agent in a particular Category or sub-Category of GMMs or their products with the potential to cause adverse health or environmental effects.

**Hazard identification:** The identification of biological agents capable of causing adverse health or environmental effects and which may be present in a particular Category or sub-Category of GMMs or their products.

**Intended effects:** Genotypic and phenotypic changes that are targeted to occur due to the introduction or inactivation of gene(s) or DNA sequences, and that fulfil the objectives of the genetic modification.

**Microorganisms:** Any microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material, including viruses, viroids, animal and plant cells in culture. For the purpose of this guidance document, microorganisms cover archaea, bacteria and eukarya. Eukarya includes filamentous fungi, yeasts, protozoa and microalgae.

**Organism:** Any biological entity capable of replication or of actively transferring genetic material.

**Post-market monitoring:** A risk management tool that provides a mechanism to monitor possible untoward consequences of the GM product included in the risk assessment.

**Prokaryotes:** A group of organisms that lack a cell nucleus, or any other membrane-bound organelles. Prokaryotes encompass all bacteria and archaea.

**Recombinant DNA:** A form of DNA that is created by combining two or more sequences that would not normally occur together.

**Recombinant gene:** A gene that is constructed from two or more sequences that would not normally occur together.

**Risk:** A function of the probability of an adverse health or environmental effect and the severity of that effect, consequential to a hazard(s) in GMMs or their products.

**Southern analysis:** Use of a DNA probe to identify, by complementarity, DNA blotted on membranes.

**Unintended effects:** Unintended consistent phenotypic changes in the GMM resulting from its genetic modification.
Categorisation of risk assessment of GMMs and their derived products

Category 1: Chemically defined purified compounds and their mixtures produced with GMMs in which both GMMs and newly introduced genes have been removed. (e.g. amino acids, vitamins);

Category 2: Complex products produced
- with GMMs in which both GMMs and newly introduced genes have been removed (e.g. most enzyme preparations); or
- from GMMs in which both GMMs and newly introduced genes are no longer present (e.g. cell extracts);

Category 3: Products produced from GMMs in which GMMs capable of replication or of transferring newly introduced genes are not present; but in which newly introduced genes are still present (e.g. heat-inactivated starter cultures);

Category 4: Products consisting of or containing GMMs capable of replication or of transferring newly introduced genes.